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血液事業部会運営委員会･安全技術調査会合同委員会提出資料

参考資料4 (BCT Japan株式会社)

文献1

MIRASOL®

病原体不活化技術：

Mirasol PRT システム

A Novel Approach to Blood Safety

前書き

海外旅行の増加は、通常の生活環境を越えて病原体の広まりを生じさせている。例えば、FDA がシャーガス病に対する初めてのスクリーニング検査を認可してから 10 ヶ月で、米国で採血された 241 件の血液はクルーズ・トリパノソーマに対し陽性であり、献血者が病原体に晒されたことを示唆していた。これは、西ナイル・ウィルスなどの新しい病原体の出現と共に、血液供給の安全性に対するリスクの増大を示している。

人々は、当然、感染症リスクの高い輸血は受けたくないと要求する。それにより、病原体不活化技術などのより改善された血液安全対策の実施に、政府と行政当局側が積極的に資金を提供するよう促している。

Gambro BCT の完全所有子会社である Navigant Biotechnologies LLC 社は、献血血液製剤の質を改善する仕事が委託されている。Navigant Biotechnologies LLC 社によって開発された Mirasol®PRT 装置は、献血血液製剤中の病原体と残存白血球を不活化するためにリボフラビンと紫外線を利用している。この装置は広範囲において試験され、信頼できる機関によって科学的に実証されている。新しい病原体が血液供給中に導入されることは無く、処理過程は安全、簡単、効果的である。

本論文より、Mirasol PRT 装置の安全性と有効性を担保する重要なデータの概観を示す。

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第 1 章

病原体不活化に関する背景情報：技術とドライバー

MIRASOL®

病原体不活化技術

病原体不活化に関する背景：技術とドライバー

Mirasol病原体不活化技術(PRT)装置は、輸血用の献血血液成分中の病原体負荷を減らし、残存白血球の不活化を意図して設計されている。本Mirasol装置は紫外線(UV)とリボフラビン（ビタミンB2）に基づく技術を応用している。血小板用のMirasol PRT装置はCEマークを受け、市場導入された最初のMirasol製品である。同じ技術は、現在、血漿と白血球適用に向けて開発中である。

Mirasol PRT 装置

血小板用の Mirasol PRT 装置は、再使用可能な照射器、および滅菌リボフラビン溶液バッグと滅菌照射・保存バッグを含むディスポーザブルセットからなる。処理の間、血液成分は照射・保存バッグに送られ、リボフラビン溶液が添加された後、照射器に移される。照射器は、マイクロプロセッサー制御式の電気機械装置であり、照射及び、血小板成分とリボフラビンの混合を調整する。照射器はまた、血小板の質が確実に維持されるように温度をモニターし調整する。照射中、血液成分は、その量にもよるが、約 6 分間から 10 分間紫外線にさらされるが、照射中の光エネルギーは 6.2 J/mL である。照射完了後、処理した血液成分は保存され、血液バンクにより管理される。

血液の安全性の簡単な歴史

歴史的に、輸血関連の肝炎のリスクはずっと以前から分かっていたことだが、ヒト免疫不全ウィルス(HIV)の出現までは、厳しい血液の安全対策が導入されることはなかった。1981 年のエイズの最初の症例の発症から数年以内に、この疾患は血液由来のウィルスが病因であることが明らかになったが、その時すでに数千人の人々が感染していた。血友病患者は、第Ⅷ因子製剤（当時は数 1,000 単位のヒト血漿を貯留して製造していた）の定期的な注射が必要であったことから、特に大きな被害を受け、米国の多数の血友病患者が HIV に感染した。

HIV の最初の血清（抗体）検査は 1985 年血液バンクによって実施されたが、献血血液や血液成分に未知の病原体が混入しているかもしれないという知識は輸血の安全性に対する態度を著しく変化させた。これらの懸念に対処するため、輸血業務では様々な方法が用いられている。

HIV 感染は、血液に由来する病原体からの血液成分の危険性を際立たせた。

血液供給の安全性の向上のための現在のアプローチ

献血者の選択

献血者の選択は、通常、血液バンクのスタッフによる面接や質問表に基づいており、伝染性病原体のキャリアーであるリスクが高い人（例えば高リスクの性的行為を行った人、静

脈麻薬を使用した人、マラリアなどの風土病が流行する地域へ旅行し、検査を受けていない人）からの献血を受け付けないことを目的としている。

過去 20 年間、この献血者の選択は汚染献血件数を減らすのに最も有効な手段であった。しかし、この方法は、献血者が除外基準を無視したり理解できないようであれば役に立たない。

血清検査と核酸増幅テスト

次の防御方法は、血清検査および／または核酸増幅テスト(NAT)による病原体についての献血血液の検査である。血清検査のルーチン使用は輸血による HIV 感染症の発症率を有意に減少させた。1990 年代後半での NAT の導入はさらに検査の精度を向上させ、潜伏期間（感染してから､検出可能な抗体またはウィルスマーカーの出現までの時間）を 22 日から 13-15 日に減少させた。しかし、検査により実質的にリスクは減少したものの、特に複数回輸血を受けた患者の、輸血を介した感染症など限定的リスクは、依然として存在する。

細菌検出

ウィルスの脅威にさらされる危険性があるにもかかわらず、（先進国における）輸血による最大の伝染性疾病リスクに細菌性敗血症がある。血小板および赤血球の細菌汚染の発生率は輸血血液 3,000 単位当たりで約 1 単位であり､また 25,000 回の血小板輸血当たり約 1 回、25,000 回の赤血球輸血当たり 1 回は、輸血に関連した敗血症を発症している。現在までに行われた、骨髄移植を受けた患者の血小板輸血に関する唯一のプロスペクティブ研究は、16 人中 1 人、輸血症例 350 症例中 1 症例、血小板 2000 単位中 1 単位に症候性菌血症のリスクがあったことを示した。これらの発症頻度は、毎年米国だけで 9 百万単位以上の血小板濃厚液が輸血されていることを考えると、無視できない値である。このリスクを減らすためには様々手段、細菌感染のリスクを考慮するような献血者選択基準を修正することや、採取した血液の処理段階および白血球不活化処理の最中に、一般向けおよび特定対象向けに衛生対策を実施することが含まれる。しかし、細菌性敗血症のリスクを減じる目的で最も広く適用されている方法は細菌検出であり、この方法によって血小板製剤は、検査結果が陰性であるということに基づき、市場に供給される。

白血球不活化

多数の国で実施されている一般的な白血球不活化処理の適用は、輸血の安全性に大きな影響を与え､ヒト白血球抗原(HLA)免疫化の発症率と患者の有害反応の発症率を劇的に減少させた。さらに、これにより細胞由来感染性病原体の伝染を減らしている。

ガンマ線照射

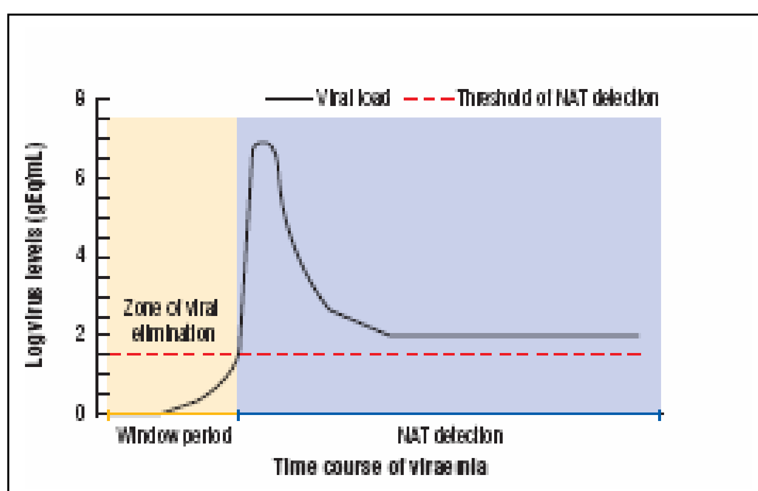
ガンマ線照射は、稀ではあるが、非常に重度の輸血合併症（例えば、同種造血幹細胞移植レシピエントなどの重度免疫障害患者で見られるが、HLA 単一同一血液成分を投与された非免疫障害患者の一部にも生じる免疫輸血関連移植片対宿主病(TA-GVHD)）を予防するのに適用されている。

血液の安全性の満たされていない要求

血液の安全性に対する現在の重層化介入アプローチは、血液成分のウィルス汚染のリスクを顕著に減少させた。しかし、細菌汚染のリスクと新しい病原体の予期せぬ出現は、未だに主要な懸念事項である。スクリーニングと検査では潜在的なヒト病原体を全て除外することはできない。血液供給の安全性を向上させるために積極的な対策を講じることが必要である。

現在の血液の安全性手順により血液と血液製剤はより安全なものとなってきたが、リスクはまだ存在している。

図 1. 核酸増幅検査（NAT）の有用性と限界



核酸増幅テストの限界

輸血による感染症の全体的リスクは低いが、有意なリスクはまだ存在している。血清学的検査は 3 週間以上の潜伏期間のために限界がある。NAT はこのリスク期間を減らしたが、それを取り除いたわけではない（図 1 参照）。さらに、NAT は少量の病原体でさえ検出することができるが、それは特異的な病原体に対してのみである。HIV、B 型肝炎ウィルス(HBV)、C 型肝炎ウィルス(HCV)などの良く知られたウィルスはルーチンに検査されているが、一連の検査に挙げられていないウィルスのリスクについて検査することは不可能である。新し

い検査法を開発することは、費用がかかり時間が要る。例えば、新しい病原体の確認から適切な検査のルーチン化までには3年かかる。さらに、検査がもっと迅速に開発できても、早期の低ウィルス負荷のウィルスはまだ検出できないかもしれない。NATは費用がかかり、比較的裕福な西洋諸国でもその費用効果性に関してまだ議論の余地がある。

新しく出現した病原体と未知の病原体のリスク

血液バンクは、血液供給の安全性に対するリスクの出現・再出現について益々慎重になってきているが、検査は、事前の策を講じることではなく、事後の処理であり、未知の病原体を検査することは不可能である。新しい病原体が確認される時までには、血液供給がすでに汚染されてしまっているかもしれない。残念ながら、そのような病原体は多くある。西ナイル・ウィルス(WNV)、クルーズ・トリパノソーマ、プラスモジウム spp、バベシア spp、パルボ・ウィルス B19、デングウィルス、変異型クロイツフェルト・ヤコブ病(vCJD)の病因となるプリオンが含まれる。ヒト・ヘルペス・ウィルス8(カポシ肉腫)、ボレリア(ライム病)、鳥インフルエンザ・ウィルスなどの他の病原体も輸血で伝染するかもしれない。

新たな病原体の危険性は、蚊由来のウィルスの顕著な地理的な広がりによって説明されている。米国で WNV によって生じた重度の神経学的疾患の発生はよく知られているが、それに加えて、以前アフリカやアジアでのみ生じたチキングニアウィルスがイタリアで2007年 9 月、突発的に発生した。血液供給経路に侵入する新生病原体や再出現病原のリスクを最小限にするために、新しい積極的な対策が必要である。

細菌検査の限界

細菌検査も完全であると言えるにはほど遠い。最初に陰性を検査する血小板の培養は、長期保存で陽性を検査できるが、それは細菌のスクリーニングが輸血由来の全ての細菌感染症を防げるわけではないことを示している。細菌検出法は血小板単位の検査合格リリースの遅れと保存期限の短縮を生じさせ、結果的に期限切れの率を増加させている。さらに、血液成分の細菌検査が陽性である場合、それはしばしばすでにリリースされて輸血されてしまっている。偽陰性の血小板もまだ生じており、致死的な敗血症の発症の原因になっている。

白血球に対する挑戦

白血球不活化法は、残存白血球と関連合併症のリスクを完全には取り除くことはできず、サイトメガロウィルス(CMV)とヒトT細胞好リンパ球ウィルス(HTLV)などの細胞関連ウィルスや潜在的に致死的な免疫輸血関連移植片対宿主病(TA-GVHD)からのリスクに抵抗力の無い患者をさらしてしまう。

血液の安全性を向上させるため、より良い技術が早急に必要である。

病原体不活化技術の見込み

病原体不活化は、血液成分輸血の安全性の向上に対する別の方法である。病原体不活化の優位性は溶剤洗浄処理の血漿分画の処理で示されてきた。しかし、この特別の処理はエンベロープを持たないウィルス（A 型肝炎ウィルスなど）には効果が無い。既知及び未知の病原体に対する広範囲に有効な病原体不活化処理は、血液の安全性の現在と将来の要求を満たすだろう。しかし、血液の安全性の向上には相当の代償を払う必要がある。PRT 技術の適用に要する費用には、ハードウエアと必要物品、他のバッグへの移し変えと混合物吸収装置の除去処理による血液成分抽出減少の可能性、より少ない修正カウント増加(CCI)や血漿タンパクで観察される成分の質、臨床的効果（より高い耐熱性や赤血球利用）への出費が含まれる。PRT 技術を適用する前に、この装置の性能と対費用効果は、特定の PRT 技術を適用することの全体的有益性が全般的経費を確実に超えないようにするために評価されなければならない（第 9 章参照）。

病原体不活化技術は、現在と将来の病原体への危険に対する保護となることによって血液供給の安全性をさらに増強し得る。

要約

- HIV 感染症は、血液由来病原体からの血液成分への危険性を際立たせた。
- 現在の血液安全性手順により血液と血液成分はより安全なものになってきたが、リスクはまだ存在している。
- 血液の安全性を向上させるため、より良い技術が早急に必要である。
  －NAT は全潜伏期間をなくす事ができない（異なる検査法には同じウィルスについて異なる潜伏期間がある）。
  －新生病原体はいつも危険となる。
  －細菌検査は細菌感染症の伝染を防ぐのに 100%効果があるわけではない。
  －残存白血球はまだ重度の有害反応を生じ得る。
- 病原体不活化技術は、現在及び将来の危険に対する保護となることによって血液供給の安全性をさらに増強し得る。

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第 2 章

Mirasol：プロセス

MIRASOL®

病原体不活化技術

Mirasol：プロセス

Mirasol 病原体不活化技術（PRT）は、光と自然界に存在するリボフラビン（ビタミン B2）を用いて血液製剤中の病原体を除去し、白血球を不活化する技術である。リボフラビンは紫外線下で核酸と反応し、不可逆的な変化を引き起こす光増感剤として用いられる。なお、リボフラビンは薬剤ではなく、薬用効果を意図して導入される訳ではない。本技術は 40 年以上もの研究を経て開発されたものであり、化学、毒物学、体外および体内でのリボフラビンの核酸への光増感反応に関する膨大なデータ蓄積に基づいている。

リボフラビン

人体における役割

リボフラビンは人体に不可欠な栄養素で、牛乳、卵、パン、葉野菜など、多くの食材に含まれている（図 1)。平均的な成人の 1 日当たり推奨摂取量は 1.3 mg [男性]／1.1 mg [女性]（授乳中の女性は最大で 1.6 mg)。そうした栄養価と毒性に関する網羅的データの蓄積も、血液製剤への利用にとって好都合である。

リボフラビンは、数多くの生化学反応に係る重要なコエンザイムの前駆体となっている。遺伝毒性が無いことは体外で実証済みで、米国食品医薬品局（FDA）により「一般に安全と認められる物質（GRAS 物質)」に分類されている。

リボフラビンは人体の生化学プロセスにおいて重要な役割を果たす、必須ビタミンである。

リボフラビンの光化学反応

リボフラビンの光増感性が最初に発見されたのは 1932 年、Warburg 博士と Christian 博士による。リボフラビンによる核酸の光増感反応は 1976 年、Speck 博士らにより提唱された。可視光･紫外線下でのリボフラビン使用によるウィルスとバクテリアの不活化は、1960 年代と 1970 年代の研究により有効性が示唆されたが、さらに 1980 年代の研究によって、病原体除去、光増感剤反応、光活性化時の核酸融合･分解におけるリボフラビンの可能性が実証された。

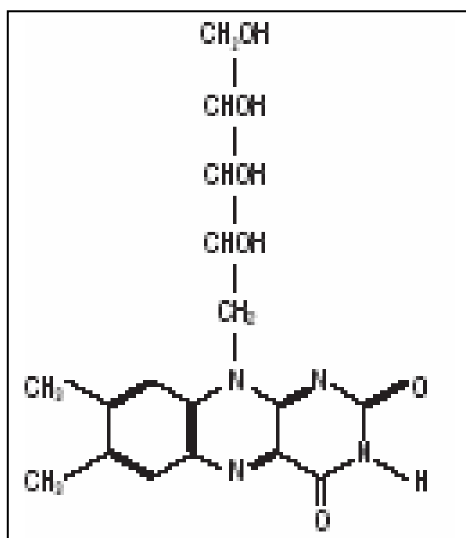


図 1. リボフラビン（ビタミン B2）必須栄養素

リボフラビンの中性水溶液に自然光を当てると、リボフラビンはルミクロム（LC）に変化する。LC は人体におけるリボフラビン代謝分解物としても知られている。フラビン体の光分解生成物はすべて特定済みで、自然界におけるリボフラビン酵素分解の代謝産物の特色も特定されている。さらに、リボフラビンの光分解生成物（主に 2'-キトフラビン、4'-キトフラビン、フォルミルメチルフラビン、LC の 4 つ）はすべて通常の代謝産物であり、未処理のアフェレーシス血小板にも含まれている。リボフラビンの代謝産物となり得るルミフラビン等は人体における通常の pH 環境では発生しない。

Mirasol PRT は 40 年以上もの研究成果に基づいて開発された技術である。

リボフラビンは 30 年以上も前から新生児黄疸の光線療法に用いられてきた。新生児に光を当てることで、黄疸の原因となるビリルビンを分解するだけでなく、新生児の血中に元から存在するリボフラビンを分解する療法であるが、臨床時における副作用の報告はこれまでなく、治療後 9 年の経過を見たレトロスペクティブ分析でも問題は報告されていない。

現時点で商品化されている不活化システムの幾つかは、有害の可能性のある光増感物質を除去するための吸収装置（CAD）を必要とする。それに対し、安全性が確立されたリボフラビンの場合、その光分解生成物も未処理の血液サンプル中に通常に存在するようなものであるため、処理後の血液製剤からこれらを除去･吸収することなく、そのまま投与することができる。

Mirasol 処理後の血液製剤は、リボフラビンとその光分解生成物を除去せずに投与できる。†

反応メカニズム

Mirasol PRT システムは照射器（および付属部品）とディスポーザブルの照射／保存キットから成る。ディスポーザブルキットは 35 ミリリットルのリボフラビン溶液と照射／保存用袋が付いている（図 2)。いずれも滅菌処理済である。

Mirasol プロセスでは、まず血液製剤にリボフラビンを混合した後に、265～370 nm 波長域の紫外線に短時間（通常 10 分未満）さらす。紫外線照射により、DNA と RNA の複製を妨げる化学反応が起き、そうしてウィルス、バクテリア、その他真核細胞（例えば、白血球）が不活化される。ゲノム核酸を持たない赤血球、血小板、血漿は影響を受けない。

リボフラビンと紫外線による核酸分解は、2 つの異なるメカニズムを通じて起きる (図 3)。

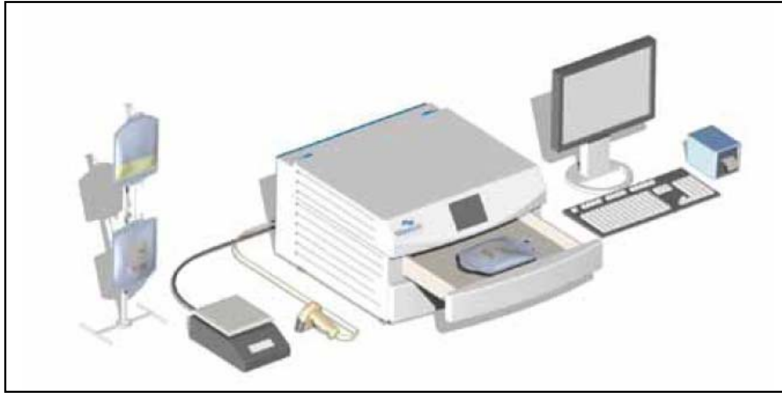


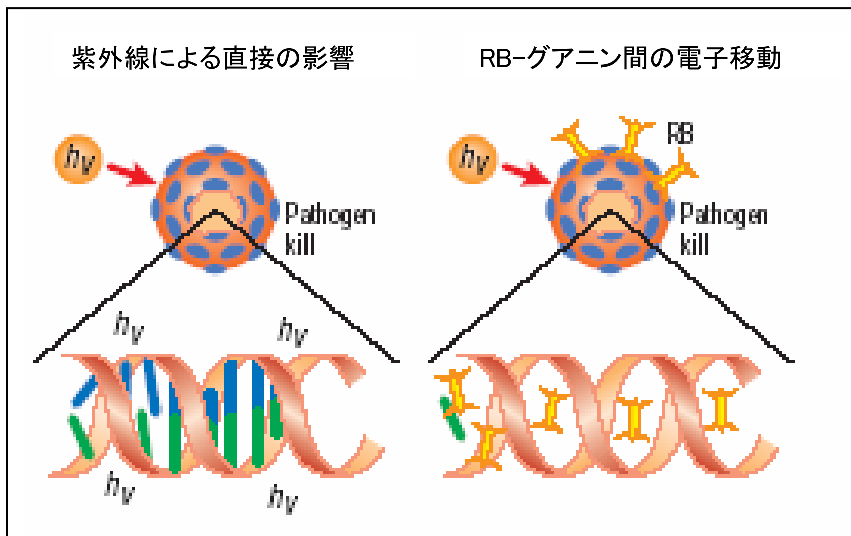
図 2. Mirasol PRT システム
主要部品：(A) 照射器、(B) 照射／保存用袋、リボフラビン溶液
付属部品：(C) バーコード･リーダー、(D) 計り、(E) Mirasol システム管理用 PC、(F) ラベル･プリンター

2 つのメカニズムとは、

・ 紫外線独自の DNA 損傷効果
・ 光励起リボフラビンと核酸塩基対との接触による電子移動反応

Mirasol RPT は 2 つの異なるメカニズムによって病原体を不活化する。

図 3. Mirasol PRT システムの 2 つの主な反応



低波長光線の吸収によって、血液製剤中のウィルス、バクテリア、寄生生物、白血球の核核酸が損傷する。

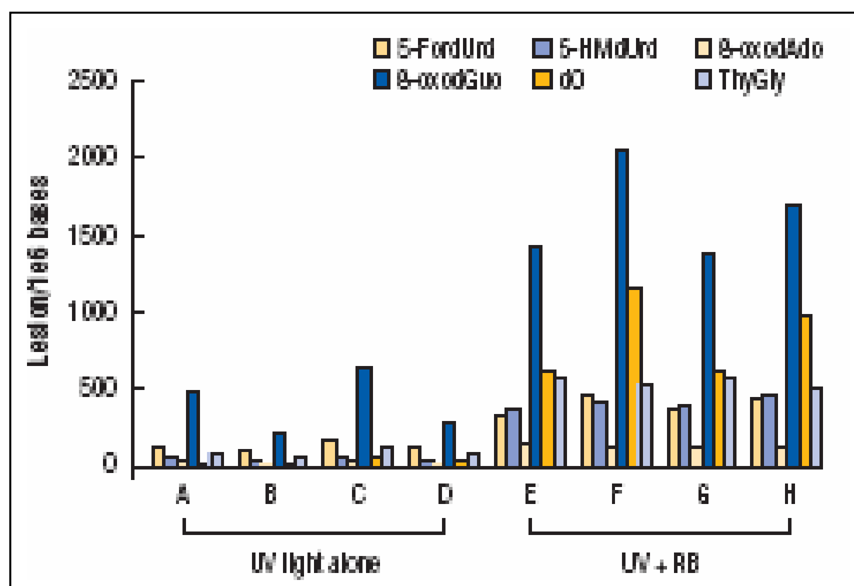
さらにリボフラビンと核酸との化学反応では、リボフラビン-グアニン間の電子移動反応によって、不可逆的な損傷が引き起こされる。

RB： リボフラビン　UV：紫外線

リボフラビンは光照射によって活性酸素種が発生する可能性もあるが、それは非特異的反応であるため望ましくない。Mirasol システムでは、処理前に袋を真空状態にし、未結合ではなく結合した状態でリボフラビンを活性化させる波長の光を選択することでこれを防ぐことができる。

リボフラビンは、塩基対（主にグアニン塩基対）の不可逆的な変形を引き起こすことで病原体の複製を阻止する。また、インターカレーション（挿入）等によって核酸と結合することも知られている。さらに、 I 型、II 型の光化学反応のいずれでも DNA 連鎖を切断することが実証されている。

図 4. 紫外線のみを使った場合とリボフラビンを併用した場合の塩基損傷



各棒グラフは個別実験の数値。損傷のパターンは似ているが、リボフラビンを併用することで損傷数が大幅に増えていることがわかる。
RB：リボフラビン　UV：紫外線

つまり、本システムにおけるリボフラビン使用は、紫外線独自の滅菌効果を増強するだけでなく、病原体が紫外線の影響をより受けるような素地を作る効果（光増感効果）をもたらす。それによって、紫外線のみを使用した場合より多くの塩基損傷を引き起こし、またその損傷を不可逆的なものにする。紫外線のみの場合、損傷の一部は DNA の修復メカニズムにより無効化される。それに対し、2 つのメカニズムを組み合わせた Mirasol PRT システムは、広範的かつ徹底的な病原体除去を可能にする（図 4）。

さらに、リボフラビンの光増感反応による微生物核酸の変性度合いについて検証を重ね、23 リボフラビンと光線への暴露がウィルス、バクテリア、寄生生物、白血球に及ぼす影響を評価した（第 4 章と第 5 章を参照）。

光増感剤としてリボフラビンを使用することで、紫外線のみを使った場合と比べて、より多くの不可逆的な塩基損傷を引き起こすことが出来る。不可逆的な塩基損傷は病原体を確実に死滅させる上で重要である。

要約

· Mirasol PRT システムはリボフラビンと紫外線を用いる
· リボフラビンは人体の生化学反応において重要な役割を果たす、必須ビタミンである
· Mirasol PRT は 40 年以上もの研究成果を基に開発された
· Mirasol 処理後の血液製剤は、リボフラビンとその光分解生成物を除去せずに投与できる
· Mirasol RPT は 2 つの異なるメカニズムによって病原体を不活化する
· 光増感剤としてリボフラビンを使用することで、紫外線のみを使った場合と比べて、より多くの不可逆的な塩基損傷を引き起こすことが出来る
· 不可逆的な塩基損傷は病原体を確実に死滅させる上で重要である

† Mirasol PRT System for Platelets に対する現行の CE マーク規格認定による。

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第 3 章

Mirasol による処理の安全面

MIRASOL®

病原体不活化技術

Mirasol による処理の安全面

血液に関する新しい安全技術に対する包括的な毒物学的評価は、その安全技術が輸血プロセスに悪影響を与えないことを実証するために不可欠である。そのため、Mirasol による処理は広範囲にわたって検査されている。紫外線に関連したリボフラビンの利用に関する大量の史料に加え、広範囲にわたる毒物学的検査によって血液成分の光化学処理の安全性が裏付けられている。

治療分野におけるリボフラビンのアプリケーション

必要不可欠なビタミンとしてのリボフラビン

リボフラビン（ビタミン B2）は 13 種類の必須ビタミンのうちの 1 つで、栄養補助食品および認可を受けた食品着色料として幅広く使用されている。リボフラビンは、欧州食品科学委員会（European Scientific Committee on Food1）により認可されており、米国食品医薬品局（United States Food and Drug Administration）により GRAS（Generally Recognized as Safe: 一般に安全であると認められる）に分類されている。リボフラビンの RDI（Recommended Daily Intake: 1 日当たりの推奨摂取量）は、平均的な成人の場合約 1.3 mg／日（男性）および約 1.1 mg／日（女性）で、授乳中の女性の場合は最大 1.6 mg／日である。リボフラビン欠乏症に対する推奨薬用量は、平均的な成人の場合≦30 mg／日。リボフラビンの安全性は、経口投与、皮下投与、腹腔内投与および静脈内投与において実証されている。

新生児黄疸の治療におけるリボフラビン

リボフラビンおよびビリルビンの光吸収スペクトルがオーバーラップするため、光線療法が促進するビリルビンの除去により新生児黄疸の新生児が一時的にリボフラビン欠乏症になる可能性がある。そのため、1970 年代までは、このように体の弱い病人には光線療法を受けている間はリボフラビンを投与することが推奨されていた。

リボフラビンおよび新生児に対する直接光線療法の毒性に関する可能性について解説されてきたが（光の存在する場で発生する可能性のある DNA への作用に関する理論的な考察および簡略化された試験管内検査システムにおける実験結果に基づく）、臨床的環境における副作用（AE）のレポートはまだない。しかし実際に、光線療法を受けた 55,000 人を超える幼児に関する大規模な遡及的分析により、平均 9 年間の追跡調査期間における小児白血病の発生率が過剰ではないということが証明され、リボフラビンの投与は、広範囲にわたる臨床治療で利用されている。

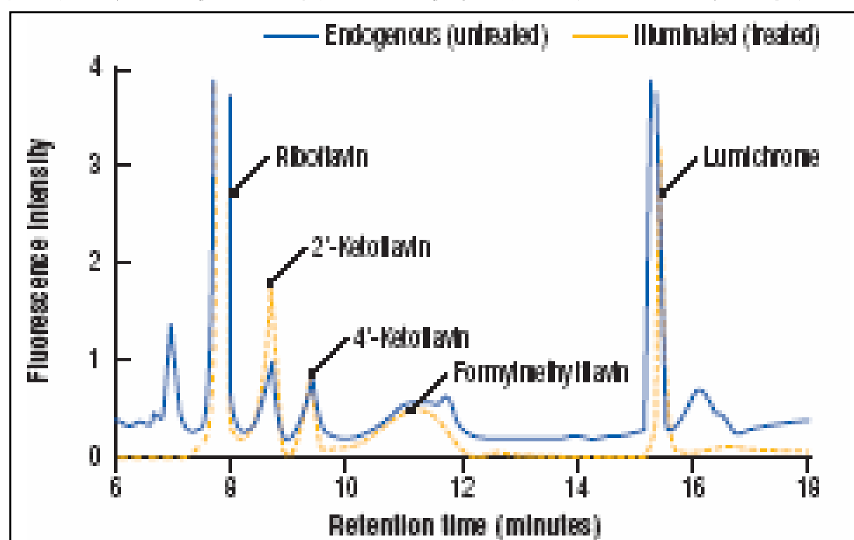
リボフラビンの安全性は、体の弱い人々に対する使用など、長年にわたる経験を通じて最終的に証明された。

リボフラビンの光化学

添加剤を利用した血液に関する新技術の導入における重大な懸念事項は、その添加物を輸血用血液に入れることによる副作用の可能性である。理論上は、添加剤がもたらすリスクが状況によっては血液成分により病気感染が発生するリスクを上回る可能性もある。Mirasol 処理で利用される添加剤は、自然に発生する物質であるリボフラビンであり、リボフラビンは、安全であると知られているにもかかわらず、光の照射を受けて多くの光化学反応の生成物へと薄められ、これらの生成物も輸血用血液に入れられる可能性がある。リボフラビンの 4 つの主要な光化学反応生成物（2'-ケトフラビン、4'-ケトフラビン、フォルミルメチルフラビンおよびルミクロム）は、分離され、その特性を示す。これらは主に親モジュールにおけるリビチル側鎖の分解を通じて形成される。光化学反応の生成物はそれぞれ標準的な代謝産物であり、各生成物は未処理のアフェレーシス血小板から検出される。

献血されたばかりで照射されていない血液成分にこれらの光化学反応の生成物が存在するという事実は、リボフラビンを基盤とする病原体不活化処理の利用によって輸血用血液に新たな化学物質が取り入れることはないということを示している（図 1 参照）。さらに、これらの生成物はすべて人間の血液からなる天然成分であるため、Mirasol 処理後にそれらを血液成分から取り除く必要はないということに注意することが重要である。

図 1. 処理済の血液および未処理の血液中のリボフラビンおよび光化学反応の生成物



未処理の血小板におけるこれらの生成物の濃度は Mirasol 処理済血小板の濃度よりも低いが、処理済血液成分の濃度は患者の血流に注入される際に 16～20 倍薄められ、循環血液中で自然発生したものに対して処理済の血小板の濃度を著しく減少させる。さらに別の光化学反応生成物であるルミフラビンが形成される可能性についても、懸念されてきた。これ

は非常に高い（非生理学的な）pH（9-3)、言い換えれば標準的な血液および血液成分の pH をはるかに超える pH 値においてのみ形成される。
ルミフラビンによりもたらされたリボフラビンの遺伝的な作用の可能性に関するレポートがあるが、これはその後の調査において再現されていない（広範囲にわたる毒物学的なデータベースの研究に基づく)。さらに、ルミフラビンは一般に普及している食品にも見られる。Mirasol 処理の毒性評価中に、ルミフラビンの活性調査は陰性だった。高性能液体クロマトグラフィー（HPLC）または質量分析法のいずれにおいても、血液成分からルミフラビンは検出されなかった。

リボフラビンが光を浴びた後に形成される光化学反応生成物は安全であり、血液や血液成分中にも見ることができる。

リボフラビンは極めて安全なプロファイルを示す

Mirasol PRT System におけるリボフラビンの暴露

ドナーの成分に対して Mirasol 処理を使用することにより、潜在的にレシピエントをリボフラビンおよびその光化学反応生成物の両方にさらす可能性がある。臨床的環境において起こりうるレシピエントに対するリボフラビンの暴露は、平均的なレシピエントの体重が 70kg、標準的なリボフラビンの溶解濃度が 500μM、平均的なリボフラビンのフォトコンバージョンが 18%、および平均的なリボフラビンの溶解体積が 35 mL であるという前提のもとで、輸血される成分単位当たり 0.077 mg/kg と算出されている。

リボフラビンのLD_{50}を定義する

人体への使用を目的とするその他の製品と同様に、LD_{50}を特定することは重要である。LD_{50}とは、試験の対象となる集団の 50%に死をもたらす服用量である。LD_{50}は重要だが、LD_{50}および患者に投与される薬用量の比率はさらに重要である。この比率が大きければ大きいほど、その製品は安全になるのである。

リボフラビンのLD_{50}を測定するため、複数の調査が行われてきた（Reddy et al.19 に概要記載)。一部の調査においてはLD_{50}の測定が可能だったが、その他の多くの調査では極めて高用量（10,000 mg/kgまたはそれ以上）の場合であっても不可能であった（表 1 参照)。輸血ごとの暴露レベルである 0.077 mg/kgを、報告されているマウスの静脈内のリボフラビンに対するLD_{50}、50-100 mg/kgと比較すると、その安全性は少なくとも 649 倍（50/0.077）になる。

表 1. リボフラビンのLD_{50}を確立するための調査概要

調査の種類	種	経路	結果
急性	ラット	腹腔内	LD_{50} = 560 mg/kg[7]
急性	ラット	皮下	LD_{50} >5000 mg/kg[7]
急性	ラット	経口	LD_{50} >10,000 mg/kg[7]
急性	マウス	腹腔内	LD_{50} >340 mg/kg[11]
亜慢性（4 日間）	マウス	腹腔内	1000 mg/kg/日[11]で死亡率 2/6

LD_{50}: 試験の対象となる集団の 50%に死をもたらすと推量される服用量。

表 2. Mirasol 処理済血液成分に対して行われた毒性評価の概要。

対象	方法	露出	結果
急性毒性	ラットおよび犬の雄および雌に対する 2 件の急性毒性調査: 死亡率、毒性の兆候、血液学、臨床化学、食料消費、一般行動および体重増加の観察。	IV 注入	毒物学的に重要な結果はなし
亜慢性毒性	犬の雄および雌に対する調査: 死亡率、毒性の兆候、臨床化学、血液学、体重、食料消費、組織の病理学および組織病理学の観察。	1 週間に 6 日間、13 週間以上投与、カテーテルと併せて IV 注入	毒物学的に重要な結果はなし
生殖毒性	ラットの胎児の発育: 臨床的観察、体重および食料消費。20 日目に検死。着床、黄体、再吸収および胎児の調査。	懐胎中に IV 注入 6～17 日	発達毒性は観測されなかった
遺伝毒性	1. 細菌を使用した復帰突然変異試験法（Ames 試験）: 細菌における生体外遺伝子突然変異 2. CHO 細胞における染色体異常: 哺乳類の細胞における生体外染色体異常誘発（染色体の崩壊） 3. 哺乳類赤血球小核試験: 大腿部から吸引された生体内骨髄に対する染色体異常誘発試験	Mirasol 処理済血小板およびルミクロムに対する Ames 試験 IP 投与	処理済血小板または制御ヒト血小板あるいはルミクロムに対する Ames 試験では変異原性は観測されなかった。 Mirasol 処理済ヒト血小板は、すべての遺伝毒性実験において陰性の結果を出した。
新抗原性試験	1.生体外実験: 血小板および血漿タンパク質に対する 14C-リボフラビン結合の評価および血小	貯蔵してから 1 日後および 5 日後のキャプチャ-P 試験	血小板または血漿成分に対するリボフラビンの結合およびその光化

	板に対するIgGの結合を評価するためのキャプチャ-P試験 2. オークタロニー法を利用した生体内試験：制御およびMirasol処理済ヒト血小板を投与したうさぎの免疫反応の比較抗体産生の評価		学反応生成物は検出されなかった 新抗原形成の兆候はなかった
血液適合性試験	1. 溶血試験: 人間の血液に直接接触 2. 血小板の機能に関する調査: 第6章に記載されている一連の実験において評価されている	該当なし	溶血は観測されなかった 第6章の結果を参照
薬物動態試験	雄のCDラットの生体内評価: 尿および糞便、全血および血漿の事前投与の検査、および実験の最後に、検死後に収集された皮膚、尾の皮膚、小腸、大腸、脾臓、腎臓、肝臓、リンパ系組織および骨髄の検査	単回投与IV注入	投与された放射能の約95%が投与後 260～275時間以内に除去された 曲線下の観測範囲に基づき、全体的な暴露は高いほうから順番に以下のとおり: 肝臓＞腎臓＞大腸＞小腸＞脾臓＞大腿部の骨髄＞リンパ管
浸出物および抽出物	血小板成分に直接入り込む可能性のある混合物を特定および定量化するために行われる分析: ガスクロマトグラフィーおよび質量分析法を利用	該当なし	治療状態にさらされる前後で著しい違いはなかった。 FTIRによりMirasol処理済または未処理の血小板抽出物から高分子材料は検出されなかった。Mirasol処理済の血小板抽出物から検出されたすべての金属は、未処理の血小板抽出物にも同様な量だけ含まれていた

CD: Crl:CDⓇ（SD)ラット; CHO: チャイニーズハムスター卵巣; FTIR: フーリエ変換赤外分光; IgG: 免疫グロブリンG; IP: 腹腔内; IV: 静脈内; NA: 該当なし。

現時点では、リボフラビンの「中毒作用が発生しないレベル」は依然として不明である。従って、Mirasol 処理済血液成分の輸血によりもたらされるリボフラビンおよびその光化学反応生成物への暴露レベルが何らかの中毒作用を引き起こすということを予測する根拠はない。てんかん発作の感作および発作を増大させる可能性について、数ヶ月（たとえば>5 mg/kg/日を 6 ヶ月以上）に渡って毎日リボフラビンを高用量投与した後に個別の題材で報告されてきたが、これらのレポートはピアレビューを受けた資料により実証されていない。20 体の人体における高用量の経口投与および親の服用に関するその他のレポートにおいて、毒性作用は記載されていない。実際に、6 ヶ月以上>200 mg/日を安全に経口服用している人々に関するレポートがあり、標準的な服用量を超えると速やかに排出されるようである。

Mirasol PRT System の安全プログラム

リボフラビンの安全性については広範囲に渡って研究され、文書によっても証明されているが、Mirasol 処理におけるリボフラビンの使用はさらなる評価を保証した。そのため、国際標準化機構（ISO: International Organization for Standardization）のガイドラインに従って Mirasol 処理に対して広範囲にわたる臨床前毒性評価が実施された。大部分の調査レポートにおける被験物質および制御物質は、Mirasol 処理済血液成分または未処理の血液成分だった。一部の調査では、混じりけのないルミクロム（リボフラビンの主な光化学反応生成物）または光分解されたリボフラビン溶液が使用された。Mirasol 処理済血液成分の検査では、リボフラビンおよび制御された光照明の追加から処理後の血液成分に対する存続可能性の評価まで、全工程を検査した。評価には、細胞および血漿蛋白質への作用、リボフラビン本体およびその光化学反応生成物の毒性、処理に使用される医療用プラスチック製コネクタおよびバッグなどのその他のシステムコンポーネントに対する影響が含まれていた。毒性の評価に使用される方法およびこれらの試験の結果の要約は、**表 2** に記載されている。

すべての調査において、ガンマ線照射の Mirasol 処理済血液成分を使用した結果は、ガンマ線非照射の Mirasol 処理済血液成分から得られる結果と同一だった。Mirasol 処理済血液成分に対して実現可能な最大限の暴露を伴う投与を繰り返した後でさえも、主な生理的システムの機能に対する有害な影響の兆候または標的臓器における毒物の生成はなかった。試験により、Mirasol 処理済血液成分または蛋白質には検出可能な抗原は存在せず、調剤によって抗体または自己抗体の形成を促すことはないということが明らかとなり、また、毒性試験により、あらゆる遺伝毒性のリスクや妊娠中の動物および胎児の発達に関する中毒作用が排除された。IV を注入した場所の部分的な耐性も良好であることが分かった。処理済血小板の調剤により、細胞毒性が示されたり、システムで使用される容器およびコネクタから不要な物質の浸出が発生したりすることはなかった。亜慢性試験やその他の試験における腫瘍発性効果を与える可能性の欠如や、リボフラビンおよびその光化学反応生成物

の遺伝毒性および化学的性質の欠乏により、さらなる発癌性試験の必要性が除去された。歴史的研究におけるリボフラビンの発癌性試験はこれらの調査結果と一致しており、また、この複合物は発癌性物質の可能性がないことを示している。

リボフラビンおよびその光化学反応生成物は、Mirasol 処理後に血液成分から除去する必要がないため、単純で簡単に使用することができる。

上記の調査結果は、Mirasol 処理を行った場合でも輸血する前に処理済血液成分からリボフラビンおよびその光化学反応生成物を除去する必要がないという事実を示す。これは、バッグの運搬および／または複合物の除去処理による成分の損失を最小限に抑え、病原体不活化処理が大いに必要とする簡易性を提供する。

要約

・リボフラビンは必要不可欠なビタミンである。

・リボフラビンの安全性は、体の弱い人々に対する使用を含む長年にわたる経験を通じて最終的に証明された。

・包括的な臨床前の毒性に関するプログラムが実施され、リボフラビンおよびその光化学反応生成物の優れた安全プロファイル、およびそれらの Mirasol 処理への使用の適合性が証明された。

・Mirasol処理済成分を受容する患者に対するリボフラビンの予想される暴露は、輸血される血液成分の単位当たり 0.077 mg/kgである。この服用量は、報告されているLD_{50}の最低値よりも少なくとも 649 倍低くなっている。

・リボフラビンが照射された後に形成される光化学反応生成物は安全であり、血液および血液成分中に自然に存在する。

・安全性に関する調査により、Mirasol 処理後に血液成分からリボフラビンおよびその光化学反応生成物を除去する必要がなく、病原体不活化処理が簡易化されているということが裏付けられた。

・毒性に関するプログラムが好ましい結果をもたらしたことにより、広範囲に渡る臨床試験プログラムの実施が可能になった。

Mirasol PRT System for Platelets の現在の CE マーク規格認定に準じる。

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第 4 章

病原体不活化の性能

MIRASOL®

病原体不活化技術

病原体不活化の性能

ウィルス、細菌および寄生虫の一般的および代表的な集団に対する、Mirasol PRT System の有効性が広範囲にわたって評価されてきた。

病原体不活化の評価

血漿から派生する生物薬剤学における病原体不活化の基準は定着しており、それに相応して血液成分における病原体不活化の評価に採用されてきた。病原体不活化の範囲は、通常「ログ・リダクション」に置き換えて表される。これは、10^x mL^{-1}の単位で表される始点力価から、同様に 10^x mL^{-1}の単位で表される処理後の力価を引いたものとして報告されている。サンプルの容量は処理の前後で一定であるため、容量の単位は相殺され、レポートにあるログ・リダクション値が導き出される。たとえば、サンプルの始点力価がmLにつき10^6個のウィルス粒子で、処理後の力価が mLにつき 10^2個のウィルス粒子である場合は、報告されるログ・リダクションは 10^4または 4 ログになると推量される。これは、ウィルスのレベルが 99.99%減少することに相当する。値はログ単位で報告されるため、100%減少することはない。

ログ・リダクション調査の結果を解釈する際には、実験的方法論を理解することが非常に重要である。

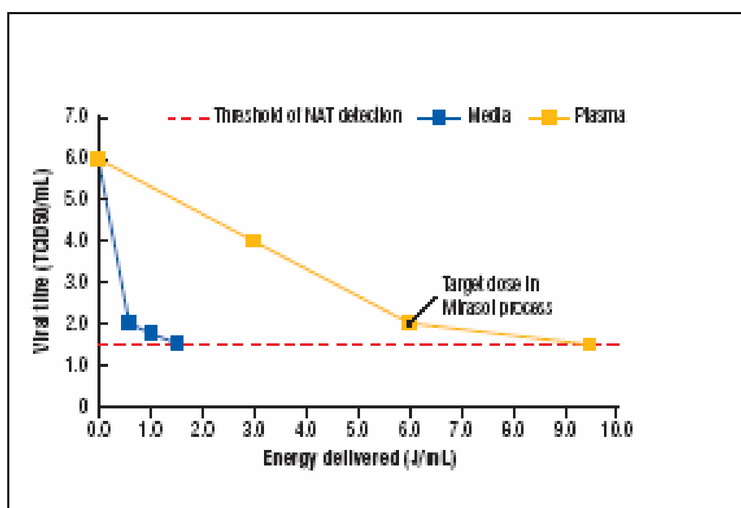

図 1.血漿および媒体における脳心筋炎(EMC)ウィルスの不活化動力学: Mirasol 処理利用時のウィルス不活化率。

たとえば、実験に使用される媒体は、実験結果に多大な影響を与える可能性がある。病原体不活化の比率および範囲のどちらとも、媒体および血漿の間で大きく変化する（図 1 参照）。Navigant Biotechnologies LLC により実施された特異ウィルス性因子、細菌性因子、寄生性因子および白血球因子に対する効能に関するあらゆる調査は、利用規格に適合する条件の下で血液成分に対して実施されていることに注意することが重要である。

さらに、ログ・リダクションの値は関連する臨床状況に分類される必要がある。図 2 は代表的なウィルス感染の経時変化を表している。感染の初期段階、特に潜伏期間における病原体不活化が重要であることは明白である。感染の段階におけるウィルス力価は低いが、それでもなお十分に感染を引き起こす可能性があり、既存の分析では検出できない場合がある。反対に、ウィルス血症が最大限の間は明白な症状が表わされる可能性が高く、ドナーに提供させないようにするほどだ。表 1 はウィルス性病原体に関するウィルス血症の様々な段階におけるウィルス力価の要約を示している。

図 2.代表的なウィルス血症期間の概要図

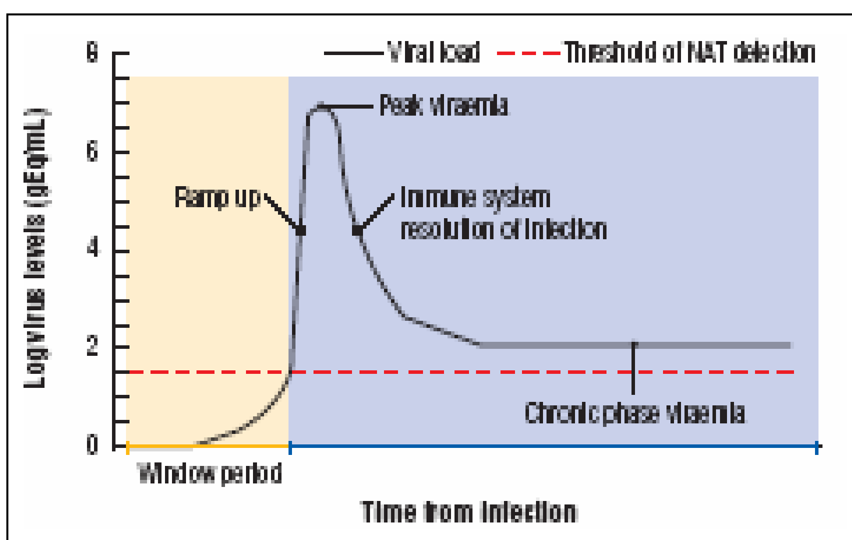

表 1. 輸血製剤のウィルス汚染レベル

作用因子	感染段階	ウィルス血症の最大値
HIV、HCV、HBV	WP w/MP-NAT WP w/SD-NAT WP wo/NAT 慢性感染症	$<10^3$ $<10^1$ 10^6～10^8 10^4～10^6
CMV、EBV HTLV	WP 慢性感染症	10^4～10^6 $<10^2$～10^4
パルボ・ウィルス	WP 慢性感染症	10^8～10^{12} $<10^2$～10^4

†mLまたは 10^6 PBMCごとのgEq。
CMV: サイトメガロ・ウィルス; EBV: エプスタインバーウィルス; gEq: 核酸相当数; HBV: B 型肝炎ウィルス; HCV; C 型肝炎ウィルス; HIV: ヒト免疫不全ウィルス; HTLV: ヒト T 細胞性白血病ウィルス; MP: ミニプール; NAT: 核酸試験; PBMC: 末梢血単核細胞; SD: シングルドナー; w:あり; wo: なし; WP: 潜伏期間.

病原体不活化処理の有効性を明確にするために必要とされる要件や性能限界を、完全に説明することのできる血液成分のウィルス不活化はない。また、ウィルス不活化の有効性が、ウィルス血症が最大限の時に、すべてのウィルスに見られるレベルに達していない場合でも、それが病気感染の可能性を減少させることのできない方法であるということにはならない。

ウィルス因子に関して重要な点は、その測定方法である。
すべての結果において、私たちは mL ごとの核酸相当数（gEq）について報告されているウィルスのレベルを示す。これは、一般に採用されている NAT 方法論でスクリーニングされる特異遺伝子配列に基づくサンプルにおいて検出される粒子の数と一致する。この特異遺伝子配列が存在するということは、ウィルス粒子またはウィルス粒子の一部が存在するということを意味し、すなわちそれは病気の指標になる。しかし、このレベルが必ずしも感染性ウィルス粒子の存在にそのまま結びつくわけではない。たとえば、このウィルス特有の特異遺伝子配列に基づいて粒子とみなされるウィルスは、完璧なゲノムを持っているかもしれないが、宿主細胞および病気の伝染に結合するために必要なウィルス・エンベロープ蛋白質が欠如している。そのため、私たちが行ったようなウィルス因子の測定は、サンプルに残されたウィルスの伝染力を大幅に過大評価する可能性がある。

以下のテキストに挙げられている実験では、ログ・リダクション因子は医薬品委員会（CHMP: Committee for Medicinal Products for Human Use、以前は Committee for Proprietary Medicinal Products [CPMP]）および US FDA のガイドラインに従って算出された。

ウィルスの不活化研究

ウィルスの選択

調査用の病原体は、 米国血液銀行協会（AABB: American Association of Blood Banks）の病原体優先順位リスト、WHO、国際輸血学会（ISBT: International Society of Blood Transfusion）、および CHMP のガイドラインに基づいて選択された。CHMP は、欧州医薬品審査庁（EMEA: European Medicines Agency）向けに人体に利用するための医薬品に関するあらゆる質問に対する見解を準備する責任がある。CHMP のガイドライン 4 は、ク

リアランス評価および処理特性調査のためにはウィルスの 3 つのカテゴリについて詳しく調査することを勧めている：

・関連ウィルス言い換えれば、細胞基質または生産処理に使用されるその他のあらゆる試薬または物質を汚染することで知られている、またはその可能性のある実際のウィルスあるいは同じ種の一種

・特異モデルウィルス言い換えれば、ウィルスとして知られているまたはその疑いがあるものと密接に関連するウィルスおよび物理化学特性を備えたウィルス

・ 非特異モデルウィルス

特異モデルウィルスは、関連ウィルスが入手不可能な場合、または研究室の状況下で適切に維持することができない場合に使用される。非特異モデルウィルスは、一般的にウィルスを除去および／または不活化するためのシステムの能力の特性を示すため、すなわちクリアランス処理の強固さの特性を示すために使用される。ウィルスクリアランス特性に関する調査は、異なる物理化学特性を備えた非特異モデルウィルスを使用して実施されなければならない。

Mirasol 処理は CHMP ガイドラインに含まれる様々なウィルスについて評価されてきた。

ウィルス不活化の調査

Mirasol PRT System は最初に優先純度の高いウィルスに対する不活化について評価される。最初に調査対象となったウィルスは、ヒト免疫不全ウィルス（HIV）、豚パルボ・ウィルス（PPV; ヒト B19 パルボ・ウィルスのモデルとして[B19]）および西ナイル・ウィルス（WNV）である。さらなる詳しい調査により、CHMP が推奨するその他のクラスのウィルスについても不活化能力が評価されている。完了済みのウィルス・ログ・リダクション調査の結果の要約は、表 2 に記載されている。

すべてのウィルス不活化調査は、Trima®アフェレーシス装置を使用したアフェレーシスにより収集されたシングルドナー血小板成分を使用して実施された。サンプルはMirasol処理の前にスパイクされる。多くの場合、血小板成分は直接ウィルスにスパイクされ、例外はすべて下記に記載されているが、サンプルは、ログ・リダクション値を計算するために使用される最初および最後のウィルス力価を得るために、照射直前および直後に試験される。感染培養液は加湿されたCO_2 5% の培養器の中で 7 日間 37℃で培養され、細胞病理学的効果について毎日検査される。ログ・リダクション結果は、感染性に関する標準的な生体外検定法により取得される（TCID50）。‡上記に記載されているように、核酸相当数により定量化された高度なウィルス力価を含む溶液には、病気を伝染させせることのできるウィルス粒子はわずかしか存在しない可能性がある。そのため、組織培養感染検査はウィルス因

子が病気を伝染および発生させる能力の間接的測定方法として使用される。

次の試験は、これらの優先度の高いウィルス、HIV、B19－モデルとして PPV を使用－および WNV、および現在の血液の安全性に関する技術に特別な課題を提示する他の 2 つの重要な病原体（A 型肝炎ウィルス[HAV]およびサイトメガロ・ウィルス）に関する、現在完了済みのウィルス不活化調査の主な結果を要約している。

‡TCID50: 組織培養検査（ウィルス因子が病気を伝染および発生させる能力の間接的測定方法）で測定される感染性のウィルス因子の数

ヒト免疫不全ウィルス（HIV）

Mirasol 処理は様々なウィルスの型に対して評価された。無細胞の HIV および細胞を有する HIV に対してシステムを評価するために、HIV に感染しているリンパ球（H9 細胞）を使用して準備されたサンプルが使用された。これらの細胞には、活発に複製を行ったり、成長媒体中で制限なく出芽しているなどの様々な型のウィルスが含まれている。プロウィルス型である細胞内 HIV（HIVi）に対する試験には、ACH-2 感染細胞が利用される。HIVi は、感染細胞のゲノムに統合されるウィルスの型である。AXH-2 感染細胞は、非複製で感染性のあるウィルス・ゲノムを含むリンパ嚢胞に血液製剤が感染している可能性のある場合の代表的なものである。Mirasol 処理は、すべての型のウィルスのウィルス力価を効果的に削減し、4.5～5.9 log/mL のログ・リダクション値を実現する（表 2 参照）。

Mirasol 処理は HIV の細胞内および細胞外の両方の型のウィルス力価を効果的に削減する。

ヒト B19 パルボ・ウィルス（B19）

B19 はプールされた血漿または血漿派生物の一般的な汚染物質で、20,000 人に 1 人のドナーの血液に存在すると予測されており、この値は流行期間には 260 人に 1 人にのぼる。この事実にもかかわらず、B19 は標準的な血液スクリーニング法の一部として定期的に試験されていない。人体におけるその存在は、免疫不全の被験者における骨髄機能不全および妊娠第二期の女性の自然流産と関連してきた。CHMPのガイドライン[4]に従い、Mirasol処理は特異モデルウィルス、豚パルボウィルス（PPV）を使用して評価された。 >5 log/mL の削減値が達成された（表 2 参照）。

Mirasol 処理の結果として、ヒト B19 の特異モデルウィルスである PPV の力価の大幅な（>5 log）削減に繋がる。

西ナイル・ウィルス（WNV）

WNV は、人間に病気や死をもたらす可能性のある蚊媒介性のウィルスである。2002 年のアメリカにおける流行期間中の輸血に関連した WNV 感染の伝染は、WNV ウィルス血症用に献血された血液のスクリーニングのための核酸試験（NAT）検定法の急速な発展を促した。試験が可能な一方で、「偽」陰性の結果に関するレポートがあり、感染をもたらしてきた。WNV（ATCC #VR-1510）に血小板濃厚液が追加され、mL 当たり 7.06±0.26 log TCID50 の初期力価を達成した。

照射前および照射後のサンプルは、連続的にハンクス緩衝食塩液で希釈され、ベロ細胞に塗布された（ATCC #CCL-81）。Mirasol を利用した処理は、5.2.のログ・リダクションを実現した。

Mirasol 処理は WNV の力価を大幅に削減する。

A 型肝炎ウィルス（HAV）

従来の病原体不活化技術（PRT）は、HAV を不活化することができない。複合物が核酸に近づくために蛋白質皮膜に浸透することが困難で、小型の HAV ゲノムは不活化の目標がほとんどないため、それは不活化するのが困難なウィルスである。この困難なウィルスの Mirasol 処理により、約 2.3 のログ・リダクション値がもたらされた。現在まで、Mirasol 処理は HAV のウィルス力価を削減すると報告されている唯一の技術である。

現在まで、Mirasol 処理はヒト HAV のウィルス力価を削減すると報告されている唯一の技術である。

表 2. ウィルス・ログの調査結果

対象ウィルス	使用モデル	種類	ログ・リダクション	モデル対象	参考
HIV 活性 HIV 潜在	細胞内のヒト HIV 細胞を有するヒト HIV	ssRNA 皮膜を有する	5.9 4.5	その他のレトロウィルス	発表済みデータ
HIV、WNV チクングニ	WNV チクン	ssRNA 皮膜を有する	5.2 計画中	その他のフラビウィルス	発表済みデータ 保留中

ヤ	グニヤ				
狂犬病ウィルス	VSV	ssRNA 皮膜を有する	>6.3	その他のラブドウィルス 大型 RNA ウィルス	発表済みデータ
HCV WNV	シンドビスウィルス BDV	ssRNA 皮膜を有する	3.2 最大 4.0 まで	その他のトガウィルス	ファイル・データ
CMV ヒト CMV	IBRV HHV	dsDNA 皮膜を有する	3.0 ～ 3.4 進行中	その他のヘルペス・ウィルス	その他のヘルペス・ウィルス ファイル・データ ファイル・データ
HBV	ヒト HBV 仮性狂犬病ウィルス	ssDNA 皮膜を有する	進行中 2.5 ～ 3.0		ファイル・データ
インフルエンザ・ウィルス 鳥インフルエンザ・ウィルス	インフルエンザ A ウィルス	ssRNA 皮膜を有する	>5.3	その他のオルトミクソウィルス	ファイル・データ
ヒト B-19 ウィルス	PPV	ssDNA 皮膜を有さない	>5.0	その他のパルボ・ウィルス	発表済みデータ
HAV	HAV EMCV BEV	ssRNA 皮膜を有さない	2.0 3.2 3.0	その他のピコルナ・ウィルス ポリオウィルス	ファイル・データ

§ファイル・データはNavigant Biotechnologies LLCが保持しており発表期限は 2008 年。

BDV: 牛下痢性ウィルス; BEV: 牛エンテロウィルス; CMV: サイトメガロ・ウィルス; EMCV: 脳心筋炎ウィルス; HAV: A 型肝炎ウィルス; HBV: B 型肝炎ウィルス; HCV: C 型肝炎ウィルス; HHV: ヒト・ヘルペス・ウィルス; HIV: ヒト免疫不全ウィルス; IBRV: 牛伝染性鼻気管炎ウィルス; PPV: 豚パルボ・ウィルス; SS: 単一ストランド; VSV: 水泡性口内炎ウィルス; WNV: 西ナイル・ウィルス

サイトメガロ・ウィルス（CMV）

CMV は血液中に存在する時は、白血球に結び付けられている。白血球除去は白血球媒介感染を削減するが、複数の発表によると、白血球除去は最高でも感染率の低下における血清 CMV 試験と同程度の効果しかないということが示唆されている。現在、様々な CMV のモデルが存在し、これらのモデルには、感染したヒト臍帯静脈内皮細胞（HUVEC）またはモデル牛伝染性鼻気管炎ウィルス（IBRV）の使用が含まれる。HUVEC は輸血における CMV 感染を引き起こすわけではないが、効果的に高レベルのウィルスを算出するため使用される。

私たちは IBRV モデルシステムを利用して Mirasol 処理を評価し、ウィルス力価が 3.0～3.4 log/mL 削減されたことを実証した。しかし、このモデルで達成されたログ・リダクションとその結果生じた白血球媒介感染の間には、説得力のある相関性はなかった。従って、Mirasol 処理による CMV の不活化の効果を実証するために CMV に感染したヒト白血球を使用したモデルの開発を進めている。

Mirasol 処理は、CMV のモデルである IBRV を効果的に不活化する。CMV に感染した白血球を使用したモデルについては調査中である。

細菌の不活化

輸血による細菌感染症（TTBI）は、輸血に関して何よりも第一に認識される致命的な危険要因である。実際に、細菌による血小板成分の汚染は、血液成分の輸血に関連する最も重大なリスクの一つとして確認されてきた。このリスクに対する懸念から、細菌に汚染された成分の輸血を削減または廃止する試みとして、細菌培養の実施およびスクリーニング方法が推奨される一方で、現在この環境で PRT の有効性を評価することに対する特定の推奨はない。

細菌の選択

現在、PRT の評価対象となる細菌種に関する標準的な指定パネルは存在しない。そのため、私たちは中心的な臨床専門家と共同で、発表されている文献または血液安全監視体制に関するレポートで特定されている主な病原体について代表的な細菌パネルを編成した。また、私たちは以下に記載されている細菌不活化性能を計測するための 2 つの補完的な試験方法も開発した。

遡及的な調査研究において、報告された事象の 70%以上を一般的な細菌である*表皮ブドウ球菌*および*黄色ブドウ球菌*の 2 種が占めていた。さらに別の 3 種、*大腸菌*、*緑膿菌*および

*セレウス菌*も、頻度は下がるが輸血に関する致命的な事象に関連していた。さらに、輸血に関連する敗血症の原因として、一般的な血小板汚染物質である*セラチア・マルセッセンス*がヘモビジランスに関する多数のレポートに見られる。これらの 6 種の細菌は、これらの研究で報告されている文書化された血小板輸血に関する敗血症の事象（およびすべての死亡事象）の約 86%に相当する（表 3 参照）。そのため、Navigant Biotechnologies LLC は、これら 6 種が Mirasol PRT System の性能を実証する対象となる受容可能な最初の細菌パネルであると考えた。現在進行中の研究では 26 種を超える幅広いパネルに対するシステム性能を評価しており、それら 26 種を合わせると輸血に関する深刻な感染の 98%を占めている。これらの追加研究の結果は、完了後に報告する。

表 3. 血小板輸血に関連する敗血症事象

有機組織体	グラム染色	特徴	観測総数	致死率	頻度（%）
表皮ブドウ球菌	グラム＋	頻繁に皮膚が感染; 輸血に関連する致死量に関与	35	1	61.4
黄色ブドウ球菌	グラム＋	体温で急速に成長 起源は多数ある；輸血に関連する致死量に関与	6	3	10.53
大腸菌	グラム－	標準的なヒトフローラ；輸血に関する致死量に関与	1	1	1.75
緑膿菌	グラム－	輸血に関する致死量に関与	2	2	3.51
セレウス菌	グラム＋	時々皮膚が感染；輸血に関連する致死量に関与	3	0	5.26
セラチア・マルセッセンス	グラム－	日和見性の環境汚染；輸血に関連する敗血症に関与	2	0	3.51
その他の全種	－		8	0	14.04
合計	－		57	7	100

細菌のログ･リダクション研究

細菌の不活化に関する研究には、適切なスパイク・レベルの選択が不可欠だ。ほとんどの研究が高度な細菌力価（例: 1000～10,000 organisms/mL）を使用するが、汚染された成分の力価が 0.03～0.3 organisms/mL（単位当たり 10～100organisms）を超えることはほとんどない。そのため、病原体不活化技術に関する最も適切な結果の基準は、高力価を不活化させる能力ではなく、低力価を不活化させて成分の有効期限を通じてこれらの微生物の成長を抑制する能力である。私たちは、細菌の負荷の削減における Mirasol システムの有効性を評価するための 2 つの補完的な試験､高スパイクおよび低スパイク試験を使用した。どちらの方法も、Mirasol 処理およびそれに続く細菌の存在の測定を受けて周知の力価の細菌を血小板成分に注入することを伴う。8　高スパイク実験の目的は、ひどく汚染された血小板成分に対するシステムの包括的な細菌不活化能力を判断することである。これらの研究は（上述されているように）臨床状況を代表するものではないが、一般的に PRT の評価に使用される。低スパイク実験の目的は、5 日間の保存期間の経過を通じて処理済血小板成分を評価し、それが培養陰性を維持して輸血の解除基準を満たすかどうかを判断することである。このアプローチでは、実際の臨床的環境において予想されるよりも実質的に高度な課題をもたらす状況下で、Mirasol 処理の細菌不活化能力を評価する。

Mirasol PRT System は、2 つの補完的な試験方法を使用して広範囲に渡る細菌病原菌を包括的に評価してきた。

高スパイク細菌力価実験

これらの実験では、Trima アフェレーシス装置を使用して収集され、5～6 log コロニー形成単位（CFU）/mL のバクテリアでスパイクしたシングルドナー血小板成分が使用される。収集されたばかりの汚染された血小板は、通常<100 CFU/成分を含んでいるため、高スパイク調査で使用されるレベルは、代表的な臨床的環境において予期されるよりも 10,000～100,000 倍高くなる。スパイク後すぐに、血小板成分は Mirasol システムで処理され、初期および最後の力価間で達成された不活化を算出することによって総合的な病原体不活化性能が判断される。これらの実験では、力価は照射の直前および直後に判断され、保管期間はない。表 4 は高スパイク細菌力価実験の公表結果を表示しており、それには多くの高耐性菌が含まれる。これらの調査は、高細菌力価における Mirasol 処理の有効性を実証された。

Mirasol PRT System は、高耐性菌を含む広範囲にわたる細菌に対して、高細菌力価を見事に削減する。

低スパイク細菌力価実験

低スパイク細菌力価調査は、保管期間中の血小板成分の無菌性を監視しながら、より低く臨床的に適切な細菌力価（0.5～2.0 log/mL）で能力を評価します。スパイク後、血小板サンプルはMirasolシステムで処理され、通常の血液を構築する状況下で保管される。最低でも 5 日間保管した後で、成分の見本が採取され培養される。サンプルは、血液成分用自動培養システム（BioMerieuxにより開発されたBacT/ALERT®）を使用して監視され、このシステムは、試験サンプルにおける細菌の存在を監視するためにヨーロッパ全域で広く使用されている。結果は、Mirasol処理は保管期間全体を通じて培養陰性成分を産出するということを実証している。

Mirasol PRT System は、通常の血液を構築する状況下で 5 日間の保管期間全体を通じて培養陰性成分を産出する。

表 4. 細菌ログ・リダクション結果

病原体	耐性菌	ログ・リダクション	種類	コメント
黄色ブドウ球菌	ATCC 25923	3.6	グラム＋	
黄色ブドウ球菌	ATCC 700787	4.8	グラム＋	MRSA 耐性菌
表皮ブドウ球菌	ATCC 12228	4.2	グラム＋	
*セレウス菌**	ATCC 7064	1.9	グラム＋	耐性菌
*セレウス菌**	NI-0001	2.7	グラム＋	献血された血液から分離
連鎖球菌	ATCC 6249	3.7	グラム＋	
緑膿菌	ATCC 43088	>4.5	グラム－	
緑膿菌	ATCC 27853	>4.7	グラム－	耐性菌
大腸菌	ATCC 25922	>4.4	グラム－	
セラチア・マルセッセンス	ATCC 43862	4.0	グラム－	

* バチルス種 頻繁に胞子形状で発生する。

MRSA: メチシリン耐性黄色ブドウ球菌

BacT/ALERT を使用した細菌培養試験における Mirasol 処理の有効性を比較する組織内データは、BacT/ALERT が理想的な状況下で実施される際にはどちらの方法も同様に効果的（約 90%）であることを示唆している。しかし、臨床環境においては、Mirasol 処理の有効性は 90%を超える状態を維持すると予測される一方で、BacT/ALERT の有効性は約 50%にまで落ち込む（表 5 および 6 参照）。

表 5. Mirasol PRT System および BacT/ALERT 方法の総合的な有効性の比較

有機組織体	ATCC 番号	発生	頻度（%）
表皮ブドウ球菌	12228 14990 700578 35984	20	33
大腸菌	25922	8	13
セレウス菌	NI-0001	7	11
黄色ブドウ球菌	29213 10832 25923 27217	6	10
B 群連鎖球菌 *連鎖球菌* *化膿連鎖球菌*	70046 6249 BAA-1064	5	8
エンテロバクター菌	29005	4	7
グラム陽性菌	51277	3	5
セラチア・マルセッセンス	43862	3	5
肺炎桿菌	8045	2	3
非発酵陰性桿菌	17961	1	2
陰性桿菌	試験対象外	1	2
エルシニア感染症	23715	1	2

表 6. BacT/ALERT 比較調査において評価された有機組織体の総括表

有機組織体のタイプ	グラム＋／－	ATCC 番号	検出までの培養時間（平均時間）	接種力価（CFU/製剤）	7 日間の積極抑制力価の成長（CFU/製剤）
非発酵陰性桿菌	－	17961	7.6±1.1	61	3.6×10^{8}
セレウス菌	＋	該当なし	4.4±1.1	103	6.5×10^{6}
エンテロバクター菌	－	29005	5.9±0.5	41.3	1.5×10^{9}
大腸菌	－	25922	6.6±2.3	65	2.7×10^{8}
肺炎桿菌	－	8045	12.6±0.7	55	6.2×10^{8}
グラム陽性菌	＋	51277	106.7±45.8	596	<1.0
セラチア・マルセッセンス	－	43862	4.3±0.2	79	$>3.0\times10^{9}$
黄色ブドウ球菌	＋	29213	9.7±1.1	75	2.2×10^{8}
黄色ブドウ球菌	＋	10832	14.4±0.9	63	3.8×10^{8}
黄色ブドウ球菌	＋	25923	9.3±0.3	68	4.3×10^{8}
黄色ブドウ球菌	＋	27217	9.0±0.6	70	5.3×10^{8}
黄色ブドウ球菌	＋	29213	12.1±2.1	14	4.0×10^{8}
表皮ブドウ球菌	＋	12228	17.3±1.7	54	3.6×10^{8}
表皮ブドウ球菌	＋	14990	19.0±1.9	57	1.2×10^{8}
表皮ブドウ球菌	＋	700578	23.2±1.7	23	2.9×10^{8}
表皮ブドウ球菌	＋	35984	19.4±1.5	39	2.7×10^{7}
表皮ブドウ球菌	＋	12228	19.1±0.8	11	1.8×10^{8}
表皮ブドウ球菌	＋	12228	該当なし	<3	1.7×10^{8}
B 群連鎖球菌	＋	700046	9.1±0.8	54	6.1×10^{6}
連鎖球菌	＋	6249	16.2±2.9	28	2.9×10^{7}
化膿連鎖球菌	＋	BAA-1064	10.2±0.6	42	4.4×10^{5}
エルシニア感染症	－	23715	10.1±2.3	76	8.5×10^{8}

CFU: コロニー形成単位; NA: 該当なし

細菌で汚染されている代表的な血小板成分には、全体で約 10～100 の有機組織体が含まれている可能性がある。高スパイク調査および低スパイク調査を合わせたデータは、実際の臨床的環境において予想されるよりも実質的に高度な課題をもたらす状況下における、

Mirasol 処理の細菌不活化能力を実証する。これらの細菌不活化レベルは、細菌感染リスクの対応不活化における非常に高い限界点を示唆している。

これらの結果にもとづき、Mirasol 処理は処理済血液成分を介した細菌感染を削減する結果をもたらすと期待されている。1:3000 という血小板成分の細菌汚染の頻度を考えると、この環境における患者の罹患率および死亡率に対する潜在的な利点は重要である。

臨床的環境において予想されるよりも大幅に高度な課題をもたらす状況下における Mirasol PRT System による細菌不活化の印象的な結果は、Mirasol 処理により輸血による細菌感染が減少するということを強く示唆している。

寄生虫の不活化

旅行や移住の増加により、熱帯地方特有の寄生虫症がそれ以外の国々の献血者にも見られる。現在では、血液成分を通じて感染する可能性のある多数の寄生虫に対する十分な献血者のスクリーニングが存在せず、シャーガス病（*クルーズ・トリパノソーマ*）、内臓リーシュマニア症（*ドノヴァン・リーシュマニア*）およびマラリア（*プラスモディウム・ファルシパルム*）を含む、輸血に関連する寄生虫感染が近年数多く報告されている。

そのため、寄生虫の不活化は PRT のもう一つの魅力的な特性であるが、細菌に関しては、PRT の試験対象となる標準的な病原体パネルが存在しない。Mirasol PRT System はどのような病原体に対しても利用される可能性があるため、寄生虫の感染を予防する効果的なオプションを提供する。私たちは現在様々な寄生虫に対してシステムの試験を行っている。現在までに得られた結果は、表 7 に要約する。

Mirasol PRT System は、輸血用血液に重大な脅威を与える様々な寄生虫を効果的に不活化させることが明らかになった。

表 7. 寄生虫のログ･リダクション結果

病気	病原体	ログ・リダクション	試験対象
リーシュマニア	*小児ドノヴァン・リーシュマニア*	>5.0[32]	血小板、血漿
マラリア	*プラスモディウム・ファルシパルム*	>2.0[2,33]	赤血球
シャーガス病	*クルーズ・トリパノソーマ*	>6.0[2]	血小板、血漿
ツツガ虫病	*オリエンティア・ツツガ*	>6.0[34]	血小板、血漿、赤血球

	ムシ		
バベシア症	バベシア・ミクロティ	>5.0[2]	血小板、血漿

††ファイル・データはNavigant Biotechnologies LLCが保持しており発表期限は 2008 年

要約

・ウィルス、細菌および寄生虫に対する Mirasol PRT System の有効性を評価するために、厳しい基準を採用した

・ガイドラインが利用可能な場合は、推奨される病原体の全機能について Mirasol PRT System の試験を実施した

・ガイドラインが利用可能でない場合は、Navigant Biotechnologies LLC は適当な血液製剤汚染に関する公開データを利用した

・Mirasol PRT System を使用した処理は、処理済血液製剤からの病気感染の可能性を減少させ、Mirasol PRT System の有効性に関する代表的な試験を保証することを目的としている

・Mirasol PRT System の有効性は細菌（グラム陽性およびグラム陰性の両方)、ウィルス（RNA および DNA、皮膜を有する場合と有さない場合）および寄生虫に対して実証されている

・Mirasol PRT System は病気感染の可能性が極めて低くなるレベルまでウィルス力価を削減する

・PPV に関する不活化調査の結果に基づき、観測されたウィルス不活化のレベルは、HIV-1 の潜伏期間および人体における全体的なウィルス血症期間を終わらせるために必要とされるレベル、およびヒト B-19 パルボ・ウィルスの慢性的段階感染の潜伏を終わらせる可能性のあるレベルを超えている

・Mirasol 処理は IBRV（CMV のモデル）ウィルス力価を大幅に削減する

・Mirasol PRT System は、HAV のレベルを削減することが証明されている唯一の技術である

・Mirasol PRT System は、高耐性菌を含む広範囲にわたる細菌の高細菌力価を削減する

・Mirasol PRT System は、通常の血液を構築する状況下における 5 日間の保管期間を通じて溶液陰性製剤を産出する

・臨床的環境において予想されるよりも大幅に高度な課題をもたらす状況下における Mirasol 処理の細菌不活化の結果は、Mirasol 処理が輸血による細菌感染を削減するということを強く示唆している

・Mirasol PRT System は、輸血用血液に重大な脅威を与える様々な寄生虫を効果的に不活化させることが明らかになった

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第 5 章

病原体不活化技術（Mirasol PRT）による白血球の不活化

MIRASOL®
病原体不活化技術

Mirasol病原体不活化技術（Mirasol PRT）による白血球の不活化

不安定な血液製剤に残存している白血球は、輸血に伴う副作用の原因のなかでももっとも一般的であり、かつ危険なものである。このため白血球は、不活化における重要なターゲットとなっている。

同種異型白血球の輸血に関連するリスク

以下に記述するのが、もっとも一般的な副作用である。また、これらをまとめて示したのが下記の表 1 である。

輸血に起因する移植片対宿主病

輸血に起因する移植片対宿主病（TA-GVHD）においては、輸血された白血球が輸血を受けた患者に対して免疫攻撃を開始する。TA-GVHDの臨床兆候としてみられるのは、肝臓、皮膚、粘膜ならびに消化管に対する選択的損傷によって引き起こされる発熱、発疹、肝機能障害、下痢などである。TA-GVHDは骨髄形成不全に関連したものであることから、これらの経過は急性であり、また劇症となる。一般的には発現の頻度は低いものとされているが、ウィルス感染や医薬品の副作用などとその他の状態と類似した特徴を示すことから、実際の罹患率は未確認である。

TA-GVHDを発症した患者は90%以上が死亡に至っており、輸血に関連する死因においては最大の割合を占めている。主な死因は、感染または汎血球減少症に由来する出血である。輸血された白血球に対し宿主対移植片攻撃を行うことができない免疫不全の患者、ならびに未熟児に主として見られる死因であるが、血縁ドナーと共有するヒト白血球抗原（HLA）のハプロタイプにヘテロ接合の免疫応答性を持った輸血を受けた患者にもみられる死因である[1]。この後者のケースにおいては、輸血を受けた患者の免疫系が移植された白血球を自身のもの以外であると認識できず、一方で移植されたドナー側の細胞がこれを認識し、輸血を受けた患者の細胞組織に対する攻撃を始めるのである。

マイクロキメリズム

マイクロキメリズムは、宿主において少数ながら宿主以外のものである細胞が安定して存在し続けることと定義される。たとえば、輸血を受けた患者の血液中において、輸血後もドナーの白血球が長期にわたって活着することなどである。こうした状態の継続は、慢性自己免疫疾患の発症と関連している可能性がある。患者のなかには、数か月間のうちにキメラ細胞を失ってしまうものもいるが、その他の患者の場合においては、ドナーの白血球数が無期限ではないにしても、何年間にもわたって維持されるのである。また、こうした現象は白血球不活化、あるいは標準的な血液製剤を使用した場合において、同程度の頻度で発生している。

同種免疫

同種免疫は、赤血球や白血球、血小板抗原に対する抗体など、抗体の発生によって特徴づけられるものである。輸血された血液製剤に含まれている白血球のHLAクラスII抗原に対する同種免疫は、以下の原因となる場合がある：

•同種抗体－同種移植片の拒絶を誘因
•同種抗体－出血傾向を増長させる血小板不応を誘因

表1. 不安定血液製剤による潜在的副作用

反応	原因物質
TA-GVHD	ドナーのT-リンパ球
マイクロキメリズム	ドナーの白血球（リンパ球）
HLA-同種免疫	ドナーの白血球（主に抗原提示細胞）
TRALI	ドナーの抗HLAクラスI抗体またはクラスII抗体、もしくは抗好中球抗体 ドナーのBRMs
FNHTR	ドナーによって生成されたサイトカイン 保存された白血球
TRIM	ドナーによる免疫修飾 T-リンパ球および血漿
輸血感染症	細胞結合型の感染物質

BRM：生物反応修飾物質、FNHTR：発熱性非溶血輸血反応、HLA：ヒト白血球抗原、TA-GVHD：輸血による移植片対宿主病、TRALI：輸血関連急性肺障害、TRIM：輸血による免疫修飾：白血球

輸血関連急性肺障害

輸血関連急性肺障害 （TRALI）は、輸血によって引き起こされる合併症のなかでも、もっとも深刻なもののひとつである。臨床的兆候には、息切れ、低酸素血症、低血圧、発熱、非心臓性肺浮腫などが含まれる。症状は通常、輸血から1–2時間以内、血漿を含有する血液製剤の輸血の場合は必ず6時間以内に発症する。たいていは一時的な症状であるが、患者の

6–10%にとっては致命的なものとなる。

TRALIの正確な発症頻度は未確認であり、TA-GVHDについては、おそらく大幅に過小評価されているとみられる。文献に報告されている罹患率は、以下のとおりである——新鮮凍結血漿の場合は7,900ユニットに1例、血液および血液製剤は5,000ユニットに1例、血漿含有血液製剤は2,000ユニットに1例、全血由来血小板は432ユニットに1例。発症数は増加しているとみられており、TRALIは輸血関連の死亡例のなかでも主要な死因となっている[5]。TRALIの病態生理学には、免疫学的および非免疫学的メカニズムのいずれもが関連しているとみられるが、明確なメカニズムは不明であり、患者によって異なるものであると考えられている。TRALIの発症においては、複数の重要な要因があると考えられている：

• 第一に、自身の白血球が対応する抗原を保有している患者に対し、抗HLAクラスI抗体、またはクラスII抗体、もしくは抗好中球抗体を輸血した場合に引き起こされる免疫反応、または抗体媒介性のメカニズムがある。抗体——抗原の相互作用が補足的に肺分画症と多形核球好中球の活性化（顆粒球）を引き起こし、その結果としてTRALIが発症する。

• 第二に、非免疫介在性の反応がある。これは、”two-event modelと呼ばれるものである。:最初の現象は、肺内皮の活性化ならびにPMN集積によって患者に発症する臨床症状である。そして、次に起こる現象が、付随的PMNを活性化し、内皮障害や毛細血管漏出、TRALIを誘発する抗顆粒球抗体、脂質、CD40リガンドなどの生物学的修飾物質（BRMs）の輸注である。

発熱性非溶血輸血反応

発熱性非溶血輸血反応（FNHTRs） は、血液成分の貯蔵中に生成されたサイトカインによって引き起こされるものである。FNHTRの臨床症状としてみられるのは、輸血後の24時間以内に起こる発熱と悪寒であり、複数回にわたる輸血を受けている患者、あるいは妊娠経験のある患者にもっとも頻繁に発生する。
FNHTRの発生率は、赤血球輸血の場合で6.8%、非白血球不活化血小板輸血の場合で30.8%と推定されている。白血球は、FNHTRの原因として重視すべきものである——保存された濃厚血小板においては、白血球細胞によってサイトカインの活性合成が起こり、白血球由来の炎症性サイトカインの含有量が増加する。濃厚血小板製剤は保存中に溶解性CD40リガンドを蓄積することがわかっており、これによって、発熱を誘引するものとして知られるプロスタグランジンE2 （PGE2）が生成されるのである。FNTHRは生死にかかわるものではないが、コスト面ならびに患者のコントロールの側面において重大な影響を持っている（血液培養に関する要件、溶血反応の可能性が否定されるまで輸血を停止する必要性、入院期間の長期化の可能性など）。

輸血関連免疫修飾

ドナーのT-リンパ球によるTRIMは、輸血後の一時的な免疫抑制によって発生するものである。これは、細菌感染ならびにガン再発のリスクの増加に関連するものである。冠動脈バイパス手術の後に同種血輸血を受ける患者の場合には、敗血症、術後感染、心臓への感染などといった感染リスクがより高くなる。

病原体の直接感染

白血球が細胞結合感染因子、なかでもとくに、サイトメガロ・ウィルス （CMV）やヒトT細胞性白血病ウィルス（HTLV）などに感染している場合もある。

血液製剤に残存している白血球は、もっとも一般的であり、また危険な輸血副作用（TRIMやTA-GVHD、FNHTRなど、そして頻度は下がるもののTRALI）の原因である。したがって、白血球は不活化における重要なターゲットである。

白血球由来の危険性削減に向けた現在の戦略における限界

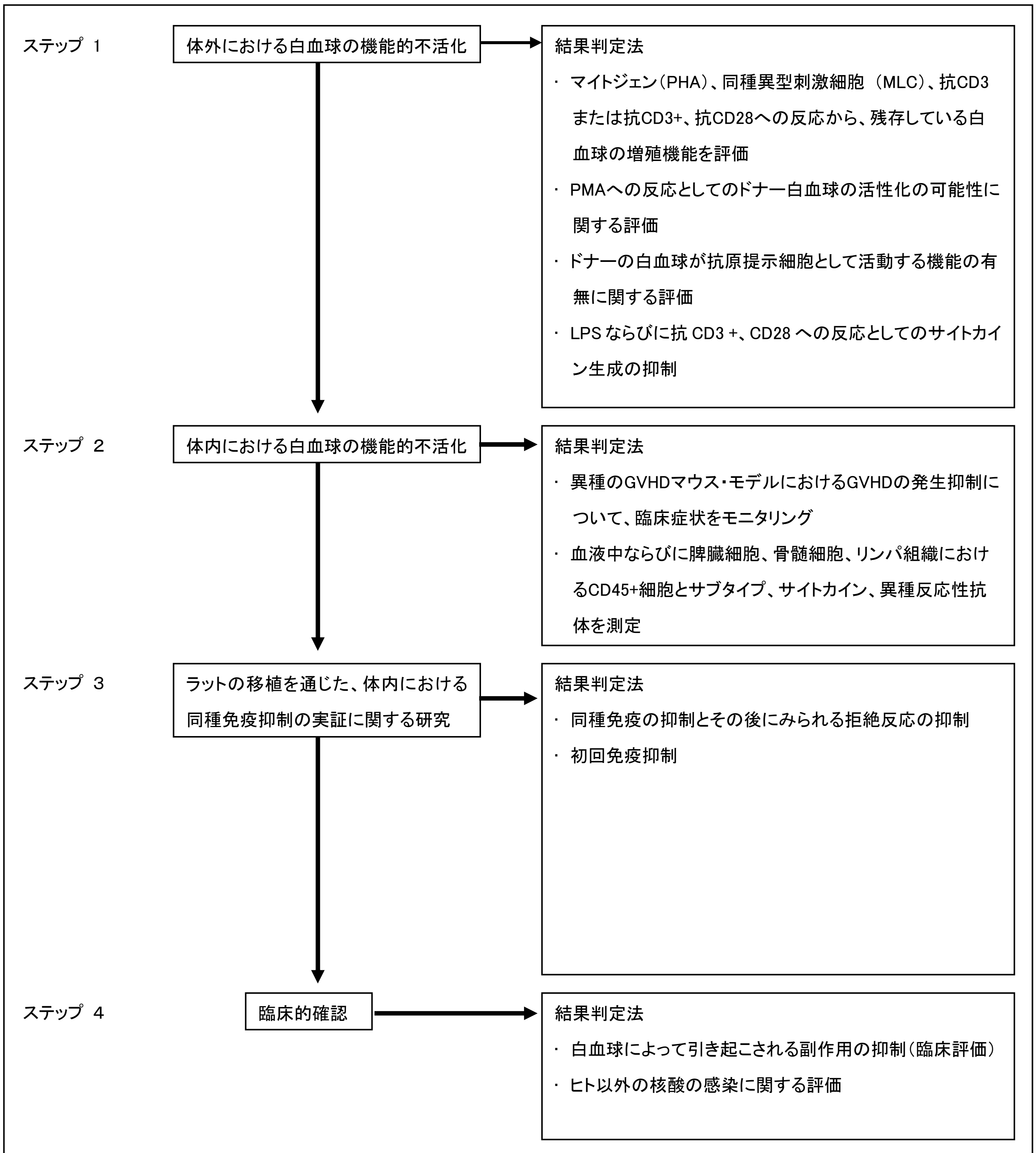
ドナーの白血球を不活化するために一般的に利用されるのが、ガンマ線照射である。適正な放射線量でこれを実施した場合には、TA-GVHDの発生が抑制される。しかしながら、使用されているガンマ線照射のレベルは、より小さく、低感受性遺伝子である非白血球性病原体（ウィルスなど）を不活化するには不十分なものである。

さらに、照射はクローン性増殖の進行を抑制し得るものの、必ずしも白血球を殺すものではない。このため、白血球はFNHTRやTRALIを引き起こすのに十分なだけのサイトカインを生成し続ける可能性がある。

リスクを軽減する目的で使用されているもうひとつの一般的な方法が、ろ過（赤血球除去または赤血球不活化）である。しかしながら、以下を抑制するためのものとしては100%有効なものではない。a) FNHTR、b) ウィルス感染、c) マイクロキメリズム。
さらに、残存している白血球には増殖機能が維持されており、ガンマ線照射をしていない白血球減損の製剤を輸血した後でのTA-GVHDが報告されている。

現在のところ、白血球由来の副作用を削減するために使用されている方法は複雑なものであり、完全に有効なものではない。そしてまた、血液製剤の製造過程を複雑なものにしている。
白血球に由来するリスクに対応するための現在の戦略は、100％有効なものではない。残存している白血球の不活化は、深刻な輸血副作用を防ぐために不可欠なものである。

図 1. Mirasol 不活化処理後における白血球由来の副作用削減についての調査



GVHD：移植片対宿主病、LPS：リポ多糖、MLC：白血球混合培養、PHA： ファイトヘマグルチニン、
PMA：ホルボールミリスチン酸アセテート、白血球：白血球

白血球に由来するリスクの軽減におけるMirasol PRTシステムの有効性

私たちは、白血球に由来する病変の発生抑制におけるMirasol処理の有効性を調査するための包括的戦略を採用した（図1を参照）。

結果：体外における白血球の機能的不活化（ステップ 1）

ステップ1では、ヒトの単核細胞に対するMirasol処理の有効性について、体外における研究を行った。それぞれの実験には、処理済み、未処理のサンプルを一組みずつ使用した。Mirasol処理は、すべての分析試験において反応を抑制した[13]。処理によって抑制された反応は、具体的には下記に示すとおりである。

- ホルボールミリスチン酸アセテート（PMA）に対する反応としての活性化
- マイトジェン（PHA）、抗CD3および抗CD3+、抗CD28、ならびに同種異型の刺激細胞に対する反応としての増殖
- 抗原提示機能
- リポ多糖（LPS）または抗CD3+ 抗CD28への反応としてのサイトカインの生成。

これらの結果は、全血ユニットにおいて確認されたものである。赤血球、血小板、または血漿製剤に対する副作用は一切発生しなかった。

Mirasol PRT処理によって、体外においては白血球を不活化することに成功した。

結果：体内における白血球の機能的不活化（ステップ 2）

ステップ 2においては、TA-GVHDのモデルとして、B-、T-、ならびにNK細胞が欠如している遺伝的免疫不全のマウスの腹膜にヒトの単核細胞（Mirasol処理済み、および未処理）を注入した。

この研究により、以下が明らかになった。：

- Mirasol処理済みの細胞を注入したグループのマウス（N=14）のうち、異種 GVHDの症状を発症したものはなかった。一方で、Mirasol未処理の細胞を注入したマウスは14匹のうち、12匹が異種GVHDの症状を発症した。後者のグループにおいてはTA-GVHD のケースと同様、細胞溶解反応に伴う末期の異種GVHDが見られた。これは、ヒトCD4+ならびにCD8+細胞の存在に特徴づけられるものである。
- 双方のグループにおいて、ドナーの白血球の分布は類似したものであった
- Mirasol未処理の細胞を注入したレシピエントのマウスの血漿においては、ヒトサイトカイン（インターフェロン-©ならびにインターロイキン-10を含む）およびヒト免疫グロブリ

ンのみが検出された
- 未処理の細胞を注入されたグループにおいてのみ、キメラ現象が検知された
- 未処理のドナー細胞は、同種異型ならびに異種の刺激細胞に対する反応として増殖することが可能であった；処理済み細胞にはみられなかった
- 未処理のグループにおいては脾臓の重量が増加し、一方でヘマトクリット（Hct）値が大幅に減少した

この研究によって、ここで採用されたTA-GVHDモデルにおいては、Mirasol処理が異種における移植片対宿主反応を排除することが示された[15]。本論の著者は、「血液製剤に対するMirasol PRT処理の実施は、TA-GVHDの発生を抑制するものである」と結論づけた。

Mirasol処理によって、ヒトのTA-GVHDのモデルとして使用したネズミの異種 GVHDに発生の抑制がみられた。

結果：同種免疫およびその他の白血球関連の副作用が抑制された（ステップ3）。

同種免疫

In vivo研究では、同種免疫の発生に対するMirasol処理の効果について調査を行った。レシピエントであるラットには複数回にわたって、ドナー・ラットから血小板50 mLおよび高濃度の白血球 [10^6/mL]を含有する輸血を行った。

以下の３つのグループについて、評価を実施した。：

- 第一グループには生理食塩液のみを与えた。
- 第二グループにはMirasol処理済みの血液を輸血した。
- 第三グループにはMirasol未処理血液を輸血した。

10週間後、ドナー・ラットの心臓をレシピエント･ラットに移植したところ、移植臓器の生着とともに免疫反応が見受けられた。：

- 未処理のグループにおいては、高度の免疫反応が観測された――IgMのピークが高水準で確認され、続いてIgG値も上昇した（図2を参照）。
- Mirasol処理済みの血液のグループにおいては、IgM反応は観測されず、１匹のマウスのみにおいて非常に軽度のIgG反応が観測された（図2を参照）。
- 生理食塩液のみのグループにおいては、何の反応も観測されなかった。
- Mirasol処理済みの血液のグループにおいては、移植を受けたすべてのマウスが生存し、未処理のグループにおいてはすべてが死亡した（図3を参照）。

本論の著者は、Mirasol処理は「血液製剤の免疫原性を減少させるという、さらに重大な利点を持つ可能性がある。感作の原因を大幅に削減することから、こうした効果はとくに移植患者にとって重要なものである」と結論づけた。

Mirasol処理は、動物モデルにおける同種免疫を抑制し、血液製剤中の免疫原性を減少させた。

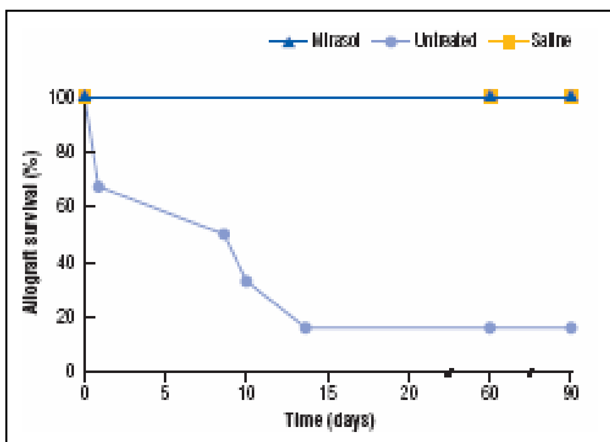


図3. ラットにおける同種免疫移植の生存率に関する研究

図2. ラットにおける同種免疫移植のIgM値およびIgG値に関する研究

2匹のラットについての結果を表すそれぞれのグラフは、IgM反応ならびにIgG反応を示したものである。右手のパネルには、未処理の血液を輸血したラットの結果が示されている——急速なIgM反応の出現と、それに続く強いIgG反応がみられる。左手のパネルは、Mirasol処理済みの血液を輸血したラットの結果である——IgM反応はみられず、6匹中1匹にのみ、後期に入ってからの軽度のIgG反応がみられた。 Ig：免疫グロブリン、UV：紫外線

表2. 白血球不活化ならびにガンマ線照射と比較した場合のMirasol PRTシステムの利点

	白血球不活化	ガンマ線照射	Mirasol PRTシステム
メカニズム	ろ過により白血球を不活化	核酸損傷を発生	リボフラビンと核酸の間に不可逆の付加体を形成
白血球不活化	不完全	>5ログ削除（25 Gy）	>6ログ削除
サイトカイン生成、発現可能性	可能性あり	可能性あり	予防された
GVHD	可能性なし	予防された	予防された
病原体感染	無細胞病原体または低レベルの細胞結合型病原体にかかる問題には対応しない	可能性あり	予防された

CMV：サイトメガロ・ウィルス、GVHD：移植片対宿主病、白血球：白血球

免疫に起因するその他の副作用

TRALIやTRIMといったその他の免疫反応を軽減させる機能に関してMirasol処理の評価を行うため、さらなる研究を実施した。血液製剤の保存期間中にはBRMsが生成された。前述のとおり、これはTRALIの発症機序に関連があるとみられている。

赤血球ならびに血小板製剤の保存中におけるこれら合成物の蓄積に対するMirasol処理の効果について、評価を行った。Mirasol処理済みのサンプルと未処理の血液製剤について、それぞれが健康なドナーから好中球の白血球遊走因子(fMLP)-誘発型呼吸バーストを増進させ

る能力を測定するため、これらの分析を行った。

赤血球および血小板のMirasol処理によって、通常は保存期間中に起こり、プライミング脂質を含めたBRMsの生成を示唆するものであるプライミング活性の生成が抑制された。細胞を含む血液製剤のMirasol処理は、TRALIに関連する病原体のひとつを不活化できる可能性がある。

現在、Mirasol処理がTRIMの発生を抑制する可能性に関して研究が進行中である。この研究実験においては、ラットに毎週一回、Mirasol処理済み全血、または未処理の全血、もしくは生理食塩液のいずれかを輸血し、その後、細菌への反応などについての調査を行う。評価項目は生存率である。

Mirasol処理は潜在的に、TRALIの発症とのかかわりが指摘されてきた生物反応修飾物質の生成を抑制するものである。

結果：臨床試験による確認（ステップ4）

現在の、そして将来における臨床プログラムによって、ヒトにおける上記のような副作用の発生、ならびに抑制の可能性に関してのデータが作成されることになるだろう。フランスでMirasolに関する臨床研究（MIRACLE試験）が実施されたが、このとき実施施設は、Mirasol処理済みの血小板を提供するにあたって、ガンマ線照射を行うか否かを自由に決定することができた。臨調研究者の大半は、ガンマ線照射を行わないことを選択した。この研究においては、TA-GVHDのケースは報告されていない。

結論

Mirasol処理が持つ白血球不活化の能力について評価を行うため、私たちは4つのステップを経る戦略を採用した。プロセスは現在も進行中であり、現在もin vivo研究ならびに臨床研究を継続している。これまでに、細胞を含有した血液製剤のMirasol処理が輸血に関連した拒絶免疫反応の排除に役立つか否かについて、in vitroならびにin vivo研究を行っている（表2参照）。

Mirasol PRTシステムを用いた処理によって、残存している白血球および関連する病原体を不活化できることが示されている。この技術は、現在の白血球抑制プロセスにおける重大な利点である（表2参照）。Mirasol処理は、生存能力を持った白血球の輸注、蓄積された炎症メディエーターによる汚染、ならびに細胞関連ウィルスへの感染の可能性を効果的に抑制する。この技術が持つこうした機能は、患者ならびに担当医にとって、臨床上の大きな利点であると考えることができる。

要約

• 残存している白血球は深刻な輸血関連の反応（TRIMやTA-GVHD、FNHTRなど、また低い頻度におけるTRALI）の抑制に不可欠である。したがって、白血球は不活化における重要なターゲットである。

• 白血球由来のリスクに対応するための現在の戦略は、100%有効なものではない。残存している白血球の不活化は、深刻な輸血関連の反応の抑制に不可欠である。

• Mirasol処理は、体内において白血球の不活化に成功した。

• ヒトTA-GVHDのモデルとしたマウスにおいて、Mirasol処理は異種GVHDの発生を抑制する。

• Mirasol処理は同種免疫を抑制し、また血液製剤の免疫原性を減少させる。

• Mirasol処理は、TRALIの発生に関連するとみられるBRMsの生成を抑制する。

• Mirasol技術は、生存能力を持った白血球の輸注、蓄積された炎症メディエーターによる汚染、ならびに細胞関連ウィルスへの感染の可能性を効果的に抑制する。こうした機能は、臨床上においても重要な利点であると考え得る。

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第 6 章

Mirasol 処理した血小板製剤の品質

MIRASOL®
病原体不活化技術

Mirasol 処理した血小板製剤の品質

血小板輸血の主目的は、患者の止血機能を回復させるために十分な機能のある血小板を供給することにある。紫外線（UV）光を利用した病原体不活化技術（PRT）はいずれも血小板の生理に影響を及ぼすので、PRT 処理した血小板が生存能と機能性を確実に保持しているようにすることが極めて重要である。採取後の血小板の二次的取扱は、in vitro 試験で認められているとおり、どんなものであっても活性化を誘発し、血小板の生化学的および細胞学的状態の複合的変化である血小板保存損傷を加速化し得る。この損傷の程度は、in vivo での血小板のリカバリーと生存性ならびに輸血後の止血活性の低下と相関するとされている。

In vitro 試験の結果

In vivo アッセイの複雑さと費用を考えると、血小板の生存能と止血機能を定期的に直接測定するためにこれを利用することは不可能である。このように定期的な直接試験を行えないということは、血液の安全性のための処理が血小板機能を損なわないことが証明されていなければならないことを意味する。私たちはこれまで、Mirasol 処理後の血小板の品質を評価するために広範にわたる細胞品質アッセイパネルを利用してきた。このパネルは部分的に、BEST（Biomedical Excellence for Safer Transfusions；より安全な輸血のための生物医学的卓越性）委員会の勧告や血小板検査に関するその他の業界ガイドラインに基づいている。

Mirasol 処理した血小板については in vitro および in vivo の評価が行われてきており、また Mirasol 処理血小板の臨床成績が現在、血小板減少症患者の大規模臨床試験で評価されている。本章で述べる結果はいずれも血漿中の血小板について得られたものであり、血小板添加溶液（PAS）中の血小板を評価する試験が現在進行中である。

血小板の品質と性能

単一の in vitro パラメータで血小板の in vivo 生存能を予測できるものは存在しないが、多くの標準的パラメータをモニターすれば、血小板品質の全体像を把握することが可能である。血小板品質を評価するための特異的 in vitro 検査の利用についてはまだコンセンサスが得られていないが、正常な円盤形からの形状変化の程度を光度計で測定した形態や低張ショック反応（HSR）といった一定の特徴は、実際に in vivo 生存能とよく相関する。これらのアッセイはまずまずの感度で、確実かつ再現性のある実施が可能であることが複数の研究によって示されている。また、乳酸塩の産生と pH が放射標識した血小板のリカバリーおよび生存性と高度に相関することが、健康な被験者において確認されている。

Mirasol処理血小板の研究は、Trima®アフェレーシス血小板濃厚液（APC）とバフィーコート血小板濃厚液（BCPC）の両方を用いての5日間の保存を通じたin vitro細胞品質試験として実施されている。実施アッセイには、平均血小板容積（MPV）の変化、血液ガス（pO_2およびpCO_2）、pH、乳酸塩とグルコースの濃度、P-セレクチン発現、血小板スワーリング、HSRおよびESCが含まれる。いずれのアッセイも、標準的なバリデーション済みのプロトコルに従って実施された。その結果を表1にまとめている。

代謝に関する結果

- Mirasol処理血小板は5日間の保存を通じて標準的なpH基準に適合する。
- Mirasol処理血小板は未処理の血小板と比較して高い代謝率を示し、グルコースの消費と乳酸塩の産生が多い。この結果はAPCとBCPCで同様である。
- Mirasol処理血小板は、pO_2レベルが大気レベルを下回り、最低 10 mmHgを超えていることで示されているとおり、酸素を消費し続け、酸化的呼吸によるATP産生能を保持する。

In vitro試験の結果は、Mirasol処理血小板が品質と性能を維持し、輸血の時点で求められるin vitroのpH基準を満たすことを実証している。

表1. 5日間保存後のin vitro結果のまとめ

細胞品質パラメータ	単位	対照 APC（N=20）	Mirasol処理 APC（N=12）	対照 BCPC（N=6）	Mirasol処理 BCPC（N=12）
代謝パラメータ					
pH（22°C）	NA	7.48 ± 0.06†	7.14 ± 0.09	7.44 ± 0.07	7.06 ± 0.23
乳酸塩産生率	$mmol/10^{12}$ /hour	0.032 ± 0.006	0.059 ± 0.012	0.023 ± 0.003	0.075 ± 0.025
グルコース消費率	$mmol/10^{12}$ /hour	0.019 ± 0.004	0.034 ± 0.005	0.04 ± 0.009	0.042 ± 0.012
pO_2	mmHg	54 ± 15	48 ± 20	61 ± 27	41 ± 22
pCO_2	mmHg	26 ± 3	28 ± 5	25 ± 6	32 ± 8
活性化パラメータ					
P-セレクチン発現	%	17.9 + 7.0	57.8 + 14.8	11.7 ± 2.6	57.3 ± 9.6
形態パラメータ					
スワーリング	NA	3 ± 0‡	3 ± 0	3 ± 0	3 ± 0
HSR	%	72.3 ± 10.9	67.0 ± 7.3	ND	65.3 ± 9.3
ESC	%	24.7 ± 4.3§	20.4 ± 4.8	ND	17.8 ± 3.8
MPV	fL	6.4 ± 0.6	6.5 ± 0.6	ND	8.1 ± 0.6

†表中の数値は平均±標準偏差である;‡N=17;§N=36。
APC:アフェレーシス血小板濃厚液;BCPC:バフィーコート血小板濃厚液;ESC:形状変化程度;HSR:低張ショック反応;MPV:平均血小板容積;NA:該当なし;ND:測定せず。

活性化に関する結果

- Mirasol 処理血小板では P-セレクチン濃度がわずかに上昇しており、これは活性化レベルの上昇を示すが、そのレベルは輸血時点で通常認められる範囲に収まっている。

他のパラメータに関する結果

- Mirasol 処理血小板の 5 日間保存後の平均 ESC 値は 15～20%である。ESC が 10～30%であれば in vivo リカバリー範囲が 40～70%となることから、Mirasol 処理血小板の in vivo リカバリーは臨床的性能の許容範囲内に収まっているといえる。
- Mirasol処理血小板と対照血小板の 5 日間保存後の平均HSR値はともに 65～70%である。40～50%のHSRは 40～70%のin vivoリカバリーと相関するため[2]、Mirasol処理血小板のin vivoリカバリーは臨床的性能の許容範囲内に収まっているといえる。

血小板の機能性

血小板が機能性を維持するためには、その凝固活性化能と同時に接着性と凝集性が保持されていなければならない（表 2 を参照）。血小板の機能性維持の調査には、フローサイトメトリーと灌流という 2 つの方法が用いられた。

フローサイトメトリーによる一次評価

この試験では、輸血に通常用いられる標準的血小板濃厚液と同等の抗原および活性化指標の変化が示された（表 2 および 3 ならびに図 1A～E を参照）。

灌流による二次評価

血小板機能の調査方法として確立している灌流試験でも血小板の評価が行われた。健康なボランティアから採取した血液に抗凝固剤を添加し、白血球と血小板を除去した。Mirasol 処理および対照血小板のサンプルを添加し、in vivo の循環条件をシミュレートするため、血液を灌流システム（酵素的に剥離した家兎大動脈の断片を備えた中心ポンプを含むチャンバー）に 10 分間循環させた。

次いで大動脈断片を組織学的に検査し、処理および未処理血小板と大動脈の内皮下層との相互作用を判定した。

その結果は以下のとおりであった（結果は血小板が血管表面を覆っているパーセンテージ

で表されている)。

- 0 日目：未処理の血小板で実施した灌流試験は、大動脈断片の血小板被覆率が 25.7%であることを示した。Mirasol 処理血小板では血小板被覆率が 5.19%のなだらかな低下を示した（図 2 参照)。
- 5 日目：Mirasol 処理血小板では未処理血小板と比較して内皮下層に対する血小板の反応性がわずかに上昇していた。

表 2. 血小板の機能性に関連する要因

特性	評価パラメータ
接着性	F21 血小板 GPIbα vWF
凝集性	血小板 GPIIb–IIIa 血小板 GPIV フィブリノーゲン vWF フィブロネクチン
凝集メカニズム活性化能	P-セレクチン（活性化抗原） LIMP（活性化抗原） アネキシン V 結合（アポトーシスマーカー） 第 Va 因子（アポトーシスマーカー）

GP：糖タンパク質；LIMP：ライソゾーム完全膜タンパク質；vWF：フォン・ヴィレブランド因子。

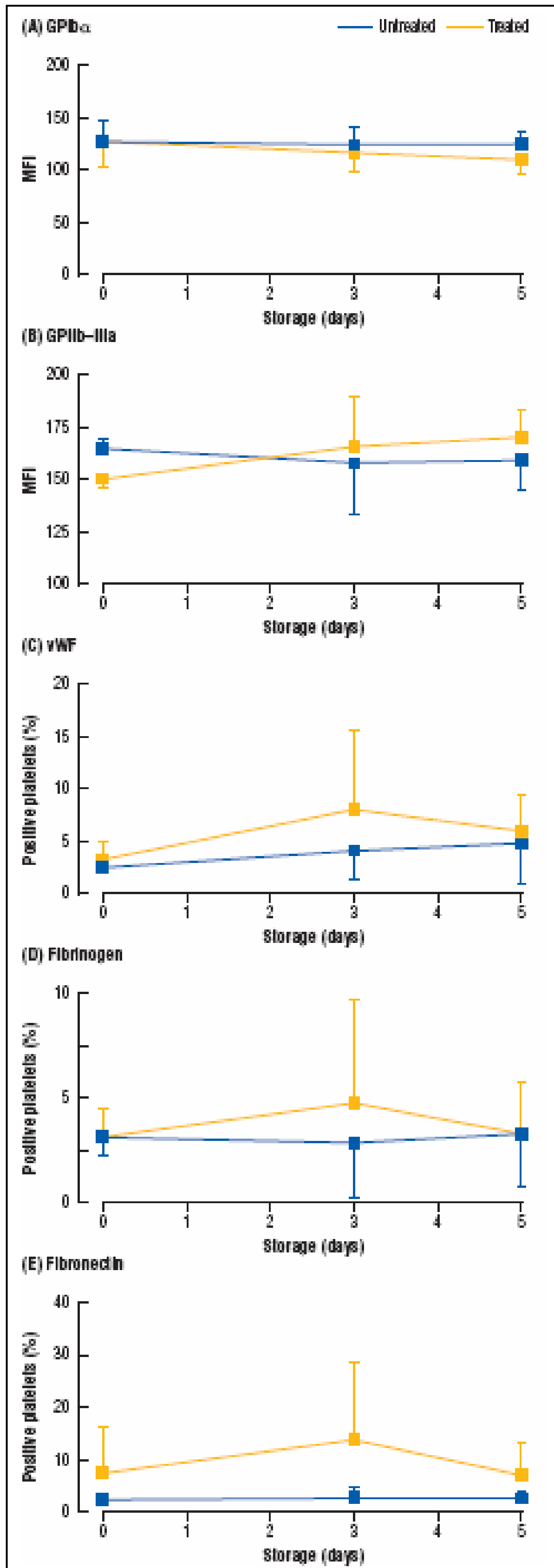

図 1A～E. 5 日間保存中の未処理および Mirasol 処理血小板に対する（A）GPIbα;（B）GPIIb-IIIa;（C）vWF;（D）フィブリノーゲン;（E）フィブロネクチン存在下でのフローサイトメトリー試験（平均±SD、n＝8）。

フローサイトメトリーと灌流試験は、Mirasol 処理血小板が未処理血小板と同等の接着性および凝集性を保持していることを示している。5 日目には Mirasol 処理血小板の接着機能が未処理血小板と比較してわずかに上昇していた。

ミトコンドリアの完全性と機能性

ミトコンドリアはアポトーシスに重要な役割を果たすことが示されており、少なくとも 1 つのアポトーシスカスケードはミトコンドリア膜電位の破綻が引き金になることが知られている。これは、血小板保存損傷に関連する多くのアポトーシス現象の基礎にミトコンドリア機能の変化が存在し得ること[9]、およびミトコンドリアの機能性維持が血小板保存損傷の最小化にとって重要な要因となることを示唆している。

Mirasol 処理は解糖流量を増加させることが以前の研究によって示されており、これはミトコンドリアの損傷および/またはアデノシン三リン酸（ATP）消費の増加が原因であるという仮説が立てられていた。これにはさらなる研究が必要であったところ、Mirasol 処理血小板におけるミトコンドリア機能と酵素活性を 7 日間という保存期間にわたり評価する試験が、いくつかの方法（JC-1 シグナル、MTT アッセイ、pH および血小板 ATP 含量）を用いて実施された。

その結果は以下のとおりであった。

- JC-1 シグナルはミトコンドリア膜電位測定値であり、分極と脱分極のパーセンテージを評価する。保存中の対照および Mirasol 処理血小板のミトコンドリア膜電位に有意差は認められなかった（図 3 を参照）。

- ミトコンドリアの酵素活性の測定に用いられる MTT アッセイでは、Mirasol 処理血小板における活性測定値が 1 日目と比較して保存終了時には 15%低下していることが示された。この低下は保存中のもっと早い時点では明らかではなかった。対照の未処理血小板に対する MTT アッセイでは、1 日目と比較して 7 日目には活性が約 10%上昇していることが示された。ただし、この結果はミトコンドリアの酵素活性の上昇を示しているのではなく、単にアッセイ方法のばらつきによるものである可能性がある。とは言え、この結果はミトコンドリアの酵素活性が Mirasol 処理の直後から最大保存 7 日目まで保持されることを示唆している（図 4 を参照）。

- Mirasol 処理血小板は、対照血小板と比較して中等度であるが有意な pH 低下促進と ATP 含量の減少を示した（表 4 を参照）。

表 3. In vitro 試験：フローサイトメトリーの結果。

因子	対照	Mirasol 処理（6.2 J/mL）	有意差
GPIbα	0 日目から 5 日目まで安定	0 日目から 5 日目まで安定	有意差なし
GPIIb-IIIa、GPIV	0 日目から 5 日目まで安定	0 日目から 5 日目まで安定	有意差なし
vWF、フィブリノーゲン、フィブロネクチン	0 日目から 5 日目まで安定	0 日目から 5 日目まで安定	有意差なし
P-セレクチン、LIMP	保存中に進行性かつ有意に上昇 曝露後に有意に上昇	保存中に進行性かつ有意に上昇	活性化パラメータ発現率が有意に上昇
アネキシン V 結合レベル	0 日目から 5 日目まで安定	0 日目から 5 日目まで安定	有意差なし
第 Va 因子	保存中に進行性かつ有意に上昇	保存中に進行性かつ有意に上昇	有意差なし

GP：糖タンパク質；LIMP：ライソゾーム完全膜タンパク質；vWF：フォン・ヴィレブランド因子。

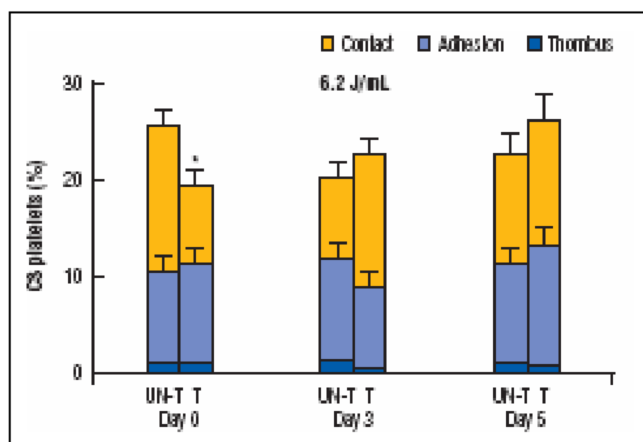

図 2. 5 日間の保存中の未処理血小板（UN-T）および Mirasol 処理血小板（T）の接触、接着および血栓形成のパーセンテージで分類した血小板による表面被覆（CS）パーセンテージ（平均±平均の標準誤差、n＝8、* UN-T に対して $p < 0.05$）。

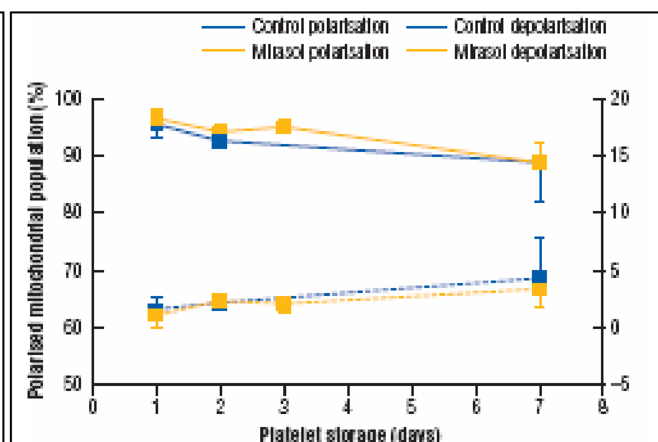

図 3. JC-1 染色アッセイを用いたミトコンドリア膜電位の測定

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以上の結果は、Mirasol 処理がミトコンドリアの構造および機能の完全性を損なわないこと、ならびに処理血小板で観察された解糖流量と酸化的代謝の増加についてはATP に対する要求の増大が推進力となっている可能性があることを示している。

続いての研究により、解糖流量の増加は保存損傷発生中の血小板の形態変化と自発的活性化の直接的な原因ではないことが示されている。その結果はまた、グルコース利用の低下は実際に保存中の血小板の損失を増大させ得ることも示唆している。

Mirasol 処理はミトコンドリアの機能的完全性と活性を変化させない。

In vivo の生存性およびリカバリー試験からの結果

あるランダム化一重盲検交差試験において、Mirasol 処理したアフェレーシス血小板と未処理血小板の有効性を比較した。すなわち、健康な被験者 24 人から採取した白血球減少アフェレーシス血小板を 5 日間保存し、処理の前後と保存期間中に微生物学的、in vitro および in vivo 分析を実施した。

放射標識して被験者に再注射した後、再注射から 3 時間以内、その後 1 週間は毎日、および再注射から 10 日目に新たな血液サンプルを採取した。In vitro分析には、血小板濃度、グルコースおよび乳酸塩濃度、単位pH、pO_2およびpCO_2、CD62/P-セレクチンの発現、ならびに血小板の形態（スワーリングスコア）が含まれた。元のドナーに再注射した血小板の生存性およびリカバリー率の計算も実施された（図 5 を参照)。

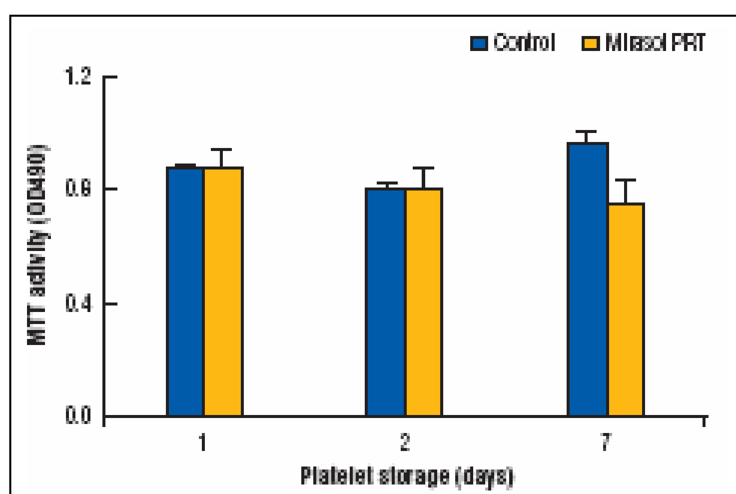


図 4. MTT 低下アッセイを用いたミトコンドリアの酵素活性の評価

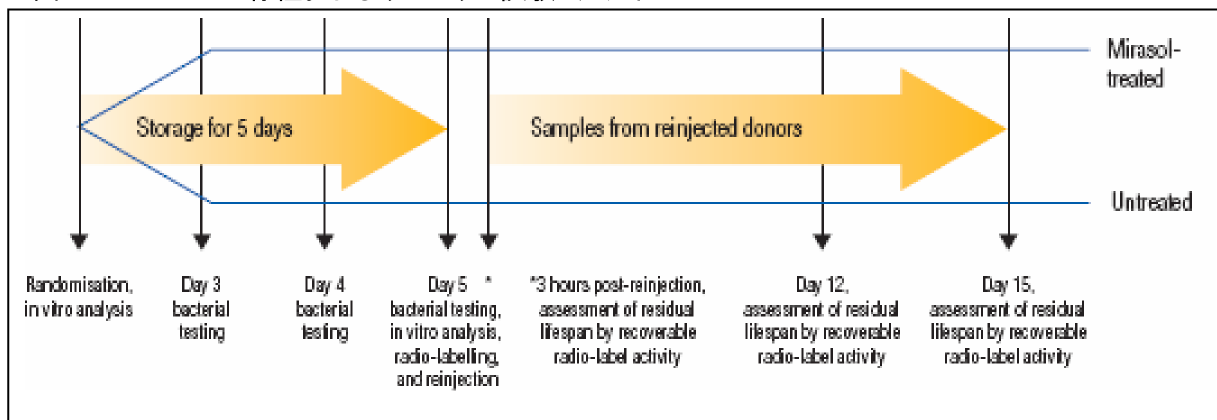
数値は平均±SD で表している。7 日目の試験サンプルと対照サンプルの間に有意差が認められた。
SD：標準偏差。

表 4. 血小板のミトコンドリアに対する Mirasol 処理の即時的効果

変数	対照（n=5）	Mirasol PRT（n=7）
pH、22°C	7.45 ± 0.02	7.51 ± 0.06
ミトコンドリア 分極（%） 脱分極（%）	 94.7 ± 1.8 1.5 ± 1.0	 95.9 ± 1.7 1.0 ± 1.2
MTT（OD490）	0.875 ± 0.027	0.885 ± 0.055
ATP（μmol/10^{11} PLT）	5.08 ± 0.72	4.64 ± 0.93

試験したいずれの変数についても、対照血小板と処理血小板の間に有意差は認められなかった。ATP：アデノシン三リン酸

図 5. In vivoの生存性およびリカバリー試験：デザイン[1]



その結果から以下のことが明らかとなった。

- いずれの被験者も試験への参加に関連した有害事象を経験することはなく、血小板の再注射はいずれも十分に忍容された。
- すべてのユニット培養は陰性で、総血小板数は無変化のままであった。
- Mirasol 処理ユニットは、グルコース消費の増加、グルコース濃度の低下および乳酸塩濃度の上昇を示した（図 6A および 6B）。
- 保存期間終了時にはすべてのユニットでグルコースの残存が認められた[1]。
- pH はすべてのユニットで 6.8 を上回っていた（図 6C）。
- Mirasol処理および未処理血小板はともに保存期間中にpO_2およびpCO_2の低下を示した（図 6Dおよび 6E）。pO_2レベルについては、未処理およびMirasol処理ユニットの間に有意差は認められなかった。処理群における処理前のpCO_2レベルは未処理群よりも有意に低かったが、保存終了時には有意に高くなった。

- P-セレクチンの発現は Mirasol 処理および未処理血小板の両方で保存期間を通じて増大しており、5 日目には Mirasol 処理ユニットの方が未処理ユニットよりも高くなっていた。
- 血小板スワーリングは保存開始時には差がなかったが、5 日目には処理ユニットの方が低くなった。

この試験により、処理血小板のリカバリーと生存性の平均は未処理ユニットより低いものの（表 5 を参照）、in vivo の有効性は十分に保持されていることが示され、臨床的有用性が推認された。

In vivo 試験の有望な結果は、Mirasol 処理血小板が十分に臨床使用できるだけの生存能と有効性を保持しており、in vivo でのリカバリーおよび生存性基準を満たしていることを示しており、これが臨床研究の根拠となった。

表 5. 放射標識試験の結果

	対照	処理	p 値
放射能収集効率（%）	64.2 ± 17.2	59.5 ± 21.2	>0.05
リカバリー（%）	66.5 ± 13.4	50.0 ± 18.9	<0.05
生存性（複数ヒット、時）	142 ± 26	104 ± 26	<0.05

^{111}In による放射標識と自家再注射の 5 日目の結果。結果は対照サイクルからの 22 所見および処理サイクルからの 24 所見を示している。

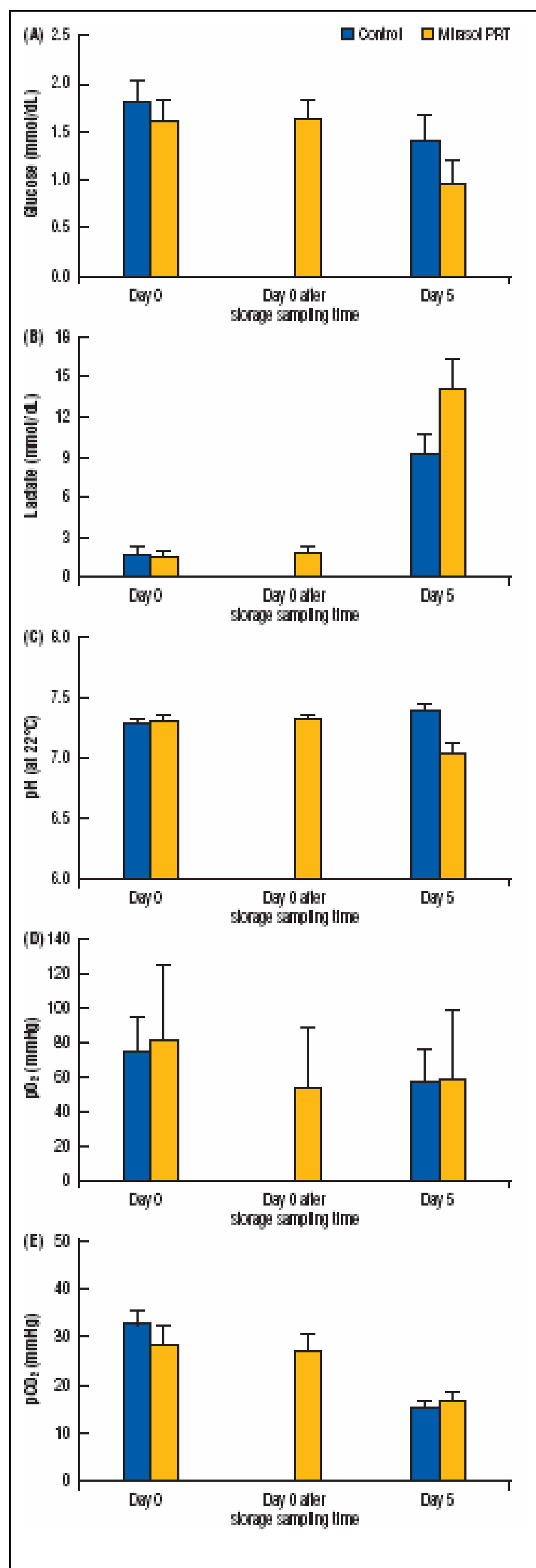
臨床試験の結果

血小板減少症患者におけるMirasol処理血小板の臨床的有効性を調べるため、多施設、非盲検、対照臨床試験が現在フランスの 6 カ所で実施されている（MIRACLE試験、第 8 章を参照）。患者には通常の臨床条件下でMirasol処理血小板濃厚液（試験コホート）または未処理の標準的血小板濃厚液（参照コホート）のいずれかを投与する。血小板成分は最大 5 日間保存することが許される。輸血から 1 時間後に測定した補正血小板増加数（$CCI_{1\,時間}$）と血小板濃厚液の注入に対する反応としての重大有害事象が、処理および未処理血小板濃厚液で比較される。輸血から 4 週間後までの、輸血関連感染症の発生など、血小板輸血に関連した有害事象による試験中止の発生率、ならびにその回数および輸血からの時間をモニターおよび比較することにより、Mirasol処理血小板の性能、安全性および忍容性がさらに評価される。

要約

- In vitro 試験の結果は、Mirasol 処理血小板が品質と性能を維持し、輸血の時点で求められる in vitro の pH 基準を満たすことを実証している。
- フローサイトメトリーと灌流試験は、Mirasol 処理血小板が未処理血小板と同等の接着性および凝集性を保持していることを示している。
- 5 日目には Mirasol 処理血小板の接着機能が未処理血小板と比較してわずかに上昇していた。
- Mirasol 処理はミトコンドリアの機能的完全性と活性を変化させない。
- In vivo 試験の有望な結果は、Mirasol 処理血小板の臨床研究の根拠となった。
- Mirasol 処理血小板は十分に臨床使用できるだけの生存能と有効性を保持しており、in vivo でのリカバリーおよび生存性の基準を満たしていた。

図 6A～E. In vitro 分析の結果



0 日目：Mirasol 処理の前に検査；0 日目の後：Mirasol 処理の直後に検査；5 日目：保存 5 日目に検査。

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第 7 章

Mirasol 処理した血漿の品質

MIRASOL®
病原体不活化技術

Mirasol 処理した血漿の品質

血漿は全血の遠心分離または単一ドナーの血漿アフェレーシスによって得られ、凍結成分として配送および保存される。全血またはアフェレーシス採取から調製され、8 時間以内に凍結保存された血漿は新鮮凍結血漿（FFP）と呼ばれる。

血漿の使用法

血漿は治療的価値の高い多様な有機および無機成分を含有し、FFP は多くの後天性凝固障害、特に複数の抗凝固タンパク質を低下させる凝固障害に最適な第一選択治療となる。FFP はまた、適当な濃厚液が存在しない単一凝固因子（たとえば第 V または第 XI 因子）欠乏症、急性播種性血管内凝固、血栓性血小板減少性紫斑病（TTP）における血漿交換、ならびにワルファリン作用の打ち消し、肝疾患、心肺バイパス、および大量輸血の一部の症例にも適応となる。しかし、第 VIII 因子、アルブミンおよび免疫グロブリンといった特定血漿成分を注射または輸血するためには分離、精製および調製が必要であるため、臨床診療における FFP の使用は制限されている。

新鮮凍結血漿に関連して起こり得る有害反応

血漿タンパク質の複雑さ、免疫グロブリン含量の不均一性、および処理と保存に関係する要因により、FFP は広範な病態生理学的反応を引き起こす可能性がある（表 1 を参照）。

FFP は多様な重大病理学的反応を引き起こす可能性がある。これを避けるためには、FFP は感染性病原体や白血球汚染物質を含んでいてはならない。

表 1. 新鮮凍結血漿に対する有害反応

免疫介在性
同種免疫：Rh 1（D）およびその他の赤血球抗原に対する抗体
アレルギー反応：IgA 欠乏症を除いて通常は原因アレルゲンが特定されない
TRALI：白血球凝集素および BRM
免疫修飾/免疫抑制
血漿汚染物質関連
ドナー特異的：投薬、感染
処理/保存特異的：サイトカイン類（FNHTR）、アナフィラトキシン
感染性汚染物質関連
非白血球関連：HIV、HBV、HCV、HAV、EBV、HHV-8、プリオン
物理化学的特徴および臨床使用関連
容量過負荷

BRM：生物反応修飾物質；EBV：エプスタイン-バーウィルス；FNHTR：熱性非溶血性輸血反応；HAV：A

型肝炎ウィルス;HBV:B 型肝炎ウィルス;HCV:C 型肝炎ウィルス;HHV:ヒト・ヘルペス・ウィルス;HIV:ヒト免疫不全ウィルス;Ig:免疫グロブリン;Rh:アカゲザル;TRALI:輸血関連急性肺障害。

血漿成分の品質要件

治療用血漿は安全である(すなわちウィルス、細菌および寄生虫を含んでいない)だけでなく、臨床的に有効であるためには、できるだけ新鮮血漿に近いレベルの凝固因子およびプロテアーゼ阻害物質を含有していなければならない。

血漿および血漿成分の品質にはいくつかの要因が影響し得る。FFP の品質は血漿の採取と保存の迅速性によって決まる。全血献血から分離され、採取から 24 時間以内に－18℃以下に凍結された血漿(FP24)は、関連凝固活性を良好に保持している。しかし、8 時間以内に凍結された FFP に関する過去の記録と比較すると、FP24 に含まれるフィブリノーゲン、第 V 因子、第 VIII 因子および第 XI 因子のレベルはそれぞれ 12%、15%、23%および 7%低下していることが示されている。

血漿の品質要件は各種ガイドラインに記されているが、それはかなり限定的なもので、主として第 VIIIc 因子と総タンパク質に関するものである。FFP が処方される患者の大部分は第 VIII 因子のレベルが正常または高いという事実にもかかわらず、これが現実である。

現在の FFP の品質管理は第 VIII 因子の測定値に基づいて行われており、欧州評議会(CE)のガイドラインはこれが 0.70 IU/mL を超えていることを要求し、英国のガイドラインはユニットの 75%がこの第 VIII 因子レベルを満たしていなければならないと規定している。

ただ、これらはガイドラインであって明確な規則ではない点に注意が必要である。欧州のガイドラインを図 1 にまとめている。ほとんどの国はこのガイドラインに従っているが、多くは各地に特有の輸血サービスのための独自の特定ガイドラインまたは勧告の参考として利用しているに過ぎない。たとえば英国の国立血液サービスは、血漿成分について完全に別個の要件リストを確立している。

さらに、市販の病原体不活化 FFP 成分にはそれぞれ特有のガイドラインが存在する(表 2 を参照)。たとえば溶剤-洗浄剤(SD)処理 FFP とメチレンブルー(MB)処理 FFP とでは性能規格が異なっている。FFP に関する CE ガイドラインは、毎月のルーチンの品質管理において第 VIIIc 因子の濃度が新鮮血漿成分の 70%(0.70 IU/mL と解される)であることを求めている。英国のガイドラインは、SD 処理 FFP については第 VIIIc 因子のレベルが 0.5 IU/mL 超であることを要求するのに対し、MB 処理 FFP についてはユニットの 75%の第 VIIIc 因子レベルが 0.5 IU/mL 超であることが必要とされている。

図 1. 新鮮凍結血漿に関する欧州のガイドライン

・ 血漿は解凍後ただちに使用する。
・ 血漿は–25°C 以下に保持するなら最大 36 カ月間保存できる。
・ 第 VIIIc 因子の平均活性は 0.70 IU/mL 超でなければならない。
・ 総タンパク質は 50 g/L 超でなければならない。
・ 適応となるのは、凝固障害における使用、血栓性血小板減少性紫斑病の治療、および血漿分画の原料としての使用である。

表 2. 新鮮凍結血漿の規格ガイドラインの比較

	EU評議会 第 13 版 FFP[4]	Paul Ehrlich Institut FFP[8]	EC指令[6]	UK FFP[5]	UK SD-FFP[5]	UK MB-FFP[5]	欧州薬局方 SD-FFP[9]	Mirasol -FFP[7]
第 VIIIc 因子	0.7 IU/mL 以上または新鮮血漿の 70%以上	新鮮血漿の 70%超	新鮮血漿の 70%以上	75% >0.7 IU/mL	>0.5 IU/mL	75% >0.5 IU/mL	≥0.5 IU/mL	75% >0.5 IU/mL
第 V 因子	NA	>70%	NA	NA	≥0.5 IU/mL	≥0.5 IU/mL	≥0.5 IU/mL	≥0.5 IU/mL
活性化凝固因子	NA	NA	NA	NA	NA	NA	150 秒以上	NA
赤血球	<6.0×10^{9} /L	<6.0×10^{9} /L	<6.0×10^{9} /L	NA	NA	NA	NA	<15×10^{9} /L
白血球	<0.1×10^{9} /L	<0.5×10^{9} /L	<0.1×10^{9} /L	NA	NA	NA	NA	<1.0×10^{9} /L
血小板	<50×10^{9} /L	<20×10^{9} /L	<50×10^{9} /L	NA	NA	NA	NA	<1.2×10^{9} /L
タンパク質	≥50 g/L	NA	≥50 g/L	≥50g/L	NA	NA	≥45 g/L	≥50 g/L

FFP：新鮮凍結血漿；IU：国際単位；NA：該当なし；赤血球。

Mirasol 処理 FFP について私たちは、SD-FFP や MB-FFP について行われてきたのと同じように、最終的に（第 VIIIc 因子、フィブリノーゲンおよび総タンパク質について）血液バンク標準品に組み込まれる成分性能に規格制限を設けている。社内品質管理で Mirasol 処理 FFP に用いている規格は以下のとおりである。

- 血漿ユニットの 75%の第 VIIIc 因子の活性が 0.5 IU/mL 超であること。
- 他のすべての因子の活性が 0.5 IU/mL 超であること。
- 抗トロンビン III、プロテイン C およびプロテイン S に有意な低下がないこと。
- フィブリノーゲン活性が 140 mg/dL 超であること。

- 総タンパク質が 50 g/L 超であること。

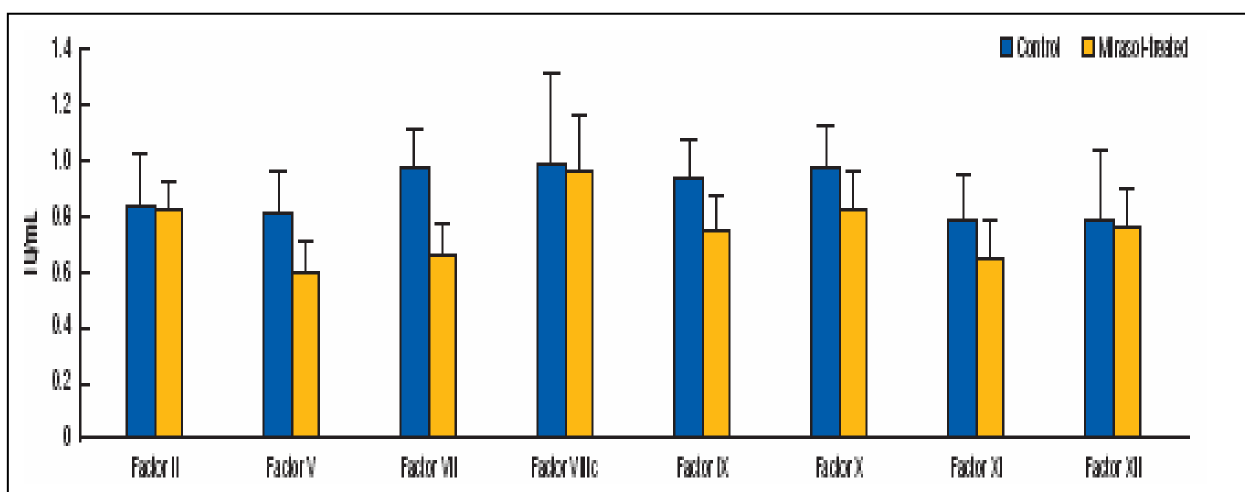
Mirasol 処理 FFP の規格は、他の病原体不活化血漿成分および未処理 FFP に用いられるものと同等である。

表 3. Mirasol 処理 FFP の保存 52 週目のタンパク質パラメータ

IU/mL	全血対照	全血処理	アフェレーシス対照	アフェレーシス処理	基準範囲
第 II 因子	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.65–1.54
第 V 因子	0.8 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.54–1.45
第 VII 因子	1.0 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.2	0.62–1.65
第 VIIIc 因子	1.1 ± 0.4	1.1 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.45–1.68 （1 段）
第 IX 因子	0.9 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.45–1.48
第 X 因子	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	0.68–1.48
第 XI 因子	0.7 ± 0.2	0.6 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.42–1.44
第 XII 因子	0.7 ± 0.3	0.7 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.40–1.52

IU：国際単位。

図 2. Mirasol 処理新鮮凍結血漿の保存 52 週目のタンパク質パラメータ



IU：国際単位。

Mirasol 処理した血漿の品質

In vitro 試験

In vitro 試験では、Mirasol 処理 FFP が欧州の FFP 基準に適合したレベルで in vitro タンパク質品質を維持していることが示されている。追加的に、Mirasol 処理したアフェレーシスおよび全血由来血漿成分の保存1年後の in vitro 血漿タンパク質活性を評価する試験も実施された（表 3、図 2 を参照）。

認定血液銀行施設でアフェレーシスおよび全血から新鮮血漿を採取し、採取後 8 時間にわたり室温で保持した。使用したのは合計 14 ユニットの処理成分であった（容量範囲は 231〜327 mL）。処理成分については、リボフラビンの最終濃度が約 50 μM となるよう、500 μM のリボフラビン溶液 35 mL と合わせて血漿を照射バックに入れた。このバッグを Mirasol 照射器に入れ、UV 光に曝露した（6.2 J/mL）。その後、血漿成分を－30℃にまで急速冷凍し、－30℃のフリーザーに 52 週間保存した。この血漿を解凍し、標準的な凝固アッセイを用いて分析した。

In vitro の血漿タンパク質品質は、次の合格基準を満たしていた。すなわち、フィブリノーゲンは平均が 140 mg/dL 以上、第 II、第 V、第 VII、第 IX、第 X、第 XI 因子は平均が 0.6 IU/mL 以上、第 VIIIc 因子は平均が 0.8 IU/mL 以上、および総タンパク質は平均が 50 g/L 以上であった。69 週目には、プロテイン S の回収率（初期値の 100%超）、プロテイン C（初期値の 90%）、抗プラスミン（初期値の 94%）、抗トロンビン（初期値の 100%）の追加アッセイを指示した。処理サンプルは補体および免疫学的活性化の徴候を示さなかった。この試験で観察された結果は、Mirasol 処理 FFP が欧州のガイドラインに記されている未処理 FFP 成分の要件を満たすか、むしろ上回っていることを示唆している（表 4 および 5 を参照）。プロテイン C、プロテイン S、抗プラスミンおよび抗トロンビンの保持レベルは、他の不活化方法で認められるものを上回っていた。Mirasol 処理 FFP を－30℃で 1 年間保存した後のフォン・ヴィレブランド因子（vWF）抗原：活性比は 1.0±0.3 であった。vWF 抗原：活性比が 1.4 未満であれば vWF の多量体分布は正常であるが、この比が 3.7 を超えると高分子量の多量体が損失していることになる（図 3 を参照）。現在進行中の試験では、vWF を切断する亜鉛含有メタロプロテアーゼである ADAMTS13（トロンボスポンジン 1 型モチーフ第 13 番を備えたメタロプロテイナーゼ・ディスインテグリン）の活性が Mirasol 処理によって有意に変化しないことが確認されている。この結果は、－30℃での 1 年間の保存後にもフィブリノーゲンと第 VIIIc 因子が良好に維持されていることを示している。総合すると、以上のデータは Mirasol 処理 FFP が 1 年間の保存後にも欧州の FFP 基準を満たすレベルで in vitro タンパク質品質を維持していることを実証している。

Mirasol 処理 FFP は未処理 FFP 成分に関する欧州の現行タンパク質品質基準を満たしてい

る。

表 4. Mirasol 処理 FFP の保存 69 週目のタンパク質パラメータ

活性	全血対照[†]	全血処理	アフェレーシス対照	アフェレーシス処理	基準範囲
プロテイン C	93.4 ± 10.6	83.0 ± 7.7	105.7 ± 27.6	96.0 ± 23.4	58–164
プロテイン S	65.0 ± 9.8	85.7 ± 18.6	92.3 ± 6.0	106.0 ± 15.1	56–168
抗トロンビン	83.0 ± 5.5	82.8 ± 6.9	87.0 ± 9.8	87.2 ± 8.4	72–145
プラスミノーゲン	80.8 ± 10.5	75.4 ± 10.3	79.7 ± 7.2	74.8 ± 6.9	68–144
抗プラスミン	99.6 ± 6.2	98.3 ± 6.2	101.3 ± 4.0	94.8 ± 6.3	72–132
プレカリクレイン	111.1 ± 16.9	62.7 ± 9.6	124 ± 17.2	52.2 ± 22.7	65–135
高分子量キニノーゲン	89.5 ± 24.6	69.0 ± 12.8	99.2 ± 7.2	69.9 ± 21.0	65–135
フォン・ヴィレブランド因子活性	125.6 ± 36.3	110.4 ± 52.4	99.2 ± 59.7	81.3 ± 39.6	50–150
フォン・ヴィレブランド因子抗原	123.8 ± 31.4	91.2 ± 47.0	141.6 ± 41.7	108.3 ± 47.3	50–150

[†]対照データは保存 52 および 69 週目の未処理成分のものである。

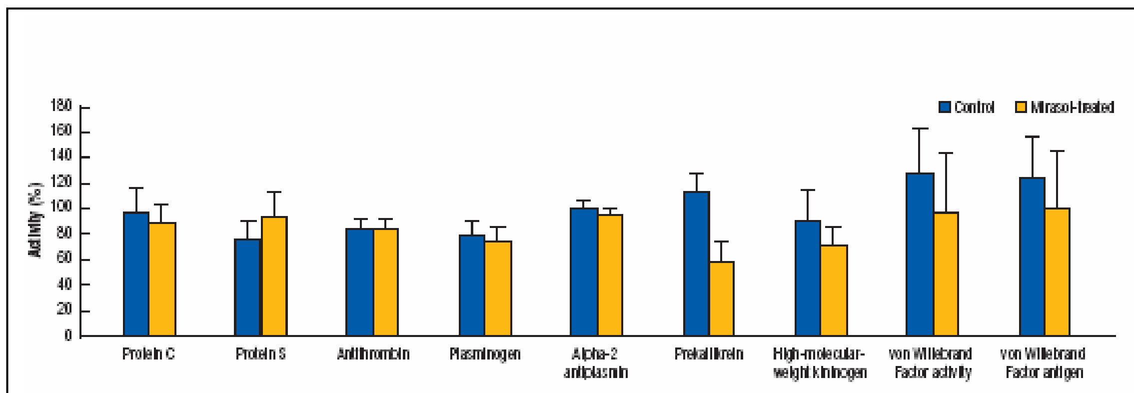


図 3. Mirasol 処理 FFP の保存 69 週目のタンパク質パラメータ

表 5. Mirasol 処理 FFP の保存 69 週目のタンパク質パラメータ

パラメータ	全血対照‡	全血処理	アフェレーシス対照	アフェレーシス処理	基準範囲
フィブリノーゲン（mg/dL）§	288.8 ± 59.6	219.9 ± 33.3	290.7 ± 4.7	211.0 ± 33.5	145–385
総タンパク質（g/L）§	47.6 ± 2.9	51.3 ± 3.1	51.9 ± 4.8	53.6 ± 3.6	48–364
PAI-1（IU/mL）	18.8 ± 12.5	7.8 ± 3.5	12.6 ± 11.4	18.8 ± 10.5	<31.1
D-二量体（ng/mL）	158.4 ± 115.3	89.0 ± 0.0	103.3 ± 24.8	132.8 ± 87.5	<256
プロトロンビン断片 1 + 2（pmol/L）	178.4 ± 61.0	161.1 ± 35.9	241.6 ± 79.9	265.8 ± 218.0	87–3325
トロンビン-抗トロンビン複合体（ng/mL）	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	<5.1
アルファ-1 抗トリプシン（mg/dL）	100.7 ± 14.7	97.5 ± 15.2	147.5 ± 9.1	110.4 ± 22.8	90–3200

‡対照データは保存 52 および 69 週目の未処理成分のものである。
§処理データは保存 52 週目の処理成分のものである。
PAI：プラスミノーゲン活性化因子阻害物質。

Mirasol 処理 FFP の機能面

さらなる研究により、Mirasol 処理 FFP が天然の抗凝固タンパク質として最も多い 3 種、すなわちプロテイン C、プロテイン S および抗トロンビンの活性を維持していることが示されている（表 6 を参照）。Mirasol 処理 FFP は抗凝固機能だけでなく免疫グロブリン（IgG と IgM）の量的および機能的活性も満足できるほどに保持しており、凝固亢進性障害のある患者の治療にも使用可能である。

Mirasol処理FFPは免疫グロブリンの機能的活性を保持していることも示されている（表 7 を参照）。この試験では、新鮮血漿を照射バッグに移して最終濃度が 50 μMとなるようリボフラビンを添加し、この溶液に 10 J/cm²のUV光を照射した。ここから照射の前後にサンプルを採取し、これをバッチ検査の前に冷凍した。このサンプルのジフテリア、破傷風および肺炎球菌の力価は基準範囲内に保持されていた（表 7 を参照）。破傷風、肺炎球菌およびジフテリアの力価の照射前：照射後の比の平均はそれぞれ 0.66、0.72 および 1 以上であり、これはMirasol処理FFPがジフテリア、破傷風および肺炎球菌の多糖体抗体の機能的活性を保持していることを示している。

Mirasol 処理には、献血時に残存していた白血球と関連病原体を不活化させる作用もある。これにより Mirasol 処理は、機能のある白血球の伝達、蓄積している炎症メディエーターへの曝露、および細胞内ウィルスによる感染を防ぐことができる（これに関する詳細は第5章を参照）。

Mirasol 処理 FFP は正常な抗凝固能と免疫能を保持している。

表 6. Mirasol 処理 FFP に含まれる抗凝固タンパク質の機能的活性（平均±標準偏差）

パラメータ	対照	処理	回収率（%）
抗トロンビン III（IU/mL）	1.01 ± 0.07	0.99 ± 0.06	98
プロテイン C（IU/mL）	1.07 ± 0.10	1.04 ± 0.15	98
プロテイン S（IU/mL）	1.04 ± 0.13	1.01 ± 0.12	97

表 7. Mirasol 処理 FFP に含まれる免疫グロブリンの機能的活性（平均±標準偏差）

抗体	処理前	処理後	正常基準範囲
ジフテリア（IU/mL）	0.46 ± 0.35	0.53 ± 0.34	>0.01
破傷風（IU/mL）	6.4 ± 6.0	4.2 ± 2.6	0.1–13.1
肺炎球菌（IU/mL）	819 ± 707	587 ± 460	>200

要約

- FFP は多様な重大病理学的反応を引き起こす可能性がある。これを避けるためには、FFP は感染性病原体や白血球汚染物質を含んでいてはならない。
- Mirasol 処理 FFP の規格は、他の病原体不活化血漿成分および未処理 FFP に用いられるものと同等である。
- Mirasol 処理 FFP は欧州の現行のタンパク質品質基準を満たしている。
- Mirasol 処理 FFP は欧州の現行の抗凝固能および免疫能基準を満たしている。

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第 8 章

臨床経験と将来における製品開発

MIRASOL®

病原体不活化技術

初期の臨床業務

初期の臨床研究は、処理済み血液製剤に関する体外のパラメータと体内におけるリカバリーならびに生存の相互関係を確立することを目的として設計されたものである。P-セレクチン発現量など、体内における血小板性能の測定値、循環における回復や生存など、体外の特徴を変更させるため、光エネルギーの放射ならびに添加剤の効果について測定を行った。処理プロセスは、この研究結果に基づいて決定されたものである。

第2回の臨床試験は、初回の研究によって確定したMirasol処理の最善のパラメータを採用することにより、血小板の回復と生存を調査することを目的として設計された。体外における測定値にはいくらかの変更が加えられたものの、この臨床試験の結果、Mirasol処理済みの血小板は臨床上において、標準濃厚血小板製剤と同様の特徴を持つことが結論づけられた。治験責任医師はこれらの2回にわたる研究に基づき、Mirasol処理済みの血小板は、患者を対象としてさらに研究を行うのが正当であると考えるのに十分なだけの実行可能性と機能性を維持していると結論づけた。Mirasol PRTシステムに関する臨床研究のうち、すでに完了したもの、ならびに現行の研究の結果を表1にまとめた。

表1.　Mirasol PRTシステムに関する初期ならびに現行の臨床研究

プロトコル	被験者	被験者	場所	報告
CTS-0001/BCT02-09PET 血小板の自己回復と活着に関するに関する評価 リボフラビン・ベースの光不活性化処理を実施	予備調査 体外における血小板パラメータと体内における血小板の回復ならびに生存率の相互関係を比較するため、血小板をTrima®アフェレーシス装置で収集し、リボフラビンを補充した光不活性化システムで加工した。その後、これに活性化抑制物質を加えたものと加えないものに分け、加工を行わない血小板とともにそれぞれ5日間保存した。	被験者 11名 サンプル 18	南アフリカ（2002年）ヨハネスブルグ	CTF-0002ならびに発行物1
CTS-0011 放射性標識血小板の回復と生存に対するMirasol処理の効果	設計検証研究 気温22℃で5日間保存した後、放射性標識管理し、Mirasol処理を行った血小板の回復と生存を評価するため、無作為化クロスオーバー単盲検を実施した。	参加者 29名 評価実施 24名	アメリカ合衆国（2003–2004年） 1) ニューハンプシャー州、レバノン	CFR-0003ならびに発行物2

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			2) バージニア州、ノーフォーク	
CTS-0028 Mirasol処理を行った血小板輸血製剤の安全性と性能に関する評価 血小板減少性疾患の患者に関する研究	MIRACLEに関する臨床研究 多施設共同無作為オープン結果遮蔽試験の臨床設定において、Mirasol PRTシステムが安全に作動し、適切な血小板の動作を維持するか否かに関して判断するための対照研究を実施した。	CEマーケティング向け：† 被験者54名 参加者総数： 被験者118名（Mirasol部門に60名、コントロール部門に58名）	フランス（2005–2007年） 1) ストラスブール 2) ブザンソン 3) ボルドー 4) グルノーブル 5) リヨン 6) ナント	2007年9月10日登録終了 データは2008中に発表の予定

MIRACLE（MIRAsol臨床評価）:

血小板減少症患者を対象とした臨床研究

MIRACLE（Mirasol®病原体不活性化技術による処理済み血小板輸血製剤の安全性、ならびに性能についての評価：血小板減少性疾患患者を対象とする）は現在、その最終段階にある。この多施設共同無作為オープン結果遮蔽試験の臨床設定の対照研究では、血小板輸血を必要とする二つの血小板減少症患者のグループの比較を行っている。

この研究の目的は、臨床設定においてMirasol処理済み血小板が安全に作用し、十分な動作を維持するか否かを判断することである。血小板輸血を必要とする血小板減少症患者を調査対象とし、輸血から1時間後に測定した補正血小板増加数($CCI1_{時間}$)と、反応として発現した重篤有害事象（SAEs）を比較することによって、これに関する評価を実施した。なお、輸血については処理済み（検査コンポーネント）ならびに未処理（参考コンポーネント）の濃厚血小板製剤を比較の対象とした。輸血から4週間後までの期間、輸血関連の感染、輸血の回数や輸血の間隔などのほか、血小板輸血に関連した有害事象（AEs）によって輸血が中止されたケースを監視し、比較することによって、Mirasol処理の動作、安全性、耐性に関するさらなる評価を行った。Gambro BCT, Inc.は、CEマークの取得申請に際して本研究の中間結果を報告している。†完全なデータセットは、2008年中に発表する予定である。

将来における臨床研究

Mirasol処理済みの不活性化病原体と白血球。

Mirasol処理は、病原体と白血球細胞（白血球）を不活性化する。Mirasol処理済みの血液製剤においては、血小板の機能性ならびに代謝活性、白血球活性の実質的な不活性化（単核細胞の増殖の抑制、サイトカイン生成の抑制、および抗原提示細胞としての活動の抑制）が維持される。体外検査、臨床前研究、ならびに患者を対象とした検査データによって、これらの効果が確認されている。これらのデータが示唆するのは、Mirasol処理を行った血液製剤は臨床上において、患者に対して適切、かつ有益な結果をもたらすと解釈し得る性能特性を示していることである。こうした結果は、臨床設定においては定量化することが難しい病原体の不活性化（病気感染ならびに感染性合併症の削減に対する潜在性など）がもたらす全般的な効果に加えて、さらに有益なものになるといえるだろう。また、これらの潜在的利点の正当性を確認するためには、具体的な結果判定法と対象患者数を定めたうえで、臨床設定においてMirasol処理済み血液製剤の性能を評価する必要があるだろう。このため、複数の研究設計を行い、患者グループを設定し、詳細な調査を実施することとする。

Mirasol処理を行った血液製剤は臨床上において、病原体除去における利益のみならず、患者に適切、かつ有益な結果をもたらすと解釈し得る性能特性を示している。

臨床設定 におけるさらなる研究には、以下を含める可能性がある

1.　白血球の不活性化による潜在的利点についての評価

a) 標準濃厚血小板製剤と比べた場合の抗HLA 抗体の生成ならびに血小板不応の抑制

b) 発熱性非溶血輸血反応（FNHTR）など、その他の輸血に関連のある疾病率、ならびに死亡率の低減、輸血後のTRIM感染性合併症の抑制

2.　アレルギー反応ならびに輸血関連急性肺障害（TRALI）に関する評価

3.　血小板代謝作用、ミトコンドリア活性の維持、血小板の機能性に関してのさらなる研究には、以下を含むものとする：

a)　活動性の出血を起こす血小板減少症の患者、もしくは血小板病症の患者における出血リスクの軽減、ならびに赤血球またはその他の血液製剤輸血の必要性の削減。

b)　大量の失血があり、血小板の使用によって血液の凝固が増進し、血小板の活性化により失血が減少するとみられ、血液製剤の輸血が必要とみられる外科的処理中の患者に関する研究。また、当該の患者の手術後の合併症に関する研究。

Mirasol PRTシステム：現在ならびに将来のアプリケーション

アフェレーシス処理と血漿の中にある血小板に関するバフィーコートの処理を目的とした初期のMirasol PRT システムは、現在はヨーロッパの大半の国、ならびに中東、アフリカで利用可能となっている‡。システムの拡充が進行中であり、利用が可能になれば、この技術を用いることによって処理可能な血液製剤の種類が大幅に増加することになる。

新たなアプリケーションによって、以下の処理が可能となる：

- 血小板添加剤溶液（PAS）における保存が可能な血漿の濃縮された（高濃度）濃厚血小板製剤：血小板の第二世代アプリケーション

- アフェレーシスによって採取した血漿、または全血に由来する血漿

血小板添加溶剤に含まれる血小板

Mirasol PRTシステムへのアップグレードによって、血漿に含まれる血小板製剤と、処理後にPASに保存される高濃度血小板ユニットの双方を処理できる設備が提供されることになる。ヨーロッパとアメリカにおいて現在、市販されているPASのうち複数のタイプに関する体外研究を通じた評価が実施されている。製品が発売されれば、PASに保存される製剤のうち認証を受けたもののいずれについても、このシステムを利用することが可能になる。

血漿

既存のMirasolシステムをさらに拡充することによって、血漿ユニットを凍結保存向けに処理することも可能になる。予備データによれば、Mirasol処理を施した血漿製剤は、タンパク質の品質ならびに抗凝固剤、免疫グロブリン機能活性に関するヨーロッパの現行基準を満たすものである（第6章参照）。

全血

現在、実施している研究は、全血の処理におけるMirasolシステムの効果を調査するためのものである。この研究には、Mirasol処理を行った全血から分離されるすべての製剤の品質、ならびに有効性の評価が含まれている。

新たなアプリケーションによって、血小板製剤をPAS、血漿、ならびに全血製剤において保

存するための処理が可能になるだろう。

要旨

- Mirasol処理を行った血液製剤は､臨床上において､病原体除去における利益のみならず､患者に適切かつ有益な結果をもたらすと解釈し得る性能特性を示している。
- 新たなアプリケーションによって、PAS、血漿、ならびに全血製剤における保存に向けた血小板製剤の処理を可能にするだろう

‡初期の血小板 Mirasol PRT システムは CE マークを取得済みであり、利用可能なものである。CE マークを承認している国々では、最終血液製剤についてその他の国家認証を取得する必要がない

‡アメリカでの販売は行われていない。

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† The Mirasol PRT System for Platelets is not available for sale in the United States.

第 9 章

血液の安全性維持にかかるコスト

MIRASOL®

病原体不活化技術

血液の安全性維持にかかるコスト

輸血によって、多数の患者の命が救われ、寿命が延長される。輸血という行為そのものが患者の健康に悪影響を与えないことを確実なものにするためには、血液供給の安全性を保証することが不可欠である。供給される血液の中からは間違いなく、病原体やドナーの白血球が発見されており、病気感染や副作用のケースに関する数多くの報告がなされている。これまでにも、血液供給の安全性を向上させるための多数の措置が導入されてきた。これらは確実に血液の安全性を高めてきたものの、一方でコストを大幅に増大させ、プロセスを煩雑化させたのである。

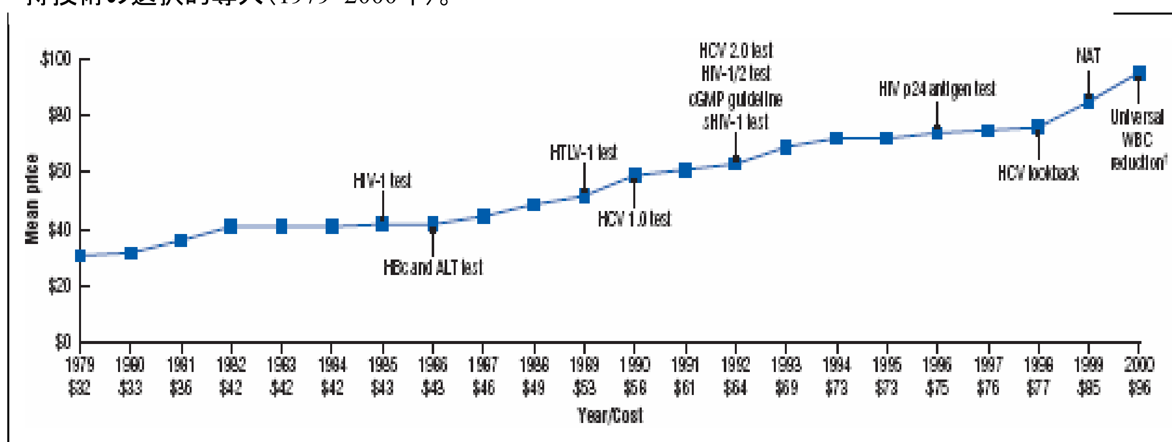
血液安全性の維持にかかる最終コスト

血液安全性の維持にかかるコスト—ならびに輸血を行う病院において血液1ユニットあたりにかかるコスト—は、ここ数十年で大幅に増加している。これは、主として新たな血液安全性メカニズムの導入によって増幅されたものである（図1を参照）。

新技術の導入によって発生したコストの評価は、クオリティオブライフを調整した生存年数（QALY：完全に健康な年を1.0 QALYとし、—輸血関連感染などで—健康を損なった年の数値を低く評価する）の評価で判断される場合が多い。共通のユニットに関して達成された平均的な健康への利点を明確にすることによって、新技術を直接に、また客観的に評価し、比較することができる。

なお、行われた比較が有効なものであり、また典型例を示すものであることを確認することが不可欠である。たとえば：

図1.　ABC加盟センターの病院向け輸血用血液1ユニットあたりの販売価格と、血液安全性維持技術の選択的導入（1979–2000年）。



†2000年12月以降、75あるABCの加盟センターのうち12施設では、白血球不活性化製剤のみが配布されている。平均価格は、これらのセンターが白血球不活性化赤血球の料金として徴収している金額から算出した。

ALT：アラニン・アミノトランスフェラーゼ、GMP：医薬品製造管理および品質管理基準、Hbc：ヘモグロビンC、HCV： C型肝炎ウィルス、HIV：ヒト免疫不全ウィルス、HTLV：ヒトT細胞性白血病ウィルス、NAT：核酸増幅テスト（NAT）、白血球：白血球

• コストの比較は、関連性のあるコストを比較対象としたものでなくてはならない。
血液の安全性に関する対策についていえば、コンパレータに医薬品—コスト効率においては計測基準が異なる—を含んではならないが、核酸増幅テスト（NAT）やガンマ線照射、白血球除去など、その他の血液の安全性維持技術はこれに含むものとする。

• 潜在的なコスト、ならびにコスト削減に関連するすべてのものを考慮する必要がある。
残存している白血球の不活性化も同時に行う病原体除去技術のコストの算出は、輸血関連の感染の削減という面のみに基づいたものであってはならない。安全性維持のための措置がもたらす利点を過小評価してしまうためである。また、同種免疫とそれに関連する反応の防止の可能性、および血小板不応も考慮すべき要因である。

• また、コスト対利益について考慮する際おいては、安全対策によって将来に生じ得る好影響（または悪影響）を考慮しなければならない。たとえば、特定の病原体を検出するために設計された血液安全性維持のための技術は、新たに出現する新興病原体に対して血液の供給を保証するものではない；すべての病原体を不活性化する潜在力を持った技術であれば、こうした新興病原体に対する新規の検査を開発し、導入しなくてはならないという要件をあらかじめ回避するものとなるだろう。

輸血の安全性維持のための措置については、QALYあたりの最終コストが高くなるものと認識されているが、コスト効率性に関する議論においては、上記の点などすべてを考慮する必要がある。

病原体不活性化システムの実質的な価値を正確に評価するためには、そのシステムがクオリティオブライフ、ならびに社会におけるコスト削減の面においてどれだけの救済となるかという点に対してのコストを算出することが不可欠である。

直接経費と間接費、病原体不活性化技術の導入によるコスト削減

新たな血液安全性維持システムの導入を検討する際には、すべての関連コスト—直接コストおよび間接コスト—、ならびにコスト削減の可能性—直接コストと間接コスト—に関する完全な理解が不可欠である（表1参照）。

表1. 潜在的な直接費用ならびに間接費、新たな病原体不活性化技術の導入によるコスト削減の可能性

直接費用	間接費
• 新規設備にかかる設備投資 • 各ユニットにかかる増分費用 • 加工にかかる時間の延長 • その他（職員研修など）	• 処理によって血小板収量や機能性に悪影響が生じ、追加ユニットが必要となる可能性
直接費用の削減	間接費の削減
• 血液の安全性維持のための対策としての要件（ガンマ線照射、新規の血清学的検査など）を事前に満たし得る • 輸血感染や輸血関連敗血症に関連したコストの排除 • 廃棄血液の削減（細菌汚染により使用不可能となるユニットの減少など）	間接費の削減 • 輸血による感染の減少を通じた入院長期化の防止 • 輸血感染の結果として起きる賠償請求件数の削減 • FNHTRや輸血関連急性肺障害（TRALI）など、白血球関連の合併症の防止 • 現在、据え置きとなっているドナーの療養> ドナー・プールの拡大> ドナー募集費用の削減 • 輸血が中断されるケースの減少 • 同種免疫の減少を原因とする不応が抑制できる可能性

FNHTR：発熱性非溶血輸血反応、TRALI： 輸血関連急性肺障害、白血球：白血球

たとえば、新技術は初期投資を必要とする一方で、既存のコスト（現行の措置などにかかるもの）を免除対象とすることができるほか、コスト効率の低いプロセスに取って代わることができる。アメリカにおいては、白血球除去（白血球除去）の場合、加工された1ユニットあたり$35–45（€24–31） の追加コストがかかり、国全体としての血液関連のコストが$5億（€3億4,000万）が上乗せされると推定されている。一方、病原体検出には加工された1ユニットあたり€20–30のコストが必要であり、ガンマ線放射には同€5–12、ウェスト・ナイル・ウィルス（WNV）血清検査には同€1–6、抗ヘモグロビンCには同€2.50、梅毒には同€4、サイトメガロ・ウィルス血清検査には同€4.4がかかると見積もられている。
これらの—そしてその他の—一部に必要とされるコストを回避できる潜在性を持った血液安全性維持のための技術は、単に追加コストを生じるものと考えられるべきものではないのである。

基準値

医薬品においては、コスト効率はQALYあたり$50,000 (€34,000)が一般的な許容範囲とされている。しかしながら、血液の安全性においてはこの基準は不適切であり、不当である。実際に、QALYあたりで推定されるヒト免疫不全ウィルス（HIV）のp24 抗原検査、Hbc抗体検査、有機溶媒／界面活性処理（SD）法、新鮮凍結血漿（FFP）のコストは$500,000–$1,000万（€339,000–u6,700,000） であり、また、これらの措置のすべてが標準的な血液プールの一部として恒常的に実施されているわけではないのである。明らかに、コスト効率に関する決定を下す際においては、血液の安全性について各項目に異なる基準を適用することが不適切であるとはいえないのである。

輸血関連の感染性合併症

コスト効率に関する推計の大半においては、病原体除去プロセスによって削減できる可能性のあるアレルギー反応やTRALIなど、非感染性輸血関連合併症が考慮されていない。実際、病原体除去技術（PRT）で処理した血漿のQALYあたりのコストは、これらの合併症を考慮した場合には大幅に減少する。例を挙げれば、SD処理済み血漿に関連したコストの分析によれば、QALYあたりのコストはUS$215万6,000（146万1,000ユーロ）- US$974万3,000（660万1,000ユーロ）である。こうした結果は、輸血伝搬性ウィルスの感染リスクが低いこと、ならびに血漿のレシピエントの大半が高齢であり、その予後診断が短期間で終わっているため不十分なものになっていることに起因したものである。しかしながら、分析において非感染性の輸血関連合併症を考慮すると、推定されるQALY値は大幅に低下することになる——TRALIの発現の減少におけるSD処理済み血漿のコストを考慮したモデルの場合、21歳以下の患者の生存年数のうち一年あたりのコストは£30,000（€42,000）未満であり、48歳以下の患者の場合は同£50,000（€70,000）である。

将来の血液提供における危機に向けた保護措置

血液供給に新たな脅威が発生する頻度についての歴史的データをみると、新たな血液の安全性維持に関する技術のコスト効率を検証するモデルはいずれも、チクングンヤなどの新興病原体やその他の未確認の有機体など、新たに発現した新興病原体を特定し、血液供給から排除するための検査方法の開発コストとして予測されるコストを考慮する必要があることがわかる。

血小板輸血における病原体不活性化のコスト

病原体除去技術（PRT）システムを通じた病原体不活性化が血小板輸血コストに与える経済的影響を評価するための研究によると、そのコスト効率はNATなどその他の安全性維持のための措置と同程度であるか、あるいはそれ以上となり得ることがわかった。PRT技術のコスト効率に関するその他の調査において本論の著者は、輸血による感染が起こり得る“[PRT]

のない世界”と感染が防がれている“ [PRT]のある世界”の比較を実施した。輸血伝搬性ウィルスへの感染によって生じる年間コストは、無症候性HIVの場合で€u2,231、AIDSの場合で€25,736と見積もられている——さらに、急性と慢性の肝炎の場合は、それぞれu2,300とu125とされる。また、敗血症の症状発現ごとのコストの総額は、€17,988と算出されている。一方、これらのすでに認識されているものに加えて、新興病原体（既知、または未知の）感染患者の看護にかかると見込まれる費用も考慮に入れられている。本論の著者は、輸血1回あたりの新興病原体への感染リスクが1/1000から1/2300以上であるとすれば、増分のコスト効率はQALYあたり€195,364であり、PRTは全体として、措置としてはコスト効率が高いものと考えられると結論づけた。

上記を考慮すると、Mirasol PRT システムの付加価値はその潜在的利点に大きく依存するものとなる：

- その他のシステムが残存している光線感作物質の副生成物や代謝産物の除去を必要とする場合についても、Mirasol PRT システムはこのステップを必要としない。‡広範な毒物学的評価と長期にわたる臨床経験によって、リボフラビンとその光分解生成物は安全であることが証明されている（第2章参照）。
このステップを排除することで技術者の作業負荷が軽減され、プロセスの処理能力が向上することから、コストと時間が削減できるのである。

- Mirasol PRTシステムは、白血球に由来する輸血副作用の発生を抑制し、追加の処理コストの回避を通じてコスト効率を上昇させる。

- Mirasol PRTシステムにより、同種免疫と血小板不応を低減できる可能性がある。

血小板輸血コストにおける病原体不活性化の経済的影響を評価するための調査によると、そのコスト効率はNATなどその他の血液安全性の維持のための措置と同程度であるか、あるいは潜在的にはより高いことが分かった。

Mirasol PRTシステムのコスト効率を確率するための新たなモデル

既存の処理方法に加え、さらなる利点を提供する新たな安全性維持のための技術が開発されていることから、コスト対利点のプロフィールを正確に評価するための新規モデルが必要となるだろう。

病原体不活性化技術に関する既存のモデルは、Mirasol PRTシステムのコストとこのシステムによって実現し得るコスト削減の側面の全般を考慮したものではないことから、Mirasol

データのみに基づく新たな、そしてより適切なモデルを開発する必要性が生じたのである。

現在、より効率の高い評価モデを開発中である。このモデルによって、Mirasol処理とその他の病原体不活性化技術、ならびに細菌類のスクリーニングや新たな核酸増幅テストなど、その他の血液安全性の維持のための措置との包括的な比較が可能になる。

Mirasol PRTシステムのさらなる強み

血液の安全性維持のための新たな技術を導入するか否かについては、導入にかかるとされているコストのみに基づいて決定を下すべきではない。その他の多くの要因についても検討する必要がある。

たとえば、細菌検査の結果が得られる前にすでに血液製剤が販売されている場合が多く、さらにその多くがすでに輸血されてしまっている場合が多い。実際、陽性であるとわかったユニットの約40%が回収されておらず、回収された血小板ユニットもその90%以上がすでに輸血に使用されたものとなっている。

血液の安全性維持のための技術の大半は本来、対処するためのものであり、そのため新しい検査を実施するには時間がかかる。一例を挙げれば、1999年にWNVが初めて検知されてから、2002年11月にその血液スクリーニングのためにNATが定期的に実施されるようになるまでには、3年の期間があった。

血液製剤の全保存期間中を通じて細菌の増殖を抑制するための技術、ならびに将来において発現し得る未知の新興病原体に対する保護機能を持つのに十分なだけの確固とした技術は、現在の血液供給の安全性向上に大きく貢献するだろう。

要旨

- 病原体不活性化システムの実質的な価値を正確に評価するためには、そのシステムがクオリティオブライフ、ならびに社会におけるコスト削減の面においてどれだけの救済となるかという点に対してのコストを算出することが不可欠である。
- 血小板輸血コストにおける病原体不活性化の経済的影響を評価するための調査によると、そのコスト効率はNATなどその他の血液安全性の維持のための措置と同程度であるか、あるいは潜在的にはより高いことが分かった。
- 現在、より効率の高い評価モデルが開発中である。このモデルにより、Mirasolとその他の病原体不活性化技術、ならびに細菌類のスクリーニングや新しい核酸増幅テストの実施などといった、その他の血液の安全性維持のための対策の包括的な比較が可能になる。

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インターセプトブラッドシステムの概要

バイオワン株式会社

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インターセプトブラッドシステム（IBS）はソラレン化合物の1種であるアモトサレン（S-59）を用いて血小板又は血漿中の病原体を不活化するシステムである。

1．本システムの構成

血小板用の本システムは，プレパレーションセット，プロセッシングセット（血小板），及びイルミネーターより構成されている.

図 1.1 プレパレーションセット

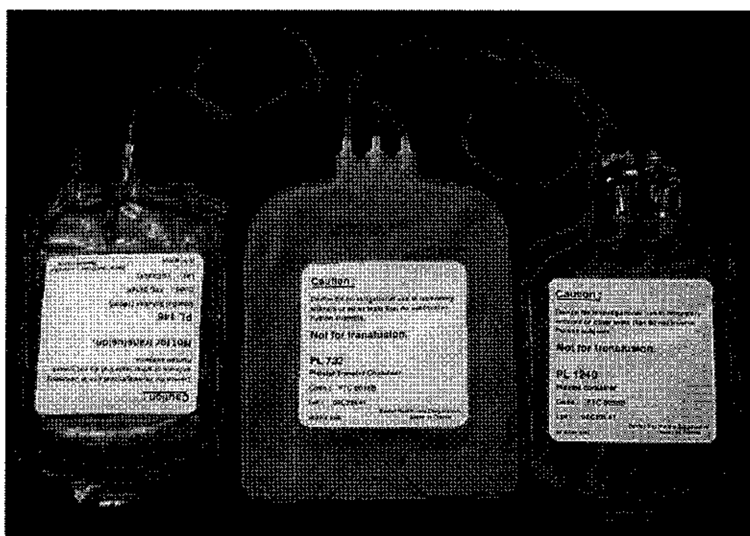


表 1.1 インターソルの組成

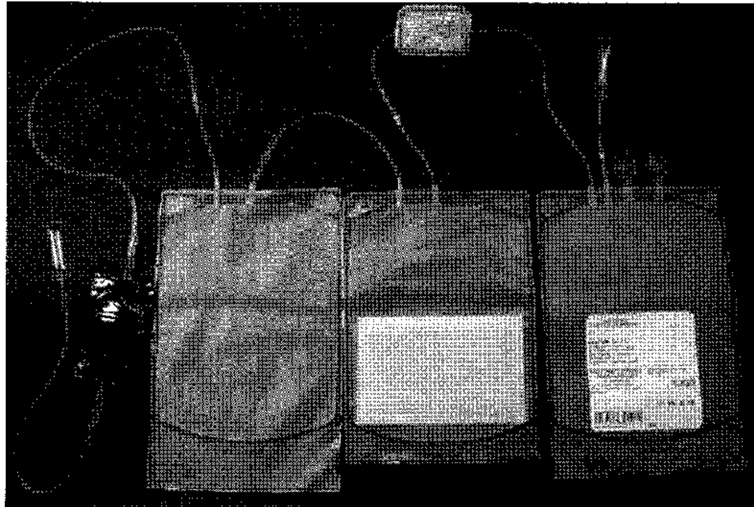
1）：日本薬局方収載品
2）：CPD，MAP，電解質輸液等の成分として既許可． EP 収載品.

成　　分	体内に入る量	処方量
塩化ナトリウム 1)	814 mg	4.52 g
酢酸ナトリウム・三水和物 1)	796 mg	4.42 g
無水クエン酸ナトリウム・二水和物 1)	572 mg	3.18 g
無水リン酸水素ナトリウム 1)	549 mg	3.05 g
リン酸二水素ナトリウム・二水和物 2)	189 mg	1.05 g
注射用水 1)	180 ml	1000 ml
pH	7.2	

【インターソル：プレパレーションセットに含まれている】

インターソルは本システムで効率的に不活化処理を行うために開発された緩衝液である．不活化処理を行う際に本緩衝液を用いて 35％血漿／65％インターソルに調整するため，血小板保存に使用されている血漿の量を減らすことができる.

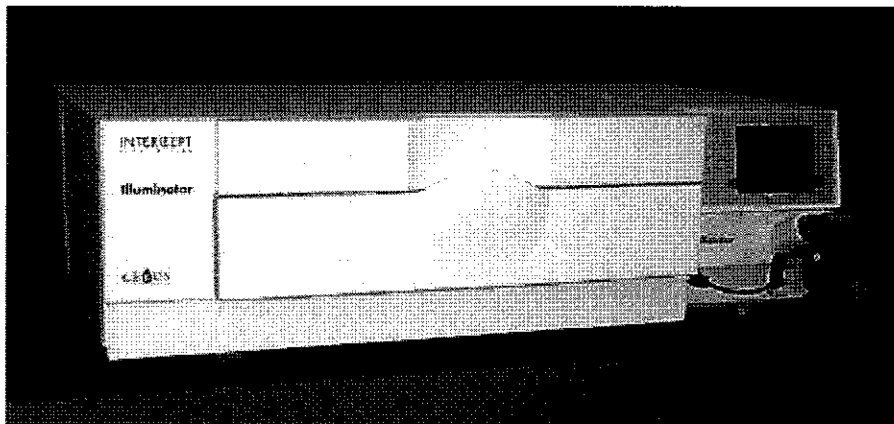
図 1.2 プロセッシングセット（血小板）



【S-59：プロセッシングセット（血小板）に含まれている】

S-59 はソラレン化合物であり，A 紫外線照射により活性化し病原因子の DNA あるいは RNA を架橋することにより病原因子を不活化する．プロセッシングセットには，3mM（1.01g/L）の S-59 溶液が 15mL 又は 17.5mL 充填されている．

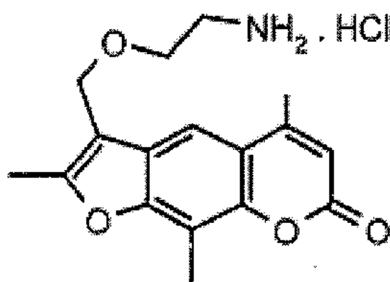
図 1.3.イルミネーター



2．S-59の作用機序

本システムにおいて病原因子の不活化にはソラレン化合物のS-59を用いる．

図2.1 アモトサレン（S-59）の構造式

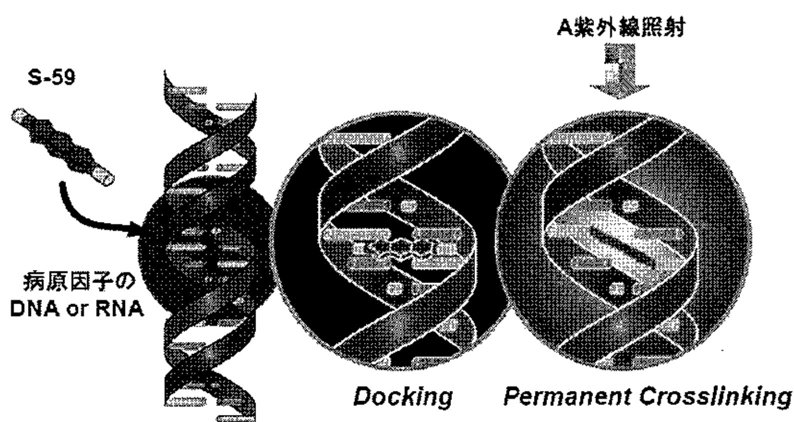


S-59の構造式

分子式：$C_{17}H_{20}ClNO_4$　分子量：337.8

ソラレン化合物は核酸を標的とする化合物の代表である(Cimino ら，1985)．ソラレン分子は光源のない状況において，感染性の病原体及び白血球のDNA及びRNAのらせん部分に可逆的に入り込む（インターカレート）．インターカレートしたソラレン分子は，A紫外線照射により活性化を受けるとピリミジン塩基と反応し永久的に共有結合を形成する．

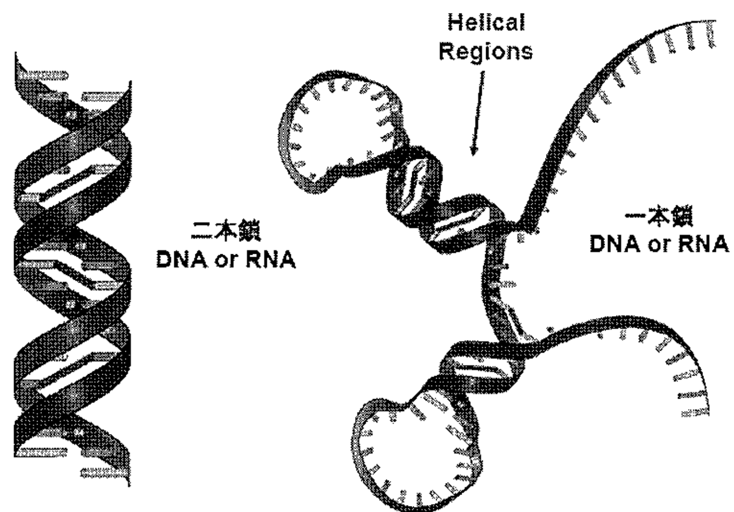
図2.2.の作用機序（その1）



ソラレン分子は2箇所の反応性部位を有するので，病原因子の遺伝子との架橋を形成することができる．ソラレンにより修飾された病原因子の遺伝子は，もはや複製することができない．対照的に血小板はそれ以上分化することがない細胞であり，その機能を発揮するために核酸の複製を必要としない．したがって血小板機能はA紫外線を用いるソラレン

化合物の活性化により影響されないと考えられる.

図 2. 3 S-59 の作用機序（その 2）



3．S-59 による病原因子の不活化能力

現在までにS-59とA紫外線を用いたて実施された血小板濃厚液中の病原因子の不活化能力を以下に示す.

表 3.1 S-59 の病原因子不活化能（1）

病原体	血小板	血漿
	対数減少値 (95%CI)	対数減少値 (95%CI)
エンベロープウイルス		
HIV-1 (cell-free)	>6.2	>5.9
HIV-1 (cell-associated)	>6.1	>6.4
HIV-1 (臨床分離株, Z84 株)	>3.4	
HIV-2 (臨床分離株,CBL20 株)	>2.5	
DHBV (HBV モデル)	>6.2	>5.1
HBV (MS-2)	>5.5	>4.5
BVDV (HCV モデル, NADL 株)	>6.0	>6.0
HCV (Hutchinson 株)	>4.5	>4.5
HTLV-I	4.2	
HTLV-II	4.6	
CMV (cell-associated, AD169 株)	>5.9	
WNV (3356 株)	>5.5	
SARS-HCoV (Urbani 株)	>5.8	
Vaccinia virus (IHD-W 株)	>4.7	
ノン・エンベロープウイルス		
Human adenovirus 5	>5.2	
Parvovirus B19	3.5 – >5.0	
Bluetongue Virus (serotype 11, Station 株)	5.6 – 5.9	
Feline conjunctivitis Virus (FC 株)	1.7 – 2.4	
Simian adenovirus 15 (AP4398 株)	0.7 – 2.3	
PPV (NADL-2 株)	0	

不活化レベルは培養細胞または動物モデルの感染性試験を用いて測定し，「対数減少値」で表した. “>”は処理後の被験試料中に生存病原因子が検出されなかったことにもとづき，不活化が検出限界未満であることを示す.
HIV : ヒト免疫不全ウイルス DHBV : アヒル B 型肝炎ウイルス HBV : B 型肝炎ウイルス
BVDV : 牛ウイルス性下痢ウイルス HCV : C 型肝炎ウイルス HTLV : ヒト T 細胞好性ウイルス
CMV : サイトメガロウイルス WNV : ウエストナイルウイルス SARS-HCoV : SARS ヒトコロナウイルス PPV : パラポックスウィルス

表 3.2　S-59 の病原因子不活化能（2）

病原体	血小板	血漿
	対数減少値	対数減少値
好気性菌		
グラム陽性菌		
Staphylococcus epidermidis	>6.6	5.7
Staphylococcus aureus	6.6	
Streptococcus pyogenes	>6.8	
Listeria monocytogenes	>6.3	
Corynebacterium minutissimum	>6.3	
Bacillus cereus (vegetative)	>5.5	
グラム陰性菌		
Escherichia coli	>6.4	
Serratia marcescens	>6.7	
Klebsiella pneumoniae	>5.6	5.6
Enterobacter cloacae	5.9	
Pseudomonas aeruginosa	4.5	
Salmonella choleraesuis	>6.2	
Yersinia enterocolitica	>5.9	
嫌気性菌		
グラム陽性菌		
Lactobacillus sp. (99A6489)	>6.4	
Bifidobacterium adolescentis (00A3154)	>6.0	
Propionibacterium acnes (00A6608)	>6.5	
Clostridium perfringens (ATCC 43150)	>6.5	
スピロヘータ		
Treponema pallidum	≥6.8 -≥7.0	
Borrelia burgdorferi	>6.9	

不活化レベルは培養細胞または動物モデルの感染性試験を用いて測定し，「対数減少値」で表した．“>”は処理後の被験試料中に生存病原因子が検出されなかったことにもとづき，不活化が検出限界未満であることを示す．

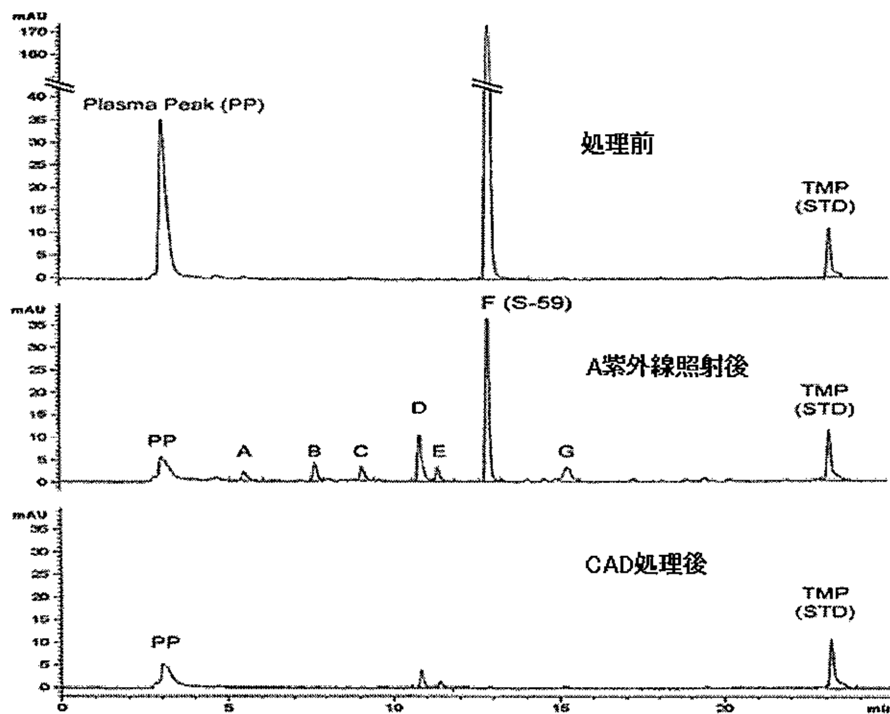
表 3.3　S-59 の病原因子不活化能（3）

原虫	対数減少値
Trypanosoma cruzi	>5.3
Plasmodium falciparum	>7.0

4．残留 S-59

不活化処理の A 紫外線照射の際に S-59 は光分解される．照射後，濃縮血小板は重量百分率で S-59 の約 53%の遊離光分解生成物，26%の共有結合した光分解生成物，及び 21%の残留 S-59 を含む．これらの遊離（非結合の）光分解生成物は非常に類似しているが，HPLC で 6 種類に分離できる．

図 4.1 不活化処理に伴う S-59 の変化



照射後，300mL の濃縮血小板は，約 3.1mg の残留 S-59,8.1mg の遊離光分解生成物及び 4.0mg の結合光分解生成物を含む．

臨床での使用前に，残留 S-59 及び遊離光分解生成物は化合物除去装置（CAD）の処理により低下させる．CAD での処理後，残留 S-59 の量は約 74～84 分の 1 に低下し，主要な光分解生成物の残留量は約 3 分の 1 に低下する．CAD 処理後，300mL の人血小板濃厚液には，約 50μg の残留 S-59，2.7mg の遊離光分解生成物，4mg（2mg は血漿の高分子に結合，2mg は血小板に結合）の共有結合した光分解生成物が含まれる．従って，体重 60kg の人では残留 S-59 とその光分解生成物の臨床暴露量は，単位体重あたり S-59 が約 1μg/kg，遊離光分解生成物が約 45μg/kg，結合光分解生成物が約 70μg/kg になると考えられる．

5．S-59 の体内動態及び代謝

S-59 のヒトにおける体内動態は Phase IB において、自己血小板濃厚液を IBS 処理し被験者に戻す検討が行なわれた。残留 S-59 の平均濃度は 0. 31 μM (25. 1 μg/body)、最高血中濃度の平均値は 1113pg/mL、半減期の平均値は 428. 1min、AUC の平均値は 15. 1ng/L・min であった。
非臨床試験において尿中および糞中排泄物を HPLC で検討した結果、糞中では多くのマイナーピークが検出され非常に高度代謝されていることがうかがえた。また尿サンプルにおいて S-59 の代謝を検討しているが、グルクロン酸抱合および硫酸抱合は関与していない。(8-MOP はグルクロン酸抱合および硫酸抱合の関連あり)。非臨床試験（ラット及びイヌ）における検討では、投与後 28 日目までに約 65%が糞中、約 10%が尿中に排泄された。
非臨床試験における検討ではラット及びイヌにおいて、投与後 48 時間目までに投与された残留 S-59 (CAD 処理済) の 57%、35%が排泄された。また、投与後 28 日目までに、それぞれ 86-87%、70-84%が排出された。一方、ラットにおいて、体内に残留した S-59 は投与後 28 日目で 6%であった。

６．血小板の機能

In vitroの試験ではS-59による不活化処理群と対象群で差がみられているがどちらもAABBの基準の範囲内であり、かつ in vivo のウサギの耳出血時間モデルでは両処理群で差が認められず、血小板止血機能に有害な影響を与えないことが判明している。
また米国の Phase III による出血の防止を End Point とした臨床試験で IBS 処理群と対象群で差がないことを実証している。
市販後の追跡調査でも IBS 処理によって血小板の登用量が増えている情報はない。
不活化処理により血小板の回収率は７－８％低下するが活性面での低下は市販後の調査結果では問題になっていない。

１）in vitroにおける血小板機能

表6.1 アフェレーシス血小板の *in vitro* 血小板機能：

検査項目	保存5日目 平均±標準偏差	
	CAD 非処理群 (N = 6)	CAD 処理群 (N = 6)
血小板数（$\times10^3/\mu L$）	1521±250	1452±234
pH	6.93±0.09	6.92±0.06
形態（0～400）	279±20*	290±20*
低張性ショック反応，HSR（%）	45±5	45±3
ATP（nmol/血小板 10^8 個）	0.7±0.1	0.7±0.2
Pセレクチン（発現率%）	51±3*	58±5*
pCO_2 (mmHg)	27.0±3.3*	23.7±3.5*
pO_2 (mmHg)	73.4±22.8*	84.8±22.5*
HCO_3^- (mM)	5.6±0.7*	4.8±0.5*
乳酸（mM）	9.9±2.2*	8.5±1.6*
グルコース（mM）	1.6±1.3*	2.1±1.1*

*： 有意差あり（Studentの paired t 検定，$p\leqq0.05$）

２）in vivoにおける止血効果

ニュージーランド白ウサギをγ線へ暴露し，続いて抗ウサギ血小板ヒツジ血清を注入し，重度の血小板減少症を誘発した．10 対の対照と不活化処理濃厚血小板（S-59 150[illegible]M＋3 J/cm² A 紫外線，CAD 処理 6 時間）を評価した．ヒト血小板（各ウサギに 2.5×10^{10} 個）の血小板減少症ウサギへの輸血により，血小板数が対照群で $6.1\pm2.0\times10^3/\mu L$ から $109\pm38\times10^3/\mu L$，不活化処理群で $6.4\pm1.5\times10^3/\mu L$ から $111\pm34\times10^3/\mu L$ へと増加した．血小

板減少症ウサギの輸血前の毛細血管出血時間は>900 秒であった．ヒト血小板の輸血後，この出血時間は対照群で 274±226 秒，不活化処理群で 259±112 秒に短縮した．この 2 群間に統計的な有意差は認められなかった．血小板数の増加は出血時間の短縮と相関した．これらの結果は *in vivo* の動物モデルにおいて不活化処理血小板の *in vivo* での止血機能が維持されていることを支持するものである．

7．毒性試験

1）毒性試験はICHの医薬品のガイドラインに従い実施した。被験試料はS-59単独のみならず、S-59で不活化処理された血小板（CAD処理、非処理）で実施されている。

表　7.1 毒性試験

試験項目	S-59混合液（A紫外線照射）		S-59単独
	CAD処理	CAD非処理	
単回投与毒性試験	実施	実施	実施
≦1ヶ月毒性試験		実施	実施
3ヶ月毒性試験	実施	実施	
がん原性試験	実施	実施	実施
遺伝毒性試験		実施	実施
生殖毒性試験	実施	実施	
安全性薬理試験	実施		
局所毒性試験		実施	

2）反復投与毒性試験

表 7.2　反復投与毒性試験の概要

試験方法	被験物質	試験結果	実施施設	資料番号
ラット 7 日間連日静脈内投与	S-59	7日間連日投与の無毒性量は, 75mg/kg/day と考えられた.	Bio Research*1	(BioResearch 53777)
ラット 14 日間連日静脈内投与	S-59	S-59, 75mg/kg/day を 14 日間連日投与した結果, 全身的な毒性は観察されなかった.	Bio Research*1	(BioResearch 54433)
ラット 28 日間連日静脈内投与	S-59	S-59, 18.8, 37.5, 75 mg/kg/day を 28 日間連日投与した結果, 全身的な毒性は観察されなかった.	Bio Research*1	BioResearch 53779
イヌ 7 日間連日静脈内投与	S-59	S-59 , 5 , 15 , 30mg/kg/day を 7 日間連日投与した結果, 毒性は観察されなかった.	Bio Research*1	(BioResearch 53778)
イヌ 28 日間連日静脈内投与	S-59	S-59, 6.25, 12.5, 25 mg/kg/day を 28 日間連日投与した結果, 全身的な毒性は観察されなかった.	Bio Research*1	BioResearch 53780
ラット 7日間連日静脈内投与 CAD 非処理	S-59 混合液	7日間連日投与の無毒性量は, 25mL/kgday と考えられた.	Bio Research*1	(BioResearch 53777)
ラット 14 日間連日静脈内投与 CAD 非処理	S-59 混合液	14 日間連日投与の無毒性量は, 25mL/kgday と考えられた.	Bio Research*1	(BioResearch 54433)
ラット 14 又は 28 日間連日静脈内投与	S-59 混合液	14 又は 28 日間連日投与の無毒性量は, 25mL/kgday と考えられた.	Bio Research*1	BioResearch 53779

CAD 非処理				
ラット 13 週間連日静脈内投与 CAD 処理/非処理	S-59 混合液	被験物質に関連する臨床的毒性，死亡及び組織の顕微鏡的所見は認められなかった．	ClinicalTrials Bioresearch*2	CTBR55260
イヌ 7 日間連日静脈内投与 CAD 非処理	S-59 混合液	イヌに CAD 非処理の S-59 混合液 5mL/kg，25mL/kg を 7 日間連日投与した結果，25mL/kg(残留 S-59 0.25mg/kg) の用量では毒性は観察されなかった．	Bio Research*1	(BioResearch 53778)
イヌ 14 又は 28 日間連日静脈内投与 CAD 非処理	S-59 混合液	CAD 非処理の S-59 混合液 5mL/kg，25mL/kg を 14 または 28 日間連日投与した結果, 25mL/kg で毒性は観察されなかった．	Bio Research*1	BioResearch 53780
イヌ 13 週間間欠静脈内投与 CAD 非処理 (3 doses/週)	S-59 混合液	CAD 非処理の S-59 混合液 25mL/kg を 13 週間(3 doses/週)投与した結果，毒性は観察されなかった．	ClinicalTrials Bioresearch*2	CTBR56418
イヌ 13 週間静脈内間欠投与 CAD 非処理 (1 dose/週)	不活化処理済血小板	CAD 非処理の血小板濃厚液 25mL/kg を 13 週間(1 dose/週)投与した結果，毒性は観察されなかった．	ClinicalTrials Bioresearch*2	CTBR56535
カニクイザル 14 日間間欠静脈内投与*1 CAD 非処理 (6 dose/14days)	不活化処理済ヒト血小板	CAD 非処理の血小板濃厚液 25mL/kg を 14 日間間欠投与した結果，毒性は観察されなかった．	BioResearch*1	(Bio Research 54359)

( )：添付資料なし．リクエストに応じて追加．
*1：Bio Research Laboratories Ltd.
87 Senneville Road, Senneville Quebec H9X 3R3, Canada
*2：Clinical Trials Bioresearch Ltd.
87 Senneville Road, Senneville Quebec H9X 3R3, Canada
*6：Battelle
505 King Ave Columbus, OH 43201-2693, U.S.A.

3）がん原性試験

表 7.3 がん原性試験の概要

試験方法	被験物質	試験結果	実施施設	資料番号
p53 遺伝子ヘテロ接合型トランスジェニックマウス 26 週間間欠静脈内投与 CAD 処理/非処理	S-59 S-59 混合液,	被験物質によるがん原性は認められなかった． ポジティブコントロール群(p-クレシジン)においては，膀胱の移行上皮の過形成，移行上皮/扁平上皮癌を誘発した．	Covance*3	Covance 6918-102

*3：Covance
9200 Leesburg Pike Vienna, VA 22182-1699, U.S.A.

4）遺伝毒性試験

表 7.4　遺伝毒性試験の概要

試験方法	被験物質	試験結果	実施施設	資料番号
エームス試験 (TA98, TA100, TA1535, TA1537, WP2 uvrA WP2)	S-59	代謝活性化系の存在に関わらず，TA1537 株で陽性を示した．その他の株では陰性であった．	Microbiological Associates, Inc.*4	(MA 59-006) (MA 59-012) MA 59-016
エームス試験 (TA98, TA100, TA1535, TA1537, WP2 uvrA WP2) CAD 非処理	不活化処理済ヒト血小板	代謝活性化系の存在に関わらず，全てのテスターで陰性であった	Microbiological Associates, Inc.*4	MA 59-009 MA 59-011
エームス試験 (TA98, TA100, TA1535, TA1537, WP2 uvrA WP2) CAD 非処理	複数回の不活化処理済ヒト血小板	代謝活性化系非存在下の TA1537 株で陽性を示した．その他の株では陰性であった．	Microbiological Associates, Inc.*4	MA 59-014 MA 59-016
マウスリンフォーマ TK 試験	S-59	代謝活性系非存在下において，7.5μg/mL 以上の濃度で陽性であった．代謝活性系存在下において，65μg/mL の濃度で陰性であった．	Microbiological Associates, Inc.*4	(MA 59-005)
マウスリンフォーマ TK 試験 CAD 非処理	不活化処理済ヒト血小板	代謝活性系非存在下及び代謝活性系存在下においても陰性であった．	Microbiological Associates, Inc.*4	MA 59-008
染色体異常試験 (CHO 細胞)	S-59	代謝活性系非存在下における最大無作用量の平均値は，2μg/mL であった．代謝活性系存在下における最大無作用量の平均値は，24μg/mL であった．	Microbiological Associates, Inc.*4	(MA 59-007) MA 59-015
染色体異常試験 (CHO 細胞) CAD 非処理	不活化処理済ヒト血小板	代謝活性系非存在下及び代謝活性系存在下においても陰性であった．	Microbiological Associates, Inc.*4	MA 59-010
染色体異常試験 (CHO 細胞) CAD 非処理	複数回の不活化処理済ヒト血小板	代謝活性系非存在下における最大無作用量の平均値は，残留 S-59 濃度 3μg/mL であった．代謝活性系存在下における最大無作用量の平均値は，残留 S-59 濃度 13μg/mL であ	Microbiological Associates, Inc.*4	MA 59-015

		った.		
試験方法	被験物質	試験結果	実施施設	資料番号
UDS 試験	S-59	34mg/kg(最高投与量)において陰性であった.	Microbiological Associates, Inc.*4	(MA 59-002)
UDS 試験 CAD 非処理	不活化処理済ヒト血小板	残留 S-59 濃度 200 μg/kg, 光反応生成物濃度 800 μg/kg (最高投与量)において陰性であった.	Microbiological Associates, Inc.*4	MA 59-004
マウス小核試験	S-59	66mg/kg(最高投与量)において陰性であった.	Microbiological Associates, Inc.*4	(MA 59-001) (MA 59-013)
マウス小核試験 CAD 非処理	不活化処理済ヒト血小板	残留 S-59 濃度 200 μg/kg, 光反応生成物濃度 800 μg/kg (最高投与量)において陰性であった.	Microbiological Associates, Inc.*4	MA 59-003

( ): 添付資料なし. リクエストに応じて追加.

5) 生殖毒性試験

表 7.5 生殖毒性試験の概要

試験方法	被験物質	試験結果	実施施設	資料番号
ラット 静脈内投与（雄性） CAD 処理/非処理	S-59 混合液	交配 28 日前より 25mL/kg を投与した. 胎児に対する影響は観察されなかった.	Argus Research Labs*5	Argus 2319-009
ラット 静脈内投与（雌性） CAD 処理/非処理	S-59 混合液	交配 15 日前より 25mL/kg を投与した. 胎児に対する影響は観察されなかった.	Argus Research Labs*5	Argus 2319-004 Argus 2319-012
ラット 静脈内投与（母体毒性と発生毒性）CAD 処理/非処理	S-59 混合液	交配後 6～17 日後に 25mL/kg を連日投与した. 母体毒性及び発生毒性は観察されなかった.	Argus Research Labs*5	Argus 2319-003 Argus 2319-013
ウサギ 静脈内投与（母体毒性と発生毒性） CAD 処理	S-59 混合液	交配後 6～19 日後に 25mL/kg を連日投与した. 母体毒性及び発生毒性は観察されなかった.	Argus Research Labs*5	Argus 2319-002
ラット 静脈内投与 (周産期, 産後の発育異常) CAD 処理	S-59 混合液	交配後 7 から授乳期までに 25mL/kg を連日投与した. F_0 及び F_1 に対する毒性は観察されなかった.	Argus Research Labs*5	Argus 2319-007

*5 : Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A Horsham, PA 19044-1297, U.S.A.

8. 臨床試験

表 8.1 不活化処理血小板の臨床試験の要約

試験名	試験デザイン	血小板投与法・量	評価項目	輸血試験回数	被験者数[a]
健常人試験					
ⅠA 試験	ランダム化 単純盲検 クロスオーバー	放射能標識自己血小板（CAD 非処理）10ml	回復と寿命	1 （1 期間当たり）	24
ⅠB 試験	ランダム化 単純盲検 クロスオーバー	自己血小板 治療用量	S-59 の薬物動態	1 （1 期間当たり）	10
ⅡA 試験	シングルアーム ⅠA 過去対照群	放射能標識自己血小板 10ml	回復と寿命	1	16
ⅡB 試験	シングルアーム ⅠA・ⅡA 過去対照群	放射能標識自己血小板γ線照射 10ml	回復と寿命	1	15
血小板減少症患者の試験					
ⅡC 試験 出血時間と 血小板数増加量	ランダム化 二重盲検 クロスオーバー	アフェレーシス血小板 2 倍治療用量 単回輸血	Template 出血時間，CI，CCI，止血能，輸血間隔	1 （1 期間当たり）	32
欧州第Ⅲ相試験 バフィーコート 血小板	ランダム化 二重盲検 並列群	バフィーコート血小板 治療用量 反復輸血 8 週間まで	CI，CCI，止血能，血小板数輸血回数，赤血球輸血回数，輸血間隔，輸血反応	反復（1 サイクル当たり 8 週間）	103
米国第Ⅲ相試験 アフェレーシス 血小板	ランダム化 二重盲検 並列群	アフェレーシス血小板 治療用量 反復輸血 4 週間まで	Grade2 WHO 出血，Grade3/4 WHO 出血，CI，CCI，止血能，血小板輸血回数，赤血球輸血回数，輸血反応	反復（1 サイクル当たり 4 週間）	645
欧州第ⅢB 相試験 バフィーコート 血小板； 一体型セット	過去対照群 シングルアーム 一施設でのオープンラベル	バフィーコート血小板 治療用量 反復輸血 4 週間まで	CI，CCI，止血能，血小板数及び赤血球の輸血回数，輸血間隔，輸血反応	反復（4 週間以上）	20[b]
欧州第ⅢB 相試験 アフェレーシス 血小板； 一体型セット	ランダム化 二重盲検 並列群	アフェレーシス血小板 治療用量 反復輸血 4 週間まで	CI，CCI，止血能，血小板輸血回数 赤血球輸血回数，輸血間隔，輸血反応	反復（4 週間以上）	42

a 試験輸血を 1 回以上受けた被験者（評価可能例）の数
b 同施設にて行われた欧州第Ⅲ相試験の 19 例を過去対照群として利用した.

これら実施した 9 件の臨床試験いずれにおいても，血小板濃厚液を不活化処理することによって，その機能及び安全性において未処理血小板に劣る結果は得られていない．また，現在実施中の大規模ヘモビジランススタディー，小児を対象にした試験においても未処理の血小板濃厚液に有効性，安全性で劣る結果は認められていない.

参考資料2

バイオワン株式会社

2008年4月１日

Mont Godinne BTC: platelet utilization during 3 years prior to and 3 years after INTERCEPT

JC Osselaer, C Doyen: BTC Mont Godinne

Parameter	Prior Period	INTERCEPT
All Patients* (n)	690	756
Platelet Transfusions (n)	6829	7538
Mean days of support	13.6	13.1
Transfusions/patient	9.9	10.0
Hematology** Patients (n)	286	359
Platelet Transfusions (n)	5846	6718
Mean days of support	29.3	25.5
Transfusions/patient	20.4	18.7

- *Includes hematology, oncology, surgery and general medical patients
- ** Hematology patients only

Residual Risk of Bacterial Sepsis

After Bacterial Culture of Apheresis Platelets:

American Red Cross — *Eder et al, 2007 (in press)*

- 20 septic reactions (3 fatal)
- 1,004,000 tested products — ~1:59,000

Canada — *Ramírez-Arcos et al, 2007 (in press)*

- 2 septic reactions (1 fatal)
- 82,004 tested products — ~1:41,000

Holland — *deKorte et al, Transfusion 2006*

- 2 septic reactions
- 113,092 tested products — ~1:56,500

Germany — *Schmidt et al, Vox Sanguinis 2007*

- 2 septic reactions (1 fatal)
- 11,037 products tested — ~1:11,000

Impact of INTERCEPT on production

	Control period	**INTERCEPT period**
Apheresis instrument	5 Amicus 3 Spectra	6 Amicus
Apheresis procedures	2,576	2,997
Mean yield	6.56×10^{11}	6.67×10^{11}
Expiration rate	9.1%*	7.2%* 1.5%†

*5-day shelf-life. †7-day shelf-life.

Osselaer JC. Presented at ISBT, Athens, Greece, 2005.

BioOne Corporation

All Patients

18 months before and after implementation of INTERCEPT Platelets

Period	Control	INTERCEPT	Δ
Platelet transfusions	3,528	3,932	+11%
Patients receiving platelets	352	400	+14%
Platelet transfusions/patient	10.0	9.8	-2%
RBC transfusions	9,506	11,471	+21%
Patients receiving RBC	1703	2031	+19%
RBC transfusions/patient	5.6	5.6	0

Osselaer JC. Presented at ISBT, Athens, Greece, 2005.

BioOne Corporation

Acute transfusion reactions

- Each transfusion was assessed for acute transfusion reactions in both the control (C-PLT and C-RBC) and INTERCEPT components (I-PLT)

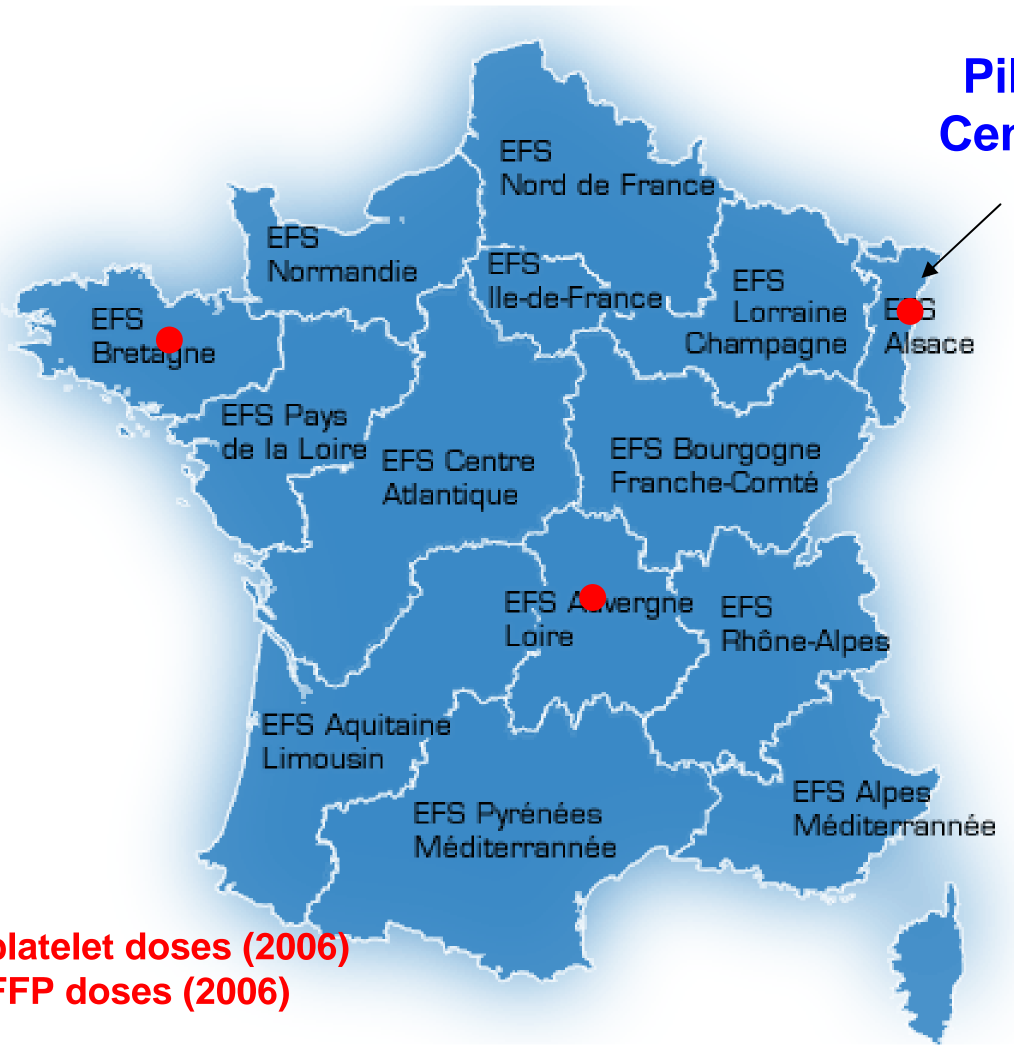
	Control period		INTERCEPT period	
Component	C-PLT	C-RBC	I-PLT	C-RBC
Months	18	18	18	18
Transfusions	3,529	9,551	4,051	11,493
Reactions	1.3%*	0.4%	0.9%*	0.4%

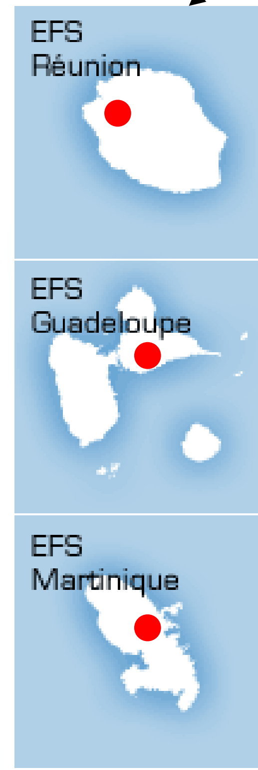
*p=0.002.

Osselaer JC. Presented at ISBT, Athens, Greece, 2005.

BioOne Corporation

Clinical experience with INTERCEPT platelets and plasma in France





Pilot Center

100% Intercept platelets

Epidemics of :

Chikungunya

Dengue fever

Chagas disease

- 232,000 platelet doses (2006)
- 292,000 FFP doses (2006)

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Demography of patients receiving platelet concentrates (PC) at EFS-Alsace

		PC (100% plasma) 1/1/2003 – 1/2/2004 99.6 %	PC (35% plasma+65% T-Sol) 1/9/2005 – 1/6/2006 95 %	PC INTERCEPT (35% plasma+65% Intersol) 1/9/2006 – 1/8/2007 99 %
Patients (n)		2,050	1,678	2,069
Age (yrs)	(median)	61	62	63
	(mini)	<1	<1	<1
	(maxi)	94	98	96
Sex	male	1,178 (58%)	1,035 (62%)	1,260 (61%)
	female	872 (42%)	643 (38%)	809 (39%)
Onco-hematology		56 %	51 %	58 %
Cardiovascular surgery		7 %	6 %	6 %
General medicine and surgery		37 %	43 %	36 %

BioOne Corporation

EFS

Platelet concentrates (PC) transfused* at EFS-Alsace

	PC (100% plasma) 1/1/2003 – 1/2/2004 99.6 %	PC (35% plasma+65% T-Sol) 1/9/2005 – 1/6/2006 95 %	PC INTERCEPT (35% plasma+65% Intersol) 1/9/2006 – 1/8/2007 99%
Patients (n)	2,050	1,678	2,069
PC transfused (n)	10,629	9,151	13,241**
Mean / patient	5.2	5.5	6.4
Median / patient	2.0	2.0	2.0
Minimum	1	1	1
Maximum	104	114	289
Platelets $x10^{11}$ / patient			
Mean	26.9	24.2	27.0
Median	10.4	8.9	8.4
Minimum	0.2	0.2	0.5
Maximum	450	445	1,149

* Ratio LR-BCPC/LR-APC : 62/38 - ** 21% : 1 PC ; 34 % : 2 PC ; 20 % : 3-5 PC ; 23 % : 6-50 PC

BioOne Corporation

Adverse transfusion reactions during platelet concentrates (PC) transfused at EFS-Alsace

	(1) PC (100% plasma) 1/1/2003 – 1/2/2004 99.6 %	(2) PC (35% plasma+65% T-Sol) 1/9/2005 – 1/6/2006 95 %	(3) PC INTERCEPT (35% plasma+65% Intersol) 1/9/2006 – 1/8/2007 99 %
Patients (n)	59	33	36
Adverse reactions (n)	67 (11 RBC Imm)	41 (16 RBC Imm)	37* (19 RBC Imm)
Adverse reactions/1000 PC (n)	5.3	2.7	1.4
Patients with reactions	2.9 %	2 %	1.7 %

Period 3 : *Fever/chills : 8 ; allergy : 3 ; TRALI : 1 ; RBC immunisations : 19

Period 1 : 1 death volume overload (2 RBCC + 2 BCPC)

All 3 periods : No bacterial sepsis

All 3 periods : 145 adverse reactions : SEVERITY grade 1 = 61 %; grade 2 = 33 % (46 RBC immunisations)

IMPUTABILITY grade 2, 3, 4 = 87 %; grade 3 and 4 = 70 %

BioOne Corporation

Red blood cell concentrates (RBCC) transfused to patients receiving PC at EFS-Alsace

	PC (100% plasma) 1/1/2003 – 1/2/2004 99.6 %	PC (35% plasma+65% T-Sol) 1/9/2005 – 1/6/2006 95 %	PC INTERCEPT (35% plasma+65% Intersol) 1/9/2006 – 1/8/2007 99%
Patients 1 PC + 1 RBCC (n)	1,715 (83.7%)	1,395 (83.1%)	1,763 (85.2%)
RBCC (n)	24,691	17,873	23,886
Mean / patient	14.4	12.8	13.5
Minimum	1	1	1
Maximum	128	155	307
PC transfused (n)	9,953	8,553	12,626
Mean / patient	5.8	6.1	7.2
Minimum	1	1	1
Maximum	104	114	289

BioOne Corporation

EFS

EFS-La Réunion : acute reactions following transfusions of INTERCEPT treated APC

(M F Angelini-Tibert and P. Rasongles)

Patient Group	Period	INTERCEPT APC transfused	Reactions per 1000 APC
All Patients	1/2005-12/2005	1194	92.0
Pediatric	1/2005-12/2005	366	218.0
All Patients	3/2006-3/2007	1948	9.8
Adults	3/2006-3/2007	1370	4.4
Pediatric	3/2006-3/2007	489	26.6
Infants	3/2006-3/2007	89	0

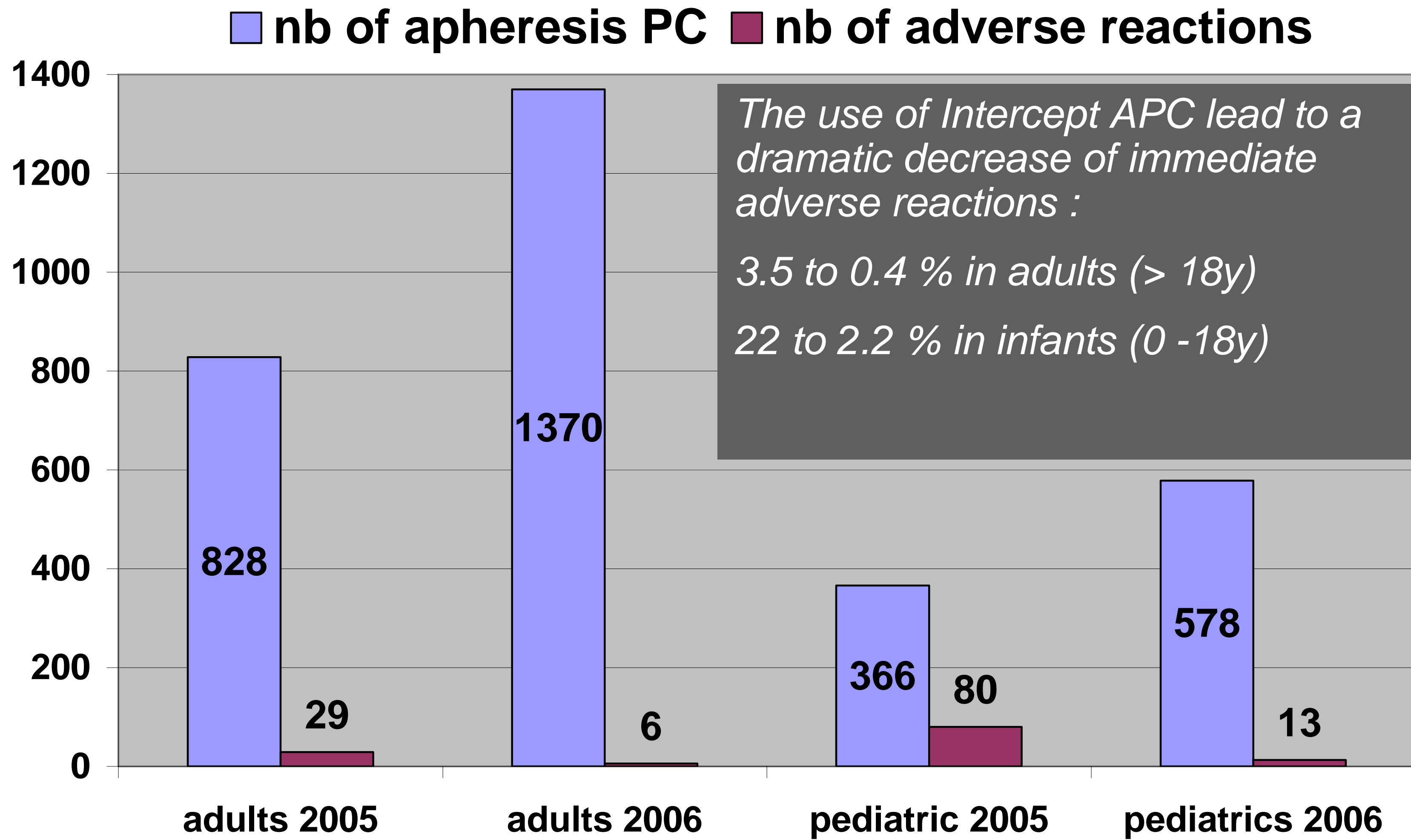
EFS-La Réunion : Adverse reactions with INTERCEPT treated Apheresis PC -1

(M F Angelini-Tibert and P. Rasongles)

year	2005	2006/3 - 2007/3
nb of patients	NA	427
nb of apheresis PC	1194	1948
nb of adverse reactions	109	19
Adverse reactions / 1000 APC	91,3	9,8

EFS-La Réunion : Adverse reactions with Intercept treated Apheresis PC -2

(M F Angelini-Tibert and P. Rasongles)

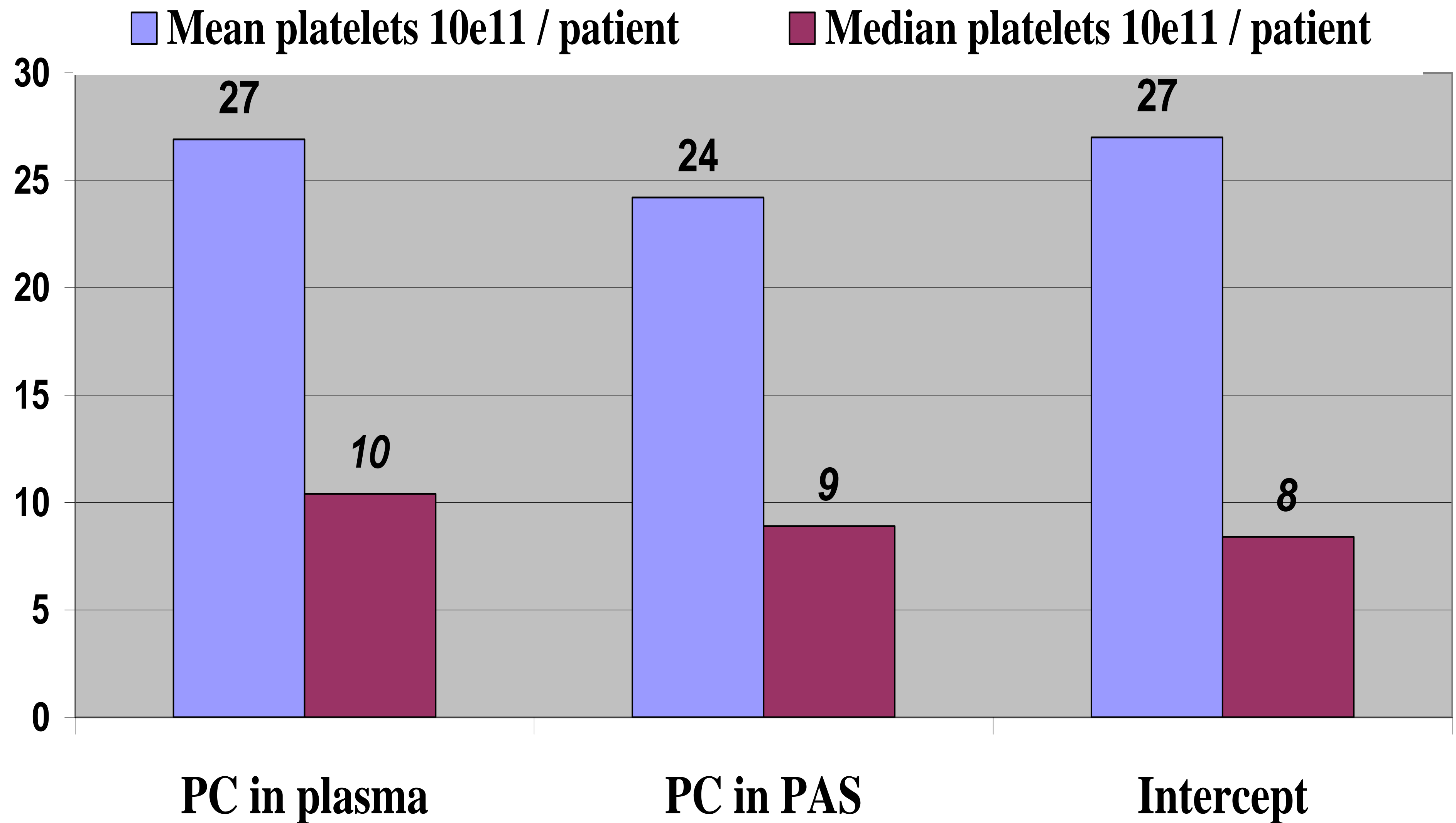


Clinical experience of Intercept at EFS Alsace-1

Intercept implementation	PC in plasma	PC in PAS	Intercept
date	2003 /1 to 2004 /1	2005 /9 to 2006 /6	2006 /9 to 2007 /8
nb of months	13	10	12
nb of patients	2 050	1 678	2 069
median age	61	62	63
Male / female	58 / 42	62 / 38	61 / 39
onco-hematology	56%	51%	58%
cardio vascular surgery	7%	6%	6%
other	37%	43%	36%
nb of PC delivered (APC/RPC=40/60)	10 629	9 151	13 241
Mean PC / patient	5,2	5,5	6,4
Median PC / patient	2,0	2,0	2,0
Mean platelets 10^{11} / patient	26,9	24,2	27,0
Median platelets 10^{11} / patient	10,4	8,9	8,4
Mean RCC / patient	14,4	12,8	13,5

INTS

Clinical experience of Intercept at EFS Alsace-2



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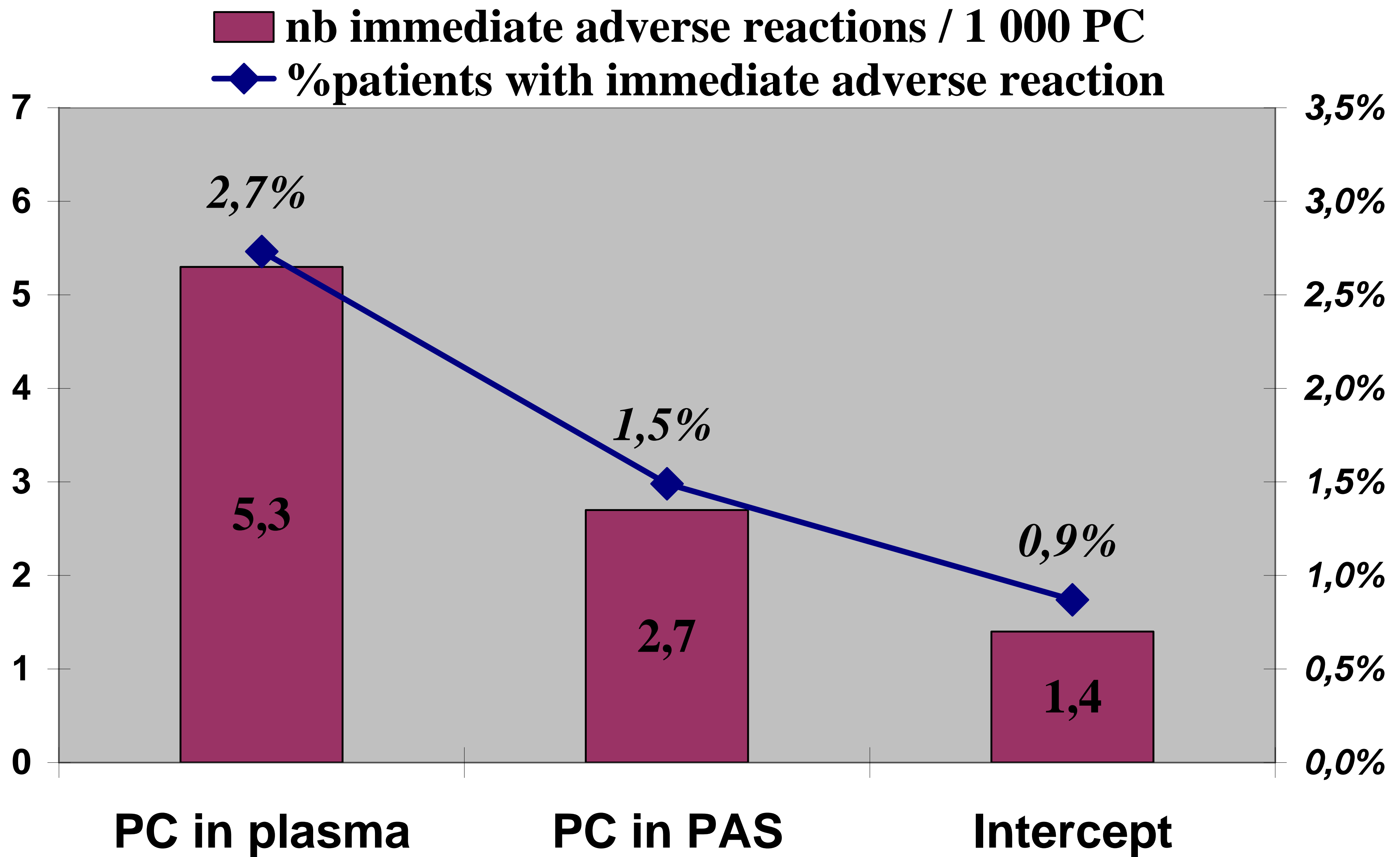
Clinical experience of Intercept at EFS Alsace 3



2月27日部会使用資料に対する追記部分

諸外国における感染因子不活化技術（S／D処理、メチレンブルー・リボフラビン・アモトサレン）の製造承認及び導入の状況

血漿の不活化については、欧州においてメチレンブルーを中心として、導入が進んでいる国もあるが、全ての血漿製剤に不活化を実施しているのは、ごく一部の国である。
またこれらの国においては、有償採血であることや、輸血用血漿製剤の使用量が我が国と比較して、1/3〜2/3と少ないなど、実施しやすい状況がある。
一方、血小板の不活化については、感染症が蔓延している地域における導入や国によっては一部試行的に導入しているところもあるが、様々な技術が開発されているところであり、一つの技術を全国的に導入すると決定している国は今のところないと聞いている。
また、多くの感染症が蔓延している国においては、NATなど高額な検査を実施できない場合もあり、広範な病原体に対して有効な不活技術のみ導入しようとする場合もある。

	資料の種類	製造承認の有無	導入状況	備考
米国	2008.2.27 部会内容	不活技術に対する承認はない	導入を検討中	様々な血液銀行による有償採血であるので、採血量の増加にも対応が可能。血漿に対する不活化技術の導入の動向はない。 新興・輸入感染症と血小板製剤に多発する細菌感染の対策として、血小板の不活化の導入を検討中。不活化血小板の承認申請審査中。 千人当りの血漿使用量は日本の3分の2程度。
	アモトサレン最新状況		**＊血小板：申請審査中。**	
フランス	2008.2.27 部会資料記述	メチレンブルーによる血漿の不活化 アモトサレンによる血小板の不活化	血漿に対しては、60%がプールした後にS／D処理、40%がメチレンブルーにより不活化処理をされている。 血小板に対する不活化については、インド洋、カリブ諸島、南米の3つの海外圏や本国の5センターでアモトサレンやリボフラビンによる処理を導入している。	フランス血液機構は、献血により採血している。 熱帯地域の海外県における感染症発生のリスクがあり、その影響で本国においても、血漿や血小板の不活化対策に取組む必要性が高い。 千人当りの血漿使用量は日本の3分の1程度。
	アモトサレン最新状況		**＊血小板：4地域で導入済。3地域で評価中。** **＊血漿：1血液センターで導入済。**	**EFS La Reunion, EFS Alsace, EFS La Martinique, EFS Guadeloupe, EFS Auvergne-Loire, EFS Bretagne, EFS Alpes Mediteranee**
ドイツ	2008.2.27 部会資料記述	メチレンブルーによる血漿の不活化 アモトサレンによる血小板の不活化	血漿に対しては、本年1月からメチレンブルーによる不活化製剤を供給。 血小板の不活化については、未導入。	ドイツ赤十字が輸血の8割を実施。 血小板の不活化として、ドイツ赤十字はアモトサレンの使用はしておらず、薬剤を用いない不活化技術（UVC）を開発中。アモトサレンを評価する計画もある。 ドイツの血漿は、有償採血のため、採血量の増加にも対応が可能。 千人当たりの血漿使用量は日本とほぼ同じ。
	アモトサレン最新状況		**＊血小板：1血液センターで導入済。10地域で評価中。** **＊血漿：3血液センターで評価中。**	**Luebeck, Mannheim, Berlin, Leipzig, Goettingen, Frankfurt, Homburg-Saar, Haema AG, Essen, Cologne, Ulm**
イギリス	2008.2.27 部会資料記述	メチレンブルーによる血漿の不活化	血漿については、小児を対象に、メチレンブルーによる不活化製剤を供給。 血小板に対する導入は行っていない。	英国の国営血液サービスは、米国で有償で採血された血漿を輸入している。 感染症のリスクを考慮して、1996年以降に誕生した子供の輸血に使用する際には、メチレンブルーによる不活化を実施している。 千人当りの血漿使用量は日本の2分の1程度。
	アモトサレン最新状況		**評価中**	
ベルギー	2008.2.27 部会資料記述	メチレンブルーによる血漿の不活化	2004年にメチレンブルーによる血漿の不活化を導入	アモトサレン及びリボフラビンによる血小板の評価試験中 アモトサレン承認申請中
	アモトサレン最新状況		**＊血小板：3血液センター導入済。ベルギー赤十字で評価中。** **＊血漿：2血液センターで導入済。1血液センターで評価中。**	**Mont Godinne, Charleroi, ABL, Gent, St Jan Brugge, Erasme, Hustin, Liege　血小板100%全国導入決定。**
ルクセンブルグ	2008.2.27部会資料記述	メチレンブルーによる血漿の不活化	メチレンブルーによる血漿の不活化を導入	
	アモトサレン最新状況			
カナダ	2008.2.27部会資料記述	不活化技術に対する承認はない	未導入	メチレンブルー不活化血漿の導入を検討中
	アモトサレン最新状況	**血小板：承認**	**評価中**	
スイス	2008.2.27 部会資料記述	不活化技術に対する承認はない	今年から、25%の血漿に対してSD処理をして供給 血小板については未導入	アモトサレンによる血小板不活化承認申請中
	アモトサレン最新状況		**＊血小板：1血液センターで評価中。**	**Basel**
オランダ	2008.2.27部会資料記述	不活化技術に対する承認はない	未導入	
	アモトサレン最新状況	**CEの承認でオーケー。**	**＊血小板：Sanquinで評価中。**	**Rotterdam, Nijmegen**
ノルウエー	2008.2.27部会資料記述	アモトサレンによる血小板の不活化	一部の血液センター・院内血液銀行でのみ導入	
	アモトサレン最新状況		**＊血小板：3血液センターで導入済。** **＊血漿：2血液センターで評価中。**	**Bergen, Trondheim, Bodo**
スペイン	2008.2.27 部会資料記述	メチレンブルーによる血漿の不活化 アモトサレンによる血小板の不活化	一部の血液センターでのみ導入	
	アモトサレン最新状況		**＊血小板：3地域で導入済。4地域で評価中。**	**Las Palmas, Tenerife, Madrid RC, Valladolid, Barcelona, Cantabria**
イタリア	2008.2.27 部会資料記述	メチレンブルーによる血漿の不活化 アモトサレンによる血小板の不活化	一部の血液センターでのみ導入	
	アモトサレン最新状況		**＊血小板：5血液センターで導入済。2血液センターで評価中。**	**Palermo, Torino, Napoli, Pescara, Bari, Milan HSR, Crotone**
ギリシャ	2008.2.27部会資料記述	メチレンブルーによる血漿の不活化	一部の血液センターでのみ導入	
	アモトサレン最新状況			
ロシア	2008.2.27部会資料記述	メチレンブルーによる血漿の不活化	一部の血液センターでのみ導入	モスクワ市内の血液センターで導入
	アモトサレン最新状況		**＊血小板：3地域で導入済。**	**Samara, St Petersburg, Moscow**
マレーシア	2008.2.27部会資料記述	アモトサレンによる血小板の不活化	一部センターで小児対象に導入	
	アモトサレン最新状況		**＊血小板：一部センターで導入済。**	

2月27日部会使用資料に対する追記部分

諸外国における感染因子不活化技術（S／D処理、メチレンブルー・リボフラビン・アモトサレン）の製造承認及び導入の状況

血漿の不活化については、欧州においてメチレンブルーを中心として、導入が進んでいる国もあるが、全ての血漿製剤に不活化を実施しているのは、ごく一部の国である。
またこれらの国においては、有償採血であることや、輸血用血漿製剤の使用量が我が国と比較して、1/3～2/3と少ないなど、実施しやすい状況がある。
一方、血小板の不活化については、感染症が蔓延している地域における導入や国によっては一部試行的に導入しているところもあるが、様々な技術が開発されているところであり、一つの技術を全国的に導入すると決定している国は今のところないと聞いている。
また、多くの感染症が蔓延している国においては、NATなど高額な検査を実施できない場合もあり、広範な病原体に対して有効な不活技術のみ導入しようとする場合もある。

	資料の種類	製造承認の有無	導入状況	備考
シンガポール	2008.2.27部会資料記述	メチレンブルーによる血漿の不活化	一部でのみ導入	アモトサレン評価試験中
	アモトサレン最新状況		**＊血小板：評価中**	
韓国	2008.2.27部会資料記述	不活化技術に対する承認はない	未導入	
	アモトサレン最新状況		**＊血小板：申請中**	
中国				
	アモトサレン最新状況		**＊血小板：申請中**	
ｵｰｽﾄﾘｱ				
	アモトサレン最新状況		**＊血小板：１血液ｾﾝﾀｰで臨床中。**	**St Anna, Vienna,**
ｽｴｰﾃﾞﾝ				
	アモトサレン最新状況		**＊血小板：2血液ｾﾝﾀｰで導入済。**	**Uppsala, Boden**
ｱｲﾙﾗﾝﾄﾞ				
	アモトサレン最新状況		**＊血小板：1血液ｾﾝﾀｰで評価中。**	**Dublin**
ｽﾛﾍﾞﾆｱ				
	アモトサレン最新状況		**＊血小板：導入決定。**	**Ljubljana**
ﾁｪｺ				
	アモトサレン最新状況		**＊血小板：選択的導入。**	**Ostrava**
ｸｴｰﾄ				
	アモトサレン最新状況		**＊血小板：100%導入済。**	

1. 感染因子不活化効果

2月27日使用資料に対する追記部分

1）論文報告（各開発メーカー資料）による評価の概要

感染因子＼不活化技術		メチレンブルー	リボフラボン	アモトサレン	アモトサレン最新報告
		血漿	血小板	血小板	血小板
ウィルス	HIV	＞5.5	＞4.4	＞6.0	＞6.0
	HBV	＞4.9	―	＞5.5	＞5.5
	HCV	＞6.2（BVDV）	―	＞4.5	＞4.5
	HPV　B9	＞4.0	―	―	―
	WNV	＞6.5	＞5.1	―	＞5.5
	SARS	―	―	―	＞5.8
	HAV	0	―	―	―
細菌	S.epidermidis	―	＞4.1	＞6.6	＞6.6
	S.aureus	―	＞3.5	＞6.5	＞6.6
	MRSA	―	＞4.9	―	―
	Y. Enterocolitica	―	―	＞5.9	＞5.9
原虫	T.Pallidum	―	―	＞6.8	＞6.8
	Leishjmania	―	＞5.0	＞5.2	＞5.2
	P.falciparum	―	―	＞7.0	＞7.0
	T.cruzi	―	―	＞5.3	＞5.3

― ： データなし

THERAFLEX UV PLATELETS: NOTHING BUT UVC LIGHT AND STRONG AGITATION

H. Mohr[1], U. Gravemann[1], F. Tolksdorf[2], W.H. Walker[2], T.H. Müller[1]

Purpose

Blood donations may not only be contaminated with viruses, e.g. HBV, HCV or HIV. In addition, they may contain bacteria. This is especially crucial for platelet concentrates (PCs), because they have to be stored at room temperature, at which bacteria can multiply to high levels [1-2].

Short-wave ultraviolet light (UVC, wavelength range: 200-280 nm) is germicidal, but low UV-permeability hampers its use for sterilizing PCs. A simple method was developed which overcomes this limitation.

Materials and Methods

Plasma-reduced PCs in storage medium SSP+ (volume approx. 350 mL, platelet concentration approx. 10^9/mL, plasma content 30-35%) were prepared from pools of 5 buffy coats [3]. PC volume was approx. 350 mL. The PCs were spiked with approx. 10^2-10^6 CFU/mL of different bacteria species or up to 10^7 $TCID_{50}$/mL of lipid-enveloped or nonenveloped viruses. Other PCs were spiked with 5×10^6/mL peripheral blood mononuclear cells (PBMC). The PCs were filled into UV-transparent plastic bags and irradiated on a device (Fig.1), equipped with mercury vapour tubes emitting monochromatic UVC-light (wavelength: 254 nm). The device was equipped with an orbital agitator. Irradiation was from both sides of the bags. UVC doses applied were up to 0.6 J/cm² (approx. 90 sec). During treatment the PCs were strongly agitated. Bacteria or virus titers, PBMC viability and platelet parameters were determined before and after irradiation. Each experiment was repeated 3-6 times. Results are depicted as mean ± SD.

Results

Pathogen inactivation was enormously enhanced when the PCs were loosely placed on a quartz plate located between the two layers of UVC tubes of the irradiation device and, in addition, strongly agitated during irradiation (Fig. 2).

UVC-light at 0.3-0.4 J/cm² (irradiation time: approx. 1 min) reduced the titers of all bacteria tested by approx. 5-6 log_{10} steps. PCs spiked with approx. 100 CFU/ml of bacteria were reproducibly sterilized (Tab.1). In one experiment with *B. cereus* the PC was sterile after 3 but unsterile after 6 days storage. This was probably due to spores of *B. cereus* that are more resistant to UVC than vegetative bacteria.

UVC sensitivity of the viruses tested was not so uniform (Table 1): The small single stranded RNA viruses VSV, Sindbis and WNV were completely inactivated at approx. 0.3-0.4 J/cm². Remarkably HIV-1 (also a small single-stranded RNA virus) was only moderately inactivated at UVC doses up to 0.6 J/cm².

The small nonenveloped DNA viruses PPV and EMCV proved to be very sensitive. Complete inactivation was achieved at 0.4-0.5 J/cm².

With the exception of HIV-1, SHV-1 was more resistant than the other viruses tested. This confirms that in general large double stranded DNA viruses are not as susceptible to UVC as smaller single stranded DNA or RNA viruses.

PBMC proved to be extremely sensitive to UVC irradiation: Complete inactivation was found at less than 0.1 J/cm² (Fig. 3)

PC properties remained almost unchanged at doses up to 0.6 J/cm². The storage stability of the treated PCs for up to 6 days after treatment (8 days after blood donation) was maintained (Table 2)

Conclusions

Irradiation with UVC under strong agitation may be used to sterilize platelet concentrates at a light dose that is not harmful to the products. The UVC dose required is 0.4 J/cm². Irradiation time is not more than approx. 1 min.

Parameter	Day 1 after irradiation				Day 6 after irradiation			
	Control	UVC dose (J/cm²)			Control	UVC dose (J/cm²)		
		0.4	0.5	0.6		0.4	0.5	0.6
Pts [x10⁸/mL]	10.8 ± 0.6	10.2 ± 0.6	9.8 ± 0.6	9.1 ± 0.9	10.1 ± 0.8	9.8 ± 0.6	9.3 ± 0.8	9.3 ± 0.9
pH	7.10 ± 0.04	7.04 ± 0.05	7.09 ± 0.05	7.05 ± 0.04	7.27 ± 0.15	7.09 ± 0.06	7.11 ± 0.10	6.98 ± 0.07
Lactate [mmol/L]	7.7 ± 1.0	8.0 ± 0.5	7.7 ± 0.5	8.0 ± 0.7	12.7 ± 1.0	14.9 ± 1.0	14.6 ± 1.4	16.7 ± 1.4
Glucose [mg/dL]	122 ± 9	117 ± 7	117 ± 6	115 ± 7	62 ± 11	43 ± 8	44 ± 11	29 ± 10
Swirling	ok	ok	ok	ok	ok	ok	ok	ok
HSR [%]	69 ± 5	66 ± 2	61 ± 6	62 ± 4	68 ± 2	65 ± 2	62 ± 3	56 ± 5
Collagen-induced aggregation [%]	95 ± 4	90 ± 5	88 ± 3	87 ± 2	62 ± 9	69 ± 8	67 ± 2	69 ± 5
CD62 [%]	36 ± 1	46 ± 3	47 ± 2	49 ± 1	29 ± 1	45 ± 8	50 ± 10	57 ± 8
Annexin V [%]	5 ± 1	6 ± 3	7 ± 4	7 ± 4	9 ± 5	8 ± 2	10 ± 2	12 ± 3

Tab. 3: Treatment of PCs with different UVC doses. Influence on platelet parameters and on storage stability. n=6, mean + SD

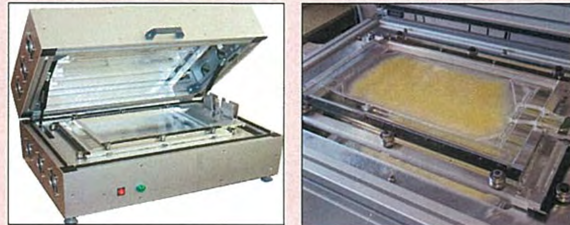


Fig. 1 : Irradiation device for UVC treatment of PCs

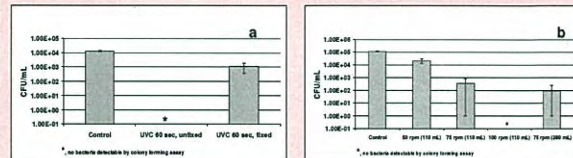


Fig. 2 : Inactivation of *St. epidermidis* in PC aliquots (110 or 280 mL) by irradiation with UV light: fixed vs. loosely placed irradiation bags (a); dependence of bacteria inactivation in loosely placed irradiation bags on the agitation speed (b). n=3, mean ± SD

Bacteria species	Characteristics	Gram stain	Number pf experiments	Spike (CFU/mL)	BacT/Alert result*	Remark
B. cereus	fac. anaerobic	pos	12	100-140	11 sterile 1 unsterile**	Spore former
E. coli	aerobic	neg	12	36-65	12 sterile	
K. pneumoniae	fac. anaerobic	neg	12	85-140	12 sterile	
P. acnes	anaerobic	neg	12	61-100	12 sterile	
S. aureus	fac. anaerobic	pos	22	60-110	22 sterile	
S. epidermidis	fac. anaerobic	pos	22	74-210	22 sterile	
Str. pyogenes	fac. anaerobic	pos	12	118-194	12 sterile	

*: Samples (2x10 mL each) were drawn after 3 and 6 days at 22°C
**: sterile after 3 days storage

Tab 1: Sterilization of PCs spiked with different bacteria species by irradiation with UVC (0.4 J/cm²)

Virus	Genome	Lipid Envelope	Model virus for	Log_{10}reduction factor
Vesicular stomatitis (VSV)	ss* RNA	X	-	≥ 6.41
Sindbis (Sindbis)	ss RNA	X	-	5.55
West Nile (WNV)	ss RNA	X	HCV	5.24
Human immunodeficency (HIV-1)	ss RNA	X	-	1.36
Suid Herpes (SHV-1)	ds** DNA	X	HBV/CMV	3.57
Porcine Parvo (PPV)	ss DNA	-	Parvo B 19	≥ 6.42
Encephalomyocarditis (EMCV)	ss DNA	-	HAV	5.73

Tab 2: Inactivation factors of viruses by irradiation with UVC (0.4 J/cm²)

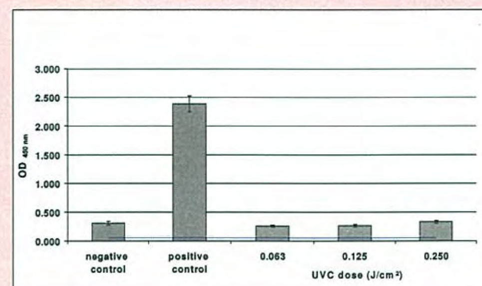


Fig. 3: Inactivation of T-lymphocytes in platelet concentrates by irradiation with UVC. Viability was assayed by mixed lymphocyte culture.

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不活化効果試験のassay methods

Pathogen		abbreviation	assay method (after illumination)	cell or animal used on the assay	initiation date	completion date	study period (days)	report number
virus	Duck Hepatitis B virus	DHBV	in vivo assay (DNA hybridization assay)	Legarth Pekin hybrid duckling	1994/10/3	1995/2/3	124	DEL 001
bacteria	Staphylococcus epidermidis		agar plate assay and liquid broth assay		1994/9/2	1995/2/13	165	DEL 002
bacteria	Klebsiella pneumoniae		agar plate assay and liquid broth assay		1994/9/2	1995/2/13	165	DEL 002
virus	Human Immunodifficiency Virus-1 (Cell associated)	HIV-1	plaque assay	MT-2 cell	1994/9/21	1995/2/18	151	DEL 004
virus	Human Immunodifficiency Virus-1 (Cell Free)	HIV-1	plaque assay	MT-2 cell	1994/9/21	1995/2/18	151	DEL 005
virus	Bovine Viral Diarrhea Virus	BVDV	plaque assay	bovine turbinate (BT) cell	1994/11/28	1995/2/24	89	DEL 006
bacteria	Pseudomonas Aeruginoza		liquid broth assay		1995/2/27	1995/6/30	124	DEL 010
bacteria	Serratia Marcescens		agar plate assay and liquid broth assay		1995/7/21	1995/9/7	49	DEL 011
WBC	T-Cell		limiting dilution analysis (LDA)		1995/8/21	1995/12/7	109	DEL 012
bacteria	Salmonella Choleraeuis		agar plate assay		1995/10/3	1996/1/15	105	DEL 016
virus	Human Cytomegalovirus (Cell associated)	CMV	plaque assay	MRC-5 cell	1996/1/16	1996/5/13	119	DEL 021
bacteria	Staphylococcus Aureus		agar plate assay and liquid broth assay		1995/11/30	1996/4/13	136	DEL 022
bacteria	Escherichia Coli		agar plate assay and liquid broth assay		1996/1/31	1996/4/24	85	DEL 023
bacteria	Yersinia Enterocolitica		agar plate assay		1996/3/15	1996/8/21	160	DEL 025
bacteria	Enterobacter Cloacae		agar plate assay and liquid broth assay		1996/5/6	1996/8/26	113	DEL 031
bacteria	Listeria Monocytogenes		liquid broth assay		1996/7/9	1996/8/8	31	DEL 032
bacteria	Corynebactrium Minutissimum		liquid broth assay		1996/7/9	1996/9/19	73	DEL 033
bacteria	Streptococcus Pyogenes		liquid broth assay		1996/7/12	1996/9/19	70	DEL 042
番外	TA-GVHD			mice	1996/9/5	1997/2/18	167	DEL 049
bacteria	Bacillus Cereus		liquid broth assay		1997/1/31	1997/4/18	78	DEL 061
virus	Bluetongue virus (Cell Free)	BV	plaque assay	bovine turbinate (BT) cell	1998/8/27	1999/10/27	427	DEL 088
virus	Feline Conjunctivitis virus (Calicvirus) (Cell Free)		plaque assay	Crandell Feline Kidney (CrFK) cell	1998/8/31	2000/11/8	801	DEL 089
virus	Simian Adenovirus (Cell Free)	SV15	evaluated for the presence or absence of cytopathic effect with the aid of a microscope	Fetal rhesus kidney (FRhK) cell	1998/11/2	2000/11/17	747	DEL 090
virus	Hepatitis B virus	HBV	in vivio assay (PCR, biopsy etc)	Chimpanzee	1997/9/10	2000/3/16	919	IACUC173
virus	Hepatitis C virus	HCV	in vivio assay (PCR, biopsy etc)	Chimpanzee	1997/9/10	2000/3/16	919	IACUC173
virus	Clinical isolated Human Immunodifficiency Virus-1(Z84 strain)	HIV-1	Mg^{++}-dependent [^{32}P]dTTP reverse transcriptase assay and P24 assay	PBMC	1996/2/16	1996/5/16	91	CP 1472
virus	Clinical isolated Human Immunodeficiency Virus-2 (CBL20 strain)	HIV-2	Mg^{++}-dependent [^{32}P]dTTP reverse transcriptase assay and P24 assay	PBMC	1996/2/22	1996/5/16	85	CP 1473
bacteria	Lactobacillus Species		agar plate assay		2001/12/3	2002/3/29	117	DEL 224
bacteria	Bifidobacterium Adolescentis		agar plate assay		2001/12/5	2002/3/29	115	DEL 225
bacteria	Propionibacterium Acnes		agar plate assay		2002/1/17	2002/5/17	121	DEL-R 00228
bacteria	Clostridium Perfringens		agar plate assay		2002/3/25	2002/10/4	194	DEL-R 00237
parasite	Trypanosoma cruzi	T. cruzi	microscopically monitoring	3T3 cell	2000/10/24	2002/4/25	549	REL 00218
parasite	Plasmodium falciparum		microscopically monitoring (smear with staining)	RBC	2001/5/21	2002/5/6	351	REL 00220
virus	Human T-cell Lymphotropic virus type I	HTLV-I	microscopically monitoring (the staining resulting from tax protein produced as a result of infection)	BHK21pA18G indicator cell line	2000/11/17	2002/4/29	529	REEL 00225-1
virus	Human T-cell Lymphotropic virus type II	HTLV-II	microscopically mongering (the staining resulting from tax protein produced as a result of infection)	BHK21pA18G indicator cell line	2000/11/17	2002/4/29	529	REL 00225-2
spirochete	Treponema pallidum		intradermal infectivity assay (observation), intratesticular infectivity assay (VDRL and FTA-ABS assay)	New Zealand rabbit	2000/8/4	2002/4/26	631	REL-R 217

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Screening platelet concentrates for bacterial contamination: low numbers of bacteria and slow growth in contaminated units mandate an alternative approach to product safety

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Vox Sanguinis

Background and Objectives We introduced 100% screening of platelets for bacterial contamination in 2005 to reduce the risk of clinical sepsis from platelet transfusion. We test all outdating units again at expiry to assess the sensitivity of the initial test.

Materials and Methods We test all platelet concentrates prior to release for clinical use using a large volume automated culture technique on the day after manufacture. All units that expire unused are retested. Platelets still in stock on day 4 of storage may have a repeat culture performed, and are returned to stock with two extra days of shelf life.

Results Of 43 230 platelet units screened, 35 (0·08%) were positive; of 8282 expired unused, 18 (0·22%) were positive; and of 3310 day-4 retests, four (0.12%) were positive. Overall sensitivity of the initial screening test was 29·2% (95% confidence interval 19·4 to 39·1%). Thirteen of the 35 positive screening tests would have been expected to grow in both aerobic and anaerobic bottles; eight grew in aerobic culture only and five grew in anaerobic culture only, indicating that the likely number of bacteria in the contaminated platelet units at the time of sampling was less than 60 colony-forming unit per platelet unit.

Conclusions Screening platelet concentrates for bacterial contamination using the most sensitive method available has a sensitivity of less than 40% because of the low numbers of bacteria in the initial contamination. Effective resolution of this problem will require a pathogen-inactivation technique.

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Introduction

Clinical disease caused by bacterial contamination of platelet concentrates has been observed to occur in between 1 in 358 platelet transfusions [1] and 1 in 73 000 [2]. The observed rate depends on the criteria and method of data collection used, and probably varies with the method of platelet manufacture: whether pooled or not, whether made by the buffy-coat method, from platelet-rich plasma, or by apheresis, and whether an initial diversion pouch is used or not [3]. Observed contamination rates in studies where the final platelet product is routinely tested by culture for bacteria give prevalence rates of culture-positive units from 1 in 5399 [4] to 1 in 125 (P Vandekerckhove, unpublished observations), and vary with the test used, the timing of the sample and the sample volume used. Bacteria that contaminate platelet units are usually derived from the skin of either the donor or the phlebotomist, less commonly from the donor's blood during asymptomatic or symptomatic bacteraemia, and rarely from contamination of the materials used in the collection process.

The natural history of bacterial contamination of blood components is highly variable. It depends on the species and strain of the contaminating bacteria, on the size of the inoculum, the component into which the bacteria are partitioned, and on the state of the recipient. Most contaminating bacteria

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fail to grow, and perish either in the collected components or in the recipient. Some do not, and may cause a severe and sometimes fatal response in the recipient. It is possible that bacteria in a platelet transfusion cause delayed rather than acute problems in the recipient – delayed sepsis, colonization of indwelling lines, or endocarditis are all possible, particularly in immunocompromised patients. However, this has never been systematically investigated, and would be difficult, but not impossible, to verify [5].

Platelets are particularly prone to problems with bacteria, mainly because they are stored at room temperature. The standard practice in the USA where platelets are made from platelet-rich plasma separated from whole blood donations, with limited pooling times, no buffy-coat concentration and no overnight hold may exacerbate the problem, while the European practice of buffy-coat pooling with or without a prolonged hold of the blood at room temperature before removing the white cells seems to reduce it [6–8].

Testing for the presence of bacteria has been widely adopted to try to address the problem [9]. Two general approaches are employed: early testing using a culture method, or late testing using some rapid detection system close to the time of issue and transfusion. Both methods have serious drawbacks. Early testing relies on culture, which is slow, necessitating that the units are released for transfusion before slower-growing bacteria can be detected in the sample; in addition, it is now apparent that the initial inoculum in a contaminated unit is often very low – perhaps as low as 10 bacteria in the unit (or larger numbers present in a few aggregates) – which requires a delay before sampling, or a very large sample, or both, to improve the chance of detection [10]. Late testing is limited by sensitivity problems: the tests generally rely on secondary characteristics of contamination such as acid generation or glucose consumption, or microscopic detection of bacteria in the component. While culture is sensitive to below 10^0 per ml [10], late testing requires 10^3 bacteria or more per ml to be useful [11–13].

These sensitivity problems are exacerbated by a desire on the part of platelet producers to store platelets for longer, to improve supply and to ease logistical problems. It may be that a platelet unit that is tested by an effective culture method near the beginning of its storage time is safer at day 7 of storage than an untested platelet unit close to expiry on day 5, but this remains to be demonstrated, notwithstanding the fact that many platelet manufacturers have made that assumption.

It was not apparent to us that even a sensitive test applied early in the shelf life of a stored platelet unit provided sufficient justification for extended platelet storage to 7 days or more: we had detected a contaminant early in an initial pilot study that consistently could not be detected before day 3 of sampling; we had observed that contaminating *Staphylococcal* species were consistently and predictably reduced in number but not eradicated during component manufacture [7], making subsequent detection even more difficult; and we had calculated that a test capable of detecting bacteria growing from a very low number after manufacture to a serious problem by day 7 could not be detected with sufficient reliability until day 4, even using the most sensitive method available [10]. Our initial experience of retesting expired units that had initially screened negative subsequently confirmed these misgivings [14].

We therefore introduced a dual approach to bacterial screening of platelets: testing by large volume culture on the day after manufacture to improve the safety of platelets stored to day 5, and a retest again using a large volume culture method at day 4 of storage to allow extended storage of platelets over day 5.

Methods

Pilot study

An initial study of buffy-coat platelets was performed to validate the hypothesis that platelets testing negative by BacTAlert (BioMerieux, Durham, NC, USA) using a 10-ml sample on day 2 would not be negative on day 4 and positive on day 7. All platelet units in the study were leucodepleted during the pooling process on day 1 and sampled 14–17 h later using a 10-ml inoculum into standard BacTAlert aerobic culture bottles. Culture was repeated as above on day 4, and again on day 7.

The 134th unit tested in this protocol was positive on day 7 only and was subjected to further analysis in spiking studies to identify growth characteristics of the contaminating bacteria.

Spiking studies

The isolate from unit number 134 was inoculated into platelet concentrates (n = 6) immediately after pooling and leucodepletion on day 2 at a concentration of 10–100 colony-forming unit (CFU) (n = 3) or 1–10 CFU (n = 3); quantitative cultures were performed using the pour-plate method as previously described [7], on days 2, 3, 4, 5, 6 and 7 (1-ml samples). BacTAlert samples were taken on days 4 and 7 postspiking (10-ml aerobic samples).

Routine platelet screening

Apheresis platelets are stored for a minimum of 12 h at 22°C after manufacture before sampling. For pooled buffy-coat platelets, manufacture takes place on the day after collection and following overnight hold of the whole blood unit at 22°C. Sampling for bacterial culture takes place at least 14 h after manufacture is completed, that is, at least 36 h after phlebotomy. A 7·5–10-ml sample is then inoculated into an aerobic BacTAlert bottle and a similar volume into an anaerobic bottle.

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For the first 10 months (15 033 units), an 8-ml inoculum in aerobic culture only was used. We changed to a two-bottle system on the basis of information from the Welsh Blood Service (G Rowe, personal communication). This blood service screens platelet concentrates prior to storage using a large sample divided between aerobic and anaerobic BacTAlert bottles. They found that a significant number of positive tests were detected in one bottle only. This suggested that our single-bottle system was probably missing a significant proportion of bacterially contaminated units. It also indicated that a two-bottle approach would provide the opportunity to quantify the size of the initial contaminating inoculum based on limiting dilution.

All units stored to expiry are retested using a 10-ml aerobic sample and a 10-ml anaerobic sample on BacTAlert. We estimate that this system can detect bacterial contamination at a concentration of 1 CFU/ml of platelet concentrate with greater than 99·5% sensitivity [10]:

- 'Confirmed positive' is a positive result on BacTAlert, a positive subculture from the BacTAlert bottle, and a further culture of the same species from the platelet unit, or, for pools, from the pool or from one other component from the donations used in the pool.
- 'False positive' is a positive signal from the bottle, but no organism detected on subculture, Gram stain and reculture of the unit.
- 'Unconfirmed positive' is a positive subculture or Gram stain from the bottle but no residual material available for culture, or all residual material negative on culture.

All platelets are leucodepleted during the manufacturing process to a residual leucocyte count of $< 1 \times 10^6$/final platelet dose, with leucocyte counting of all units before release, using flow cytometry.

Day 4 retest for extended platelet shelf life

Since November 2005, we have extended the shelf life of apheresis platelets on the basis of a second test at day 4: platelets that are still in inventory on day 4 of storage and that may not be transfused within the following 36 h (i.e. may outdate) have a repeat aerobic and anaerobic culture performed using the same protocol as for the initial test, and are returned to inventory labelled with an extra 2 days of shelf life.

Estimating the number of bacteria in the initial contamination

For most of the period of the study, we used a 15-ml test volume divided into two culture bottles. Most of the organisms detected would be expected to grow with similar ease in both bottles, and the culture system is sensitive down to 1 CFU/inoculum. Therefore, an aerobic organism in a $2 \times 7{\cdot}5$ ml sample that grew in one bottle but not the other had a probable concentration of bacteria in the platelet sample of 1·386, standard error (SE) 1·414, in 15 ml by binomial distribution.

Sensitivity of the screening test

The 'false negative rate' of the initial screening test was taken as the positive rate at outdate. The 'probable total positive rate' was calculated as the sum of the observed positive rate at screening and the 95% confidence interval (CI) of the total number of false negatives based on the observed false negative rate. The sensitivity of the screening test was calculated as the number of observed positives/probable total number of positives (%).

Statistics

Confidence intervals for the observed incidence of bacterial contamination were calculated using the single Poisson data sample module of the StatXact 8 software package (Statistical Software for Exact Nonparametric Inference, Cytel Inc., Cambridge, MA, USA).

Results

Pilot study

One hundred and thirty-four consecutive platelet concentrates made from pooled buffy-coats were tested initially on the day of manufacture, and again at days 4 and 7. All were negative on day 2 and day 4. Platelet pool number 134 was positive on day 7 having been negative on day 2 and day 4. The organism was identified as *Staphylococcus capitis*.

Spiking studies

The organism that grew in the 134th platelet unit in the pilot study was spiked into fresh platelet concentrates: 3 of 3 BacTAlert cultures (10 ml aerobic samples) were positive at days 4 and 7 of storage when a 10–100 CFU inoculum was spiked on day 2; for a 1–10 CFU inoculum, 1 of 3 BacTAlert cultures was positive at day 4, and 3 of 3 BacTAlert cultures were positive at day 7. Quantitative cultures performed using 1-ml samples taken on days 2, 3, 4, 5, 6 and 7 showed no growth in any unit until day 4 in 3 units, day 5 in a fourth unit and day 7 in the remaining 2 units. Exponential growth occurred in all units after the initial positive test (Fig. 1).

Routine platelet screening (Table 1)

In total, 43 230 platelet units have been screened prior to issue; 15 033 were tested using an 8-ml aerobic sample only; 28 197 using a 15-ml sample divided equally into an aerobic

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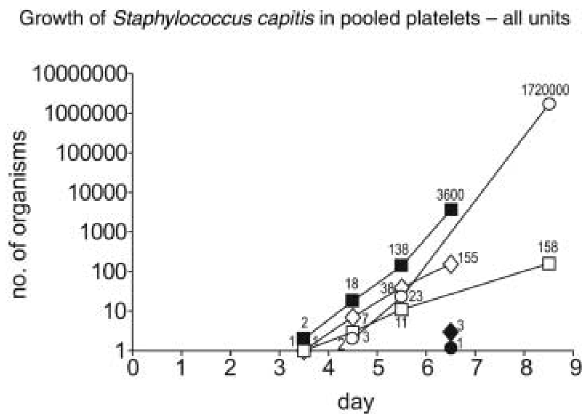

Fig. 1 An isolate of *Staphylococcus capitis* was detected from a pooled platelet concentrate during the initial pilot project. It was not detected on day 1 or day 4 of screening, but the day 7 (postexpiry) sample was positive. The isolate was inoculated at 1–10 CFU/ml (n = 3) and 10–100 CFU/ml (n = 3) into fresh platelet concentrates on day 2 of shelf life. The numbers on the growth curves indicate the concentration of bacteria (CFU/ml) in quantitative cultures from samples taken daily from day 2 onwards.

Table 1 Positive screening tests

	Confirmed positives	Unconfirmed positives	Total true positives
Apheresis platelets (n = 12 823)	4 (0·03%)	8 (0·06%)	12 (0·09%)
Pooled platelets (n = 30 407)	10 (0·03%)	13 (0·04%)	23 (0·08%)
Total platelets (n = 43 230)	14 (0·03%)	21 (0·05%)	35 (0·08%)

and an anaerobic bottle. There were 14 confirmed and 21 unconfirmed positives on the initial test, for a total positive rate of 0·08% (observed proportion = 0·001; 95% CI for the proportion: 0·001–0·001). The rate for the 8-ml sample was 11/15 033 (0·07%; observed proportion = 0·001; 95% CI for the proportion: 0·000–0·001); for the 15-ml sample it was 24/28 197 (0·09%; observed proportion = 0·001; 95% CI for the proportion: 0·001–0·001).

Twelve out of 12 823 (0·09%) were from apheresis collections; 23/30 407 (0·08%) were from pools of four buffy-coats after 22°C overnight hold of the whole blood units.

Of 43 230 platelet units, 8282 expired unused and were retested: there were 18 positives – 7 confirmed and 11 unconfirmed – for a total positive rate of 0·22% at expiry (observed proportion = 0·002; 95% CI for the proportion: 0·001–0·003).

Day 4 retest for extended platelet shelf life

Four out of 3310 (0·12%) day 4 retests for extending shelf life were positive (1 confirmed, 3 unconfirmed; observed proportion = 0·001; 95% CI for the proportion: 0·000–0·003).

Estimating the number of bacteria in the initial contaminating inoculum

Twenty-four of the 35 positive day 1/day 2 tests (confirmed and unconfirmed) were identified after the addition of the anaerobic sample to the screening test. Eleven of these were *Propionibacterium* spp. or strict anaerobes; the remaining 13 would have been expected to grow in both bottles. However, none grew in both aerobic and anaerobic bottles: eight grew in aerobic culture only and five grew in anaerobic culture only. In contrast, 12 of 18 isolates from expired units would have been expected to grow in both aerobic and anaerobic cultures: six did so; three grew in aerobic and three in anaerobic bottles only. This indicates that the mean number of bacteria in the contaminated platelet units at the time of sampling for screening was 1·386 (SE: 1·414) CFU/test volume, that is, less than 60 CFU/platelet unit in most instances [mean platelet volumes in our facility are 333 ml (SD 14 ml) for pooled platelets and 244 ml (SD 4 ml) for double apheresis units].

Sensitivity of the screening test

The false negative rate of the initial screening test was taken as the positive rate (confirmed and unconfirmed) at outdate, that is, 18/8282 (0·22%). (We considered the rate at day 4 retesting to be another, comparable estimate of the false negative rate, and not an additional component of it. The later, larger sample was considered the more accurate measurement.) The sensitivity of the screening test was calculated as the number of observed (confirmed and unconfirmed) positives/probable total number of positives (%). That is (number of positives at screening) divided by [the number of positives at outdate (95% CI) × (number of platelet units tested at screening/number of platelets tested at outdate) + number of positives at screening] %; or [35/(89·4 +35)]% to [35/(180·9 +35)] %, which gives a 95% CI of 19·35 to 39·1% for the sensitivity of the screening test.

The 57 isolates detected at screening, retesting on day 4 and at outdate are shown in Table 2. There were 20 isolates of coagulase-negative *Staphylococci*; 18 of *Propionibacterium acnes*; three each of anaerobic *Streptococcus* spp., *Bacillus* spp., and *Corynebacterium* spp.; two each of *Micrococcus* spp. and *Streptococcus* spp.; one each of *Staphylococcus aureus*, *Proteus mirabilis, Acinetobacter baumanii, Bacteroides thetaiotamicron, Leuconostoc* spec., and *Brevibacterium* spec. The interval, in days, between inoculation of the sample into the culture bottle and the positive signal from the BacTAlert device for the 57 isolates is given in Table 2.

Twelve of the contaminated platelet units were transfused before the culture signalled positive. All patients were followed up, and none had had either an acute or delayed clinical event that could be attributed to the transfusion of contaminated

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Table 2 The time, in days, between inoculation of the sample into the culture bottle and the positive signal from the BacTAlert device for bacteria isolated from platelet concentrates

Bacteria	Number of isolates (n = 57)	Detected on day 1 screening (n = 35) (time, in days, in culture bottle before detection)	Detected at day 4 retest (n = 4) (time, in days, in culture bottle before detection)	Detected at outdate (n = 18) (time, in days, in culture bottle before detection)
Coagulase-negative *Staphylococci*	20	13 (range: 0·66 to 5·8)	1 (0·75)	6 (range: 0·13 to 1·11)
Propionibacterium acnes	18	10 (range: 4·39 to 5·94)	2 (3·68, 3·51)	6 (range 4·3 to 6·57)
Anaerobic *Streptococcal* spp.	3	2 (2·05, 2·93)		1 (2·1)
Bacillus spp.	3	2 (0·88, 2.33)		1 (0·6)
Corynebacterium spp.	3	1 (1·43)		2 (4·0, 4·2)
Micrococcus spp.	2	2 (2·89, 3/03)		
Streptococcus spp.	2	2 (0·39, 0·44)		
Staphylococcus aureus	1	(3·78)		
Leuconostoc spec.	1			(0·25)
Bacteroides thetaiotamicron	1	(2·16)		
Acinetobacter baumanii	1			(0·24)
Proteus mirabilis	1	(0·85)		
Brevibacterium spec.	1		(4·8)	

platelets. The organisms cultured from these 12 platelet units were *P. acnes* (n = 6), coagulase-negative *Staphylococci* (n = 3), *Corynebacterium* spec., *P. mirabilis*, *Brevibacterium* spec. The *P. mirabilis* isolate was confirmed on both of the two non-transfused parts of a triple apheresis donation. No adverse reactions due to bacterial contamination of platelets were reported for the platelets tested in this period, in spite of an active, comprehensive, nationwide haemovigilance programme [15].

Discussion

Testing platelets for bacterial contamination has been widely adopted by blood transfusion services to try to prevent sepsis caused by transfusing such units. Bacterial contamination of blood components is inevitable from time to time – it is not possible to render the venepuncture site sterile, and in addition occasional asymptomatic bacteraemia in blood donors will result in septic collections. Cooling of red cell units before transfusion reduces the incidence of sepsis from transfusions of red cell units to a very low level. Sepsis in red cell transfusions is usually caused by psychrophilic bacteria such as *Yersinia enterocolitica*, *Klebsiella pneumoniae* or *Pseudomonas* spp. Because platelets are stored at 22°C many other species of bacteria that enter the blood collection may thrive and cause serious clinical adverse events in the recipient of the unit. Testing at day 1 or 2 of shelf life will detect many, possibly most, but not all [4] serious infections, particularly those from blood-borne bacteria that are not in lag phase, and other organisms that will grow rapidly during early storage. The longer the delay between phlebotomy and testing, the more likely the test will detect a growing, and therefore clinically relevant, contaminant. Larger volume sampling will also increase the detection capability of a test. It is difficult to determine the true rate of contamination, or to compare the rates between centres that vary in timing of sample, sample volume and detection method. Rates of approximately 1 in 5000 quoted for American studies of apheresis platelets [4] reflect the timing and the sample volume used; rates of up to 0·8% from Belgium (P Vandekerckhove, unpublished observations) may similarly reflect timing and sample size differences; the overall rate of 0·1–0·3% that we observed by combining the outdate rate with the initial screening rate after manufacture probably reflects the true rate of contamination with organisms that survive initial conditions in platelets in our system, which includes isopropyl alcohol and 0·5% chlorhexidine skin preparation, diversion of the first 40 ml collected, leucodepletion, and, for pooled platelets, overnight hold of the whole blood unit at 22°C, and buffy-coat pooling prior to leucodepletion.

Screening platelets for bacteria prior to issue, using relatively large sample volumes to increase detection rates, has a mean sensitivity in our hands of 33%. This limits the overall benefit of testing prior to release, and calls into question whether platelet shelf life can be extended to 7 days based on an initial screening test.

It is one thing to test platelets for bacteria in an attempt to reduce the incidence and severity of a serious and relatively frequent complication of platelet therapy; it is quite another to compromise the safety of patients by extending platelet

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shelf life on the basis of a test that is ineffective at detecting low-grade contamination that may have the potential to cause clinically relevant adverse events after prolonged storage. We could not convince ourselves that prolonged platelet storage could be justified on the basis of a sensitive test for bacteria test earlier than day 4, based on theoretical calculations [14], observations of the recovery of spiked *Staphylococci* over the manufacturing process [10] and the early detection of an isolate in a pilot study that repeatedly escaped detection by testing before day 4.

Using a day 4 retest prior to extending platelet shelf life is a practicable approach in our hands to the problem of low initial sensitivity of bacterial screening. Day 4 testing is relatively straightforward, and need only be performed on platelet units that are likely to expire on day 5, and whose preservation makes logistical sense based on current inventory, so that there is high return on the costs involved. Because the platelets are labelled for 7-day storage before they outdate on day 5, computer safety blocks on allowing outdated products back into inventory are avoided. The repeat sample from the platelet unit will have a 36-h minimum incubation period in the culture system before the original day 5 expiry is reached. This can be expected to detect all contaminating bacteria in the life-extended platelets, with the probable exception of *P. acnes*. *Propionibacterium acnes* is not reliably detected before transfusion by any available testing algorithm. There are no clinical reports of adverse events from the tens of thousands of *P. acnes*-contaminated platelets that have undoubtedly been transfused. However, *P. acnes* is not wholly benign [16,17], and in addition is a sentinel marker of persistent problems with bacterial testing of platelets.

A detection system with a quicker read-out and a lower sensitivity could be used at day 5 instead of the BacTAlert system at day 4 with comparable safety profile, and is worthy of further study. Nevertheless, the low utility of bacterial testing in eliminating the problem of contamination in platelet units, even using the large sample volumes that we do (which in turn indicates a high comparative sensitivity of our testing system), indicates that a pathogen-inactivation system may be needed to solve the problem.

In the studies reported here, apheresis platelets are not significantly more likely to be contaminated after collection than platelets manufactured from pooled buffy-coats made from whole blood units stored overnight at 22°C, in spite of the fewer numbers of venepunctures involved in a plateletpheresis collection. This is compelling evidence for a protective effect from overnight storage of buffy-coats at 22°C prior to platelet manufacture, as has been previous reported by ourselves and others [6–8]. It is in marked contrast to the observations by investigators who have used platelets manufactured by the platelet-rich plasma method [2], but consistent with the observations from the Serious Hazards of Transfusion programme in the UK [18] where, although the numbers are necessarily small, severe reactions attributed to apheresis platelets are over-represented compared to the relatively more frequently transfused pooled buffy-coat-derived platelets, even though the UK does not include the step of overnight warm hold of whole blood prior to making the platelets.

Twelve of the 35 contaminated platelet units were transfused. This reduced the practical effectiveness of the screening programme by a further 34%. We have not observed any cases of bacterial sepsis ascribed to platelet transfusions since the introduction of this programme, in spite of a comprehensive national haemovigilance programme [15]; this suggests that bacteria escaping detection in this system are of limited clinical significance. However, the numbers are relatively small and the natural history of low-grade contamination in platelet transfusion has not been examined in detail, especially in immunocompromised patients or in patients with indwelling lines. In addition, Eder *et al.* [4] have demonstrated, in a study of over 1 million platelet units, that contaminated platelet units escaping detection by their protocol, using a similar approach to the one reported here, could cause severe septic complications in patients, including death.

These studies indicate that the initial contamination levels in platelet concentrates are extremely low – even after a minimum of 12 h following venepuncture or leucodepletion most bacteria detected were at a concentration in the order of 1 CFU per 15 ml, that is, less than 60 CFU/platelet unit. This is consistent with earlier laboratory studies [7,10] and indicates that the sensitivity required to detect all contaminating bacteria cannot be achieved by any sampling protocol unless almost the entire unit is sampled. It will not be possible to provide complete protection from bacterial growth in platelet units by any screening test currently available. Photochemical treatment of platelets after manufacture using a sufficiently effective method will be necessary to achieve this. Such an approach will provide the added benefit of rendering all platelet units cytomegalovirus-safe regardless of serological status, graft-versus-host disease-safe without irradiation, and will also provide protection from emerging viruses, of which there are many [19].

It is generally accepted that bacterial contamination of platelets for transfusion is a serious problem requiring effective preventative action. Possible approaches include bacterial detection at the manufacturing centre (before the product is released for clinical use), bacterial screening at the clinic, shortly before the product is transfused, or treatment of the product during the manufacturing process to remove contaminating bacteria. Each of these approaches (or any combination of these) has limitations: poor sensitivity of early screening methods, and to a lesser extent limited sensitivity of near-patient tests for slow-growing, low-concentration organisms; safety and cost concerns for photochemical or other eradication methods. Platelets constitute a large-volume intravenous medicine, often given to immunocompromised

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patients, and often to patients with indwelling lines that form a prime site for colonization. It is unthinkable that a manufacturer of other intravenous medications could eschew reasonable methods to eradicate possible contamination on the basis that only organisms of questionable clinical significance persisted in the preparations infused. It is also unthinkable that end users of intravenous agents would be asked to check sterility before use, provided a reasonable alternative was available to the manufacturer. It is apparent to us that bacterial testing, whether early or late, lacks sufficient robustness of design [20] to persist as the method of choice once a method of eradication of adequate proven safety and utility is available.

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調査会合同委員会提出資料

文献4,5

資料2

委員からの質問に対する各社からの回答

○不活化技術に関するヒアリング概要

社名	マコファルマ社 株式会社アムコ	BCT Japan株式会社 （旧 ガンブロ株式会社）	バイオワン株式会社 シーラス社	（参考） シーラス社
①不活化法の種類	メチレンブルー	リボフラビン	アモトサレン（S-59）	S303
②化合物添加	要	要	要	要
③光照射	要	要	要	不要
④薬剤除去工程	要	不要	要	不要
⑤適応製剤	FFP（実用化済み）	PC （2007年実用化済み） FFP （2008年7-9月予定）	PC（実用化済み） FFP（実用化済み）	RBC （2008年5月時点で第Ⅰ相試験まで終了）
⑥不活化効果	**ウイルス**：可（HIV-1, WNV, BVDV, Influenza, Duck HBV, 等） **細菌**：不可	**ウイルス**： HIV, active 5.9,latent 4.5, HCVmodel V. 3.2, HBV 2.0-3.0, WNV 5.2, HumanB19model v .> 5.0 **細菌**： S.aureus 3.6-4.8, S.epidermidis 4.2, E.coli > 4.4, B.cereus 1.9-2.7, P. aeruginosa 4.5Parasites測定限界値まで（>3 to>6） 生体外検定法（TCID 50)による。 単位：HBV除きログ/ml, HBVはgEq/mL	**ウイルス**：HIV-1>6.2, HBV>5.5, HCV>4.5, WNV>5.5, SARS HCoV>5.8 ParvovirusB19 3.5->5.0, HTLV,CMV等可 （単位ログ/mL） **細菌**：S.epidermidis>6.6, S.aureus>6.6, E.coli>6.4, S.pyogenes>6.8, B.cereus >5.5, P.aeruginosa 4.5等, **白血球**>5.3, **原虫等** T.cruzi>5.3	**ウイルス**：HIV、ウシのウイルス性下痢ウイルス、アデノウイルス, 水泡性口内炎 **細菌**：黄色ブドウ球菌、表皮ブドウ球菌、エルシニア・エンテロコリチカ、セラチア・マルセセンス、大腸菌
⑦製剤への影響				
1）容量変化	処理する血漿製剤から約15ml減少（キット内及びキットに含まれるフィルター2個に残留するボリュームとして）	血小板製剤250mLに対し35mL（500μM)のリボフラビン溶液を加える。従って35mLの容量増加となる。	**血小板**：血漿65%を保存液インターソルで置換した血小板溶液約300mLにS-59溶液15mLを加えるが、処理ロス約7%があるので約5mL減少する。 **血漿**：血漿(385-635mL)にS-59溶液15mLを加えるが、処理ロス約7%あるので約20mL減少する。	
2）凝固因子活性低下	Fibrinogen=-14.3%/ FII=-3.4%/ FV=-21.4%/ FVII=-4.2%/FVIII=-15.4%/ FIX=-7.4%/FX=-13.3%/ FXI=-16.2%/ FXII=-11.8%/FXIII= -2.8%/vWF=-0.7%	血小板の凝固機能：5日保存でｐ－selectinは通常の輸血時同様程度の若干の上昇（17.9±7.0→57.8%±14.8）。GP1bα、GP11b－11a,GpⅣ,vWF等処理前と比較して機能性異常なし。FFP：52週保存で凝固因子Ⅷc活性0.8±0.1IU/mL,Fibrinogen活性。69週保存211±33.5mg/dl等すべて欧州ガイドラインを満たす。（資料MirasoPRTシステム参照）	**血漿**：Fibrinogen=-21%/ FII=-11%/ FV=-5%/ FVII=-18%/FVIII=-23%/ FIX=-11%/FX=-11%/ FXI=-10%/ **血小板**：**In vitro**:5日保存 pH 6.8(6.94), HSR 58.8% (58.5), Pセレクチン 51.7%(31.0), pO2 24.2mmHg(29.9), 乳酸 11.3mM(10.5) **In vivo**:輸血後のウサギ耳出血時間 259秒(274) ( )内は未処理	
3）血小板回収率		98% 程度（この処理により約2%の血小板回収率低下が想定される。	92-93%（容量減少分を含む）	（赤血球）24時間回収率＞75%
⑧安全性について	すでに臨床で広く使用されており、メチレンブルー処理された血漿製剤輸血の臨床例は累計400万件以上に上るが副作用報告は無い。現在同社製品は年間30万例以上の臨床使用が続けられている。	リボフラビンは生理物質(ビタミンB2)で、FDAにより食品着色用に安全物質（GRAS)として認知されている。輸血用血液に新たな化学合成物質が加えられることはなく、広範な生体内外の毒性試験で毒性および変異性がないことは実証されている。	ICHガイドラインに沿って全ての毒性試験を実施し安全性はEU,FDAより認められている。一般毒性は臨床量の100倍遺伝毒性は1万倍の量で安全である。臨床試験、10万例以上の臨床使用で問題は報告されていない。	安全性の実証：発がん性 遺伝毒性 安全性薬理試験 生殖毒性 操作安全性

平成20年5月23日
血液事業部会運営委員会･安全技術調査会合同委員会提出資料

資料2

委員からの質問に対する各社からの回答

○ 平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会　合同委員会
質問事項等まとめ・・・2

・　マコファルマ社回答・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・5

・　BCT Japan 株式会社回答・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・6
（(4)の質問について、4 月 8 日合同委員会後の追加説明あり）

・　バイオワン株式会社回答・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・16

○ 平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会　合同委員会後
追加質問事項1（バイオワン社のみ）・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・27

・　バイオワン株式会社回答・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・28

○ 平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会　合同委員会後
追加質問事項2・・・31

・　マコファルマ社回答・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・32
・（参考）マコファルマ社回答（英語）・・・・・・・・・・・・・・・・・・・・・・・・・34

・　BCT Japan 株式会社回答・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・37
・（参考）BCT Japan 株式会社回答（英語）、参考資料（文献2報）・・・・・・40

・　バイオワン株式会社回答・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・57

平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会合同委員会　質問事項等まとめ

1．質問事項及び議論に必要なデータ等（全ての技術に対する質問）

○不活化剤の体内動態、安全性について

（１）　不活化剤の代謝（薬剤の排泄経路）。反復投与した場合の排泄、分解、蓄積の詳細。腎臓あるいは肝障害患者の場合の配慮。代謝（排泄完了）に要する時間。

（２）　不活化剤の体内分布、特にレンズ等への蓄積など。不活化処理血液製剤を大量に使った場合の安全性は。

（３）　不活化剤を除去した後、一定濃度以下であれば安全という科学的根拠は。

（４）　他の薬剤との反応と、その問題点。

（５）　（薬剤としての）不活化剤の用法、用量、使用上の注意など（取り扱い説明書）

（６）　reduction ratio が主で、安全性試験の情報がない。

○不活化効果について

（７）　不活化効果（log をどの様にして求めたのか：使用したモデルウイルス・培養細胞など）

（８）　（3剤）不活化強度の比較。

（９）　不活化効果の評価法の妥当性（モデルウイルスのデータのみでよいか）。

（１０）　不活化の対象病原体。ウイルス（HBV、HCV、HIV、その他未知のウイルスも含む？細菌？原虫？）

（１１）　不活化法の導入で、現在の輸血用血液製剤の感染リスクのそれぞれがどれだけ減少できると考えられるか？（それぞれの病原体に対する推定値とその根拠）また、外国の導入例において、どのような輸血後感染リスクがどれだけ減少したかを比較検証（あるいは推測）できるデータがあればその提示もお願いしたい。

○不活化剤除去について

（１２）　（除去後の）不活化剤の製剤中の残存率は。不活化剤を添加した後の回収をどのように計算しているのか。

（１３）　（除去に伴う）製剤自体の減損率

（１４）　（除去に伴う）製剤の生物活性の低下率

○その他（日本、海外の状況等）

（１５）　国内、国外における治験研究の現状は。

（１６）　海外で治験（トライアル）ではなく、正式に導入している実績はどれほどか。

（１７）　危機管理体制確保の一環としての基礎的研究（治験研究）チーム設定の可能性は。

（１８）　不活化技術により現在の感染症検査のうち、何を省略できるのか。できないとしたら現在のわが国のような感染症検査が行われている国では屋上屋を架すこととならないか。また、海外で省略できている項目はあるのか。

（１９）　コストベネフィットの問題だけでなく、日本では放射線照射をしているが、不活化との相乗で製剤が使い物にならないのではないか？その治験を行えるのか。ＨＬＡの近い日本人で輸血後ＧＶＨＤ予防のために照射をやめられるか。

（２０）　薬物添加以外の方法による不活化法の検討は。γ線照射による細菌滅菌の現状は。

2．質問事項及び議論に必要なデータ等（各技術に対する質問）

1. MB

1) 処理後 C5a が 3 倍に増加している。これは処理によって補体が活性化した結果であり、血管透過性の亢進などアレルギー反応が増加する可能性がある。また、大量に投与することがあるので、投与可能な血漿量の検討はされているのか？
2) MB と結合する血漿タンパクはないのか？もし、血漿タンパクのなかで結合する因子があった場合、体内での沈着部位は確認されているのでしょうか？例えば、皮膚などにMBと結合したタンパクが存在した場合、日光によって再度、1 重項酸素が産生され、組織が破壊される可能性はないのか？
3) MBと結合した血漿タンパクが処理によって発生する1 重項酸素のために構造等が変化してネオアンチゲンとなって、血漿タンパクに対する自己抗体が産生されないのか？特に、自己免疫疾患やアトピーの患者に投与する場合は自己抗体が作られやすくなる可能性はないのか？
4) 導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由（例えば、処理能力以上にウイルス量が多かった等）も示していただきたい。
5) 今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

2 アモトサレン

6) 各種毒性試験の具体的な記載がなく、例えば、単回投与での毒性なし、といっても何頭使用して、どういう毒性を検討したのか全く不明。他の毒性試験も同様である。具体的な記載と、毒性の専門家の意見を聞きたい。
7) 不活化処理後のアモトサレン除去によっても 2mg のアモトサレンが血漿と結合して残存する。血漿タンパクと結合したアモトサレンはどのように代謝されるのか？

また、体内に結合したまま沈着する可能性を検討したことがあるか？例えば、皮膚などに MB と結合したタンパクが存在した場合、日光によって組織が破壊される可能性はないのか？

8) アモトサレンと血漿タンパクが結合して構造等が変化してネオアンチゲンとならないか？そのため、血漿タンパクに対する自己抗体が産生されないのか？特に、自己免疫疾患やアトピーの患者に投与する場合は自己抗体が作られやすくなる可能性はないのか？反復投与後の副作用として自己抗体産生の症例はないのか？

9) 導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由（例えば、処理能力以上にウイルス量が多かった等）も示していただきたい。

10) 今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

11) 血小板において 2mg のアモトサレンが血漿と結合して残存するが、新鮮凍結血漿ではさらに多くのアモトサレンが血漿に結合して残存する可能性がある。血漿のデータがないので用意していただきたい。

3.リボフラビン

12) 結合する血漿タンパクはないのか？もし、血漿タンパクのなかで結合する因子があった場合、体内での沈着部位は確認されているのか。例えば、皮膚･眼球などに結合したタンパクが存在した場合、日光によって再度、活性化され組織が破壊される可能性はないのか？

13) 導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由（例えば、処理能力以上にウイルス量が多かった等）も示していただきたい。

14) 今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

３．主な意見

（１）　議論の時間が『時間切れ』になることは好ましくなく、時間を十分確保して行うべき。

（２）　毒性学の専門家の意見を聞きたい。テルモ等、機器の滅菌を行っている人達は、病原性因子の不活化について（γ線照射など）むしろ医学関係者よりも詳しい。いずれ不活化の毒性の検討が必要。

（３）　導入にあたっては、日赤などで不活化をきちんと評価する体制が必要。

（４）　危機管理体制として不活化技術を利用するというのが現在の最も有効な利用対象ではないか。

マコファルマ社回答

回答は非公開。

BCT Japan 株式会社回答

平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会合同委員会
不活化技術を有する事業者に対する質問事項等まとめ（配布用）

1．質問事項及び議論に必要なデータ等（全ての技術に対する質問）

<u>〇不活化剤の体内動態、安全性について</u>

Mirasol PRT 製品の安全性および毒性プロファイルに関する詳細は、“MIRASOL Monograph”，第三章「MIRASOL 処置の安全性プロファイル」にその大半が記載されている。

（１）　不活化剤の代謝（薬剤の排泄経路）。反復投与した場合の排泄、分解、蓄積の詳細。腎臓あるいは肝障害患者の場合の配慮。代謝（排泄完了）に要する時間。

Mirasol PRT System は、軽質の合成リボフラビン（ビタミン B2）を使用して、ドナーから採取した血小板内の病原体数を低減させる。リボフラビンを濃縮血小板に補助的な方法で使用し、混入病原体数を減らすことで、リボフラビンそのものの効果が人体外で発揮される。なお、リボフラビンには人体への代謝的もしくは生物学的作用が皆無のため、本システムにおいてリボフラビンが医薬品として作用することはない。

リボフラビンの薬物動態調査では､血漿からのリボフラビンの除去は主として尿中排泄によって行われるが、これによるリボフラビンの半減時間はおよそ 9.9 時間であると報告されている。また、病因や治療方法がさまざまに異なる肝硬変患者間の追加調査では、リボフラビンの代謝回転数になんら変動は見られなかった。一方、光分解リボフラビンの消失半減観測時間は、対人体の約 3.8 倍の Mirasol 処置血漿を投与したラットの実験において、約 55 時間であると判定された。

亜慢性毒性試験は、対人体と同量の Mirasol 処置血漿を週 6 日、13 週間投与したビーグル犬を用いて行われ、その結果、被験犬のいずれにも被験物質毒性が認められなかった。実験群と対照群からそれぞれ採取した肝臓を顕微鏡で観察したところ、後者群の肝臓にやや強めの炎症が認められが、それ以外に特筆すべき点は何もなかった。

（２） 不活化剤の体内分布、特にレンズ等への蓄積など。不活化処理血液製剤を大量に使った場合の安全性は。

リボフラビンの LD_{50}（50％致死量）を判定するための調査がいくつか実施済みで、これらは“Reddy ら”に要約されている。LD_{50} は、一部の調査で判定できたが、その他においては、最大 10,000vmg/kg と、相当量のリボフラビンを投与しても判定が不可能であった。Mirasol 処置血小板／血漿（輸液単位）の曝露レベル値 0.077mg/kg をマウスの静脈リボフラビンの LD_{50} 報告値 50 – 100 mg/kg と比較した場合、少なくとも 649 (50/0,077)という安全係数が得られる。現時点では、リボフラビンの最大無有害性影響量は明らかではないが、かといって、MIRASOL 処置血液成分の輸血におけるリボフラビンおよびその光分解生成物への曝露レベルが有毒に作用する、との予見にはつながらない。

対 MIRASOL 処置血液成分曝露を最大可能レベルで繰り返し行ったが、主な生理機能になんら悪影響はなく、また標的臓器の毒性も検出されなかった。

Navigant による亜慢性毒性生体試験では、ビーグル犬に Mirasol 処置血漿を週 6 日 13 週間連続して投与したわけだが、検眼テストにおいても何ら異常は認められなかった。

（３） 不活化剤を除去した後、一定濃度以下であれば安全という科学的根拠は。

リボフラビンおよびその光分解生成物は、いずれも人体内で自然発生するものであり、除去する必要はない。

（４） 他の薬剤との反応と、その問題点。

テトラサイクリンとトリメトプリム・スルファメトキサゾール配合剤（抗生物質）：リボフラビンはこれらの吸収／効果を阻害するため、同時摂取してはならない。

クロロキンおよびメフロキン：リボフラビンは、これら抗マラリア医薬治療剤の効果を低減する可能性がある。

（4 月 8 日合同委員会における質問に対する追加説明）
Updated response to Question (4) from initial Q&A document:

（4）他の薬剤との相互作用とその問題について
Mirasol PRT 処理の使用に関連して、懸念を惹起するような薬物相互作用が一切ないと結論づける in-vivo の最終報告はありません。
当初、厚生労働省に提供させていただいた Tetracycline（テトラサイクリン）及びトリメトプリム・スルファメトキサゾール配合剤（Trimethoprim －sulfamethoxazole）とリボフラビン（Riboflavin）の相互作用の可能性に関する情報は、主に in-vitro 研究文献報告にもとづくものでした。それらの研究ではリボフラビン溶液が若干の潜在的影響を示唆するものでした。

とりわけ Mirasol アプリケーションに特有なこの件に関する詳細報告は、弊社とは一切関係のない毒性研究の専門家により現在準備されており、ご請求によりいつでも入手可能となります。

（５）（薬剤としての）不活化剤の用法、用量、使用上の注意など（取り扱い説明書）

リボフラビン（ビタミン B2)は 13 種の必須ビタミンのひとつで、栄養補助食品として幅広く用いられ、この認可着色剤は薬物とはみなされない。リボフラビンは、「欧州委員会科学評議会」でも認められており、またアメリカの「食品医療品局」では、“一般に安全と認められる食品”に分類されている。リボフラビンの推奨日常摂取量は、平均的成人男子で最大約 1,3 mg、同女性で最大 1.1mg、授乳中の女性で最大 1.6mg である。また、リボフラビン欠乏症のための推奨治療量は、成人で 1 日≤ 30 mg である。なお、リボフラビンの安全性は、経口・皮下・腹腔・静脈等の投与経路で実証済みである。

マルチビタミン投与の一環であるリボフラビン補給は、早産児の経静脈栄養摂取法とよく併用される。この患者集団のリボフラビンの投与レベルは次のとおりで、一般的に日常推奨摂取量（0.15 ～ 0.2 mg/kg）を超過しているが、リボフラビンによる副作用は報告されていない：0. 62 mg/kg、0.66 mg/kg、0.43～0.72 mg/kg

（６） Reduction ratio が主で、安全性試験の情報がない。

以下は、下記出版物からの抜粋である：
“Toxicity Testing of a Novel Riboflavin-Based Technology for Pathogen Reduction and White Blood Cell Inactivation; Reddy, H. et al. (2008) *Transfusion Medicine Reviews*, 22(2): 133-153

人体におけるリボフラビンおよびその光分解生成物への可能曝露レベルは0.077 mg/kg（ただし輸液単位）である。臨床条件下において受血者へのリボフラビンおよび光分解生成物への可能曝露値は、平均体重70kg、公称リボフラビン溶液濃度500 μmol/L、平均リボフラビン光変換率18%であり、公称リボフラビン溶液量35mLを前提として計算した。この曝露レベルは、生物集団の50%が生存した致死量報告地と比較するに値し、具体的には次のとおりである：マウスにリボフラビン（50-100 mg/kg）[30]を点滴投与、最低安全係数50/0.077 = 649

ただし、この安全係数計算は、人体内でのリボフラビンの中毒量について、臨床資料やしかるべき動物実験の確かなデータが存在しないため、最大無有害性影響量がいまだ不明であり、いくぶん疑似的である。
明らかに高いレベルの曝露が血小板または血漿輸血により起こらない限り、MirasolPRT処置製剤中のリボフラビン及び光生成物の非経口曝露による人体への有毒性を予見すべき根拠は何もない。

同様の理由により、血小板にMirasol PRT処理を施した後の曝露値とリボフラビンの一日の摂取許容量(ADI) 0 ～0.54 mg × kg-1 × d-1を比較した場合もまた誤解を招きかねない；ADIは生涯的かつ日常的な食物中物質への曝露値を示すものであり、仮にADI摂取を週に数回、数ヶ月間続けたとしても、短期的経口曝露の適正な比較子にはならない。

生理的必須物質にとっては正常といえない状況下、リボフラビンとその光分解生成物の生体内における毒性試験値は低い、という毒物学者の最適アドバイスがある。
Mirasol PRTシステムは、広範囲に及ぶ臨床前評価プログラムによって評価が行われており、人体における安全プロファイルを取り扱った本評価試験の結果に加え、リボフラビンとその光分解生成物の履歴資料も提供できる状況にある。本評価試験で取得・報告されたデータは、資料中のデータと整合性がとれており、かつ、輸血時の本システムの使用による毒性リスクは低いとされている。

○不活化効果について

（７） 不活化効果（log をどの様にして求めたのか：使用したモデルウイルス・培養細胞など）

“MIRASOL Monograph”第四章を参照のこと。

（８） （3剤）不活化強度の比較。

“MIRASOL Monograph”第四章を参照のこと。

（９）　不活化効果の評価法の妥当性（モデルウイルスのデータのみでよいか）。

“MIRASOL Monograph”第四章を参照のこと。

（１０）　不活化の対象病原体。ウイルス（HBV、HCV、HIV、その他未知のウイルスも含む？細菌？原虫？）

“MIRASOL Monograph”第四章および第五章を参照のこと。

（１１）　不活化法の導入で、現在の輸血用血液製剤の感染リスクのそれぞれがどれだけ減少できると考えられるか？（それぞれの病原体に対する推定値とその根拠）また、外国の導入例において、どのような輸血後感染リスクがどれだけ減少したかを比較検証（あるいは推測）できるデータがあればその提示もお願いしたい。

本件について、Mirasol 製品関連データはいまだ取得されていない。現時点で取得している限定情報をもとにモデル化を行うことはできない。

〇不活化剤除去について

（１２）　（除去後の）不活化剤の製剤中の残存率は。不活化剤を添加した後の回収をどのように計算しているのか。

MIRASOL システムでは、リボフラビンおよびその光分解生成物の残渣を除去する必要はない。本システムで使用する光感作化学物質は、全光分解生成物と共に人血中で認識されており、除去手段を必要としない。実際にリスクを解析した結果、本システムで化合物除去作業を行うと対患者リスク率が増加するであろうということがわかった。（当然のことだが、本除去作業は、未知もしくは被疑毒性プロファイルをもつ合成化合物支援システムにおいては適切なリスクの軽減手段である。）

（１３）　（除去に伴う）製剤自体の減損率

リボフラビンの吸収半減時間は 1.1 時間で、これは血液から組織またはその他の流体への吸収による半減時間をさす。この他、処置済み濃縮血小板輸血一時間後に採取した患者の血液サンプルを使った HPLC テストでは、血中のリボフラビンレベルは基準値に迅速に戻ることが示された。前述のとおり、リボフラビンの血漿からの除去は主に尿中排泄によって行われるが、これによるリボフラビンの半減時間は 9.9 時間であると実証されている。

血小板の減損率は約２％　（PLT Loss）。

血小板の品質に関する詳細については“MIRASOL Monograph”第六章を参照のこと。

（１４） （除去に伴う）製剤の生物活性の低下率

Mirasol 処置血小板は、フランスにおける血小板減少疾患患者の臨床試験でその有効性が証明されている。血漿中の血小板の生存期間は５日間、血小板添加溶液中の血小板のそれは７日であると実証された。

詳細については“MIRASOL Monograph”第六章を参照のこと。

〇その他（日本、海外の状況等）

（１５） 国内、国外における治験研究の現状は。

Navigant Biotechnologies, LLC では、日本においては臨床試験を実施していないが、フランスでは大規模な治験を最近執り行い、そのデータを解析中である。スペイン、イタリア、リトアニアでは、MIRASOL 処置血小板が輸血用として継続的に使用されている。また FDA からは、MIRASOL 処置済全血について、この全血からの赤血球分離および長期保存処置の実施許可を受領した。

（１６） 海外で治験（トライアル）ではなく、正式に導入している実績はどれほどか。

血小板向けとして､CE マーキング認識 MIRASOL システムの販売が各国で開始されている。当社では、本システムの販売許可を 2007 年 10 月後半に取得した。スペイン、イタリア、リビア、リトアニア各国の多数の血液センターでは、本システムを継続的に使用し始めている。なお、要請があり次第マニュアルを支給する予定である。

（１７） 危機管理体制確保の一環としての基礎的研究（治験研究）チーム設定の可能性は。

Gambro BCT および Navigant 間では、潜在的装置関連事故に関する情報を受領・調査する制度が確立している。この管理制度には、医師、医療従事者、科学者、技師の専門知識が必要である。Gambro BCT は、将来的に科学調査グループを設立し、PRT を専門とする品質部門を強化する予定であり、日本国内の大学や日本赤十字社と提携し、あらゆる基本臨床研究が可能になるはずである。

（１８） 不活化技術により現在の感染症検査のうち、何を省略できるのか。できないとしたら現在のわが国のような感染症検査が行われている国では屋上屋を架すこととならないか。また、海外で省略できている項目はあるのか。

当社の目的は、病原体および残余白血球を不活化する手段を提供し、最も安全な血液製剤の供給をサポートすることにある。この最終目的を達成するため、当社は、特異的試験の排除を特定の政府機関や見込み客だけに提言しない方針をとっている。Mirasol システム導入時に排除すべき試験があれば、どれを排除すべきかを決断するための支援データを顧客に提供する。また、地理情報変数（感染率、新興病原体の危険率等）をとり入れることにより、余分な試験を排除するための適正かつ各顧客に即した決定がなされることになる。

（１９） コストベネフィットの問題だけでなく、日本では放射線照射をしているが、不活化との相乗で製剤が使い物にならないのではないか？その治験を行えるのか。ＨＬＡの近い日本人で輸血後GVHD予防のために照射をやめられるか。

Navigant は、これまで広範囲に及ぶ生体内実験や動物実験を実施し、白血球細胞を不活化してγ線照射レベルと同程度、もしくはそれ以上にするための技術を実証してきた。フランスでとり行われた臨床試験では、白血球細胞レベルを落とすか、もしくはγ線照射レベルでキープするか否かの決断が治験医師にゆだねられた。一方 90%以上の症例において、Mirasol システム処理製剤の場合γ線照射を行わないという決断がなされている。ただし、臨床試験で規制対象とされている場合、大多数の患者にγ照射製剤が使用されている。

（２０） 薬物添加以外の方法による不活化法の検討は。γ線照射による細菌滅菌の現状は。

Navigant で開発中の MIRASOL システムは、現段階では薬剤を必要とせず、光線感作化学物質として自然発生ビタミンであるリボフラビンを使用している。現に、Mirasol 処置でリボフラビンが使用されていることからもわかるように、リボフラビンを薬剤とみなす規制制度が存在しないことは周知のとおりである。

リボフラビンを光感作化学物質として使用すると、病原体の核酸に回復不能なダメージが生じる。一方、リボフラビンを使用せず紫外線のみ単独で照射した場合、これらの核酸ダメージが回復可能となる、という問題が生じるため、紫外線のみに依存する前計算放射輝度伝達方法は、その効果が弱いと確信する。

γ線照射により不安定な血液製剤内の細菌を不活化する最近の技術について、弊社は詳細な知識を持ち得ない。

2．質問事項及び議論に必要なデータ等（各技術に対する質問）

1. MB

1）処理後 C5a が 3 倍に増加している。これは処理によって補体が活性化した結果であり、血管透過性の亢進などアレルギー反応が増加する可能性がある。また、大量に投与することがあるので、投与可能な血漿量の検討はされているのか？
2）MB と結合する血漿タンパクはないのか？もし、血漿タンパクのなかで結合する因子があった場合、体内での沈着部位は確認されているのでしょうか？例えば、皮膚などにMBと結合したタンパクが存在した場合、日光によって再度、1 重項酸素が産生され、組織が破壊される可能性はないのか？
3）MBと結合した血漿タンパクが処理によって発生する1 重項酸素のために構造等が変化してネオアンチゲンとなって、血漿タンパクに対する自己抗体が産生されないのか？特に、自己免疫疾患やアトピーの患者に投与する場合は自己抗体が作られやすくなる可能性はないのか？
4）導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由（例えば、処理能力以上にウイルス量が多かった等）も示していただきたい。
5）今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

2 アモトサレン

6）各種毒性試験の具体的な記載がなく、例えば、単回投与での毒性なし、といっても何頭使用して、どういう毒性を検討したのか全く不明。他の毒性試験も同様である。具体的な記載と、毒性の専門家の意見を聞きたい。
7）不活化処理後のアモトサレン除去によっても 2mg のアモトサレンが血漿と結合して残存する。血漿タンパクと結合したアモトサレンはどのように代謝されるのか？また、体内に結合したまま沈着する可能性を検討したことがあるか？例えば、皮膚などにMBと結合したタンパクが存在した場合、日光によって組織が破壊される可能性はないのか？
8）アモトサレンと血漿タンパクが結合して構造等が変化してネオアンチゲンとならないか？そのため、血漿タンパクに対する自己抗体が産生されないのか？特に、自己免疫疾患やアトピーの患者に投与する場合は自己抗体が作られやすくなる可能性はないのか？反復投与後の副作用として自己抗体産生の症例はないのか？
9）導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由（例えば、処理能力以上にウイルス量が多かった等）も示していただきたい。

10）今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

11）血小板において2mgのアモトサレンが血漿と結合して残存するが、新鮮凍結血漿ではさらに多くのアモトサレンが血漿に結合して残存する可能性がある。血漿のデータがないので用意していただきたい。

3.リボフラビン

12）結合する血漿タンパクはないのか？もし、血漿タンパクのなかで結合する因子があった場合、体内での沈着部位は確認されているのか。例えば、皮膚･眼球などに結合したタンパクが存在した場合、日光によって再度、活性化され組織が破壊される可能性はないのか？

当社が執り行った調査では、リボフラビンが血漿タンパク質、赤血球表面タンパク質、もしくはその他の表面タンパク質のいずれにも結合しないことが示された。リボフラビンは、電子転送中に電子を取得して作用するが、たとえばソラレンを使用するような競合技術では、実際のところタンパク質の二重結合や核酸に結合する。

リボフラビンは Mirasol 処置後のタンパク質とは共有結合しないため、リボフラビンの高濃度領域もしくは自然タンパク質／細胞との結合が患者への安全性に悪影響を及ぼすものとは考えられない。前述のとおり、リボフラビンには水溶性があり、人体から迅速に排出される。

赤血球は、当然ある種のタンパク質と結合しているが、皮膚や眼球への局部集中はありえない。次の出版物を参照されたい： Rao P, et al. Elevation of serum riboflavin carrier protein in breast cancer.Cancer Epidemiol Biomarkers Prev. 1999 Nov;8(11):985-90, Watson CD, Ford HC）. High-affinity binding of riboflavin and FAD by immunoglobulins from normal human serum. Biochem Int. 1988 Jun;16(6):1067-74. High-affinity binding of riboflavin and FAD by immunoglobulins from normal human serum.

13）導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由（例えば、処理能力以上にウイルス量が多かった等）も示していただきたい。

本件についてはデータはない。

14）今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

現時点までの臨床データには、装置関連の有害事象は一切含まれていない。とりわけ、血小板の不適応性や赤血球の同種免疫性を著しく高めるような事象は確認されていない。

3．主な意見

（１） 議論の時間が『時間切れ』になることは好ましくなく、時間を十分確保して行うべき。

（２） 毒性学の専門家の意見を聞きたい。テルモ等、機器の滅菌を行っている人達は、病原性因子の不活化について（γ線照射など）むしろ医学関係者よりも詳しい。いずれ不活化の毒性の検討が必要。

（３） 導入にあたっては、日赤などで不活化をきちんと評価する体制が必要。

（４） 危機管理体制として不活化技術を利用するというのが現在の最も有効な利用対象ではないか。

バイオワン株式会社回答

平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会合同委員会
不活化技術を有する事業者に対する質問事項等まとめ（配布用）

1．質問事項及び議論に必要なデータ等（全ての技術に対する質問）

IBS　　　：　InterCept Blood System の略。S-59 による不活化技術をさす。

IBS 処理　：S-59 による不活化処理をさす。

〇不活化剤の体内動態、安全性について

（１）　不活化剤の代謝（薬剤の排泄経路）。反復投与した場合の排泄、分解、蓄積の詳細。腎臓あるいは肝障害患者の場合の配慮。代謝（排泄完了）に要する時間。

*S-59 の体内動態・代謝・蓄積性。

S-59 のヒトにおける体内動態は Phase IB において、自己血小板濃厚液を IBS 処理し被験者に戻す検討が行なわれた。残留 S-59 の平均濃度は 0.31μM（25.1μg/body）、最高血中濃度の平均値は 1113pg/mL、半減期の平均値は 428.1min、AUC の平均値は 15.1ng/L・min であった。

非臨床試験において尿中および糞中排泄物を HPLC で検討した結果、糞中では多くのマイナーピークが検出され非常に高度代謝されていることがうかがえた。また尿サンプルにおいて S-59 の代謝を検討しているが、グルクロン酸抱合および硫酸抱合は関与していない。(8-MOP はグルクロン酸抱合および硫酸抱合の関連あり)。非臨床試験（ラット及びイヌ）における検討では、投与後 28 日目までに約 65%が糞中、約 10%が尿中に排泄された。

非臨床試験における検討ではラット及びイヌにおいて、投与後 48 時間目までに投与された残留 S-59（CAD 処理済）の 57%、35%が排泄された。また、投与後 28 日目までに、それぞれ 86-87%、70-84%が排出された。一方、ラットにおいて、体内に残留した S-59 は投与後 28 日目で 6%であった。

＊肝機能障害又は腎機能障害に対する場合の代謝。

血漿用 IBS の臨床試験において、肝障害患者を対象にしたランダム化比較試験が実施されているが、有害事象の発現に有意差は認められなかった。血漿用 IBS の臨床試験において、肝障害患者を対象にしたランダム化比較試験が実施されているが、有害事象の発現に有意差は認められなかった。

腎不全の患者では血小板と血漿で臨床試験をしたが蓄積性など問題はなかった。血小板の１ユニットの輸血では血中のピークレベルは1ng/ml 程度ですぐに消失した。血漿１Litter 輸血後でもピークレベルは10ng/ml ですぐに消失した。

（２）　不活化剤の体内分布、特にレンズ等への蓄積など。不活化処理血液製剤を大

量に使った場合の安全性は。

S-59 単体での代謝をラット、マウスを用いて試験したところ、半減期は 0.8 時間であり、一方血小板と一緒に投与した場合　ヒトで 6.5 時間、イヌで 7.2 時間と代謝は早い。また 28 日間の連続投与でも蓄積性は見られず、臓器蓄積性(特別な臓器のみ蓄積する)は認められていない。

C14-S-59 によりラットにおける体内分布を確認したところ、表のように脳、眼の濃度は非常に低い。

in vivo 試験では臨床使用量の x40000 をマウスに投与しても毒性は認められず、安全域は非常に広い。

臨床的には血漿交換などの治療においてほぼ同時に 100 処理分相当量以上の血漿を輸血しているが、副作用は報告されていない（17 例で 3185 血漿を輸血：平均 187 血漿/ヒト）

（３）　不活化剤を除去した後、一定濃度以下であれば安全という科学的根拠は。

欧州において 2 本の PhaseⅢB スタディが実施されている。これらの試験において、残留 S-59 量が測定されている。設定された目標値は 0.5μM（50μg/300ml）であり、結果として平均値はそれぞれ o.4μM 及び 0.46μM であった。

一方、イヌにおいて 25mg/kg、28 日間連続投与しても毒性所見は報告されていない。この量は、臨床で血小板がヒトに輸血された場合の S-59 体内混入量（0.4μg/kg）と比較した場合、60000 倍と非常に高く、安全域が大きいことを示している。

　また、本設定における安全性は、同じく欧州及び米国において実施された PhaseⅢ Study において、IBS 処理済み血小板濃厚液と通常の血小板濃厚液の間に有意差を示した有害事象は観察されなかった。また、PhaseⅢB、10000 輸血以上を集積した Hemovigilance Study、小児を対象とした Study、約 120000 輸血以上の実績において特異的な有害事象は観察されていないことから、上記の残留 S-59 量の設定が適切あるいは許容できると考えられる。

（４）　他の薬剤との反応と、その問題点。

薬剤相互作用についての試験、解析は実施されていない。しかし、臨床試験で一般的に使用されている薬剤と併用されているが、特別な問題は生じていない。

（５）　（薬剤としての）不活化剤の用法、用量、使用上の注意など（取り扱い説明書）

S-59 は人体に対する直接的な治療効果を目的とした化学物質ではないので医薬品として取り扱われることはない。

本医療機器には S-59 溶液（1.01ｇ／L）が 15mL 又は 17.5mL 含まれており、これを血小板と混合し紫外線Ａ（320－400nm でエネルギーが低く最も可視光に近い紫外線）を照射することにより病原体を不活化することが可能となる。

万一 S-59 溶液を皮膚等に付着させてしまった場合は、MSDS（Material Safety Data Sheet）の指針に従い速やかに大量の水で洗浄する。

（６）　reduction ratio が主で、安全性試験の情報がない。

前臨床試験において多くの毒性試験がICHの医薬品のガイドラインに従って実施されている。その結果、臨床使用量の 40000 倍で遺伝毒性は認められておらず、安全性は高い。また、安全性のデータは

米国FDAでは審査が終了し，EUのCEマーク、フランス AFFSAP、ドイツPEIで承認されている。

一方、本設定における安全性は、同じく欧州及び米国において実施された PhaseⅢ Study において、IBS 処理済み血小板濃厚液と通常の血小板濃厚液の間に有意差を示した有害事象は観察されなかった。また、PhaseⅢB、10000 輸血以上を集積した Hemovigilance Study、小児を対象とした Study、約 120000 輸血以上の実績において特異的な有害事象は観察されていないことから、上記の残留 S-59 量の設定が適切あるいは許容できると考えられる。

毒性試験の結果については 080401 概要の7．毒性試験　参照。

○不活化効果について

（７）　不活化効果（log をどの様にして求めたのか：使用したモデルウイルス・培養細胞など）

（８）　（3剤）不活化強度の比較。

（９）　不活化効果の評価法の妥当性（モデルウイルスのデータのみでよいか）。

（１０）　不活化の対象病原体。ウイルス（HBV、HCV、HIV、その他未知のウイルスも含む？細菌？原虫？）

(7)(9)(10)に関して

不活化効果については　080401　概要の3．S-59　による病原因子の不活化能力　及び　不活化効果試験の Assay Method　参照。

インターセプト（S-59）はウィルス、細菌、原虫のみならず白血球（T 細胞）をも不活化可能である。

不活化効果を確認したウィルスの多くはモデルウィルスであるが、HIVについてはモデルウィルス以外にエイズ患者から分離したウィルスを用いて試験を行っており、不活化効果を認めている。B 型肝炎、C 型肝炎ウィルスは in vivo で評価試験を行っている。

実験において表の数字までしか病原体を増殖することができなく、その病原体をすべて不活化できたので、もし病原体をもっと増殖させることが可能であれば、もっと数字が大きくなるかもしれないという可能性を含めて“＞”で表している。

(8)に関して

3剤を直接比較した試験結果は2005年に実施した日本赤十字社の試験以外にはまだない。

（１１）　不活化法の導入で、現在の輸血用血液製剤の感染リスクのそれぞれがどれだけ減少できると考えられるか？（それぞれの病原体に対する推定値とその根拠）また、外国の導入例において、どのような輸血後感染リスクがどれだけ減少したかを比較検証（あるいは推測）できるデータがあればその提示もお願いしたい。

臨床試験の結果、既に導入している国での使用実績ではこれまでに細菌による敗血症、GVHD，或いは他の病原による感染症の発生は報告されていない。

すでに12万回を越える輸血実績があるが、細菌検査を実施しても11、000回（ドイツ赤十字）から59、000回（米国赤十字）の輸血に1回の頻度で発生があると報告されている敗血症がIBSの使用例では細菌検査を実施していないにもかかわらず報告されていない。

フランスのLa　Union島ではChikungunyaの流行により島内での採血を中止せざるを得なくなって赤血球、血漿はフランス本土からの空輸により供給しているが、血小板は保存期間が短いためフランス本土からの供給では間に合わなくなっている。すでに導入していたストラスブールからSOPとともにIBSを

導入し2006年年初より血小板のみ成分採血により採取を開始しIBSで処理して供給している。IBS処理により新たな病原の流行があっても血液製剤の供給が確保できることが証明されている。その後北イタリアでもChikungunyaが侵入したがIBSが導入されていたため血小板の供給には支障がなかった。
輸血の副作用について米国のPhase IIIにおいてIBS処理群で副作用が少なくなる傾向が示唆された。
さらにベルギー、フランスアルサス地方、La Union島でもHemovigilanceの調査結果からIBSで処理した血小板輸血では通常の血小板輸血に比較して副作用の発生率が1000回輸血当たり1/3に減少することが報告されている。
副作用は血小板に含まれる血漿成分の減少（100%→35%）だけでも改善され IBS により不活化することでさらに改善することが示唆されている。
12万回を超える血小板輸血で1例 TRALI の発生が報告されているこの例は3回出産を経験した女性から成分採血した血小板の輸血により発生した。
海外のヘモビジランス情報はスライド 参考資料2参照。

○不活化剤除去について

（１２） （除去後の）不活化剤の製剤中の残存率は。不活化剤を添加した後の回収をどのように計算しているのか。

欧州において 2 本の PhaseⅢB スタディが実施されている。これらの試験において、残留 S-59 量が HPLC で測定されている。設定された目標値は 0.5μM（50μg/300ml）であり、結果として平均値はそれぞれ o.4μM 及び 0.46μM であった。
一方、イヌにおいて 25mg/kg、28 日間連続投与しても毒性所見は報告されていない。この量は、臨床で血小板がヒトに輸血された場合の S-59 体内混入量（0.4μg/kg）と比較した場合、60000 倍と非常に高く、安全域が大きいことを示している。
また、一方、本設定における安全性は、同じく欧州及び米国において実施された PhaseⅢ Study において、IBS 処理済み血小板濃厚液と通常の血小板濃厚液の間に有意差を示した有害事象は観察されなかった。また、PhaseⅢB、10000 輸血以上を集積した Hemovigilance Study、小児を対象とした Study、約 120000 輸血以上の実績において特異的な有害事象は観察されていないことから、上記の残留 S-59 量の設定が適切あるいは許容できると考えられる。

（１３） （除去に伴う）製剤自体の減損率

ベルギー、フランスの実績において、不活化処理による減損率は約7－8%である。

（１４） （除去に伴う）製剤の生物活性の低下率

In vitro の試験では IBS 処理群と対象群で差がみられているがどちらも AABB の基準の範囲内であり、かつ in vivo のウサギの耳出血時間モデルでは両処理群で差が認められず、血小板止血機能に有害な影響を与えないことが判明している。
また米国の Phase III による出血の防止を End Point とした臨床試験で IBS 処理群と対象群で差がないことを実証している。
市販後の追跡調査でも IBS 処理によって血小板の登用量が増えている情報はない。

不活化処理により血小板の回収率は7-8%低下するが活性面での低下は市販後の調査結果では問題になっていない。

その原因の一部は血小板輸血にあたって医療の現場では厳密には血小板数を管理していないこともあると思われる。

詳細は 080401 概要の6. 血小板の機能　参照

○その他（日本、海外の状況等）

（１５）　国内、国外における治験研究の現状は。

（１６）　海外で治験（トライアル）ではなく、正式に導入している実績はどれほどか。

導入実績は添付の資料参照：080401 導入実績

輸血に使われた血小板は 12 万ﾊﾞｯｸﾞ 以上。

＊世界中のインターセプト導入国（ルーチンユース—12 カ国）

ベルギー、フランス、ドイツ、イタリア、ノルウェー、スペイン、スウエーデン、ロシア、チェコ、スロベニア、クェート、マレーシア

＊主な既承認国での承認年月日

フランス Afssaps：2005 年 7 月 19 日
ドイツ：2007 年 1 月 29 日
イタリア：2002 年 3 月 31 日
スペイン：2002 年 3 月 31 日

＊アメリカの現状：FDA 申請中

米国では出血防止をEnd Pointにした約600人規模のDouble Blind Phase IIIまで終了。 申請書のうち前臨床の部分は既に審査が終わり問題がないとされています。Phase I, Phase II においても問題はなくPhase III の試験結果で IBS 処理した血小板を輸血した群に対象群比較して有意に肺障害の発生頻度が高いことがわかりました。この原因について追加調査が行われ追跡調査の結果 S-59 処理による障害の発生ではないと判断された。原因として考えられるのは血小板輸血が繰り返し必要になる例は白血病の治療を受けた患者、或いは骨髄移植を受けた患者が多く治療には放射線治療が入っていることが多くなります。放射線治療は肺障害が副作用として発生することが知られています。放射線の照射量を調べたところ S-59 処理群の方が前治療でより多くの放射線照射を受けていました。しかし FDA は実施した Phase III は肺障害の頻度を検出することを目的とした試験ではないので大規模な試験を実施して発生の頻度を比較すべきということで1万人規模以上の試験の提案をしました。それは S-59 開発当時は骨髄移植の前治療に放射線治療が多く使われていて副作用としての肺障害の発生も10%以上ありましたが現在は治療方法が改善されて肺障害の発生頻度は5%以下になっています。また前治療も放射線を含まない治療に変わっているため改めて同じような試験をしても結果は確認できないので FDA の担当官と協議を続けてきました。一方同じ臨床試験の報告を審査した EU, AFSAP(フランス)、PEI(ドイツ）の担当官はすべて審査の上問題はないということで承認している。 この問題についてはトロントの会議、およびMinistry of Health and Human Serviceの公開の席上でも取り上げられFDAの担当官の誤解であることがPEI,のDr. Heidenから指摘されトロント会議でも、HSSの会議でも勧告となりました。なって病原不活化は安全であることを示す十分な情報がある、一方では温暖化などにより予期していない病原の侵入がありことが起きてからではなく先取りして対応すべきということで勧告になっています。米国では欧州の Hemovigilance の情報も合わせて審査して承認する方向で動き出しています。

（１７）　危機管理体制確保の一環としての基礎的研究（治験研究）チーム設定の可能性は。

血小板を製造する施設に協力して研究チームを編成することに協力できます。しかしこの試験の実施者は血小板を製造する側にあるので臨床試験のための治験申請も製造者（日本赤十字社）になりま

す。治験申請に必要な資料については既に整っていると考えています。

（１８） 不活化技術により現在の感染症検査のうち、何を省略できるのか。できないとしたら現在のわが国のような感染症検査が行われている国では屋上屋を架すこととならないか。また、海外で省略できている項目はあるのか。

現時点では直接のコスト比較は行われていない。ただし、IBS の導入によって、新たな病原体検査の追加が不要になる可能性がある。白血球除去に関しては、IBS 処理を行えば、理論的にはなくてもかまわないが、各血液センターの判断に従う。 又、不活化処理をするため処理費用はプラスとなるが、次のような費用削減効果がフランス、ベルギーで報告されている。しかし詳細は各国の状況によって異なるであろう。フランスは導入の結果 Single NAT の導入は不要と判断している。

費用削減効果（フランス・ベルギー）
細菌試験、ガンマ線照射が不要となる。追加の病原の試験が不要になる（CMV）など。
保存期間を 7 日まで延長可能により廃棄率が減少する
血漿量を減少できるので節約した血漿を血漿製剤などに有効に利用できる。

（１９） コストベネフィットの問題だけでなく、日本では放射線照射をしているが、不活化との相乗で製剤が使い物にならないのではないか？その治験を行えるのか。ＨＬＡの近い日本人で輸血後ＧＶＨＤ予防のために照射をやめられるか。

＊GVHD の予防に関して。

γ線照射と同等あるいはそれより有効というデータに関して、臨床試験でのγ線照射との比較データがあり、サイトカイン産生能（IL-8、IL-1β）をγ線照射処理と IBS 処理で比較した試験ではγ線照射血小板より有意に抑制する結果が得られた。

また S-59 は 83 塩基対毎に結合するのに対しγ線照射は 3 万 7 千塩基対毎であることより IBS 処理はγ線照射と同等あるいはそれ以上に GVHD の発症を抑制すると考えられる。したがって IBS 処理とガンマー線処理を併用することは考えていない。（IBS 処理：S-59 による不活化処理）

一方 臨床試験で S-59 による不活化処理した血小板をガンマ線照射した例があるが、特別な問題は生じていない。

（２０） 薬物添加以外の方法による不活化法の検討は。γ線照射による細菌滅菌の現状は。

参考: スライド（080401UVC）あり。 白血球不活化程度のガンマー線照射量では Virus の不活化ができない。

UVC についてはマコファルマ社の公表試験成績がありHIV の不活化が１．３６ログであるとされています。また UVC については血小板の活性低下が報告されています。

２．質問事項及び議論に必要なデータ等（各技術に対する質問）

1. MB

1） 処理後 C5a が 3 倍に増加している。これは処理によって補体が活性化した結果で

あり、血管透過性の亢進などアレルギー反応が増加する可能性がある。また、大量に投与することがあるので、投与可能な血漿量の検討はされているのか？

2） MB と結合する血漿タンパクはないのか？もし、血漿タンパクのなかで結合する因子があった場合、体内での沈着部位は確認されているのでしょうか？例えば、皮膚などにMBと結合したタンパクが存在した場合、日光によって再度、1 重項酸素が産生され、組織が破壊される可能性はないのか？

3） MBと結合した血漿タンパクが処理によって発生する1 重項酸素のために構造等が変化してネオアンチゲンとなって、血漿タンパクに対する自己抗体が産生されないのか？特に、自己免疫疾患やアトピーの患者に投与する場合は自己抗体が作られやすくなる可能性はないのか？

4） 導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由（例えば、処理能力以上にウイルス量が多かった等）も示していただきたい。

5） 今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

2 アモトサレン

6） 各種毒性試験の具体的な記載がなく、例えば、単回投与での毒性なし、といっても何頭使用して、どういう毒性を検討したのか全く不明。他の毒性試験も同様である。具体的な記載と、毒性の専門家の意見を聞きたい。

080401 概要の7. 毒性試験結果を参照。

7） 不活化処理後のアモトサレン除去によっても 2mg のアモトサレンが血漿と結合して残存する。血漿タンパクと結合したアモトサレンはどのように代謝されるのか？また、体内に結合したまま沈着する可能性を検討したことがあるか？例えば、皮膚などにMBと結合したタンパクが存在した場合、日光によって組織が破壊される可能性はないのか？

排泄物に中間代謝物は確認されず、すべて低分子になっている。半減期、S-59 の代謝及び排泄物から推察して中間代謝物にとどまる時間は非常に短いと思われる。S-59 で処理した血小板の 28 日間連続投与の毒性試験で毒性が認められておらず蓄積性はないと考える。

8） アモトサレンと血漿タンパクが結合して構造等が変化してネオアンチゲンとならないか？そのため、血漿タンパクに対する自己抗体が産生されないのか？特に、自己免疫疾患やアトピーの患者に投与する場合は自己抗体が作られやすくなる可能性はないのか？反復投与後の副作用として自己抗体産生の症例はないのか？

免疫処理したニュージーランド白ウサギの抗体を用いて in vitro でネオアンチゲンが産生されるか

どうかの試験を実施したが、ネオアンチゲンは確認されていない。さらに、臨床試験、市販後の調査を通じても抗体産生の報告はない。

9) 導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由(例えば、処理能力以上にウイルス量が多かった等)も示していただきたい。

既に導入している国での使用実績ではこれまでに細菌による敗血症、GVHD，或いは他の病原による感染症の発生は報告されていない。

すでに12万回を越える輸血実績があるが、細菌検査を実施しても11、000回(ドイツ赤十字)から59、000回(米国赤十字)の輸血に1回の頻度で発生があると報告されている敗血症がIBSの使用例では細菌検査を実施していないにもかかわらず報告されていない。

ベルギー、フランスアルサス地方、La Union島でもHemovigilanceの調査結果からIBSで処理した血小板輸血では通常の血小板輸血に比較して副作用の発生率が1000回輸血当たり1/3に減少することが報告されている。

副作用は血小板に含まれる血漿成分の減少(100%→35%)だけでも改善され IBS により不活化することでさらに改善することが示唆されている。

12万回を超える血小板輸血で1例 TRALI の発生が報告されているこの例は3回出産を経験した女性から成分採血した血小板の輸血により発生した。

海外のヘモビジランス情報はスライド 参考資料2参照。

10) 今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

120000例以上の実績があるが、重篤な副作用報告はない。

11) 血小板において 2mg のアモトサレンが血漿と結合して残存するが、新鮮凍結血漿ではさらに多くのアモトサレンが血漿に結合して残存する可能性がある。血漿のデータがないので用意していただきたい。

後日追加。

3.リボフラビン

12) 結合する血漿タンパクはないのか？もし、血漿タンパクのなかで結合する因子があった場合、体内での沈着部位は確認されているのか。例えば、皮膚･眼球などに結合したタンパクが存在した場合、日光によって再度、活性化され組織が破壊される可能性はないのか？

13) 導入した地域･国で導入後感染症が減少したとのデータがあれば提示していただきたい。また、導入後に感染症が発生した症例があれば、その理由(例えば、処理能力以上にウイルス量が多かった等)も示していただきたい。

14) 今までの投与実績と報告された重篤な副作用例があれば示していただきたい。

3. 主な意見

（１） 議論の時間が『時間切れ』になることは好ましくなく、時間を十分確保して行うべき。
（２） 毒性学の専門家の意見を聞きたい。テルモ等、機器の滅菌を行っている人達は、病原性因子の不活化について（γ線照射など）むしろ医学関係者よりも詳しい。いずれ不活化の毒性の検討が必要。
（３） 導入にあたっては、日赤などで不活化をきちんと評価する体制が必要。
（４） 危機管理体制として不活化技術を利用するというのが現在の最も有効な利用対象ではないか。

バイオワン追加資料

１）質問(２)----参考の表 (弊社回答中、表の指定明記が無かった)

表 ラットにおける ^{14}C-S-59 又は ^{14}C-S-59 混合物投与後の組織分布

	放射能活性の最高あるいは最低であった組織		
		S-59 混合液	
	S-59	CAD 非処理	CAD 処理
最高	副腎	副腎，卵巣	副腎，卵巣
高度	肝臓，腎臓，肺，消化管，皮膚	肝臓，腎臓，肺，脾臓，小消化管	肝臓，腎臓，脾臓
最低	脳，眼，脂肪組織	脳，脂肪組織	脳，眼，脂肪組織（雄）筋肉，胸腺

２）質問(１３) 回答の訂正（減損率の言葉の意味の取り違えのための訂正）
製剤の回収率は約 7-8%低下するが、減損率（血小板製造工程での血小板パックの廃棄率）は発表されていないためわからない。

３）（各技術に対する質問）------アモトサレンに関する質問番号 11) 未回答だったため回答追加
血漿を IBS で不活化して健常人、および患者で血中の S-59 のレベルを検討している。

1． 健常人での試験

IBS で不活化処理した血漿１リットルを投与したとき投与直後にピークレベルに達しその濃度は１０ng/ml であった。S-59 は速やかに血中から消失し１６-２４時間後にはほぼ投与前のレベルに戻っている。

2． 患者での検討

患者では血漿交換の必要な血栓性血小板減少性紫斑病（TTP）の患者でも検討している。

S-59 単独での T1/2 は 41 分であり血漿或いは血小板に含まれた状態では 6.5 時間であった。

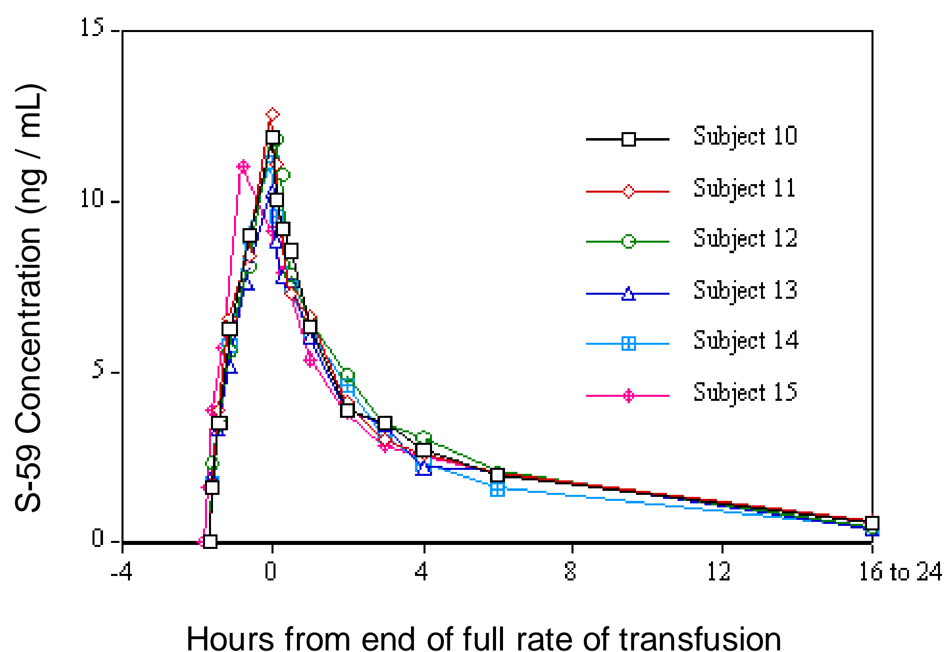
血漿蛋白が S-59 の体内での分解作用から保護的に働いていると思われる。

しかし添付したようにいずれの試験においても繰り返し輸血しても蓄積作用はみられていない。

４時間後の S-59 は 1.7-8.5 ng/ml で 24 時間後には 0.1-2.0 ng/ml であった。

参考資料

Plasma Amotosalen Levels In Healthy Subjects After 1 L FFP



Amotosalen Levels (ng/mL) In Patients

Patient Group	N	1- 4 Hr Post Transfusion	24 Hr Post Transfusion
Congenital	28	8.4 ± 2.7	0.9 ± 0.6
Acquired	57	5.4 ± 3.4	2.0 ± 2.0
Plasma Exchange	16	1.7 ± 0.6	0.1 ± 0.1

平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会合同委員会後　追加質問事項1（バイオワン社のみ）

1：追加資料として送られてきた transfusionの論文では、血小板に添加したS-59の15%が血小板に、血漿に添加したS-59の15～22%は血漿成分に結合すると記載されています。その多くは脂肪に associateしているとあります。血小板の膜は脂質を含むことから、膜にS-59が結合し、S-59がハプテンになってS-59が結合した血小板にのみ反応する抗体が産生される危険性はないのか？同様に、S-59が結合した血漿成分に対しても抗体ができる可能性があるのでは？

S-59処理したヒト血小板，又はヒト血漿をウサギに免疫した試験では明らかな 抗体の産生は認められていない、と報告されているが、試験法をよく見ると、ヒトに対する抗体を吸収するなどの前処理をおこなっており、本当に、否定して良いのか疑問が残る。適切な実験系なのか自信はないが、例えば、ウサギの血小板や血漿をS-59で処理し、ウサギに反復投与した場合にS-59処理した血小板等への抗体の有無が明らかになるのではないか？

2：S-59による新鮮凍結血漿の処理の場合、S-59を添加するだけで凝固因子のいくつかは活性が低下するが、UV 照射後にS-59を吸着させるために5時間以上（？）処理時間が必要となるが、凝固因子の活性は新鮮凍結血漿として使用できる程度に保つことは可能なのか？

バイオワン株式会社回答

平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会合同委員会後
追加質問事項1（バイオワン社のみ）

1：追加資料として送られてきた transfusionの論文では、血小板に添加したS-59の15%が血小板に、血漿に添加したS-59の15～22%は血漿成分に結合すると記載されています。その多くは脂肪に associateしているとあります。血小板の膜は脂質を含むことから、膜にS-59が結合し、S-59がハプテンになってS-59が結合した血小板にのみ反応する抗体が産生される危険性はないのか？同様に、S-59が結合した血漿成分に対しても抗体ができる可能性があるのでは？
　S-59処理したヒト血小板，又はヒト血漿をウサギに免疫した試験では明らかな 抗体の産生は認められていない、と報告されているが、試験法をよく見ると、ヒトに対する抗体を吸収するなどの前処理をおこなっており、本当に、否定して良いのか疑問が残る。適切な実験系なのか自信はないが、例えば、ウサギの血小板や血漿をS-59で処理し、ウサギに反復投与した場合にS-59処理した血小板等への抗体の有無が明らかになるのではないか？

1）動物による Neo antigenecity の検討について
　S-59 の前臨床開発に当たり Baxter, Cerus は FDA と協議の上動物実験を実施しました。
ご指摘のようにウサギを用いた試験には人血小板、および血漿を用いています。S-59 で処理した血小板、或いは血漿を用いて免疫したウサギは抗体を作りました。この抗体はいずれも Polyclonal でした。ここで得られたウサギの抗ヒト血小板抗体を S-59 で処理していない血小板と反応させた後 S-59 処理した血小板と反応させましたが反応しませんでした。推察するとウサギに生じた抗体は血小板に対する抗体で S-59 或いはその分解産物に対する抗体ではありませんでした。
同様にウサギの抗ヒト血漿抗体は血漿を認識するもので S-59 或いはその分解産物を認識するものではありませんでした。
一方内部標準として加えていたトリのアルブミンについてはウサギの抗体を血小板、或いは血漿で処理したあとでも認識しました。
この実験では人の血小板、或いは血漿を S-59 で処理した後ウサギに免疫しましたがそこで生じた Poly Clonal 抗体はいずれも S-59 処理をしていない人の血小板、或いは血漿を認識しており特別に S-59 で処理した血小板、或いは血漿を認識するものではありませんでした。
この結果から S-59 で処理した血小板、或いは血漿には新たに抗原となる変化は起きていないと推察されました。また S-59 或いは S-59 の分解産物に対する抗体の生成も認められませんでした。

ウサギの血小板、血漿を用いなかった理由は免疫を繰り返すのに十分な量の血漿、或いは血小板を

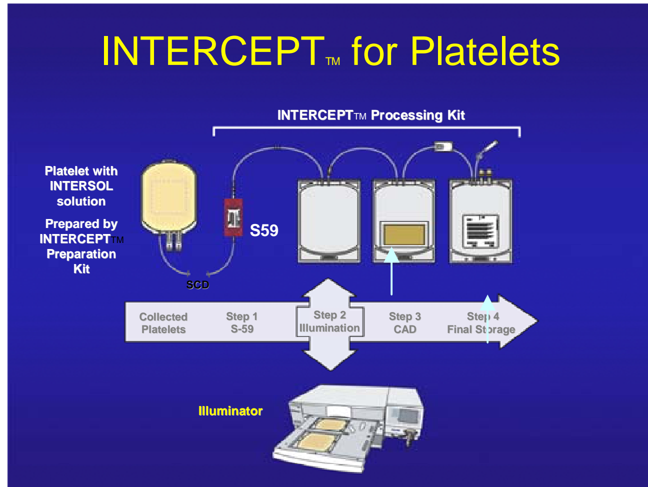
同じ固体からは採取できないことです。
S-59 に対する抗体の生成の可能性を完全に否定することはできませんがこれまでの前臨床試験、臨床試験、市販後調査の結果からは S-59 に対する抗体生成の報告はありません。
参考までに EPO, G-CSF のような遺伝子工学で製造したヒト血液ホルモンの前臨床試験、臨床試験でも抗体生成の可能性について注意深く検討していますが臨床試験の終了までに600例程度の症例を検討し抗体の生成が認められなかったため市販後には抗体の検査は行っていません。
S-59 についても臨床試験で約700例の症例について抗体生成の有無を検討していますが抗体の生成はありませんでした。
これまでのところ12万回を越える輸血に使われていますが抗体の生成は報告されていません。
2） S-59 に対する抗体の生成の可能性について
　ご指摘のように S-59 は血漿の脂質に多く結合します。この結合した状態での S-59 の半減期は約6．5時間です。（血小板中も血漿中も同じ）S-59 単独に比べると長いですが蓄積性がないことは確かめられています。血漿、或いは血小板に結合した状態でも分解は早いです。
抗体生成の可能性を完全に否定することは困難ですがこれまでのところ抗体生成を示す情報は前臨床試験、臨床試験、市販後調査を通じてありません。
8－MOP など治療に広く使われているソラレン化合物についても抗体産生の報告は現時点でありません。

2：S-59による新鮮凍結血漿の処理の場合、S-59を添加するだけで凝固因子のいくつかは活性が低下するが、UV 照射後にS-59を吸着させるために5時間以上（？）処理時間が必要となるが、凝固因子の活性は新鮮凍結血漿として使用できる程度に保つことは可能なのか？

血小板用不活化処理キットと血漿用不活化処理キットではS59吸着装置（CAD）の形状が異なり、CAD処理時間が異なります。血漿を処理する場合、長時間振盪する必要はなく、血漿全量が円形のCAD内を自然落下する時間（15～30分）のみ必要となります。（下図1参照）
前臨床試験で不活化処理前後の血漿の凝固因子活性を比較しています。目標とする凝固因子保持率はフィブリノーゲン及びファクターⅧは65％以上、ファクターⅤ、Ⅶ、Ⅸ及びXIは70％以上、他の因子については基準を設定していません。この実験では不活化処理によりいくつかの凝固因子にわずかに活性低下が認められますが、すべて目標とした基準に合致しており、不活化処理血漿は新鮮凍結血漿と同様に使用可能であります（下表2参照）。
また、すでにルーチンで使用している欧州のセンターで使用量が変化していないとの報告があります。

図1　血小板用及び血漿用不活化処理キット

血小板用不活化処理キット



血漿用不活化処理キット

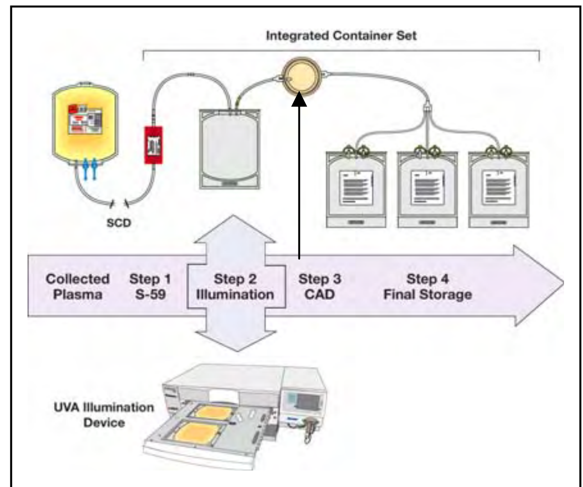


表2　in vitro インターセプトの処理前後の凝固因子の保持率

凝固因子	数	不活化処理前の活性（IU/mL）[a]	不活化処理後の活性[b]（IU/mL）	保持率
ファクターⅠ（フィブリノーゲン）	91	290±48	209±36	72±5
ファクターⅡ	59	96±11	85±11	88±4
ファクターⅤ	91	130±21	119±19	92±7
ファクターⅦ	91	123±26	95±20	78±6
ファクターⅧ	91	157±36	115±28	73±7
ファクターⅨ	91	108±19	88±16	82±4
ファクターⅩ	59	100±13	86±11	86±3
ファクターXI	91	103±22	87±18	85±4

スクリーニング試験	数	不活化処理前（秒）	不活化処理後（秒）	延長時間（秒）
PT [c]	14	11.2±0.3	11.6±0.3	0.5±0.1
aPTT [d]	13	26.9±1.4	31.6±2.1	4.7±0.8

a:フィブリノーゲンはmg/mL

b:600mL血漿に150μM S-59を添加し3.0J/cm^2 UVA照射後CAD処理

c:Prothrombin time

d:Activated partial thromboplastin time

平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会合同委員会後　追加質問事項2（各社共通）

1. EU主要国等、各社の不活化技術を導入しているそれぞれの国における供給の実態。輸血用血液製剤の全供給数（不活化技術を適用している製剤と適用していない製剤の合計）、そのうち不活化技術を適用している製剤の割合は何%か。また、供給はどのように行われているか（供給先、医師の希望により供給できるのか等）。

2. 各社の不活化技術を適用する前の製剤に白血球除去を行った場合と、不活化技術を適用した製剤に白血球除去を行わなかった場合を比較して、GVHD等の副作用の推移はどのようになっているか。

3. EU主要国における不活化技術導入後不要になった技術（白除、NAT等）

4. EU主要国における不活化技術を適用している製剤の市販後調査の実態（調査数）

マコファルマ社回答

平成20年4月8日開催　血液事業部会運営委員会・安全技術調査会合同委員会後
追加質問事項2（各社共通）

1. EU主要国等、各社の不活化技術を導入しているそれぞれの国における供給の実態。輸血用血液製剤の全供給数（不活化技術を適用している製剤と適用していない製剤の合計）、そのうち不活化技術を適用している製剤の割合は何％か。また、供給はどのように行われているか（供給先、医師の希望により供給できるのか等）。

回答

国	血漿製剤不活化の義務化	マコファルマ製品により処理された血漿製剤（本数）	マコファルマ製品により処理された血漿製剤（%）	不活化処理された血漿製剤（%）
アルゼンチン	×	2,000	―	―
ベルギー	○	70,000	100	100
ブラジル	×	2,000	―	―
フランス	○（将来的に）	150,000（2008 年予測）	60（2008 年予測）	100（2008 年予測）
ギリシャ	×	8,000	5	―
イタリア	×	34,000	5	25
マレーシア	×	300	―	―
ロシア	×	17,000	1	1
シンガポール	×	1,000	―	―
スペイン	×	105,000	44	69
イギリス	×	9,000	2	2

ベルギー及びフランスにおいては国立輸血サービス（National Transfusion Service）が血漿のみの不活化製剤を提供している（ベルギーでは 2004 年から、フランスでは 2009 年までの移行が予定されている）。その他の全ての国においては、医師並びに病院が使用する血漿製剤（不活化製剤及び検疫製剤）を自由に選択することが可能である。しかしながら欧州においては、血漿製剤並びに血小板製剤の不活化傾向が顕著である。

2. 各社の不活化技術を適用する前の製剤に白血球除去を行った場合と、不活化技術を適用した製剤に白血球除去を行わなかった場合を比較して、GVHD等の副作用の推

移はどのようになっているか。

回答

欧州全ての国において赤血球製剤並びに血小板製剤の白血球除去は、長年に亘り義務化されている。白血球除去の導入後、輸血由来による熱発例数は著しく減少した（M. M. Mueller et al., “Clinical impact of leucocyte depletion – what is the evidence?”参照）。GVHD の予防に関して、輸血製剤は依然としてガンマ線照射されている。しかしながらマコファルマ社製 THERAFLEX UVC を含む血小板製剤不活化技術は、ガンマ線照射の代替になり得ると期待されている。

3．EU主要国における不活化技術導入後不要になった技術（白除、NAT等）

回答

不活化技術が採用されたいくつかの国においては、検疫保管期間が省略された。その結果、より良好な流通、保管スペースの節約及び迅速な血漿製剤供給につながった。メチレンブルー処理の導入に伴い白血球除去並びに既存の検査を省略した国は無い。一方でメチレンブルー処理の導入により新たな検査の追加及び既存の検査感度を向上させる措置（シングル NAT 等）は行われていない。

血漿製剤 ：

欧州において、キットに採用されている 0.65μm のフィルターは血漿製剤用白血球除去フィルターより効率が良いため、THERAFLEX MB-Plasma 処理は血漿製剤の白血球除去の代替となる。

血小板製剤 ：

THERAFLEX UVC-Platelet 処理により、将来的にガンマ線照射並びに細菌スクリーニング検査の省略が期待される。現在の段階では、ウイルス NAT 検査の省略は推奨しない。

4．EU主要国における不活化技術を適用している製剤の市販後調査の実態（調査数）

回答

市販後調査は、欧州各国に導入されているヘモビジランス及び輸血サービス機関（Blood Transfusion Services）により実施されており、イギリス並びにフランスが多くの経験を有している。全ての欧州諸国がメチレンブルー処理された血漿製剤を含む全ての血液製剤に対するヘモビジランスプログラムを実施している。更に、既報の通り追跡評価の実施（フランスにおけるメチレンブルー処理血漿製剤の四相臨床治験、等）あるいは Politis 等による臨床使用経験（Vox Sanguinis, [2007];Volume 92, Issue 4, Pages: 319-326）が発表されている。

(参考) マコファルマ社回答 (英語)

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Mr. Takahiro GOTOU
c/o Blood & Blood Products Division
Pharmaceutical & Food Safety Bureau (PFS)
Ministry of Health, Labour and Welfare (MHLW)

1-2-2, Kasumigaseki, Chiyoda-ku

Tokyo, JAPAN, 100-8916

Your Reference: Your Message of: Our Reference: Dr.W./AH Date: April 30th, 2008

Committee questions from April 24th, 2008

Dear Sirs,

following you will find answers to the questions raised by you on the Theraflex MB Plasma on April 24th, 2008.

1. **Actual conditions of blood products supply in each country, where the inactivation has been implemented**
   (Please specify the total number of blood products supply with breakdown of inactivation processed and non-processed blood products in %. Also, describe, whether all supplied blood products must be processed mandatory or user (physician or hospital) can chose one).

   ANSWER:

   Supply of pathogen reduced blood products by countries:
   In our letter of 31.3.2008 to you, we indicated in Table 1) the list of countries, where the Theraflex MB Plasma has been registered and used in clinical routine. Below you will find a table of the countries with the actual quantities of plasmas treated in 2007 and the percentage of the total therapeutical plasma units treated by Theraflex and/or by other methods.

Geschäftsführer:
Peter Kremmers
Amtsgericht Offenbach HRB 33620
UST-ID.Nr. DE 119 109 470

Deutsche Bank AG
BLZ: 505 700 18 · KTO. 781 777 800
BIC: DEUTDEFF505
IBAN: DE46505700180781777800

Dresdner Bank
BLZ: 500 800 00 · KTO. 926 000 00
BIC: DRESDEFF
IBAN: DE71500800000092600000

Country	Inactivation for plasma mandatory	Quantity of plasmas treated with Theraflex MB plasma	% of Theraflex to therapeutical plasma	% of inactivated plasma to therapeutical plasma
Argentina	No	2000	-	-
Belgium	Yes	70000	100	100
Brazil	No	2000	-	-
France	Yes (in future)	150000 (in 2008)	60 (in 2008)	100 (in 2008)
Greece	No	8000	5	-
Italy	No	34000	5	25
Malaysia	No	300	-	-
Russia	No	17000	1	1
Singapore	No	1000	-	-
Spain	No	105000	44	69
UK	No	9000	2	2

In Belgium and France the National Transfusion Services offer only pathogen reduced plasma products (in Belgium since 2004, in France conversion is planned by 2009).
In all other countries the physicians and hospitals are still free to use their plasma (pathogen treated or quarantine plasma) by themselves. However, the trend in Europe goes clearly to pathogen reduced plasma products and pathogen reduced platelet products.

2. **Leukocyte reduction:**
The trend of blood transfusion related side effect occurrence rate, such as GVHD, with inactivation processed with leukocyte reduction and without leukocyte reduction.

ANSWER:
Please note, that leucodepletion of all red cells and platelets is mandatory in Europe for all countries since many years.
The number of febrile transfusion reactions has decreased since significantly after Introduction of universal leucodepletion (see f.ex. *M. M. Mueller et al.,* "Clinical impact of leucocyte depletion – what is the evidence?"; Science series (2008), 3: 85-90).
To avoid GvHD, blood products are still gamma-irradiated. However, it is expected, that platelet pathogen reduction technologies, including the THERAFLEX UVC-treatment by MacoPharma, will substitute gamma-irradiation of platelet concentrates.

3. **Test:**
The existing tests and/or processes, such as leukocyte filtration and NAT, which can be omitted after inactivation implementation.

ANSWER:

In the different countries where the process is implemented the quarantine storage is omitted. This leads to be,tter logistics, reduced storage space, and immediate provision of the plasma.
Neither leukocyte filtration nor any at the time of implementation of the MB procedure used test for infection markers was stopped in any country. Nevertheless, the implementation of new tests or tests with enhanced sensitivity, like single NAT was also not done after introduction of the MB procedure.

Plasma:
In Europe the THERAFLEX MB-Plasma Procedure substitutes leucodepletion of plasma, since the membrane 0.65 µm filter is more efficient than a plasma leucode-pletion filter.

Platelets:
We expect, that the THERAFLEX UVC-Platelet Procedure will substitute in the future both gamma-irradiation as well as bacterial screening.
We do not recommend any substitution of viral NAT-Testing for the moment

4. **Post marketing surveillance:**
   (*Specify the actual condition of the PMS including total number in each country*):

   ANSWER:
   Post market surveillance is done by Blood Transfusion Services and by Hemovigilance procedures installed in all European Countries with most experience available in the U.K. and in France.
   All European countries have ongoing Hemovigilance programmes for all components, which includes MB-treated plasma.
   Additional, we have follow-up studies (like Phase IV Clinical Study on MB-Plasma In France) or Publications on clinical use of *Politis et al.* [Vox Sanguinis, (2007); Volume 92,Issue 4, Pages: 319-326], as already mentioned in our last letter to you dated 31.3.08, under point 4.b).

We do hope to have answered clearly to all your questions arising on April 24th, 2008.
If something is still unclear, please do not hesitate to approach us.

Sincerely yours,

MacoPharma International GmbH

Dr. Wolfram H. Walker
- Scientific Director -

Dr. Stefan Reichenberg
- Project Manager Pathogen Inactivation -

Copy:
- Mr. Gus Ribeiro, General Manager, MacoPharma Asian / Pacific Region;
- Mr. Hirotaka Nagase, AMCO Inc., Tokyo / Japan;
- Mr. Kenzo Watanabe, AMCO Inc., Tokyo / Japan

BCT　Japan 株式会社回答

４月８日運営委員会･安全技術調査会合同委員会後

追加質問事項２（各社共通）

１．EU主要国等、各社の不活化技術を導入しているそれぞれの国における供給の実態。輸血用血液製剤の全供給数（不活化技術を適用している製剤と適用していない製剤の合計）、そのうち不活化技術を適用している製剤の割合は何％か。また、供給はどのように行われているか（供給先、医師の希望により供給できるのか等）。

さまざまな病原体不活化技術 Pathogen Reduction Technologies（PRT）が開発されており、ヨーロッパでは現時点で通常使用されたり、さまざまな評価段階であったりしています。

－3 種類の血漿処理のできる PRT 処理方法が現在市場で販売されています。
Solvent Detergent 処理（1990 年代初期からいろいろな国で既に使用中）
Methylene Blue（2001 年以降市場に出て、フランス、スペイン、イタリア、英国で通常使用）
Intercept Blood System（2006 年以降 CE マーク取得：通常使用についてはは未確認）
Milasol PRT System は、2008 年に血漿処理で CE マークを取得予定です。

－2 種類の血小板処理のできる PRT 法が現在販売されています。
Intercept Blood System（2002 年の CE マーク取得以降、主にフランス、スペイン、ベルギー、ノルウェーとスウェーデンの血液センターで限定的に使用中）
Mirasol PRT System（2007 年後半に CE マーク取得済み。現在アイルランド、スペイン、イタリアと中東諸国のいくつかの血液センターで通常使用に向けた評価試験を実施中）

全体として、ヨーロッパでは輸血に使用されるすべての血小板製剤の 5%に PRT 処理が施されており、これらの製剤への PRT の使用は明らかに増加傾向があると我々は考えます。とりわけ血小板製剤について高い関心および使用を予定している国々には、フランス、ベルギー、アイルランド、スペイン及び中東諸国があります。

PRT 処理済みの製剤の供給および配送に関しては、PRT 処理方法の違いにより物流方法も異なります。Mirasol PRT での血小板と血漿の処理は短時間（処理時間は 15 分以内）で、しかも簡単に処理ができることから、血液センターの通常の血液成分製剤の製造

工程の一部として組み込むことができます。 血小板の出荷直前でも PRT 処理が可能であり、血液センターは PRT 処理済みの製剤を医療機関からの需要に合わせて供給することが可能になります。

病原体不活化技術（PRT）の幅広い利益を考えますと、すべての患者様は PRT 処理済みの血液製剤から利益を享受するものと考えます。したがって、最終的にはすべての血液製剤が不活化処理されることでしょう。しかし、専門家の先生方によれば、各病院には患者様のために PRT 処理された製剤を求める緊急度、もしくは求める能力に違いがある場合があるようです。そのため各血液センターは、当面各病院のニーズに合わせて血小板の在庫の一定の部分にのみ PRT 処理を行う必要があるでしょう。

２. 各社の不活化技術を適用する前の製剤に白血球除去を行った場合と、不活化技術を適用した製剤に白血球除去を行わなかった場合を比較して、GVHD等の副作用の推移はどのようになっているか。

私達の知る限りでは、輸血後GVHD予防に関連して白血球除去対非白血球除去製剤の不活化技術の有効性について、直接比較したヒューマンデータはありません。Mirasolで不活化処理したヒトの白血球（単核球）を、遺伝的に処理した免疫不全マウス（輸血後GVHDのモデルとして）に注入した動物実験の結果は、白血球除去が施されていない血液製剤に適用された場合、輸血後GVHDの予防においてMirasol不活化処理が有効であることを間接的に示しています。
さらに、PRTの功績に関する最近の出版物の中で、Dr. H. Alter（米国　国立衛生研究所）は、PRTを実施する場合、白血球除去を行わないという選択はコスト削減の戦略の一つとなり得る、と示唆しています。白血球除去が施されていない製剤に適用されるMirasol不活化技術のさらなる確立に向けて、様々な研究が現在計画されています。

既に白血球除去が施された製剤にPRT病原体不活化技術が適用される場合の輸血後GVHDの予防という観点では、実際の臨床経験は既にInterceptの研究で示されており、Mirasol PRTにおいても同様に入手可能となりつつあります。MIRACLE（Mirasol臨床評価）、これはランダム比較臨床試験ですが、その一環で、Mirasol処理された製剤の大多数(160/168[4])は輸血前にガンマ線照射は施されておらず、輸血後GVHDの危険性があると見なされた患者グループでは、リファレンスの輸血製剤（Mirasol処理されていない製剤）の大多数(122/166)にガンマ線照射が施されています。どちらの患者グループからも輸血後GVHDは報告されておりません。MIRACLEトライアルの最終結果は、今年の後半に提示される予定です。

3. EU主要国における不活化技術導入後不要になった技術（白除、NAT等）

この 20 年間もしくはそれ以上の年月の間に、血液安全性を主導する技術は数多く導入されてきました。その技術とは、ガンマ線照射、白血球除去、新しいマーカーに対する血清学検査、現存のマーカーに対する改良された血清学検査、NAT、バクテリア・スクリーニングです。一度検査が導入されると、通常はその検査を撤廃することはできません。一つの例外は P24 抗原分析の撤廃で、HIV 検査用の NAT 導入に伴い FDA により許可されました。これは、大規模な多施設臨床試験での入念な分析により達成されました。

この 20 年余りの間に導入されたこれら技術の多く、あるいは幾つかの技術は、長い年月をかければ、PRT により置き換えることは可能と考えられます。血液の安全性および安定供給に関するアドバイザリーコミッティー（米国　保健社会福祉省）は、ガンマ線照射とバクテリア検査はその候補になり得ることを示唆しています。さらに、過去 10 年以上の間に、NAT 検査のプールサイズは著しく減少し、10 以下となりました。全ての血液製剤において PRT が一般的に導入されると、各国政府はコスト削減に関連して、NAT 検査のプールサイズを以前のような大きさに戻すことを考慮し始めるかもしれません。

技術の撤廃や修正を行っていくにあたり、市販後調査や臨床試験の実施は必要となりましょう。

4. EU主要国における不活化技術を適用している製剤の市販後調査の実態（調査数）

Navigant Biotechnologies LLC 社と Gambro BCT 社は、Mirasol PRT 処理を行った製品の輸血の安全性を継続的にモニターするという市販後調査の実施を約束しています（CE マークの承認の一部として）。2007 年 11 月にヨーロッパ、中東各国とアフリカで Mirasol PRT System が公式に導入されて以降、各施設で Mirasol PRT 処理を行った 100 以上の血小板製剤の輸血に関しての安全性データが収集されており、今後もこの活動は継続されます。さらに、輸血業務における Mirasol PRT 処理製剤の臨床使用に関する重要なデータを記録することを目的に、電子データ収集システムが開発されました。最後になりましたが、我々は Mirasol の不活化処理に関心の深い国々の政府機関と協力し、各国の必要に応じた適切な安全性のモニタリングが確実に実施されるよう作業をすすめとおります。

（参考）BCT Japan 株式会社回答（英語）、参考資料（文献2報）

Additional questions after the Joint Session of April 8 (same questions to each companies)

1. Update the current use status of each major EU nations where pathogen reduction technology is known to be available： Total quantity of blood products for transfusion (ratio of the total number of PRT-Pathogen Reduction Products- supplied vs. non PRT).　How PRT products are supplied and distributed? (i.e. can they supply PRT based on the demands from physicians or end users?)

Various Pathogen Reduction Technologies (PRT) have been developed and are in various stages of evaluation and/or routine use in Europe.
- Three PRT methods are currently marketed for treatment of plasma: Solvent Detergent (in use in various countries since early 1990's[1]), Methylene Blue (marketed since 2001 and routinely used in France, Spain, Italy, UK), and the Intercept Blood System (CE marked since 2006; status of routine use unknown). The Mirasol PRT System is expected to receive CE mark for treatment of plasma in 2008.
- Two PRT methods are being marketed for treatment of platelet concentrates: the Intercept Blood System (CE Marked since 2002 and in routine use in a limited number of blood centers primarily in France, Spain, Belgium, Norway, and Sweden), and the Mirasol PRT System (CE Marked since late 2007 and currently under evaluation for routine implementation in several blood centers in Ireland, Spain, Italy, and the Middle East).

Overall, we believe that approximately 5% of all platelet products transfused in Europe are PRT-treated, with a clear trend towards an increasing use of PRT for these products. Countries with a high interest in and/or commitment to PRT for platelets include France, Belgium, Ireland, Spain, and various countries in the Middle-East.

In terms of supply and distribution of PRT-treated products, the logistics vary by PRT method. Because the Mirasol PRT procedure for platelets and plasma is quick (<15 min. total processing time) and easy to perform, the treatment may be conducted as part of the routine component manufacturing process in the blood center, or may be performed immediately prior to issue of platelets and so allow a blood center to supply PRT-treated components on demand. Given the broad benefits of PRT, all patient groups would benefit from receiving PRT-treated components, therefore, ultimately universal conversion to PRT is likely. However, expert opinion suggests that different hospitals may have a different sense of urgency and/or ability to request PRT treated products for their patients, hence blood centers may initially be required to perform PRT on a portion of their platelet inventory to meet various

[1] Pelletier et al., Best Practice and Research Clinical Haematology (Elsevier), Vol 19: 205-242, 2006

hospitals' specific needs.

2. Is there any difference in the frequency of the occurrence of the transfusion related side effects such as GVHD by the use of the pathogen reduced product manufacturing methods when leukocyte reduced products and non leukocyte reduced products are compared.

To our knowledge, no human data are available providing a direct comparison of the effectiveness of PRT treatment of leukoreduced vs. non-leukoreduced components in preventing transfusion-associated (TA-) GVHD. Results from an animal model in which human white blood cells (mononuclear cells) treated with the Mirasol PRT System were injected into genetically immune-deficient mice (as a model for TA-GVHD), indirectly support the effectiveness of the Mirasol PRT system in preventing GVHD when applied to non-leukoreduced blood components[2]. Additionally, in a recent publication[3] on the merits of PRT, Dr. H. Alter (National Institute of Health, US) indicated that discontinuation of leukoreduction was one of several potential cost-saving strategies when implementing PRT. Various studies are planned to further establish the Mirasol system performance when applied to non-leukoreduced components.

In terms of prevention of TA-GVHD when applying PRT to already leukoreduced components, actual clinical experience has been presented for the Intercept system, and is now becoming available for the Mirasol PRT system as well. As part of the MIRACLE (Mirasol Clinical Evaluation) trial, a randomized controlled clinical trial, the majority (160/168[4]) of Mirasol-treated products were not gamma-irradiated prior to transfusion, whereas the majority of reference (untreated) products (122/166) were treated with gamma-irradiation, confirming that the patient group studied was considered at risk for TA-GVHD. There were no reports of TA-GVHD in either patient group. Complete results from the MIRACLE trial will be presented later this year.

3. Is there any technology becomes obsolete after introduction of Pathogen Reduction Technology?

There are a number of blood safety initiatives that have been introduced over the last 20 or more years: Gamma irradiation, leukocyte reduction, serology testing for new markers, enhanced serology testing for existing markers, NAT testing, Bacterial testing. Typically, once a test is introduced it has not been allowed by regulators to be removed; one exception is the dropping of P24 antigen assay that was allowed by the FDA upon the introduction of NAT testing for HIV. This was achieved through careful analysis of a large multi-center clinical trial.

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[2] Fast, et al., *Transfusion* 2006; 46: 1553-1560.

[3] Alter, *Transfusion Medicine Reviews*, Vol 22(2): 97-102 (relevant statement on bottom of page 100)

[4] Data submitted to Notified Body (KEMA). Based on this data the Mirasol System was granted CE Mark for application to platelets on October 5, 2007.

Potentially many or some of these technologies introduced over the last 20 years could over time be replaced by PRT. The indications of the Advisory Committee on Blood Safety and Availability (Department of Health and Human Services, US)[5] suggested that Gamma irradiation and Bacterial testing were likely candidates. Additionally, over the last 10 years the pool size for NAT testing has decreased significantly down to < 10. With the general introduction of PRT for all components, countries may consider a return to larger pool sizes for NAT testing with the associated reduction in costs.

For any of the technologies to be dropped or their use to be modified it is likely that Post Market surveillance and clinical trials will need to be conducted.

4. The investigation status on the post market approval status on the Pathogen Reduced Blood Products in the major EU nations. Reality of the post market approval investigation (number of investigated cases).

Navigant Biotechnologies, LLC and Gambro BCT Inc. have a commitment (as part of the CE Mark certification) to conduct Post-Market Surveillance studies to continue to monitor the safety of Mirasol-treated products transfused on a routine basis. Since the Mirasol PRT system was officially launched in Europe, the Middle East and Africa in November 2007, safety data have been collected on over 100 routine transfusions of Mirasol-treated platelet products at multiple sites, and this activity continues. Additionally, an electronic data capture system has been developed to allow transfusion services to record important data regarding the clinical use of Mirasol-treated products. Finally, we will be working with the authorities of countries interested in adopting the Mirasol process to ensure adequate safety monitoring is in place according to local country needs.

Updated response to Question (4) from initial Q&A document:

(4) Reaction of the medical agent with other medical agent and its issues.

There have been no conclusive reports from in-vivo studies on drug interactions that would raise any concerns related to the use of Mirasol. The information initially provided to MHLW regarding a possible interaction of Riboflavin with Tetracycline and Trimethoprim-sulfamethoxazole was based only on a review of the literature in which primarily in-vitro studies suggested some potential effect with Riboflavin solutions. A detailed report on this matter, specific to the Mirasol application, is being prepared by independent toxicology experts and will be made available upon request.

[5] Thirty-third meeting of the ACSBA meeting, Washington, DC, January 9-10.

Mirasol PRT treatment of donor white blood cells prevents the development of xenogeneic graft-versus-host disease in Rag2$^{-/-}\gamma c^{-/-}$ double knockout mice

Loren D. Fast, Gilbert DiLeone, Gene Cardarelli, Junzhi Li, and Raymond Goodrich

BACKGROUND: Mirasol PRT (Navigant Biotechnologies) treatment utilizes exposure to light in the presence of riboflavin to introduce irreparable lesions to nucleic acids thereby inhibiting pathogen and WBC replication. The ability of Mirasol PRT–treated mononuclear cells (MNCs) to generate xenogeneic graft-versus-host disease (GVHD) responses was used to model transfusion-associated GVHD (TAGVHD).

STUDY DESIGN AND METHODS: Pairs of human MNCs from five different individual donors that had or had not received Mirasol PRT treatment and contained 30×10^6 CD3+ cells were injected intraperitoneally into sublethally irradiated (350 cGy) Rag2$^{-/-}\gamma c^{-/-}$ double-knockout mice. Recipient mice were weighed and observed regularly and euthanized when they exhibited symptoms of GVHD or at termination of the experiment. Recipient lymphoid compartments were collected and phenotyped for the presence of human lymphoid cells. The presence of human cytokines and/or immunoglobulins in the recipient plasma was also used to detect the presence of human cells.

RESULTS: Twelve of 14 mice injected with untreated cells developed xenogeneic GVHD, whereas 0 of 14 mice injected with Mirasol PRT–treated cells developed xenogeneic GVHD. End-stage xenogeneic GVHD in the recipients of untreated cells was characterized by the presence of splenic human cytolytic CD4+ and CD8+ cells, with high levels of interferon-γ, interleukin-10, and xenoreactive antibodies in the plasma.

CONCLUSION: Mirasol PRT treatment of the donor MNCs abolished xenogeneic GVHD responses, indicating that the use of Mirasol PRT treatment of blood products should prevent the development of TAGVHD.

The presence of white blood cells (WBCs) in transfused blood products is a significant contributor to the immunologic consequences of transfusion.[1,2] The immune responses resulting from transfusion include donor antirecipient responses such as transfusion-associated graft-versus-host disease (TAGVHD), graft-versus-tumor responses, and production of cytokines. Recipient antidonor responses can be induced by direct presentation of antigen by donor WBCs or indirectly after processing of the donor cells by recipient antigen-presenting cells.[3] These responses include elimination of donor cells, production of alloantibodies, and the induction of immunoregulatory immune responses that result in increased infection, increased risk of tumor relapse, and increased survival of transplanted organs. Because TAGVHD is almost always fatal, the emphasis has been placed in the development of protocols that prevent TAGVHD. The severity of TAGVHD has made it impossible to design clinical trials to test the ability of different protocols to inhibit the development of TAGVHD. As a result, models of human in vivo immune responses are being developed to test the effectiveness of various protocols in preventing TAGVHD.

ABBREVIATIONS: PBST = phosphate-buffered saline containing 0.5 percent Tween 20; TAGVHD = transfusion-associated graft-versus-host disease.

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TRANSFUSION 2006;46:1553-1560.

Although a number of in vitro assays have been developed to measure the functionality of human WBCs, it would be useful to develop a model that more closely mimics the in vivo responses resulting in TAGVHD. Recent studies have utilized the xenogeneic GVHD responses of human cells when injected into immunodeficient murine recipients as a model of TAGVHD.[4,5] Initial studies found that human WBCs injected into SCID mice lacking both T and B lymphocytes were rejected by the murine NK cells and macrophages still present in these SCID mice especially following intravenous injection of the human mononuclear cells (MNCs).[6-9] Depletion of murine NK cells or macrophages from the SCID mice before injection of the human cells resulted in improved engraftment of intraperitoneally injected cells. If a sufficient number of cells were injected, the human lymphocytes were able to overcome the recipient mediated rejection and attack the recipient resulting in xenogeneic GVHD.[5] Recent studies have shown that the use of Rag2$^{-/-}\gamma c^{-/-}$ double-knockout mice that lack B, T, and NK cells as recipients accelerated the xenogeneic GVHD response as a result of increased human T-cell engraftment.[4]

Several different approaches are being developed for inactivation of pathogens that could be present in blood products. Mirasol PRT technology (Navigant Biotechnologies, Lakewood, CO) accomplishes pathogen inactivation by introducing irreparable nucleic acid lesions by exposure to light in the presence of riboflavin.[10] An initial study found that riboflavin plus light exposure functionally inactivated WBCs when the WBCs were tested with a panel of in vitro assays.[11] The ability to generate xenogeneic GVHD responses was utilized to test the functional ability of Mirasol PRT–treated WBCs in vivo and as a surrogate indicator for the efficacy of this treatment on TAGVHD prevention. Pairs of Mirasol PRT–treated or control untreated WBCs were injected into sublethally irradiated Rag2$^{-/-}\gamma c^{-/-}$ double-knockout mice, and the development of xenogeneic GVHD was monitored by regular observation of the mice and further immunologic analysis was conducted when the mice exhibited symptoms of GVHD or at the termination of the experiment.

MATERIALS AND METHODS

Preparation and characterization of human WBCs

WBCs were obtained from the leukoreduction chamber of an automated blood collection machine (Trima, Gambro BCT, Lakewood, CO) after standard apheresis collection of platelets (PLTs) from five different volunteer donors. The cells were separated into the MNC fraction with Ficoll-Hypaque (Pharmacia, Piscataway, NJ) discontinuous centrifugation and then placed equally into two PLT bags containing autologous plasma. The test cells received the Mirasol PRT treatment in 10 to 15 minutes after cell preparation as described.[11] Control cells received no treatment. The treated and untreated cells were then washed three times with phosphate-buffered saline (PBS) containing 0.1 percent fetal calf serum (FCS) and resuspended in RPMI 1640 containing 10 percent FCS. The cell populations were sent via overnight courier to Rhode Island Hospital. Upon receipt, the cell populations were assessed for number and viability with trypan blue staining (all cells were viable). The cells were also stained with a panel of antibodies to CD45, CD3, CD14, CD19, and CD56 (BD Biosciences, San Jose, CA) as previously described[11] to characterize the distribution of WBC subpopulations present in each of the treated and untreated donor populations.

In addition, the ability of the donor cells to proliferate in response to mitomycin C–treated allogeneic peripheral blood mononuclear cells (PBMNCs) as previously described[12] or to mitomycin C–treated xenogeneic C57BL/6 spleen cells was tested in triplicate. The xenogeneic MLC was set up by mixing 100 µL of donor MNCs at 4×10^6 MNCs per mL with 100 µL of mitomycin C–treated C57BL/6 spleen cells at 4×10^6 cells per mL in MLC medium.[13] The cells were cultured for 5 days, and then the cells were pulsed with 1 µCi of [^{3}H]thymidine for 4 hours before harvesting the wells to measure incorporation of the [^{3}H]thymidine as a measure of proliferation.

Mice

Six- to eight-week-old Rag2$^{-/-}\gamma c^{-/-}$ double-knockout mice were obtained from Taconic (Germantown, NY). These mice were housed in the special suite for immunodeficient mice in the Central Research Facility at Rhode Island Hospital. C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME).

Analysis of GVHD response

The recipient mice received 350 cGy gamma irradiation the night before the injection of cells. Each recipient mouse was injected intraperitoneally with a treated or untreated cell population containing 30×10^6 CD3+ cells from a single donor (three mice per group). Recipient mice were weighed twice per week and observed regularly. One recipient mouse in the untreated group developed an inner ear problem and was euthanized because it was unable to get its head off the bedding. One recipient mouse in the treated group died unexpectedly without any symptoms of GVHD as evidence by weight loss or splenomegaly when autopsied. There was no evidence suggesting the death was related to injection of treated cells. Recipient mice that demonstrated a dramatic weight loss (usually >20%) and exhibited lethargy, hunched posture, and ruffled fur were euthanized. Blood was collected by cardiac puncture with a heparinized syringe. The blood

was centrifuged, the hematocrit (Hct) level was recorded, and the plasma collected and stored at –20°C. The buffy-coat cells were collected, and the remaining RBCs were lysed with RBC lysis solution (Gentra, Minneapolis, MN). In addition, spleen cells were obtained by dissociating the spleen in PBS after weighing the spleen, marrow cells were obtained by flushing marrow from the femurs with PBS containing 1 percent BSA, and cells were obtained from intestinal lymphoid tissue by dissociation in PBS. The liver MNC population was obtained from the dissociated liver cells by centrifuging the cells over a Ficoll-Hypaque discontinuous gradient and collecting the MNCs at the interface and washing them twice with PBS. All mice that did not exhibit GVHD symptoms were euthanized by Day 63 and a similar analysis to that described above was conducted on these recipient mice.

Analysis of human cell chimerism

Cells from the various tissues were initially stained with PECy5 or PE anti-human CD45 or isotype controls (BD Biosciences) and then analyzed immediately for the presence of human CD45+ cells on a flow cytometer (FACScan, BD Biosciences). If the number of CD45+ cells present were equal to or less than the number of positive cells in the isotype control, the result was recorded as 0.0. If human CD45+ cells were detected and sufficient cells were present in a cell population, a second battery of staining was done in which the expression of WBC subpopulation markers including CD3, CD4, CD8, CD14, CD19, and CD56 (BD Biosciences) was measured. The populations containing T cells were also stained for the presence on CD107a on the cell surface, an indicator that the cells were experienced cytolytic cells because this marker is expressed on the cell surface after granule exocytosis.[14,15]

Measurement of cytokines

The level of cytokines in the plasma samples were measured with the CBA human TH1/TH2 kit I and the CBA human inflammation kit according to manufacturer's instructions (BD Biosciences).

Measurement of immunoglobulin levels

Ninety-six well flat-bottom microtest plates (BD Labware, Franklin Lakes, NJ) were coated with goat anti-human IgG-IgM-IgA-light chains(Biosource, Camarillo, CA) at 5 µg per mL in 0.1 mol per L bicarbonate buffer, pH 9.6. The plates were incubated for 4 hours at room temperature and then stored at 4°C until used in the assay. On the day of assay, the plate was washed three times with Dulbecco's PBS containing 0.5 percent Tween 20 (PBST). Human IgG and IgM standards were prepared from purified IgG and IgM (Calbiochem, La Jolla, CA). Serial twofold dilutions of the standards were prepared in PBST starting at 500 ng per mL and ending with 4 ng per mL. The standards or plasma diluted 1:4 or 1:8 were added to designated wells (100 µL) and incubated at room temperature for 2 hours. After the plate was washed three times with PBST, 100 µL of horseradish peroxidase–goat anti-human IgG or IgM (Biosource) diluted 1:16000 in PBST were added. The plates were incubated for 2 hours at room temperature. After washing 3× in PBST, 100 µL of 1× 2,2'-azinobis-(3-ethylbenzothiazoline sulfonate (Zymed, San Francisco, CA) diluted in 0.1 mol per L citrate buffer containing 0.3 percent hydrogen peroxide was added to all wells. The plates were read at 405 nm on a enzyme-linked immunosorbent assay (ELISA) plate reader after a 20-minute incubation at room temperature. The levels of IgG and IgM in the plasma samples were determined by comparison to the values obtained for the standard curves for IgG and IgM.

RESULTS

The paired Mirasol PRT–treated and control untreated donor MNCs were stained with trypan blue upon receipt to measure viability. All cell preparations were found to be 100 percent viable. The cells were then stained with a panel of antibodies to define the distribution of different subpopulations in these donor WBC populations. Although there was variation in the donor cell subset distribution from donor to donor, no differences in the number of CD3+ cells were observed when untreated ($62.9 \pm 8.3\%$) were compared to treated groups ($64.3 \pm 10.6\%$). The donor MNCs were also characterized by testing their ability to proliferate in response to allogeneic and xenogeneic murine stimulator cells. The results (Fig. 1) show that control donor cells that had not received Mirasol PRT treatment were able to proliferate in response to both allogeneic and xenogeneic stimulator cells and these proliferative responses as well as nonstimulated responses were completely abrogated by Mirasol PRT treatment.

Recipient $Rag2^{-/-}\gamma c^{-/-}$ double-knockout mice that had received sublethal irradiation (350 cGy) the evening before were injected intraperitoneally with a treated or untreated donor cell populations from individual donors (n = 5) that contained 30×10^6 CD3+ cells. Recipient mice were euthanized when they demonstrated symptoms of xenogeneic GVHD such as more than 20 percent weight loss (Fig. 2), hunched posture, and ruffled fur or when the experiment was terminated. Assessment of clinical parameters in recipient mice including spleen weight, Hct, and the presence of human CD45+ cells in various lymphoid compartments indicated that 12 of 14 recipient mice injected with untreated cells displayed symptoms of xenogeneic GVHD and 12 of 13 expressed varying degrees

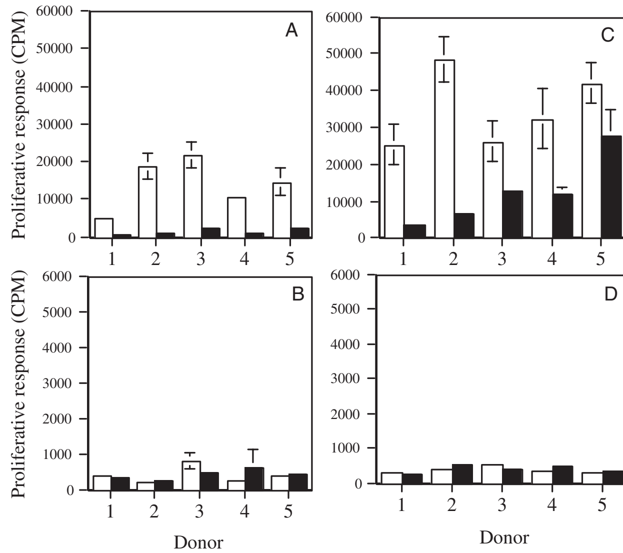

Fig. 1. Proliferative responses of Mirasol PRT–treated and untreated donor cells to allogeneic or xenogeneic stimulator cells. Five different paired sets of MNCs that had received Mirasol treatment (B and D) or no treatment (A and C) as responders were incubated with mitomycin C–treated allogeneic PBMNCs (A and B, ☐) or xenogeneic C57BL/6 spleen cells (C and D, ☐) or medium alone (■) for 5 days, and [^{3}H]thymidine incorporation was measured on Day 5.

of human cell chimerism (Table 1). In addition to exhibiting human cell chimerism, the recipients injected with untreated donor cells also exhibited increased spleen weight (splenomegaly) and significantly decreased Hct levels (Table 1).

In the cell populations from the recipient mice that contained sufficient numbers of human CD45+ cells to analyze, samples of cells were stained with a panel of antibodies to define the distribution of various WBC subpopulations in the lymphoid compartments. The results (Table 2) demonstrated that human T cells were the cells that were primarily present in the spleen, blood, and marrow. B cells were primarily found in the liver and intestinal lymphoid tissue. With the exception of one recipient mouse in which CD56+ cells were observed, no reconstitution with CD14+ cells (macrophages) or CD56+ cells (NK cells) was observed in any lymphoid compartment.[4] The cells were also stained with antibodies to CD4 and CD8 and the ratio of the percentage of T cells that were CD4+ to the percentage of T cells that were CD8+ T cells was determined. The CD4:CD8 ratios in the recipients of untreated cells from the different donors varied from 0.38 ± 0.08 to 1.82 ± 0.8 in a donor-dependent fashion.

Acute GVHD and especially TAGVHD are characterized by cytolytic responses. To determine whether the human T cells found in these mice exhibited properties of cytolytic cells, the T-cell subsets from a subset of recipients were dual-stained with anti-CD107a, a marker of cells

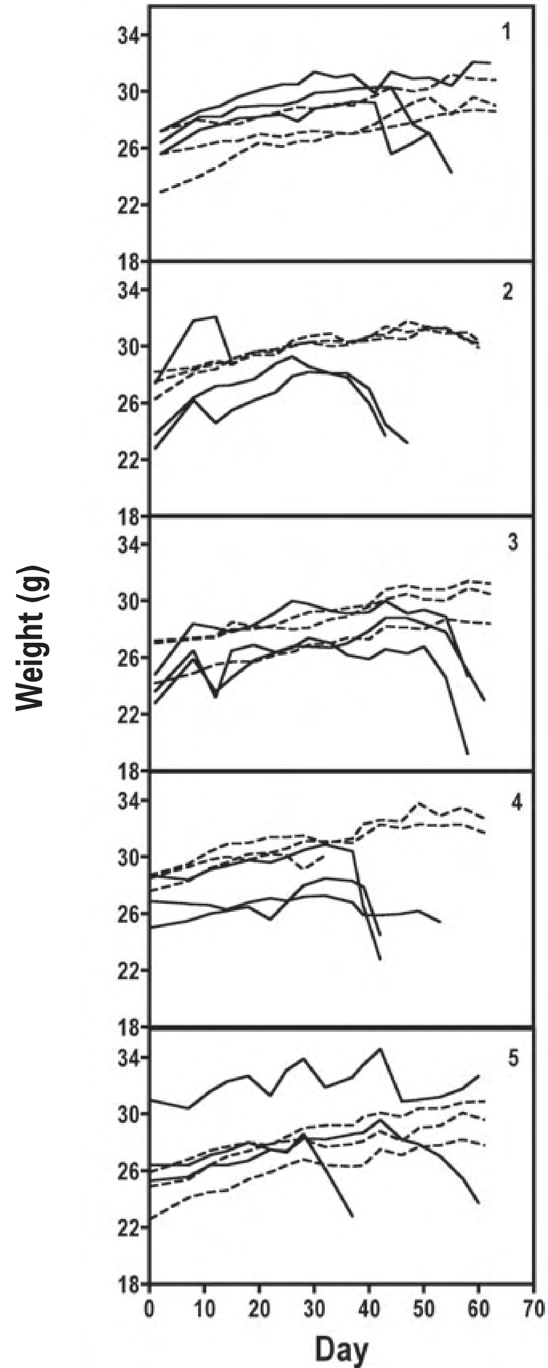

Fig. 2. The weight of recipient mice injected with untreated or Mirasol-treated donor cells. The weights (g) of recipient mice (3 mice per group) injected on Day 0 with Mirasol-treated (- - -) and untreated (—) cells from each of five donors (Panels 1-5).

that have previously mediated exocytosis of cytotoxic granules.[14,15] This staining procedure identified 41.1 ± 16.7 percent of human CD4+ cells (n = 8) and 45.5 ± 20.2 percent of human CD8+ cells (n = 8) as expressing CD107a. This would suggest that both CD4+ and CD8+

TABLE 1. Characterization of immune responses and reconstitution in mice injected with untreated or Mirasol PRT–treated MNCs

Parameter	Untreated donor cells	Treated donor cells
Number of mice with GVHD symptoms	12/14*	0/14*
Day euthanized	51.8 ± 8.2 days	Not applicable†
Number of mice with human cells	12/13‡	0/14
Spleen weight (gram)	0.27 ± 0.27	0.07 ± 0.07
Hct (%)	27.9 ± 16.9	53.9 ± 2.9
Percentage of CD45+ cells in		
Spleen	26.8 ± 19.8	0.0
Blood	4.6 ± 4.7	0.0
Marrow	4.9 ± 5.6	0.0
Intestinal lymphoid tissue	58.4 ± 28.4	0.0
Liver	20.3 ± 20.7	0.0

* One mouse was lost from a total of 15 recipient mice for unrelated reasons as detailed under Materials and Methods.
† None of the recipient mice in this group exhibited GVHD symptoms so mice were euthanized at the end of the experiment.
‡ One mouse that exhibited GVHD symptoms died before analysis could be conducted.

TABLE 2. The human lymphocyte subpopulations present in recipient lymphoid compartments

		Total number of human CD3+/CD19+ cells ($\times 10^6$)*				
Donor	Mouse	Spleen	Blood	Marrow	Liver	Intestinal
1	4	65.0/0.0	0.3/0.0	5.4/0.0	3.2/8.8	0.0/0.0
1	5	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
1	6	0.4/0.2	0.01/0.06	0.03/0.4	1.5/6.1	0.7/0.2
2	10	2.8/0.02	0.04/0.1	0.1/0.2	0.2/1.1	0.1/0.5
2	12	1.5/0.4	0.2/0.6	0.7/0.7	0.3/1.2	0.7/5.3
3	16	0.08/0.4	0.06/0.07	0.1/0.2	0.2/2.1	0.0/0.0
3	17	0.09/0.4	0.04/0.06	0.1/0.1	0.5/2.7	0.04/0.4
3	18	0.04/0.01	0.02/0.1	0.08/0.1	0.1/0.5	0.0/0.0
4	22	1.3/0.4	0.1/0.02	0.2/0.1	2.0/6.6	0.4/0.01
4	23	4.2/0.3	0.2/0.01	0.2/0.01	0.0/0.0	0.3/0.01
5	28	5.7/0.7	0.5/0.5	0.9/0.09	5.5/5.3	0.5/0.6
5	29	3.5/0.2	0.2/0.05	1.8/1.0	0.0/0.0	0.1/1.0
5	30	4.3/0.09	0.3/0.0	0.4/0.0	0.4/1.1	0.0/0.0

* Each number is the product of the cell recovery in millions for each lymphoid compartment and the percentage of human CD3+ or CD19+ detected by flow cytometric analysis in each population.

cells have mediated cytolytic activity via granule exocytosis. CD4+CD25+ have been recently shown to contain granzymes and mediate cytolytic activity against autologous cells.[16,17] Staining of a limited number of samples with anti-CD25 found that less than 5 percent of the CD4+ cells expressed CD25, suggesting that the human CD4+ cells present in the spleen were not T regulatory cells.

Measurement of the levels of human cytokines in the plasma of the recipient mice was used as another approach to assess the possibility that human cells were present but not located in the lymphoid compartments. The plasma samples from recipient mice that had been injected with Mirasol PRT–treated cells did not contain any human cytokines. In contrast, the plasma samples of mice injected with untreated donor cells (n = 13) contained very high levels of interferon-γ (IFN-γ); high levels of interleukin (IL)-10; low levels of IL-5, IL-1β, tumor necrosis factor-α (TNF-α), IL-8, and IL-12p70; and no IL-2 or IL-4 (Fig. 3).

The measurement of human immunoglobulin levels in the plasma was a third approach to measure the presence of human lymphoid cell chimerism. An ELISA to measure the levels of human IgG and IgM in the plasma of the recipient found that human immunoglobulins were only detected in the plasma of recipients injected with untreated cells (Table 3). To test for the specificities exhibited by the human immunoglobulin, C57BL/6 spleen cells were stained with a 1:10 dilution of the recipients' plasma followed by fluorescein isothiocyanate–labeled anti-human IgM or IgG. Flow cytometric analysis of WBCs, RBCs, and PLTs was conducted by gating on the different sized populations. The results of this experiment (Table 3) indicated that there were high levels of IgM antibodies and lower levels of IgG antibodies binding to murine RBCs and PLTs with very low levels of antibodies binding to WBC.

DISCUSSION

Mirasol PRT is a novel technology for pathogen reduction that has been shown to effectively preserve PLT viability in a PLT recovery and survival clinical trial.[18] The validation clinical trial for its efficacy and safety in thrombocytopenic patients has just been initiated in Europe. During Mirasol PRT treatment, blood products such as PLT concentrates are exposed to light in the presence of riboflavin resulting in inactivation of a wide range of pathogens.[19-23] Because this process introduces irreparable lesions on nucleic acids,[20] the treatment was also expected to affect the function of WBCs. In a previous study, it was shown that Mirasol PRT treatment of WBCs prevented their ability to be activated, to proliferate in response to various stimuli, and to induce proliferation of normal PBMNCs.[11] The conclusion of these studies was that Mirasol PRT treatment caused a complete functional inactivation of WBCs. Several studies had previously shown that inhibition of in vitro proliferative responses correlated with an inability to generate GVHD.[13,24] To confirm the inactivation of WBC by Mirasol PRT treatment observed with in vitro assays also prevented in vivo GVHD responses, the ability of Mirasol PRT–treated cells to induce xenogeneic GVHD responses when injected into $Rag2^{-/-}\gamma c^{-/-}$ double-knockout recipient

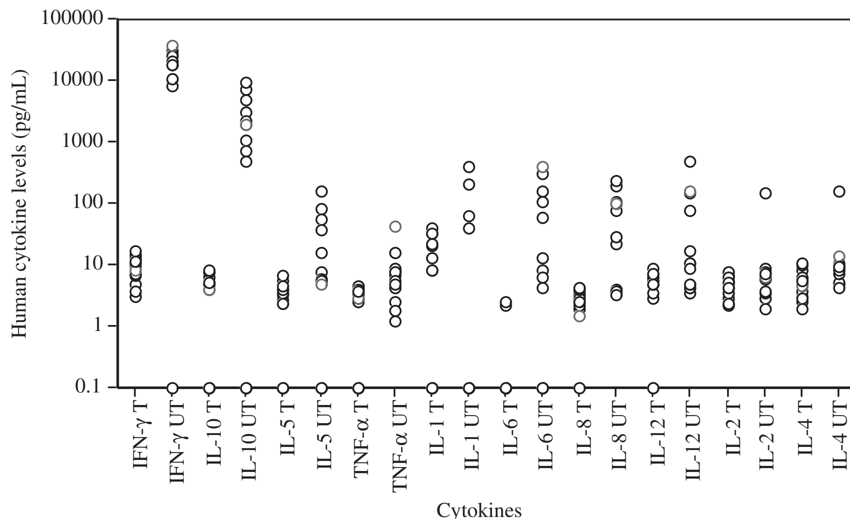

Fig. 3. Human cytokine levels in recipient mice plasma. Plasma samples obtained from recipient mice injected with Mirasol-treated cells (T) or untreated cells (UT) were tested for the concentrations of the indicated cytokines. The data show the results when undiluted plasma was tested except for the levels of IFN-γ and IL-10, which were obtained with plasma diluted 1:10. Samples in which no cytokine was detected were recorded as 0.1 pg per mL.

TABLE 3. Amount and specificity of human immunoglobulin present in the plasma of recipient mice injected with untreated MNCs

Antibody isotype	Antibody specificity	Untreated cells	Treated cells
IgG	Not tested	5981 ± 2781*	0.1 ± 0.2*
IgM	Not tested	1390 ± 845*	0.3 ± 0.8*
IgG	Mouse RBCs	16.1 ± 17.9†	0.3 ± 0.4†
IgM	Mouse RBCs	30.8 ± 21.7†	0.2 ± 0.5†
IgG	Mouse WBCs	5.1 ± 6.1†	0.5 ± 0.3†
IgM	Mouse WBCs	8.9 ± 7.3†	0.2 ± 0.4†
IgG	Mouse PLTs	28.2 ± 17.8†	0.3 ± 0.6†
IgM	Mouse PLTs	25.6 ± 23.5†	0.0 ± 0.1†

* ng/mL.
† Percent positive cells.

mice was tested.[4] These results indicated that Mirasol PRT–treated cells were unable to generate xenogeneic GVHD responses. Thus, it would be predicted that Mirasol PRT treatment would prevent the development of TAGVHD after the transfusion of treated blood products.

The standard current protocol for prevention of TAGVHD is to expose blood products to 25 Gy of gamma irradiation. Although this dose of gamma irradiation has been shown to be effective in preventing the proliferation of irradiated WBC, subsequent studies have indicated that 25 Gy of gamma irradiation is not sufficient to prevent the presentation of antigens by these cells.[13,25] In contrast, Mirasol PRT treatment is able to completely inhibit all functions of WBCs including the ability to directly present antigen and induce proliferation of normal PBMNCs in an MLC assay.[11] This would suggest that Mirasol PRT treatment is able to prevent at least direct antigen presentation by the treated cells, indicating that Mirasol PRT treatment could potentially prevent the induction of alloantibodies after the transfusion of treated blood products. This would distinguish this treatment from gamma irradiation, which does not interfere with alloimmunization and the induction of alloantibodies.[13,25]

These studies also provide additional information about the xenogeneic GVHD response in $Rag2^{-/-}\gamma c^{-/-}$ double-knockout recipient mice. A previous study had shown that injection of human PBMNCs into $Rag2^{-/-}\gamma c^{-/-}$ double-knockout recipient mice resulted in enhanced peripheral reconstitution with human T cells.[4] Separation of donor PBMNCs into different subpopulations showed that both CD4+ and CD8+ cells were required to induce lethal GVHD in this model.[4] Although it took longer to develop xenogeneic GVHD than had been published, it was confirmed that it was primarily human T cells including both CD4+ and CD8+ cells that reconstituted the peripheral lymphoid compartments. One explanation for the delayed kinetics may be due to intraperitoneal injection instead of intravenous injection of the donor cells. Another possibility is that we did not bleed our recipient mice weekly, which could induce leukopenia and facilitate reconstitution with the human donor cells. Human CD19+ cells were the primary cells found in the liver and in any intestinal lymphoid tissue that was observed. The preferential location of B cells in the peritoneal cavity could represent an inability of the B cells to home to other sites in the murine recipient after intraperitoneal injection. Another possibility is that this tissue could represent the beginnings of an expansion of Epstein-Barr virus–seropositive B cells that has been previously observed in studies of immunodeficient SCID mice.[6,26] These cells represent an uncontrolled expansion of these cells as occurs in posttransplant lymphoproliferative disorder.[27]

Characterization of human cytokines present in the serum at early time points after injection of the human cells into $Rag2^{-/-}\gamma c^{-/-}$ double-knockout recipients found high levels of IL-1β, IL-2, IL-15, and IL-18; intermediate levels of IL-6 and IL-10; and very low levels of IL-4, IL-8, IFN-γ, and TNF-α.[4] The lack of human cytokines in the plasma of recipients of cells treated with Mirasol PRT was consistent with the lack of human cell chimerism observed with other approaches. Measurement of the

level of cytokines in the plasma of recipients with end-stage GVHD contained a very different mixture of cytokines because very high levels of IFN-γ, high levels of IL-10, and low levels of IL-5, TNF-α, IL-1β, IL-6, IL-8, IL-12p70 were detected. Concordant expression of high levels of both IL-10 and IFN-γ have been observed in autologous GVHD in humans.[28] Autologous GVHD is induced by a short course of cyclosporine in recipients of autologous transplants. Autologous GVHD is the result of cytolytic CD8+ cell responses whose T-cell receptors recognize MHC class II molecules containing the CLIP peptide. Polymorphisms in the IL-10 promoter were also found to correlate with the ability to generate autologous GVHD responses in patients.[28] Thus the xenogeneic GVHD responses in these recipients may be useful for modeling autologous GVHD responses.

The results indicate that both human CD4+ and CD8+ T cells are found in the recipient mice injected with untreated cells and that a large percentage of these cells express CD107a, indicative of CD4+ and CD8+ cells that had previously carried out granule exocytosis. There is evidence indicating that CD4+ cells can act as cytolytic cells in murine GVHD responses.[29] In these studies, the cytolytic CD4+ cells appeared to lyse peripheral blood progenitor cells in the marrow. This would provide one explanation for the decrease in Hct levels observed in the recipients of untreated MNCs. Although the antigens recognized by human T cells present in the Rag2$^{-/-}$γc$^{-/-}$ double-knockout recipient mice undergoing GVHD have not been determined, previous studies have suggested that human T cells recognize xenogeneic H-2 antigens expressed by murine antigen-presenting cells.[30,31]

Another possible explanation for the decreased Hct levels in the recipients of untreated MNCs could be the production of xenoreactive antibodies by the human cells. Published studies had indicated that the injection of human cells results in production of xenoreactive antibodies that bind to multiple antigens expressed by murine RBC.[32] We also observed that there were high levels of human immunoglobulins in the serum of the recipients, and that these immunoglobulins contained primarily IgM antibodies with specificity toward murine RBCs and PLTs but not WBCs. The activation of complement by the IgM bound to the RBCs could facilitate the elimination of the murine RBCs.

In summary, these experiments demonstrate that the injection of untreated MNCs into Rag2$^{-/-}$γc$^{-/-}$ double-knockout recipient mice induces a xenogeneic GVHD response that resembles the responses seen in TAGVHD. Mirasol PRT–treated donor cells are unable to generate xenogeneic GVHD when injected into Rag2$^{-/-}$γc$^{-/-}$ double-knockout recipient mice. This finding is consistent the previous finding that the Mirasol PRT–treated cells are functionally inactive in in vitro assays.[11] These findings suggest that a single treatment would be able not only to inactivate pathogens present in blood products but also prevent the immunologic consequences such as TAGVHD that can occur after blood transfusion.

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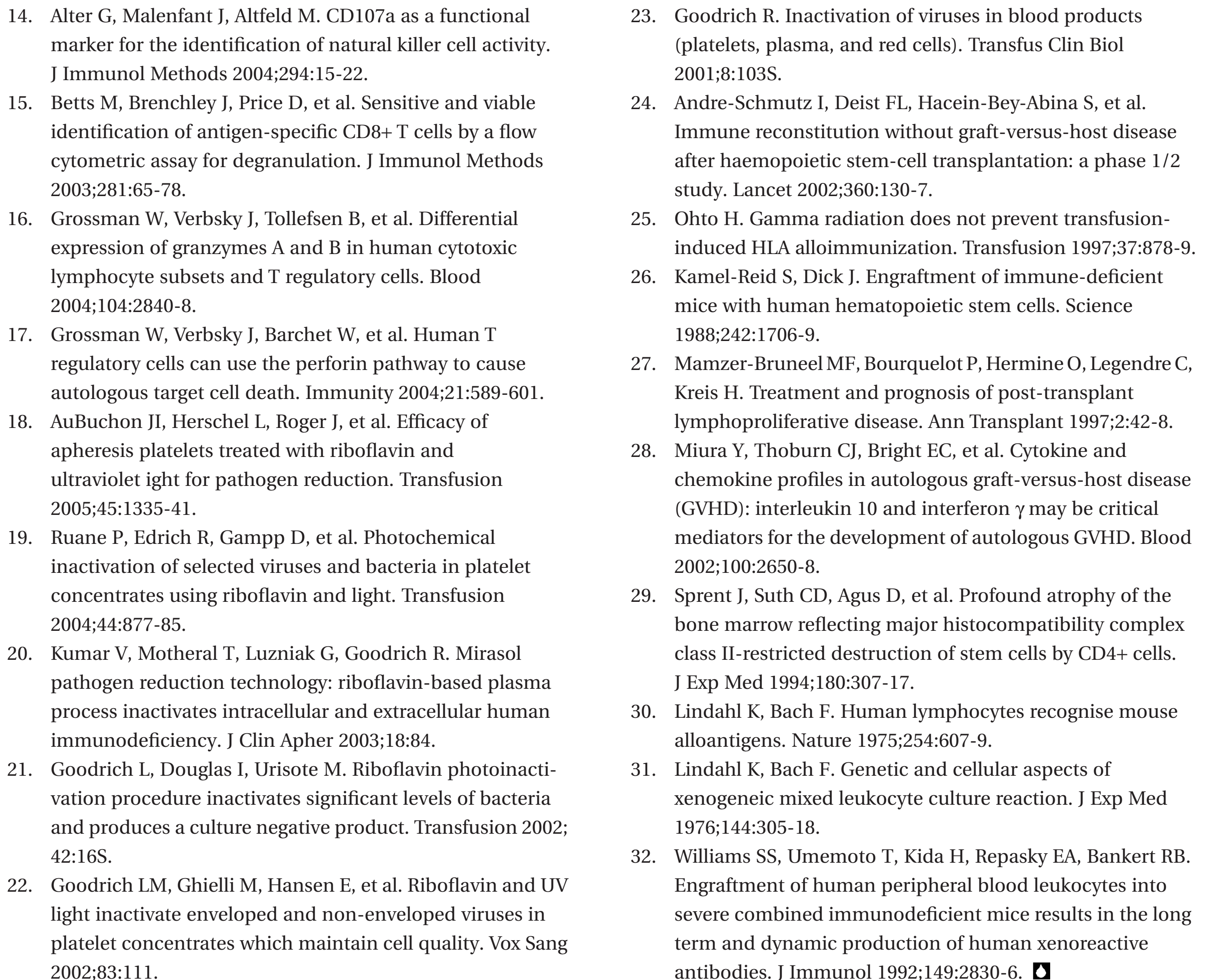

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TRANSFUSION MEDICINE REVIEWS

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Pathogen Reduction: A Precautionary Principle Paradigm

Harvey J. Alter

Although remarkable advances have been made in the prevention of the major transfusion-transmitted diseases, long intervals have transpired between the first recognition of transfusion risk and the implementation of a preventive strategy. For hepatitis B virus, that interval was 30 years; for non-A, non-B/hepatitis C virus, 15 years; and for human immunodeficiency virus, West Nile virus, *Trypanosoma cruzi*, and bacteria, 3, 4, 5, and 18 years, respectively. In our existing reactive approach, there is a fundamental and inevitable delay before we can react; and thus, infections are destined to occur. The continued emergence or reemergence of transfusion-transmitted infections calls for a new paradigm of preemptive pathogen reduction (PR). Two PR systems, psoralen/UV-A and riboflavin/UV-A, have shown efficacy and safety for platelets and plasma; and psoralen/UV-A technology has been successfully implemented for platelets in Europe. Pathogen reduction can eliminate or reduce the risk for any nucleic acid containing agent, including bacteria, and thus will be effective for all but prion diseases. It is possible to introduce PR for platelets and plasma now and to concentrate resources on developing PR for red cells. This will require an intellectual and financial commitment from the National Institutes of Health, the Food and Drug Administration, industry, and the blood bank establishment, just as occurred for nucleic acid testing (NAT) technology. This can be done if there is sufficient will to do it.
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IN THE WAKE of the human immunodeficiency virus (HIV) tragedy, the Food and Drug Administration (FDA) and US blood establishments have endorsed the *precautionary principle* that states that "for situations of scientific uncertainty, the possibility of risk should be taken into account in the absence of proof to the contrary." As a corollary, the precautionary principle asserts that "measures need to be taken to face potential serious risks." Yet pathogen reduction (PR), perhaps the quintessential example of the precautionary principle, has not been embraced. Pathogen reduction calls for a new paradigm in transfusion safety, namely, the transition from a reactive to a proactive and preemptive strategy for the prevention of transfusion-transmitted diseases. Pathogen reduction may initially add cost and complexity to blood processing, but ultimately will not only offer maximum safety, but also will prove to be cost-neutral and possibly cost-saving.

The decline in transfusion-associated hepatitis incidence from 30% to near zero over the course of 3 decades has been considered one of the major triumphs in transfusion medicine.[1] However, viewed in the present context, it can also be seen as one of the major failures of transfusion medicine because no preemptive viral reduction strategy was in place and because decades passed before the extent of the hepatitis risk was defined, the causative agents discovered, and proper testing strategies implemented. A vast number of cases occurred before we could, or chose to, do anything about it. Based on prospectively determined

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Table 1. Time Interval Between Recognition of Risk and Implementation of a Donor Screening Assay

Agent	Recognized as a transfusion risk	First screening assay	Interval (y)
HBV	1940	1970	30
NANB/HCV	1975	1990	15
HIV	1982	1985	3
WNV	2002 (1999)*	2003	1 (4)*
Chagas	2002	2007	5
Bacteria	1986	2004	18

Abbreviations: HBV, hepatitis B virus; NANB, non-A, non-B agent; HIV, human immunodeficiency virus; WNV, West Nile virus; Chagas, *T cruzi* infection.

* Suspected, but not proven, in 1999.

hepatitis incidence rates[1] and the number of blood transfusions nationwide in the United States, it can be estimated that there were 4.8 million transfusion-transmitted hepatitis C virus (HCV) infections between 1970 and 1990; and based on a 20% incidence of severe outcomes, these might have resulted in 768 000 cases of cirrhosis. Clearly, not all these cases of cirrhosis occurred because many transfusion recipients died of underlying diseases before the severe chronic manifestations of hepatitis could ensue. This does not diminish the fact that allogeneic transfusions placed these recipients at potential risk for a fatal disease. These projections are sobering, even if they are off by a factor of 10.

Historically, there has been a very long interval between the first recognition that a disease is transfusion-transmitted and the eventual implementation of a donor-screening test to prevent that transmission (Table 1). For the hepatitis B virus (HBV), the interval between recognition of transfusion-transmission and implementation of the first assays for HBV, the Australia antigen, was approximately 30 years; and for non-A, non-B/HCV hepatitis, it was 15 years. For HIV, the interval was reduced to 3 years; however, in that comparatively brief interval, more than 14 000 transfusion-transmitted, predominantly fatal HIV infections occurred in the United States alone.[2] We generally consider that the interval from recognition to implementation for West Nile virus (WNV) was from 2002 to 2003; however, retrospective analysis of the 1999 WNV epidemic in New York City made it highly probable that WNV could be transmitted by transfusion,[3] so that the true implementation interval was approximately 4 years. In addition, testing donations for *Trypanosoma cruzi* (Chagas disease) was recommended by the FDA's Blood Products Advisory Committee in 2002; and yet the first reliable test was not introduced until 2007. Furthermore, the relatively frequent transmission of bacteria by room temperature–stored platelets was evident by 1986; but it was not until 18 years later (2004) that routine bacteriologic testing of platelets was introduced in the United States. The inherent problem is that in a reactive strategy to pathogen risks, there is a fundamental and inevitable delay between the recognition of risk and the prevention of that risk. Thus, transfusion-transmitted infections are destined to occur before we can adequately react. Perhaps William Murphy phrased it best when he stated, "this effect, that a new or poorly understood disease with a long preclinical phase can be spread among humans by transfusion for many years, and perhaps extensively, before the consequences become apparent is a fixed and inevitable property of transfusion medicine."[4]

Nonetheless, on a more positive note, based on prospectively determined hepatitis incidence figures,[1] the introduction of anti-HCV screening in 1990 is projected to have prevented 1.2 million cases of transfusion-associated hepatitis and 192 000 potential cases of cirrhosis in the decade of the 1990s. Furthermore, increasingly sensitive serologic and nucleic acid tests for HCV and HIV have reduced these risks dramatically. In the United States, the risks for the 3 major transfusion-transmitted viruses in 2006 were 1 in 1.6 million for HCV, 1 in 1.8 million for HIV, and 1 in 269 000 for HBV, as calculated by the incidence rate–window period model and approximately the same using the minipool nucleic acid testing (NAT) yield model.[5] In Canada, O'Brien et al,[6] using the incidence rate–window period model, reported a residual risk of 1 in 7.8 million for HCV, 1 in 2.3 million for HIV, and 1 in 153 000 for HBV. Although HBV transmissions occur much more frequently than HCV or HIV, this estimate is based on antibody to hepatitis B core antigen (anti-HBc) seroconversion and not on the development of the hepatitis B surface antigen (HBsAg) carrier state or clinical disease. Actual cases of transfusion-associated hepatitis B are quite rare.

Currently, the major infectious risks are zoonotic in origin, wherein animal viruses have been introduced to humans as an incidental host, either through the food chain, as in variant Creutzfeldt-Jakob disease, or through vector transmission. Mosquitoes, ticks, and other insect vectors are

injecting diseases into us at alarming frequency; and most, if not all, of these are then secondarily transmitted by transfusion. These vector-borne and transfusion-borne diseases include malaria, dengue fever, WNV-associated fever and meningoencephalitis, babesiosis, ehrlichiosis, Colorado tick fever, and Chagas disease. Conclusive evidence is missing; but Lyme disease, visceral leishmaniasis, and flavivirus-induced encephalitis might also prove to be transfusion-transmitted.

The history of transfusion-transmitted WNV infection is a case in point. Before the implementation of WNV testing in 2003, there were 23 documented transfusion-transmitted clinical cases reported in the United States; and based on the Centers for Disease Control and Prevention conversion factor of 140 asymptomatic cases to each clinical case,[7] it can be estimated that there were an additional 3200 subclinical transfusion-transmitted cases in 2002. It is commendable that WNV NAT testing began under IND only 8 months after the first documented transfusion-transmission. However, in retrospect, it was projected from the New York City epidemic in 1999 that transfusion-transmitted WNV cases were likely.[3] However, because the evidence was indirect and WNV epidemics were known to be intermittent in nature, no definitive action was taken at that time. Overall, since 2002, there have been 32 documented transfusion-associated cases of clinical WNV infection and 4480 projected transmissions in the United States. Remarkably, none of these cases would have occurred had a PR system been in place at the time, as evidenced by the absence of cases associated with the transfusion of solvent-detergent–treated plasma. Once implemented in 2003, WNV testing of donor blood has been highly effective. In the American Red Cross experience, WNV NAT prevented the release and transfusion of at least 1000 potentially infectious components from 519 confirmed positive blood donors in 2003 and 2004.[8] There have been only 9 WNV transmissions since testing was implemented, each representing a window period donation that failed to be detected in the minipool testing format being used.

There is current concern that the WNV story may be replayed by the dengue fever virus (DFV) because the agents and predisposing elements are similar. Globally, DFV causes 100 million cases of dengue fever, 250 000 cases of dengue hemorrhagic fever, and 25 000 deaths annually. It is transmitted by the *Aedes* mosquito and is found in more than 100 countries with a rapidly expanding distribution that could easily follow the path of WNV to North America. The DFV has a median viremia of 5 days, and most cases are asymptomatic; RNA levels range from 10^5 to 10^9 copies per milliliter. Thus, dengue has all the characteristics of a transfusion-transmitted agent; and its vector is already present in North America. Thus far, there have been 2 transfusion-transmitted cases, 2 transplant-related cases, and 7 nosocomial dengue transmissions that have been documented.[9] Undoubtedly, many more transfusion-associated dengue cases occur in endemic areas; but these are masked by the high background infection rate. Fortunately, the epidemiologic pattern of this agent differs from WNV in that it does not have an intermediary bird host that would facilitate its spread.

An agent of particular current interest is human herpesvirus (HHV)–8, important not only for its potential to transmit Kaposi sarcoma (KS) but also because it is critical to decisions regarding the donor reentry of men who have sex with men (MSM). The prevalence of HHV-8 antibody is 2% to 4% in blood donors,[10] 2% to 10% in the general population, 12% to 16% in HIV-negative MSMs, 40% to 50% in HIV-positive MSMs, and >95% in patients with KS (Dollard SC, personal communication). There have been several case reports and epidemiologic insights to suggest that HHV-8 can be transfusion-transmitted, but the best data indicating the transfusion-transmission of HHV-8 are in the study by Hladik and associates at the Centers for Disease Control and Prevention[11] who followed 1811 transfusion recipients in an HHV-8 endemic area of Uganda. The risk of seroconversion was significantly higher among recipients of seropositive blood (excess risk, 2.7%; $P < .005$); and the increase was seen primarily in those who seroconverted for anti-HHV-8 between 3 and 10 weeks posttransfusion, an interval highly suggestive of transfusion-transmission.

In the final analysis, any agent that even transiently traverses the human circulation during an asymptomatic phase of infection is a threat to be transfusion-transmitted. The likelihood of that transmission is highly dependent on the duration of viremia ("agentemia"), and the level of concern is dependent upon the severity of the ensuing disease. Agents for which there are no routine screening

measures in place and that pose a documented or potential transfusion risk in this model include malaria sp, dengue and other arboviruses, HHV-8 (KS virus), cytomegalovirus (CMV) and other herpes viruses, parvovirus B-19 (erythrovirus), human papilloma virus, Colorado tick fever virus, *Babesia microti* (*Babesia*), *Ehrlichia* and other *Rickettsia*, *Borrelia burgdorferi* (Lyme disease), chikungunya virus, hepatitis A virus, and variant Creutzfeldt-Jakob disease. A comprehensive analysis of 68 potential/known transfusion-transmitted agents is being prepared for publication by a task force of the American Association of Blood Banks (AABB) and should be accessible sometime during 2008 (Stramer S, personal communication).

This vast array of potential microbiological threats requires continuous surveillance, clinical assessment of the magnitude of each identified risk, and then, where possible, testing or other strategies to limit risk of the most clinically significant agents. This is thus an agent by agent process that is inefficient, insensitive, often controversial in its decisions, and inevitably applied only after clinical disease has occurred. It is a reactive strategy that requires demonstrable risk before preventive measures can be implemented. A more encompassing, efficient, and intuitively appealing option is a preemptive approach that includes PR. Almost all of the aforementioned agents and many others can be reduced to nonpathogenic levels by nucleic acid intercalating agents such as the psoralens[12] and riboflavin[13] in the presence of UV-A light. Shown in Table 2 are known log reductions for psoralen/UV-A, and much the same could be shown for riboflavin/UV-A.

Pathogen reduction has multiple proven advantages in that it: (1) effectively inactivates most clinically relevant viruses whether RNA or DNA, single-stranded or double-stranded, enveloped or nonenveloped, and intracellular or extracellular; (2) inactivates clinically relevant gram-positive and gram-negative bacteria; (3) inactivates all the spirochetes, *Rickettsia*, and protozoa of known transfusion relevance; (4) inactivates lymphocytes and thus prevents transfusion-associated graft-versus-host disease (GVHD); and (5) offers probable preemptive protection against pathogenic, potentially lethal agents that will inevitably emerge in the future.

There are also impediments to the implementation of PR that to this point have limited its widespread application, as follows: (1) decreased product yield for platelets in the range of 10% to 15%; (2) insufficient kill of some high-titer, nonenveloped agents such as hepatitis A virus (HAV) and parvovirus B-19. However, antibodies to these agents are common in the recipient population, and documented transmissions are exceedingly rare; (3) concern over potential toxicity, although none are known for riboflavin and toxicity of the psoralens is theoretical at the low residual doses transfused. For both agents, there appears to be a wide safety margin; (4) most significantly, there is at present no single PR system that can be applied to all blood products and, particularly, no proven system for PR of whole blood and packed red cells; and (5) anticipated high cost.

Although there are no methods for the PR of red blood cells (RBCs) that have been proven effective and safe in appropriate clinical trials, if the evolving technologies establish their efficacy and multicomponent PR can be implemented, then there are many potential savings that would offset the cost of the process itself, including (1) reducing or eliminating the future need for additional donor screening assays such as testing for HHV-8, *Babesia*, *Ehrlichia*, dengue, and malaria; (2) eliminating some current assays including anti-HBc, WNV, *T cruzi* (Chagas), and syphilis; (3) eliminating the bacterial testing of platelets; (4) discontinuing irradiation of blood products; (5) discontinuing leukoreduction; (6) allowing for the continuation of

Table 2. Inactivation of Infectious Agents in Plasma and Platelets Using Psoralen/UV-A

Classification	Agents	Log reduction
Virus (enveloped)	HIV-1/2, HTLV-I/II, HBV, DHBV, HCV, BVDV, WNV, CMV, SARS-CoV, vaccinia	>4.5 to >6.8
Virus (nonenveloped)	Human adenovirus-5, bluetongue, parvovirus B-19, HAV	>5.1 to >6.8
		3.5 to >5.0
Bacteria	Gram + and gram−	>7.3
Spirochetes	*Treponema pallidum*, *B burgdorferi*	>5.9 to >10.6
Protozoa	*Plasmodium falciparum*, *T cruzi*, *B microti*	>5.0 to >6.9

Abbreviations: HTLV, human T lymphotropic virus; BVDV, bovine diarrheal virus.

minipool testing rather than the probable evolution to individual donor testing; and (7) reducing donor exclusions based on geography (malaria). Cumulatively, these measures could result in vast savings that should offset the implementation costs of PR. However, these savings can only be realized after procedures for the PR of RBC products are fully operational.

Perhaps the key immediate issue is not the efficacy of PR, where the evidence is substantial, and not even its safety, where toxicity remains theoretical, but rather whether we should introduce PR reduction for platelets and single-donor plasma before a system is in place to inactivate pathogens in RBC products. This is a difficult conundrum, and opinion on this issue is sharply divided. However, it is known that many patients receive repeated, often-daily platelet transfusions that are only intermittently accompanied by RBC transfusions and also that many centers pool platelet products, vastly increasing the recipient exposure risk.

It is also probable that if we wait for the complete PR package, another 5 to 10 years will elapse before licensure and implementation; and in that time, innumerable platelet and plasma infusions will continue to transmit infections to recipients that could have easily been prevented. If such transfusion-transmission infections are accompanied by significant disease, then further transfusion-transmitted tragedies could ensue. I believe that the precautionary principle and the moral imperative dictate that we implement what we have, even if they are less than perfect. Admittedly, there is the other side of the coin in that platelet and single-donor plasma inactivation, in the absence of RBC inactivation, will not reap the financial offsets of a complete PR system and in that RBC products will continue to transmit disease. Furthermore, it is known that transfusion-transmitted pathogens are only one part of the risk equation and, now, not even the largest part. However, I would counter that preventing human error, controlling transfusion-related acute lung injury, and PR are not mutually exclusive and all ways of reducing transfusion risk should be pursued with equal vigor.

The solvent-detergent treatment of plasma and its derivatives has established the principle that PR of even a single blood component is highly beneficial and has established the enormous value of a preemptive PR strategy. Universal inactivation of plasma derivatives has rendered the formerly highest-risk blood products now to be the safest. As blood transfusion services scrambled to meet the threat of WNV, how reassured plasma manufacturers were to know that they had this agent preemptively covered, as they would for DFV or any other lipid-encapsulated agent that threatens the blood supply. Had solvent detergent treatment of plasma derivatives been implemented in the early 1980s, most HIV and HCV cases that devastated the hemophiliac population could have been avoided. This statement is being made not to cast retrospective blame, but to take a lesson from history and to illustrate the value of having a protective preemptive mechanism in place before the next agent strikes.

There is great potential risk in delaying implementation of PR while waiting for absolute evidence and a perfect system to be put into place. Waiting is a calculated risk that defies the precautionary principle. Can we chance the possibility that a new lethal agent will enter the blood supply and replay the HIV tragedy? Can we face future generations and say we did all that we could do at the time? I believe the time has come to act. At present, there are 2 technologies, psoralen/UV-A and riboflavin/UV-A, that would bring the same level of safety to platelets as currently exists for commercial plasma and its derivatives. Evidence for the efficacy and safety of PR for platelets and single-donor plasma products is sufficient,[14,15] if not overwhelming; and the European experience with psoralen/UV-A–treated platelets has proven the practicality and safety of this approach to transfusion-transmitted disease prevention.[16] Whether or not one concurs with the need to rapidly introduce PR for platelets and nonfractionated plasma in the United States and Canada, the more important and, I believe, crucial message is that we have to establish a mind-set that says PR of *all* blood products is a laudatory and achievable goal and that we need to invest ourselves emotionally, intellectually, and financially to make this happen. The blood bank establishment, the National Institutes of Health, the FDA, and industry have to make this concept a priority and then work in concert to devote substantial resources and energy to achieve this goal, just as they did for viral nucleic acid testing. Only then will it happen!

I was in that packed hotel ballroom in 1994 when Dr David Kessler urged blood banks to develop NAT for routine donor screening. His talk raised

eyebrows and great skepticism; but because of his position of authority, it drove the system, generated government-industry collaboration, and resulted in the remarkably rapid development of practical NAT assays that have been an enormous addition to blood safety. I am no David Kessler; but I admonish and encourage you to say this is the right thing to do and that we have to find a way to do it. We have to bite the bullet. Fortunately, in this case, it is a magic bullet.

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バイオワン株式会社回答

4月8日運営委員会・安全技術調査会合同委員会後

追加質問事項2（各社共通）

インターセプトブラッドシステム（S-59）　バイオワン株式会社

1．EU主要国等、各社の不活化技術を導入しているそれぞれの国における供給の実態。輸血用血液製剤の全供給数（不活化技術を適用している製剤と適用していない製剤の合計）、そのうち不活化技術を適用している製剤の割合は何％か。また、供給はどのように行われているか（供給先、医師の希望により供給できるのか等）。

国	血小板供給数		血漿供給数	
	全数	不活化率	全数	不活化率
ベルギー	70,000	100%＊1	110,000	
フランス	230,000	約10％[2007年]	245,000	100%＊2
ドイツ	400,000		1,200,000	
イタリア	120,000		605,000	
スペイン	215,000		275,000	
スウェーデン	35,000		120,000	
ノルウェイ	20,000		30,000	
ロシア	75,000		260,000	

＊1：ベルギーは血小板の 100％不活化導入が決定されているが、未政府状態が続いており、大臣就任後 100％実施になる。保険適用についても実施が遅れている。血漿についてはすでに2センターで IBS の日常的使用を開始。

＊2：フランスは規制により血漿の 100％不活化を要求されている。IBSによる不活化率は未確認(アルサスなど3か所で MB から IBS に変更)

＊3．ドイツではリューベック、フランクフルトで血小板用 IBS の日常的使用開始。

＊4．イタリア、スペインでは地域により採用が始まっている。採用の判断は血液センターによる。昨年北イタリアで Chikungunya が発生した際には既に IBS を採用していたため血小板の供給に支障は生じなかった。

＊5．ノルウェー、スエーデンでは血液センターにより採用の判断をしている。不活化の費用は全体治療に必要な保険の範囲内で負担可能。

＊6．ロシアは医師、患者の判断により不活化した製剤を血液供給者に要求。

供給方法

1）ベルギー

　その地域の血液センターが提供する血液製剤をそのまま使用。血液センターによって供給する製剤が不活化されているかいないか異なっているが2008年中に100％不活化した血小板が供給される予定。不活化は100%IBS による。

2）フランス

採用する血液センターは100％採用になっているため地域によって不活化されているかされていないか決まる。

3） ドイツ

血液製剤はドイツ赤十字(75-80%)、病院/大学（10-15%）、供給民間会社（10%以下）から供給されている。血小板は原則 Buffy Coat 血小板で成分採血血小板は理由をつけて医師の要求がある場合に限られる。ガンマー線照射も骨髄移植患者など特に必要な患者に限って実施している。現在は成分採血の際に IBS を採用しているが(リューベック)今後は Buffy Coat にも IBS の採用を広げている予定です。フランクフルト血液センターで Hemovigilance の体制を整えて実施予定。

2. 各社の不活化技術を適用する前の製剤に白血球除去を行った場合と、不活化技術を適用した製剤に白血球除去を行わなかった場合を比較して、GVHD等の副作用の推移はどのようになっているか。

現在　白血球除去を行わずに不活化処理を実施している国はない。
白血球除去導入実態
ベルギー、フランス、ドイツ、スェーデン、スペイン、ノルウェイ　100%
イタリア　　約70%
ロシア　　約 20%

白除と、白除＋不活化の副作用の比較は下記情報がある。

InterCept による不活化導入前後の副作用の比較（Dr. Osselaer　ベルギー）

	対照期間(不活化処理前)		INTERCEPT 不活化処理期間	
血液製剤	血小板(未処理)	赤血球(未処理)	血小板（不活化）	赤血球(未処理)
調査期間	18 ヶ月	18 ヶ月	18 ヶ月	18 ヶ月
輸血数	3529	9551	4051	11493
副作用	1．3%	0．4%	0．9%	0．4%

血小板では、不活化処理前の期間は細菌検査、γ線照射、CMV検査を実施していたが、不活化処理導入後これらの検査、処理は実施していない。
赤血球は不活化処理を行っていないので、両期間とも副作用の発生率に変化はない。
一方、血小板は不活化処理導入後、上記検査、照射を廃止したにもかかわらず、副作用の発生率が減少し統計的に有意差が認められている（p　0.002）

Alsace（フランス）

Adverse transfusion reactions during platelet concentrates (PC) transfused at EFS-Alsace

	(1) PC (100% plasma) 1/1/2003 – 1/2/2004 99.6 %	(2) PC (35% plasma+65% T-Sol) 1/9/2005 – 1/6/2006 95 %	(3) PC INTERCEPT (35% plasma+65% Intersol) 1/9/2006 – 1/8/2007 99 %
Patients (n)	59	33	36
Adverse reactions (n)	67 (11 RBC Imm)	41 (16 RBC Imm)	37* (19 RBC Imm)
Adverse reactions/1000 PC (n)	5.3	2.7	1.4
Patients with reactions	2.9 %	2 %	1.7 %

Period 3 : *Fever/chills : 8 ; allergy : 3 ; TRALI : 1 ; RBC immunisations : 19
Period 1 : 1 death volume overload (2 RBCC + 2 BCPC)
All 3 periods : No bacterial sepsis
All 3 periods : 145 adverse reactions : SEVERITY grade 1 = 61 %; grade 2 = 33 % (46 RBC immunisations)
BioOne Corporation
IMPUTABILITY grade 2, 3, 4 = 87 %; grade 3 and 4 = 70 %

La Reunion（フランス）

EFS-La Réunion : Adverse reactions with INTERCEPT treated Apheresis PC -1

(M F Angelini-Tibert and P. Rasongles)

year	2005	2006/3 - 2007/3
nb of patients	NA	427
nb of apheresis PC	1194	1948
nb of adverse reactions	109	19
Adverse reactions / 1000 APC	91,3	9,8

HV Intercept G Andreu 2008 02 29 Frankfurt

3．EU主要国における不活化技術導入後不要になった技術（白除、NAT等）

国	不要になった技術
ベルギー	細菌検査、γ線照射、CMV検査、導入予定の個別NATの導入中止
フランス	γ線照射、導入予定の個別NATの導入中止
ドイツ	γ線照射
イタリア	
スペイン	
スェーデン	
ノルウェイ	
ロシア	

東欧のIBS導入センターでは導入予定の白除の導入中止を検討しているが、まだ決定していない。

4．EU主要国における不活化技術を適用している製剤の市販後調査の実態（調査数）

1）血小板

<u>ヘモビジランス① （2003 年 10 月～2005 年 12 月）</u>

ベルギー、イタリア、スペイン、ノルウェイ

輸血数 5106PC、患者数 651 人、1 人当たりの輸血数：1～156 回 平均 7.8±16.2 回

97.3%の血小板製剤はγ線照射なし

輸血関連の有害事象（可能性がある、おそらく関連、関連ある）

グレード1：41 輸血、グレード2以上：1 輸血 （全有害事象 42 例、0.8%）

感染、TRALI及び死亡はない。

<u>ヘモビジランス② （2005 年 5 月～2007 年 1 月）</u>

ベルギー、フランス、スペイン

輸血数 7437PC、患者数 1400 人、1 人当たりの輸血数：1～129 回 平均 5.3±10.8 回

98.9%の血小板製剤はγ線照射なし

輸血関連の有害事象（可能性がある、おそらく関連、関連ある）

グレード1：55 輸血、グレード2以上：０ （全有害事象 55 例、0.7%）

感染、TRALI及び死亡はない。

<u>ヘモビジランス③ フランス（Ile de La Reunion）</u>

Ile de La Reunion では 2005 年から 2006 年にかけてチキングニャが大流行し島内採血を禁止した。急遽、本国で導入されているIBS不活化技術を導入後、島内採血を再開し血小板を確保した。

輸血数 1948PC、有害事象 0.98%

チキングニャ感染例はない

<u>ヘモビジランス④ フランス（Alsace）（2006年 9 月～2007 年 8 月）</u>

輸血数 13241PC, 患者数 2069 人、1 人当たりの輸血数：1～289 回 平均 6.4 回

有害事象 37 輸血（0.14%）

TRALI 1 例、感染、死亡は報告されていない。TRALI は出産経験が3回ある女性の成分献血による。

<u>ヘモビジランス⑤</u>

輸血数 4000PC 2008 年 6 月 International societ of blood transfusion で発表予定

<u>ヘモビジランス⑥ ドイツ</u>

輸血数 5000PC 2008 年より開始

2）血漿

<u>ヘモビジランス①</u>

輸血数　3000PC　 2008 年 6 月 International societ of blood transfusion で発表予定

なおバイオワンとシーラスでは Hemovigilance に当たる市販後調査を継続して実施する予定です。アジアにおいても欧州と同じ基準により実施しますが日本は日本赤十字社のご判断によると思います。

文献6

The role of toxicology assessment in transfusion medicine

Vic Ciaravino, Tim McCullough, and George Cimino

A toxicology program is a planned process conducted during pharmaceutical development to provide safety data and to support a clinical research program. Toxicology testing is initiated during the preclinical stage of drug development to evaluate potential adverse health effects of new chemical entities (NCEs) and provides regulatory authorities with quantitative evidence of potential effects on human health. In turn, this permits an informed decision about whether an NCE should be tested in humans. Toxicology testing continues throughout clinical development to support an NCE through to commercialization and after-market surveillance. Appropriate studies are specified by international guidelines put forth by the International Conference on Harmonisation (ICH) tripartite (US, Europe, and Japan). This review focuses on the science of toxicology and how it was applied to the development of a blood system for PLTs (the INTERCEPT Blood System for PLTs, Cerus Corp., Concord, CA).

PATHOGEN INACTIVATION AND DEVELOPMENT OF AMOTOSALEN

Since the recognition of HIV in the blood supply, addressing viral risk has become an important component of transfusion medicine and science. Not only the risk posed to the blood supply by viruses, bacteria, and parasites, but also the potential risk of as yet unknown infectious agents has led to investigation of blood component pathogen inactivation (PI).[1] The active component in the INTERCEPT photochemical treatment system is a synthetic psoralen, amotosalen. Amotosalen is specifically designed to reversibly intercalate into helical regions of DNA and RNA (Fig. 1). Upon illumination with UVA light, amotosalen reacts with pyrimidine bases to form both covalent monoadducts and crosslinks (diadducts). Infectious pathogens and WBCs whose genomes are crosslinked or contain monoadducts are unable to replicate, a process termed PI.

To add to the safety of the blood supply, PI should not exchange one type of risk (pathogens) for another (toxicity). The toxicology program for INTERCEPT was conducted according to international standards for drugs, which are the highest standards for NCE testing. Amotosalen was tested as though it were a drug, even though it is a processing chemical that is largely removed before transfusion.

AREAS OF TOXICOLOGY AND RISK ASSESSMENT

The professional activities of toxicologists are interrelated and vitally important to chemical risk assessment (Fig. 2).[2] A *mechanistic toxicologist* identifies the cellular, biochemical, and molecular mechanisms by which chemicals exert toxic effects. A *descriptive toxicologist* is concerned directly with toxicity testing, which provides information for safety evaluation and regulatory requirements. A *regulatory toxicologist* decides whether an NCE poses a sufficiently low risk to be marketed for a stated purpose. Sound risk assessment ensures public protection and allows the use of products whose benefits outweigh their risks.

There are four components of *risk* assessment.[3] *Hazard identification* is an evaluation of the adverse health effects a NCE may cause (What are the potential effects of residual PI agents once transfused?). *Dose-response assessment* is a determination of the quantity of an NCE required to cause a toxic effect and a prediction of exposure levels likely to be negligible or nonexistent (Can safety margins be established with PI technology?). *Exposure assessment* evaluates what quantity of an NCE patients might be exposed to under various conditions (What is the residual amount of amotosalen in a PLT transfusion unit?). *Risk characterization* is an integration of this information to characterize the risks to the exposed population (What is the likelihood of target organ toxicity, phototoxicity, or

ABBREVIATIONS: CAD = compound adsorption device; CHO = Chinese hamster ovary; ICH = International Conference on Harmonisation; NCE(s) = new chemical entity(-ies); PI = pathogen inactivation; TRAP = Trial to Reduce Alloimmunization to Platelets.

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TRANSFUSION 2003;43:1481-1492.

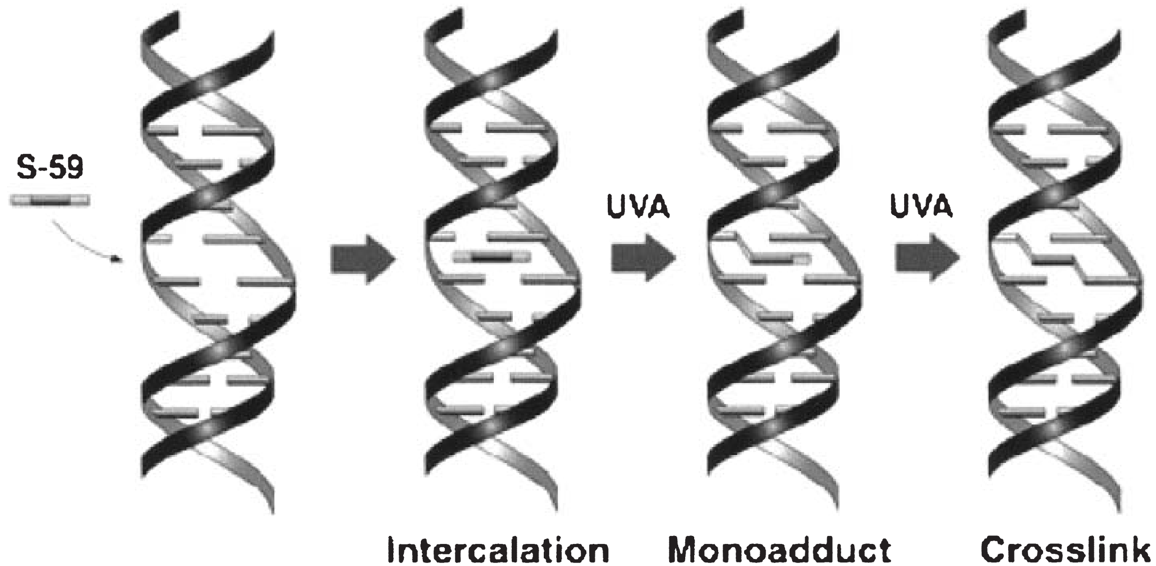

Fig. 1. Mechanism of action of PI. Amotosalen (S-59) is a synthetic psoralen that inhibits nucleic acid replication through UVA light-mediated covalent addition to nucleic acids. 1) The molecule is designed to penetrate cells, viruses, bacteria, or other pathogens and seek out DNA or RNA. 2) Amotosalen then intercalates between the base pairs. 3) Once illuminated by UVA light, amotosalen forms monoadducts between pyrimidine bases. 4) Another photon of light enables the molecule to form crosslinks (diadducts) between DNA or RNA strands.

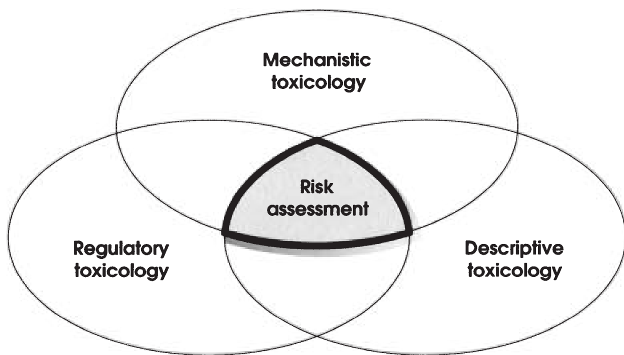

Fig. 2. The interconnections between different areas of toxicology.

carcinogenicity after repeated PLT transfusions containing amotosalen?).

Safety assessment can be expressed in more than one way. *Therapeutic index* is an approximation of relative safety, which is the ratio of the toxic dose to the therapeutic dose. An example of a therapeutic product is an antibiotic that is dosed to achieve a threshold concentration in plasma to eliminate an infection. Interestingly, this concept is not applicable to the INTERCEPT Blood System because residual amotosalen has no intended pharmacologic effect and is not considered a therapeutic product.

A more applicable assessment of safety pertaining to the INTERCEPT Blood System is the *margin of safety*. This is an indicator of the magnitude of the difference between estimated exposure to a population and the highest nontoxic dose determined in experimental animals. The estimated exposure used as the denominator in calculating safety margin is a 300-mL PLT transfusion administered to a 60-kg individual containing approximately 50 μg of residual amotosalen. The exposure, thus, is approximately 1 μg per kg. Safety margins for the photochemically treated test articles are expressed in terms of the residual amotosalen because that is the active agent in the photochemical treatment process. As an example of a calculated safety margin, the highest amotosalen dose in the carcinogenicity study was approximately 1 mg per kg, and no toxicity or carcinogenicity was found. This is approximately 1000 times the amount administered to a patient receiving one 300-mL PLT transfusion. Thus, the margin of safety in this study is at least 1000 because no toxicity was observed at the highest dose used.

Safety margins are expressed on a per-dose basis. Further comparison to clinical exposure would take into account that for a repeated-dose study, daily dosing for 13 weeks or 78 doses in 26 weeks (carcinogenicity study) is more frequently dosed than the clinical regimen of 14 transfusions over an 8-week period.[4]

TOXICOLOGY TESTING STANDARDS

In 1962, the US Congress authorized the FDA to exempt investigational drugs from premarket approval require-

ments to encourage clinical testing, subject to conditions appropriate to protect human subjects.[5] One condition was that investigational new drugs first must undergo evaluation in preclinical studies. The current regulations specify the type of tests that are to be performed and the design they are to follow. NCEs that are candidates for use as human drugs also must meet the standards set by the FDA's Good Laboratory Practices (GLP) regulations, which apply to all laboratories in which studies are conducted. The work of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) highlights the trend toward international agreement on test methods. In 1994 the ICH, composed of the European Union, Japan, and the US, issued six draft guidelines on various toxicology testing methods for human drugs.[6]

Maintenance of the ICH Guideline on Non-Clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals,[7] outlines toxicology studies for the marketing approval of a pharmaceutical agent. Nonclinical safety studies include single- and repeated-dose toxicity studies, reproduction toxicity studies, genotoxicity studies, local tolerance studies, and an assessment of carcinogenic potential for drugs that have special cause for concern or are intended for a long duration of use. Other nonclinical studies include pharmacology studies for safety assessment and pharmacokinetic studies (absorption, distribution, metabolism, and excretion).

The preclinical safety studies, although limited to the beginning of clinical development, should be adequate to characterize potential toxic effects under the conditions of later-stage drug development. The guidelines recommend the minimum duration of repeated-dose toxicity studies relative to clinical trial duration. Nevertheless, PLT concentrates are administered episodically and intermittently. To determine an appropriate minimum duration for these toxicology studies, the treatment of acute myeloid leukemia was used as a model because it probably represents the most extensive clinical use of PLTs. In the US, the Trial to Reduce Alloimmunization to Platelets (TRAP) studied PLT usage in acute myeloid leukemia.[4] Based on the TRAP data, the mean number of PLT transfusions per patient was 14 PLT transfusions spread over an 8-week period. Technically, based on patients receiving 14 transfusions, 1-month toxicity studies would be of sufficient duration to adequately assess potential patient toxicity. Nevertheless, the maximum duration of toxicity studies for the INTERCEPT toxicology program was set at 3 months to ensure that long-term toxicity was thoroughly assessed.

TOXICOLOGY PROGRAM FOR THE INTERCEPT BLOOD SYSTEM FOR PLTs

The preclinical testing of this novel agent, which can be viewed as a processing chemical, a potential pharmacologic, and a medical device, raised unique questions about how to design and conduct toxicity and related preclinical studies. In the photochemical treatment process with the INTERCEPT Blood System for PLTs, a transfusion unit of PLTs is suspended in 35 percent plasma and 65 percent InterSol, a buffered PLT storage solution. The PLTs comprise 3 percent (vol/vol) of the transfusion unit. The initial processing mixture contains 150 μ*M* amotosalen (15.2 mg in a 300-mL apheresis unit) and is exposed to a 3 J per cm^2 treatment of long-wavelength ultraviolet light (UVA; 320-400 nm) for 3 to 6 minutes to inactivate pathogens. By-products of the amotosalen photochemical treatment process consist of residual amotosalen as well as the formation of free and covalently bound photoproducts.

During processing, levels of residual amotosalen are reduced 74- to 84-fold and the free photoproducts are reduced approximately 3-fold by compound adsorption device (CAD) treatment. The clinical exposure to amotosalen and its photoproducts for a 60-kg individual from a 300-mL PLT concentrate would therefore be less than 1 μg per kg amotosalen, 45 μg per kg free photoproducts, and 70 μg per kg bound photoproducts equally divided between plasma macromolecule and PLT-bound products. Toxicology studies with photochemically treated 35 percent plasma consisted of a mixture of residual amotosalen and photoproducts.

Because positive findings were observed with amotosalen alone at high doses, safety margins have been expressed on the basis of the residual amount of amotosalen in the test article. To provide a safety factor for photoproducts, which are present in the photochemically treated mixtures, the cumulative exposure to photoproducts in toxicology studies was expressed as the corresponding equivalent number of PLT transfusion units that would be administered in a study. For example, in the 3-month rat study, the cumulative exposure to photoproducts is 1440 and 810 PLT unit equivalents, for free and bound photoproducts, respectively. For the p53 mouse carcinogenicity study, the cumulative exposure to photoproducts is 860 and 270 PLT unit equivalents, for free and bound photoproducts, respectively. No evidence of toxicity was observed in these studies.

STUDY DESIGN

In toxicology testing, the most relevant test article was the photochemically treated mixture because it is administered clinically. Procedures were developed to increase the levels of residual amotosalen administered in the treated mixtures and to thus increase safety margins. Toxicology studies were also conducted with amotosalen alone at relatively high doses in the absence of photochemical treatment and consequently in the absence of photoproducts.

The toxicology studies were designed to test the photochemically treated mixtures at the maximum feasible

dose or volumes that would not compromise the studies by inducing protein-load-related renal and cardiovascular impairment. For example, in animal studies, acute doses delivered over a 1-hour period were about twice the plasma volume of the animal and the daily doses for the multidose studies were equal to 50 percent of the plasma volume of the test species. The volume of PLT concentrate administered to humans is approximately 5 mL per kg. In the toxicology studies, a dose volume of 25 mL per kg was used. If CAD treatment was employed, the 25 mL per kg doses provided mean amotosalen exposures of 7.5 µg per kg, and if no CAD treatment was employed, mean amotosalen exposures were approximately 350 µg per kg. The clinical human exposure is approximately 1 µg per kg.

Studies conducted in rats (Sprague-Dawley), dogs (beagle), and rabbits (New Zealand White) employed photochemically treated mixtures containing 35 percent homologous plasma and 65 percent InterSol. A vehicle control of 35 percent homologous plasma was used in all studies. Species-specific plasma was used, but homologous PLT concentrates could not be prepared for laboratory animals, with the exception of the use of photochemically treated canine PLTs in dogs. Human PLTs were used in monkeys. The highest dose in each study was without CAD and the maximum feasible dose volume in that species (as discussed previously) based on the results of preceding studies. The remainder of this review will provide a description, method, and significance for the types of studies shown in Fig. 3.[8] For the studies with amotosalen parent compound, the highest dose level in each study was selected as being the maximum tolerated dose in that species based on the results of the preceding studies.

Acute toxicity

Acute toxic effects are determined in vivo after the administration of a single high dose. Typically, males and females of one rodent and one nonrodent species are used. After dosing, animals are observed for a 14-day period. In addition to mortality, daily examinations of test animals are conducted for signs of toxicity, lethargy, behavioral modifications, morbidity, body weight, and food consumption. At the end of the 14-day period, a gross necropsy is conducted to identify gross lesions, and tissues are collected for histologic evaluations. The most meaningful scientific information derived from acute toxicity tests comes from clinical observations and postmortem examination of animals rather than a specific LD_{50} value. The utility of the acute study for purposes of establishing an LD_{50} has been an area of controversy and is of limited significance in this setting.[9] Nevertheless, acute toxicity studies are essential for characterizing the toxic effects of NCEs.

Acute studies for the INTERCEPT program used photochemically treated 35 percent plasma and amotosalen alone and were conducted in the rat (five rats/sex/group) and dog (one dog/sex/group). The results are summarized in Tables 1 and 2.

Repeated-dose toxicity (subacute and subchronic)

Repeated administration in subacute studies, which are of 14 days duration, obtain additional information on the toxicity of an NCE and aid in establishing dosing parameters for subchronic studies, which are 30 to 90 days in duration. A subchronic study is usually conducted in both sexes of one rodent and one nonrodent species with animals observed once or twice daily for signs of toxicity. Clinical laboratory evaluations (hematology, chemistry, and urinalysis) and ophthalmologic examinations (indirect and slit lamp) are completed at scheduled intervals. In dogs, electrocardiographic and tail cuff blood pressure recordings are made before dosing and at scheduled intervals. Blood is taken after the first dose and during the last week of dosing for evaluation of drug levels in the plasma (toxicokinetics). At the completion of dosing, gross pathologic examinations are made and tissues from specified organs are evaluated microscopically. In subchronic studies, a subset of animals is designated to an observation period and remains on study undosed for an additional 4 weeks to assess for latent toxicity or toxicity reversal. The same set of clinical observations, clinical pathology, and gross and histopathologic evaluations are made on these animals.

Repeated-dose studies for the INTERCEPT program were conducted in the rat (5-20 rats/sex/group), dog (3-8 dogs/sex/group), and monkey (2-3 monkeys/sex/group).

Acute (single-dose)
Repeated-dose
Safety pharmacology
Reproductive
Genotoxicity
Carcinogenicity
Phototoxicity
Pharmacokinetics

Fig. 3. Studies conducted in the preclinical safety evaluation of the INTERCEPT Blood System for PLTs.

Studies were conducted with photochemically treated 35 percent plasma (rat and dog), photochemically treated canine PLTs in 35 percent plasma (dog), and human PLTs in photochemically treated 35 percent human plasma (monkey). Studies were conducted with amotosalen alone in rats and dogs. The results are summarized in Table 1. Repeated-dose toxicity tests indicated an absence of toxicity at relatively high exposure levels. In addition, administration in excess of recommended guidelines over long periods of time established long-term safety of the INTERCEPT Blood System for PLTs.

Safety pharmacology

Safety pharmacology studies are recommended in the ICH guidelines[10] and include an assessment of effects on function of vital organ systems. Doses selected for these studies mirror anticipated clinical doses and are conducted in a single species by the intended route of administration.

Administration of photochemically treated 35 percent plasma to rats (10 males/group) did not result in any functional effects on the central nervous system, gross pathologic changes in the brain, or any functional or gross pathologic changes in the renal system. Administration of photochemically treated human PLT mixture with CAD treatment to cynomolgus monkeys (six males) for 1 hour did not result in any electrocardiographic or hemodynamic effects on the cardiovascular system. In a cardiac arrhythmia study, nine monkeys were administered 1-hour transfusions of a control human PLT mixture or photochemically treated human PLTs with or without CAD treatment three times within 13 hours at an infusion rate of 25 mL per kg per hour. Each monkey was given infusions with each mixture at weekly intervals. There was no evidence of a drug-related effect on the incidences of cardiac arrhythmias or on hemodynamic parameters. Although no specific studies were conducted, neither respiratory nor gastrointestinal effects were detected in acute toxicity studies after intravenous infusion of 80 mL per kg photochemically treated 35 percent plasma with CAD (rats) or without CAD (rats and dogs).

TABLE 1. Summary of findings with photochemically treated test articles

Study type	Findings	Dose frequency	Safety margin*
Acute toxicity	None	Once	>1120
Repeated-dose toxicity	None	Up to daily for 3 months	>350
Reproductive toxicity	None	Daily for 12 to ~30 days	>350
Genotoxicity			
In vitro	None	Once	>2000
In vivo	None	Once or twice	>200
Carcinogenicity	None	3/week for 6 months	>350
Phototoxicity	None	3/week for 1 month	>40

* Safety margin calculated with clinical exposure of 1 μg per kg for in vivo studies.

Reproductive toxicity

The ICH guideline *Detection of Toxicity to Reproduction for Medicinal Products*[11] encourages a full assessment of the effects of NCEs on the development of offspring. The actual testing strategy is determined by:

- Anticipated NCE use, especially in relation to reproduction;

TABLE 2. Summary of findings with amotosalen alone

Study type	Findings	LOEL* (mg/kg)	Safety margin†
Acute toxicity	Mortality (rat)	250	250,000
	Electrocardiographic effects (dog)	45	45,000
	Central nervous system effects (dog)	40	40,000
Repeated-dose toxicity	Mortality (rat)	150	150,000
	Central nervous system effects (dog)	30	30,000
Reproductive toxicity‡	None	75	>75,000
Phototoxicity	Dermal	1	1,000
	Ocular	10	10,000
Genotoxicity—in vitro			
Bacterial mutagenicity assay	Positive	44§	44,000
Mouse lymphoma assay	Positive	7.5§	7,500
Chromosome aberration assay	Positive	5§	5,000
Genotoxicity—in vivo			
Mouse micronucleus assay	None	66	>66,000
Unscheduled DNA synthesis assay	None	34	>34,000
Carcinogenicity			
p53+/− mice	None	1	>1,000

* LOEL = lowest observed effect level or highest dose tested when no positive findings were observed.
† Safety margin calculated with the human C_{max} levels of 1 ng per mL for in vitro genotoxicity studies and the clinical exposure of 1 μg per kg for in vivo studies.
‡ Histopathology; not functional testing.
§ Data are μg/mL.

- The form of the substance and the route of administration intended for humans;
- Existing data on toxicity, pharmacodynamics, kinetics, and similarity in structure or activity to other compounds.

The combination of studies selected should allow for exposure to mature adults and all stages of development from conception to sexual maturity. To detect immediate and latent effects of exposure, observations should be continued through one complete life cycle, that is, from conception in one generation through conception in the following generation.

Reproductive toxicity assessments for INTERCEPT included male and female reproductive studies in the rat, teratology studies in rats and rabbits, and a peripostnatal developmental toxicity study in rats. Assessments were made with the photochemically treated product with and without CAD treatment. Studies were conducted with 25 males or females per sex per group except for the rabbit study with 20 female rabbits per group. No male or female reproductive toxicity, teratology, or developmental reproductive toxicity was observed. Additionally, no histologic evidence of effects on reproductive organs was seen in any subchronic or chronic toxicology study.

Genotoxicity

Genotoxicity tests can be defined as in vitro and in vivo tests designed to detect induced genetic damage. Compounds that induce damage have the potential to be human carcinogens and/or mutagens. A causal relationship between chemical exposure and carcinogenesis is established; however, a relationship between chemical exposure and heritable diseases has been difficult to prove. Thus, genotoxicity tests have been used mainly for screening as predictors of carcinogenicity.

Registration of a pharmaceutical requires comprehensive assessment of its genotoxic potential. No single test is capable of detecting all relevant genotoxic agents. Therefore, the usual approach is to conduct a battery of in vitro and in vivo tests for genotoxicity. The following standard battery is recommended by the ICH guideline[12] and a description of each test is given in Table 3:

- A test for gene mutation in bacteria;
- An in vitro test with cytogenetic evaluation of chromosome damage with mammalian cells or an in vitro mouse lymphoma thymidine kinase assay;
- An in vivo test for chromosome damage with rodent hematopoietic cells.

In the INTERCEPT Blood System for PLTs, with one exception (discussed later), photochemically treated human PLTs without CAD treatment did not induce genotoxicity in any in vitro or in vivo assay. Nevertheless, in these studies the residual amotosalen levels were far below the threshold concentrations for positive effects observed with amotosalen alone (≥5 µg/mL). To increase residual amotosalen and photoproducts in a PLT concentrate unit, amotosalen addition and UVA illumination were repeated 25 times in a single sample. These human PLTs, multiply processed by photochemical treatment without CAD treatment, were genotoxic both in the bacterial mutagenicity assay (one of six strains) and in the (CHO) cell assay. The effects were attributed solely to the residual amotosalen and did not reflect anticipated clinical exposures (positive at 35,000 times the clinical exposure in the bacterial mutagenicity assay and 7,500 times clinical exposure in the CHO assay). All positive controls in the studies induced a significant genotoxic response.

TABLE 3. Description of genotoxicity studies*

Bacterial mutagenicity assay—The bacterial mutagenicity assay detects point mutations in a series of histidine-requiring auxotrophs of *Salmonella typhimurium and Escherichia coli*. Tester strains are reverted from histidine dependence to histidine independence either by frameshift mutagens or base pair substitutions. The strains have different mutations in their histidine genes and thus have different sensitivities to mutagens.

Chromosome aberration assay—This assay detects chromosome damage. The Chinese hamster ovary (CHO-K_1) cell line, with a well-defined karyotype, was used. The cells were primarily assessed for structural chromosome aberrations after exposure to the test agent.

Mouse lymphoma assay—This assay detects point mutations in the mouse lymphoma L5178Y cell line at the thymidine kinase (TK) gene. This assay relies on the fact that mutant TK cells are resistant to the addition of the pyrimidine analog, trifluorothymidine (TFT). Nonmutant cells die in the presence of TFT. If exposing cells to the test agent causes expression of the mutation, placing the cells in TFT permits only mutant cells to survive.

In vivo mouse micronucleus assay—This assay detects DNA breakage or a lagging chromosome. Polychromatic erythrocytes (PCE), precursor cells to circulating RBCs, are assessed for the presence of nuclear material after genotoxic interaction with the test agent. These micronuclei represent parts of chromosomes or whole chromosomes left behind as the PCE ejects its nucleus in normal development to a mature erythrocyte.

In vivo unscheduled DNA synthesis assay—This assay detects DNA repair in targeted rat hepatocytes. Normally, liver cells do not undergo DNA synthesis or division with radiolabeled [^{14}C]thymidine, no significant incorporation of label will occur unless the test agent damages the DNA and stimulates the repair system.

* Vehicle and positive controls were included in all studies. In vitro genotoxicity studies were conducted in the absence and in the presence of metabolic activation. This activation involves cotreatment of the test article with a rat liver microsome preparation to assess whether the test article is metabolized to products that are more or less genotoxic.

Amotosalen alone was genotoxic in vitro in one of six strains in the bacterial mutagenicity assay, in the mouse lymphoma TK assay, and in the CHO cell assay. Because amotosalen interacts with DNA and RNA by intercalation, these in vitro genotoxicity results were anticipated as characteristic of intercalators. Nevertheless, metabolic activation reduced the genotoxic potential of both amotosalen, even at high intravenous doses, and the multiply processed photochemically treated human PLTs without CAD treatment in all in vitro genotoxicity studies. These results were consistent with those from in vivo (intravenous) genotoxicity assays (hepatic unscheduled DNA synthesis assay in Fischer 344 rats and micronucleus assay in ICR mice), in which, when mammalian metabolic pathways were intact and functional, amotosalen was not genotoxic. All positive controls in the studies induced a significant genotoxic response.

The ratio between the doses of amotosalen that were genotoxic and expected clinical peak plasma level after transfusion (1 ng/mL) were very high in all the assays. Among the in vitro assays in which a positive result was obtained, the lowest ratios were found in the CHO cell studies (2000-fold without metabolic activation and 24,000-fold with metabolic activation). The in vivo assay results were negative; the ratio of the highest nongenotoxic dose to the expected clinical peak plasma level was 40,000-fold.

Carcinogenicity

The strategy for testing the carcinogenic potential of an NCE is developed after obtaining key information such as the intended patient population and clinical dosage regimen, the pharmacodynamics of the NCE, and the results of genetic toxicology assessments and repeated-dose toxicity studies. Short-term mutagenicity studies are used to aid in the identification of potential carcinogens; however, these methods are of limited use in directly establishing the estimated risk posed to a human population. Genotoxicity tests are typically used as screens to indicate if the NCE has the capacity to induce mutations and/or DNA damage in cells in vitro or in vivo. If an NCE is identified as a genotoxin, the question remains—does it possess the ability to be a genotoxic carcinogen? The process by which this could take place is shown in Fig. 4.

Neoplastic induction in rodents has been the basis of the testing strategy for the medium to long-term approach to carcinogenicity testing. The administration of chemicals in the diet for long periods was introduced in the 1930s[13] and this assay is still the basis for the chronic bioassay of carcinogenicity.[14,15] Additionally, various organ-specific model systems, multistage models, and the use of transgenic animals in carcinogen testing have been employed.[16]

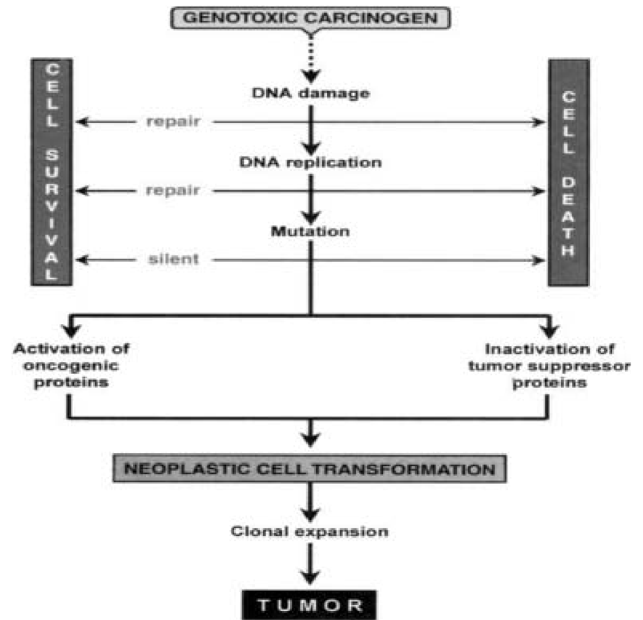

Fig. 4. The process of carcinogenesis initiated by genotoxic carcinogens.[27]

One of the transgenic models for carcinogenesis is the $p53^{def}$ mouse. The $p53^{def}$ mouse line has one functional wild-type p53 allele and one inactivated allele. The p53 gene is critical to cell cycle control and DNA repair and is often found to be mutated or lost in human and rodent tumors.[17] Mice with a single copy of the wild-type p53 allele ($p53^{+/-}$ heterozygous) offer a single target for mutagens, a condition analogous to humans with some heritable forms of cancer. These transgenic mice have increased sensitivity to genetic carcinogens.[18-20] In a study comparing mice homozygous versus heterozygous for the deletion,[18] approximately half of the homozygotes developed tumors by 4.5 months and all of the mice died or developed tumors by 10 months of age. In contrast, at 12 months of age, 8 percent of p53 heterozygotes developed spontaneous tumors. Nevertheless, after 12 months of age the rate of tumor development accelerated and by 18 months over 50 percent of the mice succumbed to tumors. Wild-type control mice did not develop tumors over the 18-month period (Fig. 5).

Amotosalen was specifically tested for potential carcinogenicity in the INTERCEPT program because it directly interacts with DNA. A panel of internationally recognized experts on genotoxicity and carcinogenicity was convened to conduct an in-depth review of the genotoxic and carcinogenic potential of amotosalen photochemically treated PLTs. The panel of experts concluded that "the CAD-treated illuminated amotosalen human PLT mixture did not present a significant genotoxic risk to patients and that no further mutagenicity or carcinogenic-

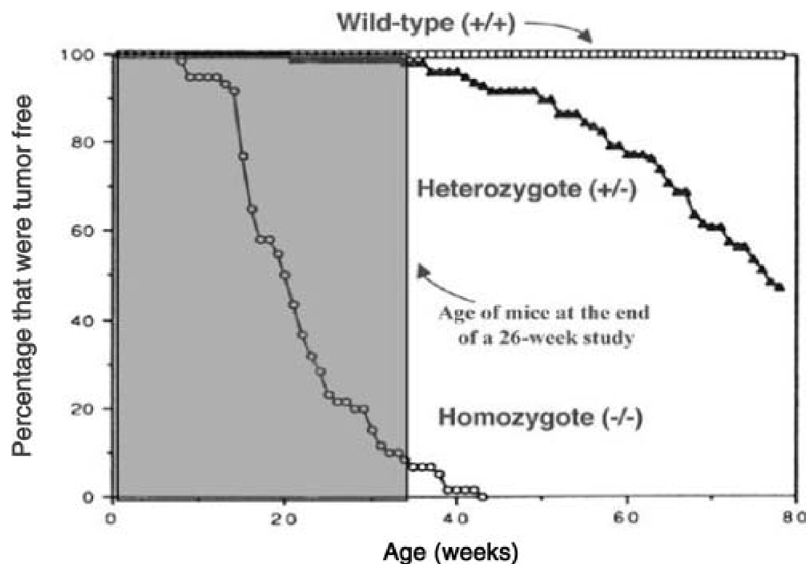

Fig. 5. Spontaneous tumor incidence in p53-deficient mice. (□) surviving wild type mice (p53$^{+/+}$); (▲) heterozygous mice (p53$^{+/-}$); and (○) homozygous mice (p53$^{-/-}$).[18]

ity testing was warranted." Key observations in forming that conclusion were the following:

- PLT concentrates are administered relatively infrequently.
- Each PLT concentrate contains only about 50 µg of residual amotosalen. (In comparison, dietary intake of psoralens in the US is reported by the FDA to be about 1300 µg per day.[21])
- Amotosalen is rapidly metabolized and excreted.
- Photochemically treated PLT concentrates were nonmutagenic, even when tested without CAD treatment.

Moreover, in PLT concentrates photochemically treated 25 times without any CAD treatment, mutagenicity corresponded to the amotosalen levels, indicating no additional mutagenicity from the photoproducts. In addition, amotosalen was rapidly metabolized and excreted, and the mutagenicity of amotosalen was reduced in all assays by metabolic activation. Finally, amotosalen was nongenotoxic in the in vivo mouse micronucleus and rat unscheduled DNA synthesis genotoxicity assays, even at doses that produced some lethality.

The ratios of the highest nongenotoxic concentrations of amotosalen to the peak plasma level anticipated in clinical use are extremely large. Nevertheless, because the carcinogenic potential of photochemically treated PLTs remained a critical factor in defining the risk-to-benefit ratio of the product, the FDA requested conduct of a carcinogenicity assay in p53 transgenic mice (strain C57BL/6TacfBR-[KO]p53N5(+/–)) to assess further the carcinogenic potential of amotosalen and its photoproducts.

The study evaluated the potential oncogenicity of amotosalen alone and amotosalen photochemically treated 35 percent plasma before and after photochemical treatment and with and without CAD treatment when administered to groups of 20 mice per sex by IV infusion three times per week for 26 weeks. The dose of amotosalen ranged from approximately 1 µg per kg to approximately 1 mg per kg (1000 times the clinical exposure). A control group received p-cresidine, a known carcinogen, by daily oral gavage. Mice were observed for evidence of morbidity and mortality and body weights were measured weekly. After 26 weeks of treatment, the positive control group showed urinary bladder cell dysmorphology and evidence of carcinoma, findings consistent with previous bioassay data for p-cresidine. No amotosalen-related toxicities or evidence of amotosalen-induced carcinogenicity were observed.

The margin of safety of approximately 1000-fold is based on a single dose. The cumulative exposure in the carcinogenicity study far exceeded the anticipated clinical exposure for most patients receiving 14 transfusions over 8 weeks, based on the TRAP study,[4] compared to 78 doses over 26 weeks in the carcinogenicity study.

Phototoxicity

Phototoxicity studies are not typical in preclinical safety programs. Their conduct is based on the nature of the NCE and whether it falls into a class of compounds having the ability to induce a photoreaction. Some psoralens are known to elicit dermal and ocular phototoxicity in the presence of UV light, including sunlight.[22-25] With this fact in mind, several phototoxicity studies were conducted to assess the phototoxic potential of amotosalen photochemically treated 35 percent plasma and amotosalen alone.

In the INTERCEPT program, four studies, ranging from a single-dose study to a 1-month study, were conducted in rats (five rats/sex/group) to evaluate the phototoxic potential of photochemically treated 35 percent plasma. No evidence of ocular phototoxicity was found in any experiment. Dermal phototoxicity (erythema and/or edema) was present in the initial single-dose studies but did not occur in the subsequent multidose experiments. Considering dose volume and amotosalen concentration, the high dose in the 1-month study provided more than 40 times the clinical exposure from an amotosalen photochemically treated PLT concentrate. In all studies, evi-

TABLE 4. Toxicokinetics of amotosalen*

Species	Test article†	Amotosalen dose (µg/kg)	Ratio to human	C_{max}‡ (ng/mL)	Ratio to human	AUC§ (ng·hr/mL)	Ratio to human	t_{50} (hr)
Rat	PCT** 35% plasma, no CAD	347	868	30	33	112	187	7.2
Dog	PCT 35% plasma, no CAD	240	600	34	38	133	222	9.1
Dog	PCT PLTs, no CAD	190	475	43	48	188	313	7.2
Human	PCT PLTs, with CAD¶	0.4	1	0.9	1	0.6	1	6.5

* Rat and dog data are from the 3-month toxicity studies; human data are from the Phase 1b clinical trial.
† Dose volume in toxicology studies was 25 mL per kg. Values for Day 1 and Week 13 were the same; thus, values presented are for Day 1.
‡ C_{max} = maximum concentration of amotosalen in plasma.
§ AUC = area under the concentration-time curve.
¶ Clinically, subjects were given a single 300-mL PLT unit.
** PCT = photochemically treated.

dence of dermal phototoxicity was present in positive control groups administered a known phototoxic psoralen (8-methoxypsoralen).

Rats were also administered amotosalen alone by a 1-hour IV infusion followed by UV exposure to a dorsal skin site and both eyes within 15 minutes after dosing. Dermal responses of erythema, edema, or flaking occurred at 10 mg per kg amotosalen in males and 1 and 10 mg per kg amotosalen in females; ocular responses of keratitis, miosis, or chemosis occurred at 10 mg per kg amotosalen. No treatment-related dermal changes were present in rats given 0.1 mg per kg amotosalen, and no treatment-related ophthalmologic effects were present in rats given 1 mg per kg amotosalen. The overall phototoxicity findings for amotosalen and photochemically treated 35 percent plasma indicated only a minimal potential for such a response to photochemically treated PLTs under the intended conditions of clinical use.

Pharmacokinetics

The quantitation and determination of the time course of absorption, distribution, biotransformation, and excretion of chemicals are referred to as pharmacokinetics. Determination of the disposition of chemicals (t_{50}, elimination rate constants, tissue profile, etc.) in a repeated-dose toxicology study is referred to as toxicokinetics. Calculations with various mathematical models enable the toxicologist to characterize chemical disposition, which is essential for the assessment of toxicity.

Examination of species differences and species-specific metabolic pathways permits a prediction of the disposition of a compound and its role in toxicity during human exposure.[26] In chronic toxicology studies, quantitation of plasma drug levels is determined after the first dose and during the last week of dosing. The results indicate if drug accumulation occurs over the duration of the study and may enable corroboration of toxicities with accumulation. The pharmacokinetics of amotosalen and photochemically treated 35 percent species-specific plasma, with and without CAD treatment, were evaluated and the results have been summarized elsewhere.[8]

In 3-month toxicity studies, rats were dosed once daily and dogs were dosed three times per week with photochemically treated 35 percent plasma with and without CAD treatment. In another study, dogs were dosed once weekly with photochemically treated canine PLTs without CAD treatment. In each of these studies, blood samples were collected at scheduled intervals before and after dosing on the first day and during the last week of dosing to enable a complete pharmacokinetic analysis of amotosalen. This permitted a comparison to human pharmacokinetic data from a Phase 1b safety and tolerance trial (Table 4).

DISCUSSION

Toxic effects of a new drug occur when the parent molecule or its metabolites reach target tissues at a concentration and remain for a length of time sufficient to produce a toxic result. Figure 6 represents exposure to a compound and the resulting response. At very low levels, no pharmacologic response is elicited. As exposure increases, a pharmacologic response is observed. With a blood pressure medication, for example, the pharmacologic response takes place at a threshold plasma drug concentration. If the pharmacologic concentration is exceeded, an exaggerated clinical response may occur and blood pressure may be lowered to dangerous levels. The situation with amotosalen is very different. There is no pharmacologic range because amotosalen has no intended or observed pharmacologic action.

Safety margins for amotosalen in the various toxicology studies have been shown to be very large (>350-fold in repeated-dose, reproductive, safety pharmacology studies; >1000-fold in the carcinogenicity study). When compared to prescription pharmaceuticals, these safety margins appear more pronounced. Pharmaceuticals may have very low safety margins (<1, anticancers) to safety margins of less than 10 for cardiovascular agents, anti-infectives, and anti-inflammatory agents. Even a com-

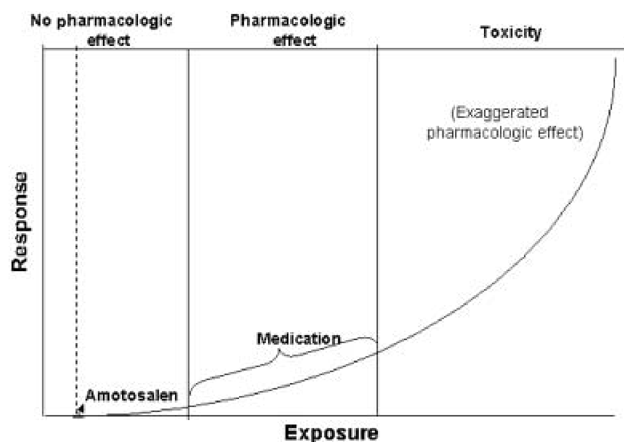

Fig. 6. Biologic response of a NCE ranges from no pharmacologic effect to desired pharmacologic action to toxicity. Amotosalen has no pharmacologic effects when administered at large multiples of clinical exposure.

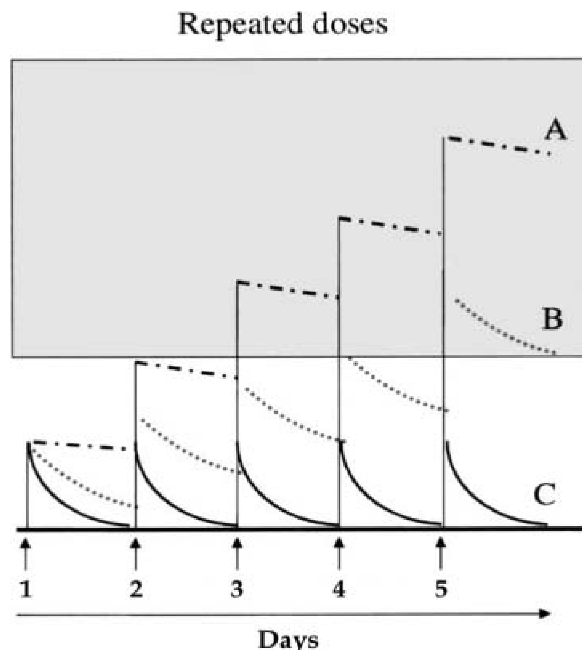

Fig. 7. Relationship between dose and concentration at the target site under different conditions of elimination rate. Lines A, B, and C represent chemicals with different t_{50} ranging from a long t_{50} to a short t_{50}, respectively. Shaded area is representative of the concentration of a chemical at the target site necessary to elicit a toxic response. Owing to the relative short t_{50} of amotosalen, no toxic response is elicited after repeated doses.

monly used over-the-counter compound such as acetaminophen has a safety margin for hepatotoxicity of approximately 5-fold.

The toxicology studies with amotosalen demonstrated that toxicity only occurs after many orders of magnitude above the residual level in one transfusion unit. Yet the question remained, can amotosalen levels in patient's plasma be reached that could elicit a toxic response? Figure 7 illustrates that, for compounds with different t_{50}, accumulation in plasma varies. Repeated doses of a compound with a long t_{50} can result in toxic levels within two to three daily doses. A compound with an intermediate t_{50} would require five to six doses before eliciting toxicity. Amotosalen, with a comparatively short t_{50} is eliminated before the next dose and no accumulation occurs. The toxicokinetic data demonstrate that amotosalen's t_{50} is relatively short and steady state in plasma is reached after the first dose. Moreover, the data indicate there is no accumulation after 3 months of dosing in rats or dogs.

Safety margins in toxicology studies obtained with the INTERCEPT Blood System for PLTs were evaluated as multiples of the clinical dose (Fig. 8) of amotosalen (1 µg/kg). For repeated-dose studies, reproductive toxicity studies, and safety pharmacology studies, amotosalen doses up to 350 µg per kg were administered in photochemically treated 35 percent plasma studies without CAD treatment. CAD treatment sometimes was omitted and increasing dose volumes were implemented to evaluate safety margins at higher exposures. In the carcinogenicity study, the highest dose of amotosalen was 1 mg per kg, approximately 1000 times the clinical exposure. For in vivo genotoxicity studies, the mean highest nongenotoxic dose level of amotosalen administered was more than 40,000 times the clinical dose. Amotosalen and photochemically treated formulations demonstrate central nervous system and electrocardiographic toxicity only at nonclinical doses, more than 30,000-fold expected clinical exposures. Based on the extremely large safety margins, these observations are not considered to be of toxicologic relevance. Similarly, no genotoxicity or photoxicity was observed up to 17,000 and 1,000 times the clinical exposure, respectively. The results of the toxicokinetic analyses from 3-month rat and dog studies with photochemically treated formulations with or without CAD treatment were compared with clinical data. These data indicate that the test articles used in the key toxicology studies provided large multiples of the clinical exposure to amotosalen, whether the comparison was based on dose, C_{max}, or area under the concentration-time curve.

The results of this comprehensive series of studies demonstrated no toxicologically relevant effects of the INTERCEPT Blood System for PLTs and demonstrate the utility of toxicology testing in the drug development process.

In conclusion, toxicity testing is often poorly understood, but is a vital component of the drug development process. Toxicology testing is designed to investigate the preclinical safety before an informed decision about the risk a particular compound poses before human testing. In latter stages of the development process, toxicity testing

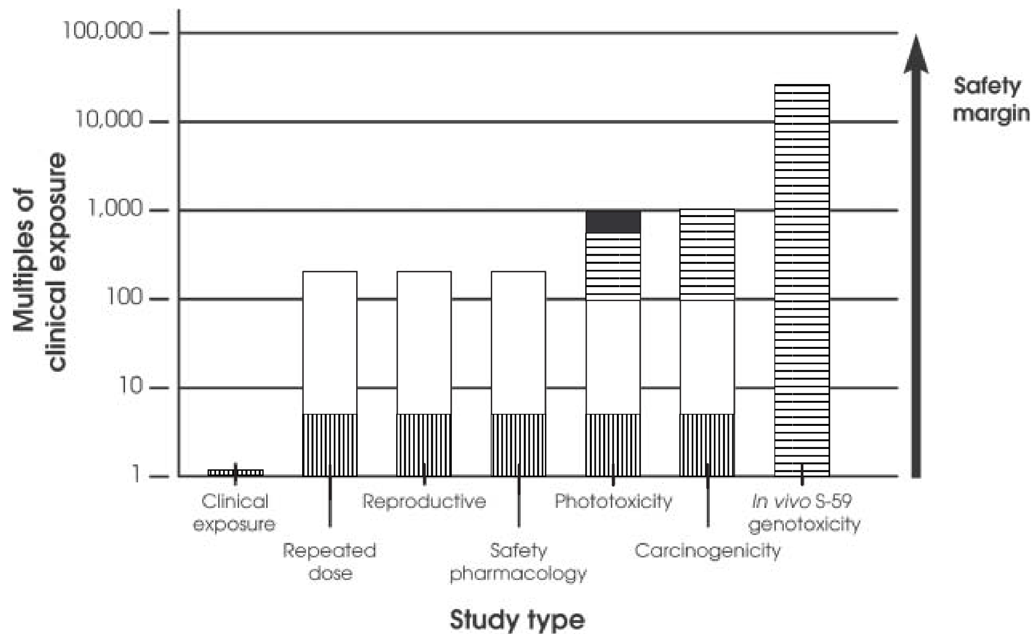

Fig. 8. Safety margins from toxicology studies compared to the clinical exposure. (■) Toxicity observed; (▤) no UVA; (□) no CAD; (▥) with CAD.

can address special populations such as pediatric populations. Moreover, only by embarking on this organized and extensive series of tests can regulatory bodies and clinicians make reasonable decisions regarding the risks and benefits of a therapeutic agent.

The preclinical safety program for the INTERCEPT Blood System for PLTs encompassed a comprehensive set of disciplines that established the safety margins for amotosalen. Studies were conducted that assessed the potential toxicity of a single dose or repeated doses, safety pharmacology, genetic toxicology, reproductive toxicity, carcinogenicity, and phototoxicity of amotosalen by itself and in the formulation of the photochemical treatment process.

This review was designed to clarify the role of toxicology, implementation of toxicology testing standards, and determination of risk assessment as it pertained to the INTERCEPT Blood System for PLTs. The results presented in this review are critical in ensuring safety in the development of a new PI technology for the blood transfusion community.

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文献7

Transfusion of platelet components prepared with photochemical pathogen inactivation treatment during a Chikungunya virus epidemic in Ile de La Réunion

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BACKGROUND: During the Chikungunya virus (CHIKV) epidemic on Ile de La Réunion, France, more than 30% of 750,000 inhabitants were infected. Local blood donation was suspended to prevent transfusion-transmitted infection (TT-CHIKV). To sustain the availability of platelet (PLT) components, the Établissement Français du Sang implemented universal pathogen inactivation (INTERCEPT, Cerus Europe BV) of PLT components (CPAs). The study assessed the safety of PLT components treated with pathogen inactivation transfused in routine clinical practice.

STUDY DESIGN AND METHODS: This was a retrospective observational study using patient medical records and the AFSSAPS hemovigilance database (eFIT) to identify TT-CHIKV and adverse events (AEs) classified as acute transfusion reactions (ATRs) to PLT components prepared with pathogen inactivation.

RESULTS: During 1 year, 1950 INTERCEPT-CPAs were transfused to 335 adult, 51 pediatric, and 41 infant patients. Nineteen AEs were observed in 15 patients and 10 were classified as ATRs. Eight ATRs occurred in 6 pediatric hematology-oncology patients. No ATRs were observed in infants. The most frequently reported signs and symptoms were Grade 1 urticaria, itching, chills, fever, and anxiety. No cases of transfusion-related acute lung injury, TT-sepsis, or TT-CHIKV were detected.

CONCLUSIONS: INTERCEPT-CPAs were well tolerated in a broad range of patients, including infants. ATR incidence was low and when present ATRs were of mild severity.

Starting in 2005, an epidemic of Chikungunya virus (CHIKV) in the overseas French department of Ile de La Réunion, an island in the South Indian Ocean, resulted in the infection of more than one-third of the 750,000 inhabitants by early 2006.[1] CHIKV is an enveloped single-stranded alpha virus from the Togaviridae family transmitted by *Aedes* mosquitoes. It generally causes a mild febrile illness characterized by arthralgias lasting up to 10 days, but the recent epidemic was associated with myalgias, dermatitis, hemorrhage, meningoencephalitis, respiratory failure, cardiovascular decompensation, and fulminant hepatitis with persistent arthralgias in some patients.[2] Subsequently, more than 700 cases of CHIKV infection were reported in metropolitan France among returning travelers, and 1 infection

ABBREVIATIONS: AE(s) = adverse event(s); ATR(s) = acute transfusion reaction(s); CHIKV = Chikungunya virus; CPA(s) = apheresis platelet component(s); CRF(s) = case report form(s); EFS = Établissement Français du Sang; SAE(s) = severe adverse event(s); TT = transfusion transmitted.

From the EFS Ile de La Réunion and CHR Centre Hospitalier Départemental Felix Guyon, St Denis, Ile de La Réunion, France; CHR Groupe Hospitalier Sud Réunion de St Pierre, Ile de La Réunion, France; Cerus Corporation, Concord, California; EFS Alsace, Strasbourg, France; Cerus Europe BV, Amersfoort, The Netherlands; the University of California School of Medicine, San Francisco, California; INSERM U.311 and Université Louis Pasteur, Strasbourg, France.

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after needle stick of a health care worker.[3,4] Owing to the high prevalence of CHIKV infection and the potential for transfusion-transmitted (TT) infection, the Établissement Français du Sang (EFS [French National Transfusion Service]) suspended blood donation on Ile de La Réunion to prevent TT-CHIKV.[1] To meet the requirements for safe blood components on Ile de La Réunion, red blood cells and plasma components (fresh-frozen plasma) were supplied by EFS from metropolitan France. Because of the limited shelf life (5 days) of platelet (PLT) components, EFS-La Réunion implemented pathogen inactivation preparation of apheresis PLT components (CPAs) to maintain local PLT component supplies.[5]

Prior research studies had demonstrated that CHIKV was inactivated by photochemical treatment with amotosalen HCl and UVA light (INTERCEPT Blood System for platelets, Cerus Europe BV, Amersfoort, The Netherlands).[6] In addition, this system had been shown to inactivate high levels of a broad spectrum of viruses, bacteria, protozoa, and white blood cells (WBC) in PLT components.[7-9] The INTERCEPT system received CE Mark registration as a Class III drug device and as of 2005 received approval from the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS, French Agency of Medical Safety of Health Products) for use with both apheresis- and whole blood–derived PLT components in France.

The INTERCEPT Blood System was implemented in routine practice as of March 13, 2006, by EFS-Ile de La Réunion. To date, approximately 4000 INTERCEPT-CPAs have been administered to a broad range of patients on Ile de La Réunion. After the first year of routine use of pathogen inactivation to prepare PLT components, we conducted a retrospective analysis of the response to transfusion of 1950 components to determine the incidence of acute transfusion reactions (ATRs) and serious adverse events (SAEs) attributed to use of this novel component. In addition, we determined the incidence of TT-CHIKV infection for the first year after implementation of pathogen inactivation treatment during the CHIKV epidemic.

MATERIALS AND METHODS

Collection of PLT components

Before introduction of the INTERCEPT system, CPAs were the sole type of PLT component provided by EFS-La Réunion. All CPAs were collected in donor plasma with integral filtration leukoreduction (Haemonetics, Braintree, MA). After introduction of INTERCEPT, PLTs were collected in approximately 40% donor plasma and 60% PLT additive solution (InterSol, Fenwal, La Chatre, France) from donors with PLT counts of 250×10^9/L or more using a blood component collection system (Haemonetics MCS+ system with the CSDP software) to allow automatic addition of InterSol. The targeted PLT dose per collection was 4.0×10^{11} or greater. WBC contamination was reduced by filtration with an integral WBC filter (Haemonetics). In addition to standard viral screening tests, donors were tested for CHIKV infection by an investigational reverse-transcriptase polymerase chain reaction assay (RT-PCR).[3,10]

Pathogen inactivation treatment of PLT components

CPAs containing 2.5×10^{11} to 6.0×10^{11} PLTs in 300 to 390 mL of approximately 40% plasma and 60% InterSol were prepared with pathogen inactivation using the INTERCEPT processing system (INT2202, Cerus Europe BV) according to manufacturer's instructions for use. Briefly, a unit of CPA was mixed with amotosalen (nominal final concentration of 150 µmol/L) and illuminated with long-wavelength ultraviolet UVA (320-400 nm) light for a 3 J/cm^2 treatment. The illuminated PLT mixture was incubated in a compound adsorption device in a temperature controlled PLT shaker/incubator (22 ± 2°C) for 6 to 16 hours before transferring to the final storage container. Treated CPAs were stored for up to 5 days under standard blood bank conditions before issue for transfusion.

Hemovigilance surveillance

General study design

This was a retrospective analysis of data recorded prospectively in primary care medical records and as part of the AFSSAPS active hemovigilance surveillance program.[11] There were no patient inclusion or exclusion criteria other than the requirement for PLT transfusion. All patients who received PLT transfusion support during the defined study period were included in the analysis. Case report forms (CRFs) were used to collect patient data[12] on each transfusion of INTERCEPT-CPAs between March 13, 2006, and March 13, 2007, regardless of whether an adverse event (AE) was reported.

The primary endpoint of the study was the proportion of transfusions with ATR after administration of PLT components. ATRs were defined as AEs possibly related, probably related, or related to a PLT transfusion. SAEs were defined as AEs that were fatal, life-threatening, or disabling; resulted in or prolonged hospitalization or morbidity; or were incapacitating. Secondary endpoints included evidence of acute TT-CHIKV infection (based on nucleic acid amplification of viral sequences). All transfused patients were monitored for 7 days after each transfusion for potential TT-CHIKV infection using standard EFS operating procedures.[10] Data also were collected on use of INTERCEPT-CPAs by patient primary diagnosis category and clinical indication for transfusion.

Data collection methods

All patients transfused with PLTs prepared by EFS-La Réunion from March 13, 2006, through March 13, 2007, were identified from the EFS-La Réunion electronic database for the collection, production, and issuance of blood components. Each patient was identified with a unique study number to preserve anonymity. The following data were collected: PLT product code; patient unique identification number associated with the component, patient demographics (age, sex), and primary diagnosis based on clinical care area; primary therapy (chemotherapy, hematopoietic stem cell transplant); surgery (cardiovascular or organ transplant); or other (general medical or multisystem organ failure).

Primary care medical records of each patient were reviewed for the 24 hour period before each transfusion to establish a baseline profile of the patient's clinical condition, for 7 days after each PLT transfusion to identify new AEs arising after transfusion, and to record the relationship of AEs to PLT transfusion in the primary medical record as assessed by primary care physicians. This review was conducted by an observer without knowledge of AEs reported in the AFSSAPS hemovigilance system (eFIT).[11] For the 24 hours before and for the 7 days after each PLT transfusion, medical records were specifically reviewed for evidence of clinical conditions that could be attributed to transfusion-related reactions, including fever (increase in temperature of 2 or 1°C with chills), chills, nausea, skin rash, urticaria, dyspnea, bronchospasm, tachycardia or bradycardia (change in heart rate by >25 bpm), hypotension or hypertension (decrease or increase in systolic or diastolic blood pressure >30 mm Hg, respectively), hemoglobinuria, hemolysis, and change in general well-being. Specific criteria were provided for the diagnosis of transfusion-associated acute lung injury (TRALI).[13] Clinical microbiology laboratory records were reviewed for documentation of transfusion-associated sepsis. The diagnosis of transfusion-associated sepsis required the isolation of the same bacteria species from the patient and the implicated PLT component.

Transfusion CRFs were completed for each PLT transfusion regardless of whether or not an AE was noted in the medical record. In case of the occurrence of an AE, additional clinical and biologic information as well as test results for CHIKV infection (nucleic acid testing [NAT] by RT-PCR) were collected. These data were used by the medical record reviewer for assessment of causality and severity based on the medical record. Clinical severity of AEs was classified according to the following scale: Grade 0 = isolated dysfunction without clinical or biologic manifestation; Grade 1 = absence of immediate or long-term life-threatening effects; Grade 2 = long-term life-threatening effects; Grade 3 = immediate life-threatening effects; and Grade 4 = death. The relationship of AEs to the most proximate PLT transfusion was classified using the same criteria as used by the AFSSAPS hemovigilance system.[11]

The standardized CRFs had been validated in a prior hemovigilance study.[14] Data from the CRF were entered into an independent electronic database used for postmarketing hemovigilance programs[12,14] and reviewed by the principal investigator for incomplete data. At the conclusion of the study, AEs classified as transfusion reactions based on review of the primary care medical records were compared against AEs previously reported under the AFSSAPS hemovigilance program recorded in the eFIT database[11] to determine the total incidence of AEs attributed to PLT transfusion. These data were then analyzed to determine the incidence of ATRs.

Statistical analyses

A statistical analysis plan for the study was prepared and approved before analysis. All statistical analyses, summary tables, and data listings were generated using computer software (SAS, Version 8.2, SAS Institute, Cary, NC). The primary assessment was the incidence of transfusion reactions. The number and proportion (%) of transfusions and the proportion of patients with one or more transfusion reactions were summarized overall, by seriousness and by relationship to PLT transfusion. Corresponding 95% confidence intervals (CIs) for the binomial proportion were calculated using the F distribution method. The 95% CI were based on number of patients with any AE/ATR and the number of transfusions associated with any AE/ATR. In addition, the patient population profile, the characteristics of the PLT components, and the characteristics of the AEs after PLT transfusion were analyzed. Analyses to identify risk factors potentially associated with transfusion reactions were conducted using multivariate logistic regression analysis and by assessing association at a 10% significance level. Data were analyzed on a per-transfusion and a per-patient basis. All INTERCEPT PLT components administered to patients were part of the full analysis population and were analyzed, whether or not an AE was observed. All analyses were conducted using this full analysis population.

RESULTS

PLT component characteristics

Each CPA was treated with pathogen inactivation using the INTERCEPT Blood System on either Day 0 or Day 1 after PLT collection and stored for up to 5 days before release for transfusion. PLT components were released after completion of serologic and NAT. Pathogen inactivation treatment was used without bacteria detection other than routine quality control (QC) assays. Pathogen inactivation treatment replaced cytomegalovirus (CMV)

serology for patients who required CMV-safe PLTs and replaced gamma irradiation for prevention of transfusion-associated graft versus host disease.

The INTERCEPT process resulted in a mean PLT loss of 7.8% due to volume loss during container transfers. The mean PLT yield of INTERCEPT-CPAs was $4.2 \times 10^{11} \pm 0.7 \times 10^{11}$ PLTs per component. The residual WBC count met the national QC requirement ($<0.5 \times 10^6$/unit). Approximately 15% of PLT components were divided into 2 units before transfusion to fulfill clinical demand. The proportion of split PLT components was similar to that in the period before implementation of pathogen inactivation.

Patient demographics

Between March 13, 2006, and March 13, 2007, a total of 1950 INTERCEPT-CPAs were transfused to 427 patients (Table 1). Each patient received at least one INTERCEPT-CPA. The patient population consisted of 335 adult patients (>18 years), 51 pediatric patients (≥1 to <18 years), and 41 infants (<1 year). There were more male patients in each age group (Table 1).

Hematology-oncology disorders treated with chemotherapy and stem cell transplantation constituted 29.0% of the primary diagnoses among the transfused patient population and these patients received 61% of the PLT components (Tables 1 and 2). The largest patient group supported with PLT components was the general medical population (58.5%), but they received only 30% of the PLT components. A number of patients receiving PLT transfusions (12.2%) underwent major surgical procedures including cardiovascular surgery or solid organ transplantation. Among the pediatric patient group, the proportion of hematology-oncology patients (66.7%) was significantly higher ($p = 0.001$) than among the adult patient group (26.3%).

Approximately half of the patient population (51.5%) received transfusions in intensive care units and the other half (48.5%) were transfused on non–intensive care hospital services (Table 1). There were no outpatient transfusions in the current surveillance program. Subgroup analysis showed that, while most of the pediatric patients (78.4%) were transfused in non–intensive care hospital wards, the majority of infants (90.2%) were transfused on intensive care units.

PLT transfusion exposure

Approximately 53% of patients had a prior history of transfusion exposure to some blood component. The median number of PLT transfusions per patient was 2.0 (range, 1-66; Table 2). Of 1950 PLT transfusions, 1372 transfusions were administered to adult patients while 487 and 91 transfusions were administered to pediatric patients and infants, respectively. Based on the respective patient population, 36 to 47% of patients received two or more PLT transfusions. The number of transfusions per pediatric patient (9.5 ± 14.7) was significantly higher ($p < 0.001$) compared to those in the adult population (4.1 ± 6.2) while the opposite was true for infants (2.2 ± 2.4, $p < 0.002$). Based on primary diagnosis category, hematology-oncology patients in all age groups received a higher proportion of PLT transfusions per patient than those in other diagnosis groups (Table 2).

TABLE 1. Demographics of patients transfused with INTERCEPT-CPAs*

Demographic	Patients (n = 427)	Adult (n = 335)	Pediatric (n = 51)	Infants (n = 41)
Gender				
Male	262 (61.4)	202 (60.3)	35 (68.6)	25 (61.0)
Female	165 (38.6)	133 (39.7)	16 (31.4)	16 (39.0)
Age (years)				
Mean ± SD	42.4 ± 24.8	52.6 ± 17.1	9.4 ± 5.3	NA†
Median	46.0	53.0	10.0	NA†
Range	<1 to 87	>18 to 87	1 to 18	<1
Care location				
Intensive	220 (51.5)	172 (51.3)	11 (21.6)	37 (90.2)
Nonintensive	207 (48.5)	163 (48.7)	40 (78.4)	4 (9.8)
Hematology-oncology primary therapy	124‡ (29.0)	87 (26.3)	34 (66.7)	3 (7.3)
Conventional chemotherapy	102 (82.2)	69 (79.3)	30 (88.2)	3 (100)
Stem cell transplant	14 (11.3)	10 (11.5)	4 (11.8)	0 (0)
Surgery	52 (12.2)	48 (14.3)	3 (5.9)	1 (2.4)
Cardiovascular	49 (94.2)	45 (93.8)	3 (100)	1 (100)
Solid organ transplant	3 (5.8)	3 (6.2)	0 (0)	0 (0)
General medical	250 (58.5)	199 (59.4)	14 (27.4)	37 (90.3)
Missing diagnosis	1 (0.2)	1 (0.3)	0 (0)	0 (0)

* The number of patients (n) and the proportion (%) within each category are presented.
† Age for infants was only recorded as <1 year. NA = not applicable.
‡ Eight adult patients had no active therapy specified at time of transfusion.

TABLE 2. Transfusion exposure among patient populations

Population	All patients (n = 427)	Adult patients (n = 335)	Pediatric patients (n = 51)	Infant patients (n = 41)
All patients				
Transfusions (n)	1950	1372	487	91
Mean ± SD	4.6 ± 7.7	4.1 ± 6.2	9.5 ± 14.7	2.2 ± 2.4
Median	2.0	2.0	4.0	1.0
Range	1-66	1-46	1-66	1-11
Hematology-oncology				
Transfusions (n)	1192	738	446	8
Mean ± SD	9.6 ± 11.7	8.5 ± 8.9	13.1 ± 16.8	2.7 ± 2.9
Median	6.0	6.0	6.5	1.0
Range	1-66	1-46	1-66	1-6
Surgical				
Transfusions (n)	149	135	8	6
Mean ± SD	2.9 ± 3.6	2.8 ± 3.7	2.7 ± 1.5	6.0 ± 0.0
Median	2.0	2.0	3.0	6.0
Range	1-24	1-24	1-4	6.0
General medical				
Transfusions (n)	596	486	33	77
Mean ± SD	2.4 ± 3.6	2.4 ± 3.8	2.4 ± 2.8	2.1 ± 2.3
Median	1.0	1.0	1.0	1.0
Range	1-37	1-37	1-11	1-11
Missing diagnoses				
Transfusions (n)	13	13	0	0
Mean ± SD	13.0 ± 0.0	13.0 ± 0.0		
Median	13.0	13.0		
Range	13	13		

TABLE 3. Clinical characteristics of AEs and ATRs among patient populations*

	All patients (n = 427) Transfusions (n = 1950)		Adult patients (n = 335) Transfusions (n = 1372)		Pediatric patients (n = 51) Transfusions (n = 487)	
Characteristic	Any AE	ATRs	Any AE	ATRs	Any AE	ATRs
Patients with 1 or >AE	15 (3.5)	8 (1.9)	6 (1.8)	2 (0.6)	9 (17.6)	6 (11.8)
Transfusions with 1 or >AE	19 (1.0)	10 (0.5)	6 (0.4)	2 (0.1)	13 (2.7)	8 (1.6)
Signs/symptoms per transfusion†						
Fever	5 (0.3)	1 (<0.1)	2 (0.1)	1 (<0.1)	3 (0.6)	0
Chills	7 (0.4)	2 (0.1)	4 (0.3)	2 (0.1)	3 (0.6)	0
Itching	5 (0.3)	4 (0.2)	1 (<0.1)	0	4 (0.8)	4 (0.8)
Urticaria	7 (0.4)	6 (0.3)	1 (<0.1)	0	6 (1.2)	6 (1.2)
Dyspnea	1 (<0.1)	0	1 (<0.1)	0	0	0
Anxiety	4 (0.2)	0	2 (0.1)	0	2 (0.4)	0
Other	6 (0.3)	2 (0.1)	1 (<0.1)	0	5 (1.0)	2 (0.4)
Signs/symptoms per patient†						
Fever	4 (0.9)	1 (0.2)	2 (0.6)	1 (0.3)	2 (3.9)	0
Chills	5 (1.2)	2 (0.5)	4 (1.2)	2 (0.6)	1 (2.0)	0
Itching	5 (1.2)	4 (0.9)	1 (0.3)	0	4 (7.8)	4 (7.8)
Urticaria	5 (1.2)	4 (0.9)	1 (0.3)	0	4 (7.8)	4 (7.8)
Dyspnea	1 (0.2)	0	1 (0.3)	0	0	0
Anxiety	4 (0.9)	0	2 (0.6)	0	2 (3.9)	0
Other	6 (1.4)	2 (0.5)	1 (0.3)	0	5 (9.8)	2 (3.9)

* Data are reported as number (%). No AEs were reported for infant patients; thus, these patients and transfusions are not included in this table.
† Number of signs/symptoms can exceed number of AEs due to multiple observed signs/symptoms per AE.
ATR = causal relationship that an AE was possibly related, probably related, or related to INTERCEPT-CPA transfusion.

AEs and ATRs after PLT transfusion

The incidences of AEs and ATRs were evaluated on a per-transfusion as well as per-patient basis (Table 3). On a per-transfusion basis, 19 transfusions (95% CI, 1.0%-1.5%) were associated with an AE. Of these AEs, 10 (95% CI, 0.5%-0.9%) were classified as ATRs possibly, probably, or related to INTERCEPT-CPA transfusion. No SAEs, no cases of TT-sepsis, no cases of TRALI, and no deaths due to INTERCEPT-CPA transfusions were reported. On a per-patient basis, 15 patients (95% CI, 3.5%-5.7%) who received at least one transfusion of INTERCEPT-CPAs experienced an AE after PLT transfusions (Table 3). Only 8 patients (95% CI, 1.9%-3.6%) experienced an ATR attributed to INTERCEPT-CPA transfusion (Table 3).

Overall patient population: characteristics of clinical signs and symptoms associated with PLT transfusion

Of all AEs, on a per-transfusion basis, the most frequently observed symptoms/signs (0.3%-0.4% of 1950 transfusions) were fever, chills, itching, and urticaria (Table 3). Anxiety (0.2%) was the second most frequently reported symptom/sign. Only one incident of dyspnea was reported. Additional symptoms in the category of "other" included tachycardia, facial flushing, body pain, and cough, but with an individual incidence of 0.1% or less of transfusions. Most of the ATRs were described principally as Grade 1 urticaria (0.3%) and itching (0.2%) with all other symptoms/signs observed at a rate of 0.1% or less of transfusions.

On a per-patient basis, the most frequently observed symptoms/signs (1.2% of 427 patients) were chills, itching, and urticaria (Table 3). Fever and anxiety (0.9%) were the second most frequently observed symptoms/signs. One patient (0.2%) experienced a single episode of dyspnea. Additional symptoms in the category of "other" included tachycardia, facial flushing, body pain, and cough, each with an individual incidence of 0.5% or less on a per-patient basis. Most of ATRs were described as Grade 1 itching (0.9%), urticaria (0.9%), and chills (0.5%) with all others observed at a rate of 0.2% or less per patient.

Characteristics of AEs and ATRs in pediatric patients

Pediatric patients experienced a higher incidence of AEs than adult patients (Table 3). On a per-transfusion basis, 13 AEs (2.7%) and 8 ATRs (1.6%) occurred in pediatric patients compared to 6 AEs (0.4%) and 2 ATRs (0.1%) in adult patients. On a per-patient basis, 9 pediatric patients (17.6%) experienced at least 1 AE compared to 6 adult patients (1.8%). Similarly, 6 pediatric patients (11.8%) experienced at least 1 ATR compared to 2 adult patients (0.6%).

For all AEs reported in pediatric patients, the symptoms/signs were predominantly Grade 1 in severity consisting of fever, chills, itching, urticaria, anxiety, tachycardia, and facial flushing (Table 3). For pediatric patients experiencing ATRs, the symptoms/signs included itching, urticaria, tachycardia, and facial flushing, none of which were reported in adult patients. No AEs were associated with the 91 INTERCEPT-CPA transfusions administered to 41 infants who required PLT support.

Characteristics of AEs and ATRs associated with transfusion of split components

Of the 1950 transfusions, 540 INTERCEPT-CPAs were obtained from a split PLT component. The rates of AEs and ATRs on a per-transfusion basis for split components were 0.4 and 0%, respectively, compared to 1.2 and 0.7% for whole components. Of the 19 AEs reported, only 2 AEs (one in a 77-year-old male patient and one in a 16-year-old male patient) were associated with transfusion of a split INTERCEPT-CPAs (Table 4).

TABLE 4. Effect of split components on the frequency of AEs and ATRs*

Component	Transfusions	AEs	ATRs
Split INTERCEPT-CPAs	540	2 (0.4)	0 (0)
Whole INTERCEPT-CPAs	1410	17 (1.2)	10 (0.7)
Total	1950	19 (1.0)	10 (0.5)

* Data are reported as number (%).

Incidence of TT-CHIKV

A substantial proportion of transfusions were administered to hematology-oncology patients treated with potentially immune-suppressive therapy. There were no cases of TT-CHIKV reported in this survey based on the test results using an investigational assay for viral nucleic acid or posttransfusion clinical observation for signs and symptoms of CHIKV infection.

DISCUSSION

CHIKV resulted in an epidemic on La Réunion Island in which approximately 41% of the population was infected. Serologic and epidemiologic surveillance studies estimated the prevalence of asymptomatic infection at 15% of total CHIKV infections.[1] Efforts to identify infected blood donors with either serologic assays or CHIKV specific nucleic acid amplification assays have shown considerable variability and suboptimal sensitivity.[15] The mean risk of contamination of a blood donation throughout the epidemic was estimated at 132 per 100,000 donations, and at the peak of the epidemic, the risk was estimated at 1,500 per 100,000 donations.[1] At the time of the current study, optimal methods to detect infected donors with low viral titers were not available, and a NAT with sensitivities of 40 to 350 copies/mL was only developed later.[16] In the period of this study, collection of CHIKV-contaminated PLTs from asymptomatic donors was plausible. During the epidemic before use of pathogen inactivation, two cases of TT-CHIKV were suspected, but neither case could be conclusively proven.[10] At least one blood-borne transmission due to a needle-stick has been documented.[4]

This study accomplished multiple objectives. Foremost, it provided hemovigilance data to evaluate the effectiveness of the INTERCEPT Blood System to prevent PLT TT-CHIKV during an epidemic. These data are especially relevant given the specific association of CHIKV with

PLTs,[17,18] which could lead to low detection sensitivity for serum-based tests. In addition to evaluating the efficacy to prevent TT-CHIKV, this study provided an opportunity to extend the safety profile of INTERCEPT PLTs transfused to a broad patient population. Finally, this study permitted an evaluation of the operational logistics of the INTERCEPT PLT system implemented under emergency conditions.

Data provided by EFS-La Réunion for the years 2004 and 2005 with conventional PLT components suspended in 100% plasma indicated an ATR incidence of 2.2 and 5.4% of PLT transfusions among heavily transfused pediatric oncology-hematology patients, respectively.[19] In comparison, this study demonstrated a lower incidence (1.6%) of ATRs per PLT transfusion. These results are consistent with reported ATR frequencies reported for INTERCEPT PLT components in routine use from multiple European centers,[12,14] but lower than the frequencies reported for treated PLT components in the EuroSprite (6%) and the SPRINT clinical trials (3%).[20,21] The higher incidence of ATRs observed in the clinical trials may have been due to differences in patient populations, which in the clinical trials consisted largely of heavily transfused hematology-oncology patients undergoing hematopoietic stem cell transplantation. Similar to previous studies, all of the ATRs observed in the current survey were of mild severity, and none were indicative of clinical CHIKV. It is relevant to note that the size of this study was insufficient to characterize the incidence of septic transfusion reactions, although none were reported.

The clinical symptoms of CHIKV infection include fever, severe polyarthralgia, myalgia, dermatitis, hemorrhage, meningoencephalitis, respiratory failure, cardiovascular decompensation, and fulminant hepatitis with a mortality rate of one in 1000 during the La Réunion epidemic.[22] Thus, review of primary medical records should have been sufficiently sensitive to detect TT-CHIKV. No TT-CHIKV cases were detected in the patient population monitored in this study after implementation of the INTERCEPT Blood System for PLTs.

The retrospective surveillance described in this report provided an opportunity to evaluate the sensitivity of the AFSSAPS active hemovigilance system[11] to detect transfusion-related AEs. We did not detect any additional transfusion-related AEs in our independent review of primary medical records compared to the AFSSAPS/eFIT database for transfusion-related incidents. This limited experience is consistent with the sensitivity of the AFSSAPS hemovigilance system in detecting transfusion-related AEs.

This study included a substantial number of pediatric patients, some of whom were infants. None of the prior studies with INTERCEPT PLT components included a substantial infant patient population. Interestingly, pediatric patients had the highest rate of AEs and ATRs after transfusion of INTERCEPT-CPAs. This finding may not be surprising because the proportion of hematology-oncology patients and the levels of PLT component exposure were higher among pediatric patients. On the other hand, no AEs or ATRs were observed in infants who received INTERCEPT-CPA transfusions largely for nonmalignant medical disorders, but this population was of limited size and less intensively transfused.

The study also provided experience with the implementation and operational logistics of the INTERCEPT system in a remote, small regional blood center. EFS-Ile de La Réunion performs approximately 100 to 150 apheresis PLT collections per month.[23] Complete conversion to pathogen inactivation of PLT components was achieved in 2 weeks. In routine operation, no additional personnel were required after implementation of the INTERCEPT system.

This is the first study to demonstrate the utility of pathogen inactivation as a proactive approach to prevent a potentially TT infection during an epidemic. The technology facilitated the availability of PLT components that otherwise were in limited supply. This experience is relevant given the observation of imported cases of CHIKV infection in metropolitan France, Germany, the United Kingdom, Belgium, Norway, the Czech Republic, Canada, and the United States[3,24,25] and the autochthonous outbreak of CHIK infection in the Emilia-Romagna region of Italy.[26] The success of EFS-La Réunion in implementing the INTERCEPT Blood System demonstrates the utility of pathogen inactivation to support the availability of labile blood components during an epidemic.

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CONFLICT OF INTEREST

Three authors (DS, LL, and LC) were affiliated with and held stock or stock options in Cerus Corporation during the conduct of this study. MJ was a consultant to Cerus Corporation, and CC received a research grant from Cerus Europe BV for conduct of this study. JPC received research support and serves on Scientific Advisory Boards for Cerus Corporation.

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SaBTO

Advisory Committee on the Safety of
Blood, Tissues and Organs

Summary of the Ninth Meeting, 27 January 2010

1. Transfusion practice and haemovigilance

Members received reports and updates from three organisations with remits closely aligned to the work of the committee, namely The Chief Medical Officer's National Blood Transfusion Committee (NBTC), Serious Hazards of Transfusion (SHOT) and Serious Adverse Blood Reactions and Events (SABRE).

2. Evidence base for exclusion of high-risk donors

Members discussed the Public Meeting on this subject held in October 2009, agreeing that the range of views from stakeholders was extremely valuable. Members also reconsidered the most recent evidence concerning exclusion of high-risk donors and considered looking at several new potential approaches to assessing and managing risk, including time-limited deferral, operation of donor sessions, and obtaining data more specific to sexual practice. Given the number of issues that continue to arise in this area, and the relative infrequency and workload of full committee meetings, a working group with wide-ranging membership will be formed to explore all of the issues in detail, before production of a full report by autumn 2010. Members reiterated the importance of the research into compliance, expected to report in July 2010, which will inform the final committee advice.

3. Pathogen inactivation of platelets

The Platelets working group had met previously to discuss the current evidence, mainly concerning one pathogen inactivation method for which there exists data concerning safety and efficacy, and their views were communicated to members. Pathogen inactivation of platelets would be an expensive measure, even after taking into account the potential ancillary benefits and possible underreporting of transfusion-transmitted infections. Results from a clinical trial, recently published in abstract form, have suggested that patients receiving pathogen-inactivated platelets are at increased risk of bleeding. In addition, those patients receiving pathogen inactivated platelets show a reduced increase in platelet count. This contrasts with previous studies, which do not raise similar concerns. The uncertainties around patient safety, increased donor exposure and efficacy of pathogen-inactivated platelets lead SaBTO to conclude that this technology should not be implemented at this time. The situation will be monitored by the secretariat.

4. Consent for transfusion update

The consultation of healthcare professionals and those interested in patient safety on the "Introduction of informed consent for transfusion" will commence in early March 2010, through the SaBTO website. The consultation is expected to run for 12 weeks.

5. Revision of MSBTO Guidance on Microbiological Safety of Transplantation of Organs, Tissues and Cells – update

A draft will be shared with relevant professional groups by the end of February.

FDA Liaison Meeting – 1/7/2010

Current FDA Initiatives and Priorities

Current AABB Initiatives and Priorities

Specific Topics of Discussion with FDA

- Interpretation of Recommendations Contained in Guidance Documents
- Statistical Sampling Plans for Product Quality Control
- Pathogen Inactivation
- Infectious Disease Testing
- Disasters and Pandemic Preparedness
- Blood Pressure and Pulse Measurements in the Allogeneic Blood Donor Population
- Abbreviated Donor History Questionnaire
- Plasma Obtained From Whole Blood Donors for Further Manufacturing Use
- Requirements for Human Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use, Proposed Rule, November 2007
- Publication of Documents

Participants

CURRENT FDA INITIATIVES AND PRIORITIES

Food and Drug Administration Initiatives and Priorities presented by Jay Epstein, MD, director, Office of Blood Research and Review, Center for Biologics Evaluation and Research, and Leslie Kux, deputy director, Office of Compliance and Biologics Quality, included information on the FDA Transparency Initiative initiated in January 2009, guidance documents issued in 2009 and those that OBRR is giving high priority to in 2010, blood supply monitoring during the H1N1 influenza pandemic, current concerns and initiatives in addressing emerging infectious agents, an update on the OBRR Quality Program, and an introduction to new staff working in OBRR and the Division of Blood Applications. In addition, Kux provided information on FDA's agencywide initiatives related to compliance and enforcement. Of particular interest is the requirement that an establishment respond to a 483 within 15 days if the response is to be evaluated prior to any additional action by the agency.

CURRENT AABB INITIATIVES AND PRIORITIES

Jacquelyn Fredrick, president of AABB, introduced the discussion of AABB Initiatives and Priorities and reviewed the association's Statement of Intent in support of the strategic plan that is focused on patient and donor safety through the practice of transfusion medicine and cellular therapies. Five critical focus areas for

achieving the goal of improved patient and donor health include member engagement, excellence in medicine, knowledge translation, regulatory advocacy, and standards and accreditation.

SPECIFIC TOPICS OF DISCUSSION WITH FDA

Interpretation of Recommendations Contained in Guidance Documents

Guidance documents are published with a statement that the contents are nonbinding and that alternative approaches can be used if they satisfy the regulations and statutes.Generally, the alternative approaches require validation to show they are equally effective. Blood establishments have had different experiences at the time of inspection when they have implemented a validated alternative approach, and some examples were discussed. FDA was asked to clarify its policy on review and acceptance of validated data to support implementation of an alternate approach to nonbinding recommendations contained in a guidance document.

In general, properly validated alternatives that meet the published recommendations should be acceptable. However, the alternatives should not be a deviation from manufacturer's instructions, and in the case of equipment, it is important to ensure the equipment is working properly. A discussion with the consumer safety officer, or CSO, before implementing the alternative procedure may be helpful, and Richard Davey, MD (301-827-2763), and Leslie Holness, MD (301-827-6115), made their phone numbers available to establishments that would like to call them.

Statistical Sampling Plans for Product Quality Control

The Code of Federal Regulations written specifically for blood components (21 CFR 600 series) requires that modest numbers of blood components be tested on a monthly basis to satisfy quality control, or QC, requirements. However, current good manufacturing practices, or cGMP, that have broad applications for blood components (21 CFR 200 series) require the use of statistically significant sampling plans. In recent years, the blood community and FDA have discussed several approaches. When the December 2007 guidance for collection of Apheresis Platelets was published, a binomial sampling plan was recommended. (Prior to that, the potential use of scan statistics had been introduced but at that time was untested in the blood center environment and was essentially abandoned.) Many blood establishments continue to struggle with issues related to correct use of a statistically significant sampling plan, particularly when the institution does not collect enough platelets to use the binomial sampling plan.

Several sampling plans were reviewed, including hypergeometric distribution statistics, which are more adaptable to facilities that collect and process smaller numbers of units. The hypergeometric plan supports sample sizes of $n<60$ for QC purposes. However, it is not applicable to undefined population sizes and

therefore is not valid for process validation. (Tables based upon hypergeometric distribution have been prepared by the Office of Biostatistics and Epidemiology, CBER, and are expected to be made publicly available in the future.) Clusters of failures at the endpoints of the sample size may be masked when using binomial or hypergeometric approaches, but such clusters could be identified through use of scan statistics. Scan statistics is conceptually and computationally complex but software now exists to manage the use of this method. References for the various statistical approaches were provided on the final slide. When asked how an establishment would get FDA acceptance of a statistical process that has not been recommended by FDA, the committee was reminded that any sound and "known" process should be acceptable. Novel approaches might also be acceptable and a pre-implementation conversation with the CSO could answer all questions.

Pathogen Inactivation

The Blood Products Advisory Committee recently advised the FDA to place certain boundaries around a phase III clinical trial for pathogen inactivation of human platelets, effectively requiring a three-fold increase in the size of the trial and perhaps rendering it financially infeasible. The committee believes it is important that the inactivation processes not only be effective against disease agents but that they also result in a safe product for patient transfusion. However, there is concern following discussions at the November 2009 BPAC meeting that approval of any pathogen inactivation process or product is moving further into the future. FDA was asked about current considerations of this issue, particularly in the context of the July 2008 request from the assistant secretary for health to further the progress of pathogen reduction in the U.S.

FDA participants responded that patient safety and efficacy of the transfused product remain of paramount concern for them, and they remain very interested in the prospects for pathogen inactivation processes coming to the U.S. market. Agency representatives continue to engage the trial sponsor in conversations about possible trial designs. The Laboratory of Cellular Hematology in the Division of Hematology is conducting research on the action of UV light on platelets and red cells. Committee members reiterated concerns that pathogen inactivation appeared unlikely to be a potential tool for use with emerging infectious disease agents in the U.S. in the event that infectious disease tests become increasingly difficult to obtain or become unavailable.

Infectious Disease Testing

The lack of availability of tests is not restricted to those that are thought to be useful only in selective testing situations (dengue, Babesia). Universal testing volumes — such as for human T-cell lymphotropic virus I and II — alone also do not create a market incentive, and the confirmatory test market will always be small. The committee's discussions with FDA included:

How to obtain testing (low level or universal) against a lack of market incentive — transfusion medicine infectious disease tests are a very low percentage of the in vitro diagnostic market.

HTLV-I/II testing is required by regulations, and there is concern that in 2010, the one licensed test distributed in the U.S. may at some point in time not be universally available because of lot issues relating to manufacturing.

Re-entry of some donors, especially those deferred in high numbers for markers that have low prevalence in the U.S. population, is very desirable, yet there are no licensed tests available. Are there other options for re-entry?

FDA participants stated they share the same concerns and said they have interacted with several AABB task forces and manufacturers in an effort to support products coming to market.
Committee members and FDA agreed there are shared responsibilities and opportunities to resolve some of the issues. The problems have been described multiple times, and the time appears ripe for a roundtable summit.

Disasters and Pandemic Preparedness

The transfusion medicine community acting through the Interorganizational Task Force on Domestic Disasters and Acts of Terrorism and other working groups has met repeatedly over the past few years and engaged with government liaisons and participants in an effort to establish robust preparedness plans for unplanned events. The goal of such planning is to ensure an adequate and safe supply of blood (and tissues) for the patient community in the event of a disaster or pandemic. A final unresolved issue for blood establishments is that of pre-determining which processes and procedures may be necessary to vary from during the manufacturing process. One area of focus is relaxation of certain donor requirements in order to ameliorate catastrophic shortages. Preparation of a robust preparedness plan is hampered by a facility's inability to write the necessary standard operating procedures and train staff under acceptable cGMP conditions during an emergency.

The agenda submitted in advance to FDA asked for a discussion of options available to blood establishments including, but not limited to, alternative procedures/variances, as well as areas where enforcement discretion might be more appropriate in the event of an unexpected, sudden expansion of a pandemic. Clarification of existing regulations with regard to emergency situations would be helpful. The goal is to find concrete steps that would enable establishments to have a fully robust preparedness plan that could be initiated rapidly in the event of need.

FDA participants acknowledged their understanding of the need to preplan and have attempted to provide some additional guidance in the form of the draft guidance issued in November 2009: "Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus."

Blood Pressure and Pulse Measurements in the Allogeneic Blood Donor Population

At the November BPAC meeting the committee considered evidence of currently available data for blood pressure and pulse measurements as predictors of risk for adverse reactions to blood donation and whether data support specific ranges for these measurements as predictors of adverse reactions. BPAC advised that while blood pressure measurements are not predictors of adverse reactions, pulse appears to have relevance. Committee discussion further suggested that measurement of blood pressure and pulse may be of value even if FDA does not retain them as donor eligibility determinations.

Following the BPAC discussion, FDA participants were asked about their current considerations for the use of blood pressure and pulse measurements in the allogeneic blood donor setting. Several items were noted:

- BPAC's concern with the paucity of data on donors with blood pressures >180.
- Knowledge gaps creating an opportunity for surveillance.
- Establishing limits in regulations may not be ideal.
- Having no requirements may not be ideal.

Abbreviated Donor History Questionnaire

BPAC advised that the use of the abbreviated donor history questionnaire (DHQ) is desirable and suggested that a post-implementation study/data collection would be appropriate. The AABB Donor History Task Force developed a study (with the advice of FDA liaisons to the task force) and submitted it to FDA in April 2008. The task force has been told on several occasions that the plan submitted satisfactorily addressed all outstanding issues.

FDA was asked to explain the pathway for advancing the abbreviated donor history questionnaire and responded that the necessary guidance document is in an editing stage. The post-implementation plan will likely be referenced in the guidance. This draft guidance is on OBRR's priority list for 2010, but the time frame is not definitely known.

Plasma Obtained From Whole Blood Donors for Further Manufacturing Use

A regulatory framework for plasma obtained by apheresis that could be used for further manufacture was proposed for BPAC's discussion last April.

FDA was asked for an update on current considerations for these blood components and responded that staff within OBRR continue to look at the issues raised by the BPAC discussion and remain in contact with the AABB task force. There was nothing definite to report.

Requirements for Human Blood and Blood Components Intended for Transfusion or for Further Manufacturing Use, Proposed Rule, November 2007

The committee was interested to hear an update on progress regarding reconciling comments received to the proposed rule and to know if it is likely to be reissued as a proposal or to be published as a final rule.

FDA continues to look at the many proposals put forth in the document and has engaged BPAC in discussions on some of the subjects (i.e., hemoglobin and blood pressure/pulse). It took more than 10 years to draft the document, and it likely will not be reissued as a draft. However, it is also likely that all proposals may not be issued as final at the same time.

Publication of Documents

The FDA Liaison Committee remains concerned with the significant delay in publishing documents that are of critical importance to blood establishments. Updates to several documents provided by discussions at the meeting are included in the list below. FDA participants stated that although they have assigned the documents priority for 2010, they are not in control of the overall process and timelines involved.

- Guidance document that would recognize the abbreviated DHQ – A draft is on the 2010 priority list for OBRR.
- Guidance document that would recognize v1.3 of the DHQ – A guidance with final recommendations is on the 2010 priority list for OBRR. The AABB Donor History Task Force has decided to proceed with posting v1.3 on the AABB Web site along with instructions for submitting the materials to FDA as a Prior Approval Supplement to the establishment's Biologics License Application before implementation.
- Nucleic Acid Testing (NAT) for Human Immunodeficiency Virus Type 1 (HIV-1) and Hepatitis C Virus (HCV): Testing, Product Disposition, and Donor Deferral and Reentry (draft guidance, July 2005) – A guidance with final recommendations is on the 2010 priority list for OBRR.
- Pre-Storage Leukocyte Reduction of Whole Blood and Blood Components Intended for Transfusion (draft guidance, January 2001) – Reissuance as a draft guidance is a priority for OBRR in 2010.
- Revisions to Labeling and Storage Requirements for Blood and Blood Components, Including Source Plasma (proposed rule, July 2003) – No update was provided.
- Safety Reporting Requirements for Human Drug and Biological Products (proposed rule, March 2003) – No update was provided.

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LE08 **PATHOGEN INACTIVATED PLATELETS AND PREVENTION OF IMMUNOLOGICAL ADVERSE REACTIONS: THE ITALIAN PLATELET TECHNOLOGY ASSESSMENT STUDY (IPTAS)**

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Introduction

Although extremely safe, platelet (PLT) transfusion still carries measurable risks of pathogen transmission, allo-immunization to human leucocyte antigens (HLA) carried by viable white blood cells (WBCs) present in platelet concentrates (PCs) -in turn causing refractoriness to random donors in 10-15% of recipients- and transfusion associated graft vs host disease (TA-GvHD). Traditional approaches to prevent these untoward effects include improved donor screening procedures, bacterial detection assays, WBC removal by filtration and γ-irradiation. Recently, two commercial photo-chemical technologies "Intercept Blood System", by Cerus and "Mirasol Pathogen Reduction Technology", by Caridian, previously named Navigant Biotechnologies/Gambro, have been developed which not only inactivate pathogens but also prevent WBC replication, thus offering the potential additional benefit of reducing immunological side effects. These pathogen inactivation (PI) systems use UV-A light to irradiate PLTs in the presence of a photo sensitizer (amotosalen, S-59, in Intercept Blood System; or riboflavin - Vitamin B_2, in Mirasol Pathogen Reduction Technology). Clinical studies[1-7] show safety of both procedures and non inferiority of PI treated PLTs vs standard PLTs in regard to bleeding prevention[8]. Data from published preclinical studies show that transfusion of pathogen inactivated PLTs can prevent presensitization to transplants[9]. Moreover, using PI in place of γ-irradiation to prevent TA-GvHD was authorised by some organizations and regulatory bodies including the Paul Ehrlich Institut.

In view of broad clinical use of expensive PI procedures in transfusion medicine, technology assessment (TA) studies including cost to benefit evaluations can provide suitable information for evidence based decisions in health care[10].

Blood components are highly complex mixtures of plasma proteins and cells. During the production, inactivation, and storage of PCs there is the risk of changes in their integrity, especially at the protein level. It is therefore a major challenge to identify significant alterations of these products. At present, blood component quality control is mainly focused on standardized quantitative assessments providing relatively limited information about products. Proteomics allows a comprehensive study of protein modifications, qualitative and quantitative analysis, and high-throughput protein identification, thus potentially enabling a global assessment of processing, inactivation and storage methods, as well as of possible contaminants and neoantigens that may influence the immunogenic capacity of blood-derived therapeutics[11-14].

The Italian Platelet Technology Assessment Study (IPTAS)

IPTAS will provide new information on the safety and efficacy of PCs prepared with standardized procedures using WBC inactivation in place of filtration to reduce anti-HLA allo-immunization and acute transfusion reactions. Incidence of both side effects and the cost impact of PI technology (net balance between added and avoided costs) will be compared for buffy coat (BC) PLTs and for leuco-reduced apheresis (AP) PLTs, which represent the prevalent types of PCs used in Italy. Data from this study will support the analysis on whether PI can be used to replace WBC filtration, γ-irradiation and donor CMV serology.

Proteome analysis will provide additional in vitro data on the possible mechanisms of inactivation of residual WBCs in pathogen-inactivated PCs and new insights on the impact of PI processes on the global proteome profile of PCs, including functional studies on PLT mitochondria and the potential to identify markers of PLT lesion and activation, both in treated and untreated PLTs. Therefore, proteomics will offer new approaches for PC quality control.

IPTAS objectives

The primary objective of the clinical arm of this study is to determine: 1) if pooled whole blood (WB) PLT components prepared by the BC method without leuco-filtration and with photochemical PI treatment and 2) if AP PLT components prepared with leuco-reduction and photochemical PI treatment are associated with similar or lower incidence of HLA-alloimmunization compared to non PI treated PLTs transfused to onco-haematology recipients.

Secondary objectives are: 1) to compare the incidence of acute transfusion reactions as a measure of safety; 2) to compare the cost of photochemical PI treated vs untreated PLT components; 3) to implement an active haemovigilance program for all types of PLT components, and to evaluate the effectiveness of this haemovigilance program to monitor transfusion safety in multiple blood transfusion services (as recommended in the EUCOMED Position Paper "Health Technology Assessment for Medical Devices in Europe"; www.assobiomedica.it).

The primary objective of the proteomic study is the comprehensive assessment of protein modifications, with high coverage, through qualitative and quantitative analysis of BC versus AP pathogen-inactivated PCs. Secondary objectives are: 1) to improve understanding of PLT storage lesions; 2) to identify potential storage lesion biomarkers; 3) to study molecular mechanisms of the elimination of proliferative and functional capacities of WBCs; 4) to compare mitochondrial mechanisms of energy production in inactivated versus standard PCs.

Clinical study design and interventions

The clinical arm of IPTAS will be a multicenter, non randomized, prospective controlled study involving 6 large blood transfusion services (BTS). Three BTSs will use the Cerus technology (CeT) vs local standard (STD) PLTs while 3 BTSs will use the Navigant-Gambro technology (NGT) vs local STD PLTs. PI and STD laboratory procedures and protocol deviations will be recorded. The PLT dose will be determined in all PLT components. Each BTS will allocate consecutive patients (PTs) alternatively to PI treated or STD PLT components. PI treatment will be used in place of γ-irradiation and CMV serology following ethical approval.

Patient population, analytical procedures and outcome indicators

The PT population will be haematology-oncology adult PTs expected to require 2 or more PLT transfusions (TXs). The study will include both new and previously transfused PLT recipients, but transfused PTs with historical documented record of 2 or more 1-hour post-TX PLT count increments <5,000/μL will be excluded. Presence of HLA antibodies (Abs) on admission will not be an exclusion

criteria. PTs will be transfused with the PLT component assigned by the BTS until: 1) PLT TX independent; 2) detection of refractoriness; 3) discharge or death, for a maximum of 8 weeks.
PLTs will be transfused according to the American Society of Clinical Oncology guidelines[15] and to local policies. While this study will not be stratified with respect to primary diagnoses or therapy, these data will be collected to permit analysis of the distribution of primary diagnoses and therapies among the treatment groups.
For each PLT component administered, the response to TX will be evaluated using standardized case report forms recording PT demographics, primary diagnosis and therapy. For each TX the type of PLT transfused will be indicated together with the requirement for: 1) TA-GVHD prevention; 2) CMV Ab negative components; 3) HLA-matched or cross-matched PLT components. Following each PLT TX, PTs will be evaluated for adverse events (AEs) for a period of 24 h. AEs will be recorded using a standardized form, assessed for relation to the PLT TX and graded for clinical severity. TXs with AEs possibly, probably, or definitely related to PLT TX will be defined as TXs with an acute reaction. PTs will be assessed for HLA allo-immunization using conventional assays at each study site. As a minimum, each PT will be evaluated for anti-HLA Ab status on a serum sample collected at enrolment and within 2 days of the conclusion of participation in the study. HLA matched or cross-matched PLT TXs will be specifically recorded.
A PT daily record will be used including morning PLT count, haemoglobin, no. of RBC and PLT TXs, PLT age, type and dose, post-TX PLT count, body temperature, presence/absence of sepsis, WHO bleeding grade, bleeding type/organ/system and outcome, occurrence and type of post-TX reactions, drugs negatively impacting on PLT transfusion.

Sample size and statistical analysis
In the TRAP study it was estimated that 570 PTs were necessary for a statistically significant reduction of HLA allo-immunization from 40% to 20% with type I error = 0.05 and power = 0.92[16]. In the IPTAS study, according to the formula described by AR Feinstein[17], the required sample size, for type I error = 0.05 and power = 0.80 of detecting a reduction of HLA-alloimmunization from 40% (control) to 20% (treatment), is 162 PTs per each comparison, i.e. 162 for the comparison of CeT treated PLTs (81 PTs) vs STD PLTs (81 PTs) in 3 BTSs and another 162 PTs for the comparison of NGT treated PLTs (81 PTs) versus STD PLTs (81 PTs) in the other 3 BTSs. To take into account PT drop out, we aim at collecting 100 PTs per arm (total: 400 PTs). We expect that IPTAS will include about 500-1,000 CeT PLT TXs, 500-1,000 "local" control TXs in the 3 BTSs using CeT, 500-1,000 NGT PLT TXs, 500-1,000 "local" control TXs in the 3 BTSs using NGT, for a total of 2,000-4,000 PLT TXs.
All data will be analysed according to an intention to treat model. The proportions of PTs who develop HLA Abs and their 95% CI will be determined. Comparisons between CeT treated PLTs vs local STD PLTs and comparisons between NGT treated PLTs vs local STD PLTs will be made using the χ^2 or the Fisher's exact test with p = 0.05. Similar comparisons will be made for the incidence of acute TX reactions on a per TX basis and a per PT basis. Descriptive statistics will be used to report number of TXs, PLT doses, pre- and post-TX PLT counts.

Cost analysis
Direct costs of collection and preparation of WB PLTs prepared without WBC filtration and PI treated will be compared with those of PI treated AP PLTs and those of conventional PLTs prepared with WBC reduction, γ-irradiation, and CMV serology. The cost per PT for HLA matched or cross-matched PLTs will be compared between treatment arms. The costs of γ-irradiation and CMV serology per PLT component transfused and per PT in each treatment arm will be compared between treatment groups. The cost impact related to treatment of acute TX reactions will be determined and compared between treatment groups.

Proteomic study
Proteomic analysis will be used to compare the effects of CeT and NGT on the proteome profile of AP and BC PCs and of WBCs. To this aim, 10 AP and 10 BC non inactivated units and, for each PI method, 10 AP and 10 BC inactivated units will be studied. Moreover, up to 18 WBC samples will be studied: 9 harvested from pooled BCs and 9 from Gambro Trima AP devices. Non treated WBCs and WBCs treated with both PI methods will be compared using procedures based on published methods[18,19].

Discussion
The results from this study will be applicable to estimates related to the routine use of PI treatment for all platelet components on a national basis. These data will provide a balanced economic rationale to regional healthcare administrators and hospital managers to evaluate whether or not to introduce blood component pathogen inactivation, extensively or for selected categories of patients. Moreover, additional scientific data will be made available about outcomes of low cost (BC) vs high cost (AP) PCs, thus allowing suitable clinical governance of this specific aspect of blood therapeutics. The implementation of an active haemovigilance system to monitor the safety of transfusion of platelet components in this study will provide a system for monitoring safety of all platelet components and ultimately all other labile blood components. This study will provide clinical and technical staff at study centres with training and experience on active haemovigilance. This experience could be easily transferred to other blood centres not participating in this study.
Proteomic technologies will provide new comprehensive information about changes occurring during processing and storage of blood products, introducing a novel approach to qualitative and quantitative assessment of blood components.
The proteomic study will potentially provide data to optimize PLT production processes and/or pathogen inactivation systems, thus increasing present safety and standard of care in PLT transfusion therapy.
The combination of data from the in vivo and the in vitro parts of the study will hopefully allow an original and useful impact on the comprehensive clinical setting of blood transfusion.

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Disclosures
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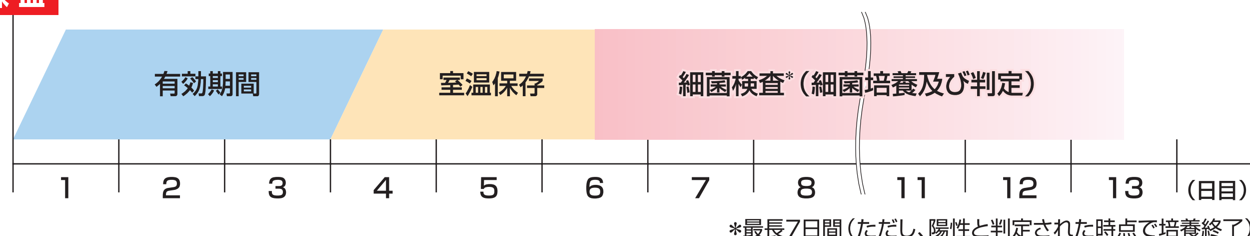
血小板製剤への初流血除去導入の効果について

日本赤十字社では2006年10月26日採血分から細菌混入を低減する目的で、血小板製剤の初流血除去を開始しました。採血時の皮膚消毒では殺菌効果が届きにくい毛嚢等を含む皮膚片の製剤への混入を防ぐことが目的です。この細菌混入低減効果を評価するため、期限切れ血小板製剤を用い、初流血除去導入前後で細菌陽性率*を比較しました。その結果、初流血除去導入により細菌陽性率*（*Propionibacterium acnes*以外の細菌）が0.06%から0.02%に減少していることが分かりました。

*細菌陽性率：細菌検査の結果、細菌が検出され陽性と判定された割合。

【評価方法】

期限切れとなった血小板製剤を採血後6日目まで室温（20〜25℃）で保存しました。細菌検査には全自動血液培養装置（BacT/ALERT）を用い、好気的、嫌気的条件それぞれで10mLの製剤を最長7日間培養し、細菌の有無を判定しました。



*最長7日間（ただし、陽性と判定された時点で培養終了）

【評価結果】

	初流血除去導入前 2005年5月〜2006年4月	初流血除去導入後 2006年12月〜2008年 3月
培養実施数	21,786	21,783
陽性数（陽性率）	36*（0.17%）	11（0.05%）
P. acnes	24（0.11%）	7（0.03%）
P. acnes 以外の細菌	13（0.06%）	4（0.02%）

＊1例は *P. acnes* と non-*P. acnes* の重複混入

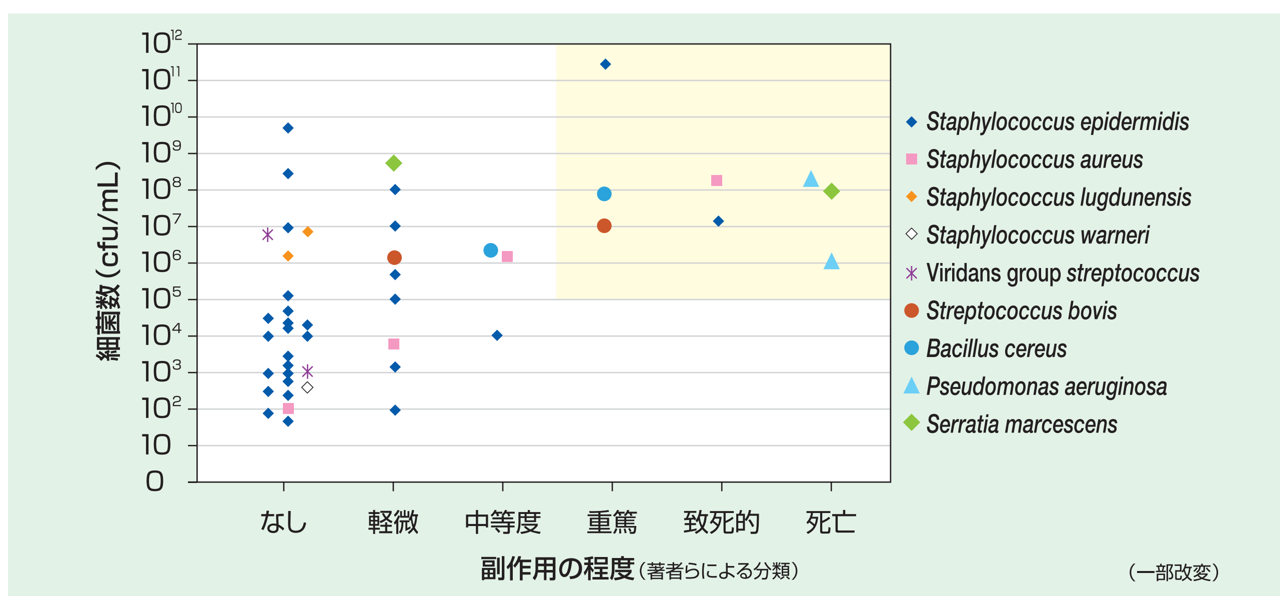
検出された細菌（*P.acnes*以外）

細菌の由来（推定）	初流血除去導入前	初流血除去導入後
皮　膚	*Staphylococcus epidermidis*（4） *Staphylococcus* sp.（1） CNS（Coagulase-negative *Staphylococcus*）（1） *Staphylococcus saccharolyticus*（1） Gram(+) *bacillus*, 非芽胞菌（1）	*Staphylococcus epidermidis*（1）
皮膚または血液	*Staphylococcus aureus*（2）	*Staphylococcus aureus*（1）
血　液	*Streptococcus constellatus*（1） *Salmonella choleraesuis*（1） *Eikenella corrodens*（1）	*Streptococcus dysgalactiae subsp.equisimilis*（1） *Escherichia coli*（1）

初流血除去導入前後でそれぞれ2万本を越す期限切れ血小板製剤について細菌検査をした結果、陽性と判定されたのは、初流血除去導入前は0.17%、初流血除去導入後は0.05%でした。*P.acnes*を除くと、細菌陽性率は導入前が0.06%、導入後は0.02%でした。*P.acnes*は、血小板製剤中では増殖が極めて遅く、また陽性判定された製剤を輸血しても副作用がなかったと報告されています[1]。*P.acnes*も含め、検出された菌種はこれまでに論文等で報告されているものでした。また、細菌陽性率も海外での報告とほぼ同程度です[2]。今回の評価では、細菌の検出率を高めるために、血小板製剤を6日目まで室温で保存しました。細菌陽性率は保存期間と共に高くなるため[2]、有効期間の4日間での細菌陽性率は今回の結果よりも低いと予想されます。

臨床的に重篤な副作用は細菌が10^5CFU/mL以上含まれる製剤が原因だとされています[3]（下記「細菌数と輸血副作用」参照）。初流血除去により、最も細菌濃度が高い皮膚片が除かれる[4]ので、初流血除去した製剤は細菌陽性率だけでなく、細菌数も少なくなると推測され、副作用の危険性は細菌陽性率の減少以上に軽減されていると考えています。

細菌数と輸血副作用[3]



（一部改変）

日本赤十字社では、血小板製剤の有効期間を諸外国より短く設定し、さらに初流血除去により皮膚常在菌の混入数をできる限り少なくして、細菌汚染に対する安全性を高めています。しかしながら、細菌の混入が全く無くなるわけではありませんので、輸血前の血液製剤の外観検査と輸血中、輸血後の患者さんの注意深い観察をお願いします。

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輸血による細菌感染が疑われる症例が発生した場合には、直ちに輸血を中止して適切な処置をするとともに、使用された製剤バッグを適切に（衛生的かつ冷所）*に保管し、赤十字血液センター医薬情報担当者までご連絡ください。
また、原因究明のために、使用された製剤バッグ、患者さんの検体、さらに血液培養試験をはじめとする臨床検査関連情報等の提供をお願いします。

*輸血セットのクランプを固く閉め、輸血部門に返却願います。その後、点滴筒の上下をチューブシーラーでシール（チューブシーラーがない場合は鉗子等で確実に結紮）し、ビニール袋に入れて冷蔵保存してください。

医療関係者向け製品情報サイト
URL http://www.jrc.or.jp/mr/top.html

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＊お問い合わせは、最寄りの赤十字血液センター医薬情報担当者へお願いいたします。

0903

Protein quality in Mirasol pathogen reduction technology–treated, apheresis-derived fresh-frozen plasma

James Smith and Gail Rock

BACKGROUND: The Mirasol pathogen reduction technology (PRT) system for plasma is based on a riboflavin (vitamin B_2) and ultraviolet (UV) light treatment process resulting in pathogen inactivation due to irreversible photo-oxidative damage of nucleic acids. The purpose of this study was to evaluate the in vitro protein quality of apheresis-derived plasma treated with riboflavin and UV light in comparison with untreated fresh-frozen plasma (FFP).

STUDY DESIGN AND METHODS: Twenty apheresis plasma samples (270 ± 10 mL) were combined with 35 ± 5 mL of riboflavin solution (500 μM), yielding a mean 60 μM final riboflavin concentration, and then exposed to UV light (6.24 J/mL). Riboflavin and UV light–treated plasma was then flash frozen, within 8 hours of collection, generating treated FFP. Treated FFP was thawed and analyzed using standard coagulation assays, and the percent retention of protein activity was reported, relative to untreated, paired controls.

RESULTS: Plasma proteins demonstrated different sensitivities to riboflavin and UV treatment. The amount of total protein remained unchanged. After treatment, fibrinogen (antigen) showed 99% retention; Factor (F)XII, FXIII, ADAMTS-13, and von Willebrand factor (ristocetin cofactor) 96% to 100%. Fibrinogen retained 77% activity, FII 80%, FVIIIc 75%, and FV 73% after treatment. Antithrombin, protein S, plasminogen, and α_2-antiplasmin retained between 91 and 100% activity.

CONCLUSION: The results from this study demonstrate that coagulant and anticoagulant proteins in riboflavin and UV light–treated (PRT) apheresis plasma are well preserved.

Blood transfusion is not a risk-free procedure even though concerns have been reduced dramatically with recent improvements in blood donor selection procedures and implementation of sensitive screening tests for known blood-borne pathogens. However, tests are not available for all known pathogens, and additional risks come with newly emerging agents.[1-5] Detection is also complicated because of a latent period between actual infection and detectable antibody formation in the donor. Increases in the amount of world travel are a rising concern for disease transmission through transfusions, due to migration of agents from one geography to another. As a result there is a growing interest in the implementation of pathogen reduction technologies (PRTs) to increase the safety of blood components used in transfusion.

All PRTs have advantages and disadvantages. The important goal is to simultaneously improve transfusion safety while maintaining efficacy after treatment. Among the methods that have been employed successfully are solvent/detergent (S/D) and methylene blue (MB) treatment, both of which are used to treat plasma and to manufacture fractionated and purified blood-derived products. These treatments are primarily effective against enveloped viruses. They also yield some reduction in protein quality due to the treatment.[6,7] A psoralen compound (amotosalen, S-59) with ultraviolet (UV) light–based technology has also been developed for the reduction of pathogens

ABBREVIATIONS: HMW = high molecular weight; MB = methylene blue; PRT(s) = pathogen reduction technology(-ies).

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TRANSFUSION **;**:**-**.

and inactivation of white blood cells (WBCs) in platelet (PLT) preparations and in plasma.[8-10]

Another method is riboflavin and UV light treatment, which inactivates viruses, bacteria, parasites, and WBCs.[11-14] Riboflavin, a naturally occurring vitamin (vitamin B_2), has the advantage of a well-characterized safety profile.[15-17] Exposure to UV light activates the riboflavin and causes a chemical alteration of guanine residues of the nucleic acids causing irreversible damage and making the pathogens unable to replicate.[14,18,19] This chemistry involves oxygen-dependent and oxygen-independent processes. The Mirasol PRT system is designed to minimize the effect of reactive oxygen by specific light wavelength selection and by minimizing residual air in the component bag.

This study evaluated the protein quality of apheresis-derived plasma products treated with riboflavin and UV light. Results were compared to paired, untreated products handled under the same conditions.

MATERIALS AND METHODS

Collection and preparation of plasma samples

A total of 20 apheresis-derived plasma products were collected in ACD from the following blood types: 35% group O, 45% group A, 10% group B, and 10% group AB. The blood types were selected to be representative of typical donor populations present in the United States and Western Europe. All blood products were collected at an accredited blood banking facility. Units were held at 22 ± 2°C before processing. Each apheresis unit was split into one control unit (nontreated) and one test unit (riboflavin and UV light treated).

Product treatment

A total of 270 ± 10 mL of plasma was transferred to an illumination bag and 35 ± 5 mL of 500 μM sterile riboflavin solution was added using a sterile connector (Terumo TSCD sterile tubing welder, Terumo Medical Corp., Elkton, MD) yielding a mean 60 μM final concentration. The set was placed into the illuminator and exposed to UV light with a linear agitation of 120 cpm. Product temperature and light dose delivered were monitored continuously throughout processing. The bag was removed after the illuminator delivered the necessary dose of UV light (6.24 J/mL) based on product volume and measured flux rate.

Sampling and storage preparation

Treated and control aliquots were frozen concurrently, within 8 hours of collection and stored at –70°C, for at least 24 hours before plasma protein quality evaluation.

Plasma proteins assays

Treated fresh-frozen plasma (FFP) was thawed and analyzed using standard coagulation assays. Fibrinogen activity, Factor (F)II, FV, F IX, FX, FXI, FXII, FXIII, antithrombin, protein C, protein S, and total protein were evaluated in Regional Medical Laboratory (Tulsa, OK). Fibrinogen antigen, FVIIIc, von Willebrand factor (VWF) activity, and α_2-antiplasmin were measured at Quest Diagnostics Nichols Institute (San Juan Capistrano, CA). ADAMTS-13 activity was determined at Montefiore Medical Center/Albert Einstein College of Medicine (Bronx, NY) and VWF multimers at McMaster University (Hamilton, Ontario, Canada). Details are as follows:

The majority of clotting and chromogenic assays were performed on the Diagnostica Stago STA-R Evolution (Stago, France). FVIIIc was measured using a FVIII chromogenic assay from Siemens on the Behring coagulation system, extra performance (Siemens Healthcare Diagnostics, Newark, DE).

Antithrombin, α_2-antiplasmin, and FXIII analyses were done using a colorimetric assay of antithrombin III activity (Stachrom ATIII, Diagnostica Stago-US, Parsippany, NJ), with Berichrom α_2-antiplasmin and Berichrom FXIII (Siemens Healthcare Diagnostics), respectively.

Plasma levels of FII, FV, and FX were determined using functional assays based on the prothrombin time with human FII-, FV-, and FX-immunodepleted plasma samples. Similarly, plasma levels of F IX and FXI were determined using human F IX- and FXI-immunodepleted plasma samples, based on the activated partial thromboplastin time. Proteins C and S were determined using clotting assays (StaClot protein C and StaClot protein S, STA-Compact, Diagnostica Stago, France).

Fibrinogen antigen was determined by nephelometry. Fibrinogen activity was determined with the Clauss kinetic clotting method. The fibrinogen in plasma before and after riboflavin and UV treatment was characterized using 4% to 20% Tris-HCl criterion gels (Bio-Rad, Hercules, CA). Human fibrinogen (Calbiochem, San Diego, CA) was applied on gels as a control for plasma samples. Untreated control plasma and fibrinogen samples contained an amount of saline equal to the volume and amount of riboflavin in the treated samples. Gel images were obtained using a commercial system (GelDoc XR, Bio-Rad). Area (mm^2) analyses of fibrinogen in plasma samples before and after riboflavin and UV treatment were performed on a high resolution gel imaging and documentation system (Molecular Imager GelDoc XR, Bio-Rad).

The total protein content was measured by the biuret assay using a chemistry analyzer (Olympus, Center Valley, PA). Ristocetin cofactor activity of VWF was measured using a von Willebrand reagent containing human stabilized PLTs from Siemens Healthcare Diagnostics.

The VWF multimers were analyzed using sodium dodecyl sulfate (SDS)-multimer gels containing 1.25% agarose (SeaKem LGT (p), FMC Bioproducts, Rockland, ME) and 1% acrylamide.[20] After electrophoresis (20 hr at 35 V), the proteins were transferred onto nitrocellulose membranes and incubated with peroxidase-conjugated rabbit anti-human VWF antibodies (Dako D/S, Glostrup, Denmark). The VWF multimer patterns were developed using Western blotting chemiluminescent substrate (ECL, GE Healthcare, Piscataway, NJ).[21]

Internal standards on each gel included normal pooled plasma and plasma deficient in large-molecular-weight multimers of VWF (Type 2A VWD). The multimer migration of each sample was compared to the normal pool. Intermediate bands were considered to be 5 to 10 subunits and high-molecular-weight (HMW) bands were considered to be greater than 10 subunits.

ADAMTS-13 activity level was measured using guanidine-HCl–treated VWF multimers as the substrate as previously described[22] with the proteolytic fragments generated after incubation analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting.

Statistical analysis

Arithmetic means and standard deviations (SDs) were calculated for each of the variables listed and reported in the final analysis. To analyze the effect of photodynamic treatment on the coagulation factors, the control coagulation factor levels (mean ± SD) and percent retention (mean ± SD) for riboflavin and UV light–treated plasma are reported.

RESULTS

Plasma proteins had different sensitivities to riboflavin and UV treatment as shown in Table 1. The amount of total protein remained unchanged. Fibrinogen had 99% antigen and 77% activity retention after treatment. Results from gel electrophoresis (Fig. 1) confirmed that after riboflavin and UV treatment the electrophoretic patterns of fibrinogen were unchanged.

FXII retained 96% activity and FXIII remained normal. FII showed 80%, FVIIIc 75%, and FV 73% activity retention, respectively, after riboflavin and UV treatment. ADAMTS-13 and VWF fully retained activity. The data demonstrated a variable decrease in the HMW multimers of VWF in the postillumination samples, with some samples having normal patterns (Fig. 2).

All anticoagulant and inhibitor proteins, antithrombin, protein S, plasminogen, and α_2-antiplasmin showed retention of activity between 91 and 100% (Table 1). Protein C was more sensitive and retained 81% activity after treatment.

DISCUSSION

Plasma proteins

The existing PRTs show different levels of loss or degradation of plasma proteins after treatment.[6,7,23-30] The level of these reductions and the particular factors that are affected vary from method to method. For example, S/D-treated plasma shows an overall reduction of the activity and concentration of coagulation factors, inhibitors, immunoglobulins, and other plasma proteins of approximately 5% to 20%; IgM antigen isoagglutinins are reduced

TABLE 1. Protein retention for riboflavin and UV light–treated apheresis plasma*

Plasma protein quality variable	Before illumination	After illumination†	Percent recovery, before illumination vs. after illumination‡
Fibrinogen activity (mg/dL)	345 ± 80	267 ± 64	77 ± 4
Fibrinogen antigen (mg/dL)	344 ± 90	339 ± 87	99 ± 4
FII (IU/mL)	1.1 ± 0.2	0.9 ± 0.1	80 ± 6
FV (IU/mL)	1.1 ± 0.2	0.8 ± 0.1	73 ± 8
FVIIIc (IU/mL)	1.3 ± 0.5	1.0 ± 0.3	75 ± 16
F IX (IU/mL)	1.4 ± 0.4	1.0 ± 0.2	76 ± 6
FX (IU/mL)	1.1 ± 0.2	0.9 ± 0.2	80 ± 7
FXI (IU/mL)	1.2 ± 0.2	0.8 ± 0.1	67 ± 5
FXII (IU/mL)	1.3 ± 0.3	1.3 ± 0.3	96 ± 14
FXIII (IU/mL)	All normal	All normal	NA
VWF activity (U/mL)	0.7 ± 0.3	0.8 ± 0.3	117 ± 10
ADAMTS-13 (% activity)	107 ± 41	103 ± 36	100 ± 25
Protein C (U/mL)	1.4 ± 0.5	1.1 ± 0.3	81 ± 11
Protein S (U/mL)	1.1 ± 0.2	1.0 ± 0.2	91 ± 9
Antithrombin (U/mL)	1.0 ± 0.1	1.0 ± 0.1	99 ± 4
α_2-Antiplasmin (U/mL)	1.0 ± 0.1	0.9 ± 0.1	93 ± 4
Total protein (g/L)	58 ± 3	58 ± 3	99 ± 1

* Protein retention in treated plasma is reported as the percent retention relative to the paired, untreated control products at the same storage interval. All values are mean ± 1 SD. Values are reported for a total of 20 apheresis-derived paired plasma units.
† Corrected for dilution.
‡ Calculated as mean percent retention of individual paired units.

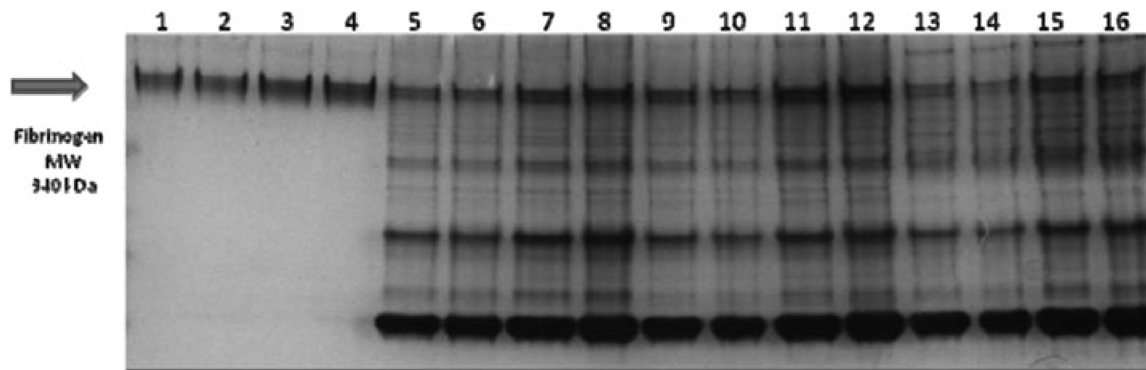

Fig. 1. Gel electrophoresis demonstrating fibrinogen patterns in riboflavin-treated and untreated plasma. The gels show riboflavin-treated plasma samples versus untreated and human plasma fibrinogen with saline as a control on 4% to 20%Tris-HCl native gel. Lane 1 = fibrinogen 3 μg with saline; Lane 2 = fibrinogen 3 μg with riboflavin; Lane 3 = fibrinogen 4 μg with saline; Lane 4 = fibrinogen 4 μg with riboflavin; Lane 5 = pretreated plasma with saline, Unit 1 (2 μL); Lane 6 = posttreated plasma with riboflavin, Unit 1 (2 μL); Lane 7 = pretreated plasma with saline, Unit 1 (4 μL); Lane 8 = posttreated plasma with riboflavin, Unit 1(4 μL); Lane 9 = pretreated plasma with saline, Unit 2 (2 μL); Lane 10 = posttreated plasma with riboflavin, Unit 2 (2 μL); Lane 11 = pretreated plasma with saline, Unit 2 (4 μL); Lane 12 = posttreated plasma with riboflavin, Unit 2 (4 μL); Lane 13 = pretreated plasma with saline, Unit 3 (2 μL); Lane 14 = posttreated plasma with riboflavin, Unit 3 (2 μL); Lane 15 = pretreated plasma with saline, Unit 3 (4 μL); Lane 16 = posttreated plasma with riboflavin, Unit 3 (4 μL).

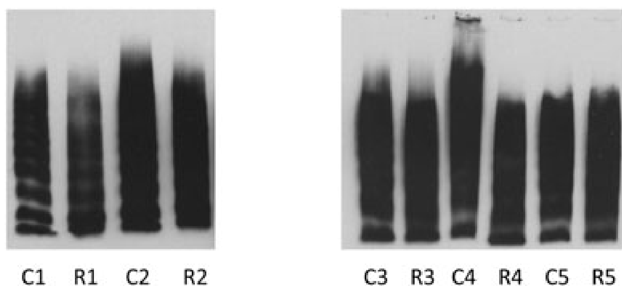

Fig. 2. VWF multimer analysis of individual samples before and after riboflavin and UV light treatment. VWF multimer patterns of untreated (C) and riboflavin-treated (R) plasma samples. Results from five paired sets are shown.

by 30%; and protein S and α_2-antiplasmin are decreased by more than 40%.[6,23,24]

According to various studies, riboflavin and UV treatment significantly reduces infectious levels of viruses, bacteria, and parasites in treated FFP.[11,13,14,19,31,32] This study addresses the protein quality of apheresis-derived plasma treated with this process.

FVIIIc, being one of the most labile proteins in plasma, is usually the most affected by the various pathogen inactivation processes. Riboflavin and UV light–treated plasma also showed reduced levels of FVIIIc (75% retention). In comparison, MB-treated plasma has been reported to show 67% retention of FVIIIc after treatment.[7,26]

Other factors were generally well preserved in riboflavin and UV light–treated plasma. The total amount of protein is fully retained; FXII, FX, FII, and F IX retained 96, 80, and 75% activity, respectively. However, FXI was sensitive to the treatment and retained only 67% activity.

These values are comparable to the data obtained by Hornsey and colleagues[33] from whole blood–derived plasma treated with riboflavin and UV light.

The fibrinogen antigen assay showed 99% retention, which was confirmed by PAGE results and quantitation of the bands. However, the fibrinogen activity was affected more by the treatment than the antigen itself. Additional studies are in progress to characterize the functional activity of the protein. In any case, the riboflavin-treated plasma showed higher retention of fibrinogen activity (77%) compared to MB, which has demonstrated retention of 64% to 69% for fibrinogen activity after treatment.[26-28] Further, the pre- and posttreatment gels show similar bands indicating that there was no structural alteration of the protein as has been reported when MB is used.[34]

ADAMTS-13, the VWF-cleaving metalloprotease cleaves the ultralarge multimers of VWF, which form PLT thrombi in patients with thrombotic thrombocytopenic purpura.[35,36] It is considered that the replacement plasma used to treat thrombotic thrombocytopenic purpura should contain adequate levels of ADAMTS-13 activity to ensure multimer breakdown. Plasma treated with either MB or S/D retains normal levels of ADAMTS-13 activity.[36] Similar results were obtained after riboflavin and UV light treatment (Table 1). It is noted that, in this study, the plasma samples were all tested after a relatively short period of storage; however, the data from Bihm and colleagues[37] examined riboflavin-treated plasma stored for 1 and 2 years and showed similar results.

There is a tight association between ABO blood groups and the level of VWF. VWF levels are much lower in O group individuals due to the higher rate of proteolysis and in a rank of order for other groups: O < B < A < AB.[38] Results from this study also show the dependence of the amount of VWF on ABO groups. There were seven individuals of a total of 20 in the study who were group O, and five of these seven had fewer HMW VWF multimers. All nine individuals with group A and two individuals with group B had normal patterns of VWF multimers. However, among the two individuals with group AB, one had normal VWF multimers, as expected, and the other one had a reduction of the HMW forms before treatment. After the riboflavin and UV treatment, 13 of the 20 plasma samples had lower amounts of the HMW multimers in comparison with controls. The reason for the variable loss of some HMW multimers after treatment is not known but may be due to different susceptibilities based on glycosylation of the VWF. The complete loss of HMW forms has been reported for the Vitex solvent detergent plasma (Raritan, NJ).[39]

Inhibitors of coagulation and plasminogen and markers of activation of fibrinolytic proteins and thrombin activation were well preserved in riboflavin and UV light–treated apheresis plasma. Antithrombin, α_2-antiplasmin, and protein S retained 91% or more activity. These proteins have been reported to be significantly reduced in S/D-treated plasma.[24] Protein C was more sensitive to the treatment and retained 81% activity.

In conclusion, the percentage of protein retention of the proteins in apheresis-derived plasma after riboflavin and UV light treatment compares favorably to those reported with other pathogen inactivation methods for plasma presently in clinical use.[40-42]

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 文献13

Photochemical treatment of plasma with amotosalen and long-wavelength ultraviolet light inactivates pathogens while retaining coagulation function

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BACKGROUND: The INTERCEPT Blood System, a photochemical treatment (PCT) process, has been developed to inactivate pathogens in platelet concentrates. These studies evaluated the efficacy of PCT to inactivate pathogens in plasma and the effect of PCT on plasma function.

STUDY DESIGN AND METHODS: Jumbo (600 mL) plasma units were inoculated with high titers of test pathogens and treated with 150 µmol per L amotosalen and 3 J per cm^2 long-wavelength ultraviolet light. The viability of each pathogen before and after treatment was measured with biological assays. Plasma function was evaluated through measurement of coagulation factors and antithrombotic protein activities.

RESULTS: The levels of inactivation expressed as log-reduction were as follows: cell-free human immunodeficiency virus-1 (HIV-1), greater than 6.8; cell-associated HIV-1, greater than 6.4; human T-lymphotropic virus-I (HTLV-I), 4.5; HTLV-II, greater than 5.7; hepatitis B virus (HBV) and hepatitis C virus, greater than 4.5; duck HBV, 4.4 to 4.5; bovine viral diarrhea virus, 6.0; severe acute respiratory syndrome coronavirus, 5.5; West Nile virus, 6.8; bluetongue virus, 5.1; human adenovirus 5, 6.8; *Klebsiella pneumoniae,* greater than 7.4; *Staphylococcus epidermidis* and *Yersinia enterocolitica*, greater than 7.3; *Treponema pallidum*, greater than 5.9; *Borrelia burgdorferi*, greater than 10.6; *Plasmodium falciparum*, 6.9; *Trypanosoma cruzi,* greater than 5.0; and *Babesia microti*, greater than 5.3. Retention of coagulation factor activity after PCT was expressed as the proportion of pretreatment (baseline) activity. Retention was 72 to 73 percent of baseline fibrinogen and Factor (F)VIII activity and 78 to 98 percent for FII, FV, FVII, F IX, FX, FXI, FXIII, protein C, protein S, antithrombin, and α2-antiplasmin.

CONCLUSION: PCT of plasma inactivated high levels of a wide range of pathogens while maintaining adequate coagulation function. PCT has the potential to reduce the risk of transfusion-transmitted diseases in patients requiring plasma transfusion support.

Approximately 3.3 million units of fresh-frozen plasma (FFP) and 816,000 units of cryoprecipitate are transfused annually in the United States.[1] FFP is indicated for treatment of congenital and acquired coagulation factor deficiencies, coagulopathy resulting from liver disease, massive blood loss, and thrombotic thrombocytopenia purpura. In addition, FFP may be used to prepare cryoprecipitate for fibrinogen replacement and treatment of von Willebrand's disease. Typical therapeutic use of FFP for correction of coagulopathy requires transfusion of approximately 10 to 20 mL per kg FFP per transfusion episode, necessitating exposure to multiple donors.[2] Plasma exchange therapy for thrombotic thrombocytopenia purpura patients may require repeated large volume FFP transfusions with even greater donor exposures.

ABBREVIATIONS: APTT = activated partial thromboplastin time; BVDV = bovine viral diarrhea virus; CAD = compound adsorption device; DHBV = duck hepatitis B virus; FACT = factor assay control plasma; PC = protein C; PCT = photochemical treatment; PS = protein S; PT = prothrombin time; SARS-CoV = severe acute respiratory syndrome coronavirus; UVA = long-wavelength ultraviolet (light); VWF:RCo = von Willebrand factor:ristocetin cofactor; WNV = West Nile virus.

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Donor screening and postdonation testing have greatly reduced the risk of transfusion-transmitted diseases in patients requiring transfusion of blood products. Residual risk persists, however, because only a limited number of pathogens are routinely screened,[3] new blood-borne organisms continue to emerge,[4] and in many cases, the diagnostic tests available are insufficiently sensitive to detect low-level contaminants and infections in the "window period."[5,6] Even though quarantine plasma could potentially eliminate the window period by repeated donor testing within 4 to 6 months, the safety of this approach still depends on test sensitivity. Furthermore, testing remains a reactive approach to blood safety. The contaminating organisms must be identified before screening tests can be developed.

In contrast, pathogen inactivation is a proactive approach to blood safety. Since the 1990s, the transfusion community has become more receptive to this new approach provided that the method is efficacious, safe, easy to implement, and cost-effective. For plasma, two inactivation technologies, methylene blue treatment[7,8] and solvent/detergent (S/D) treatment,[9] have received regulatory approval and are currently in clinical use in several countries in Europe. Neither of these methods, however, has a broad range of effectiveness against pathogens, because S/D treatment only inactivates lipid-enveloped viruses,[10] and methylene blue is ineffective against intracellular viruses.[11] Furthermore, S/D treatment has been shown to compromise some of the in vitro coagulation function of plasma and is contraindicated by the FDA for patients undergoing liver transplant.[12]

The INTERCEPT Blood System for platelets (PLTs) is the only pathogen inactivation technology for blood cellular components that is CE Marked and in clinical use in several countries in Europe.[13] The INTERCEPT Blood System is a photochemical treatment (PCT) process that utilizes amotosalen (also known as S-59) and long-wavelength ultraviolet(UVA; 320-400 nm) light to permanently cross-link helical regions of DNA and RNA.[14] PCT has been shown to inactivate a broad range of viruses,[15-17] bacteria,[18] and protozoa,[19,20] as well as white blood cells (WBCs)[21] in PLT concentrates. Because plasma has similar optical properties to PLT concentrate, it is expected that the PCT process developed for use with PLTs is applicable to plasma; thus supporting the synergy of use of the same PCT technology for two blood components in a blood center. These studies evaluated the efficacy of PCT of plasma for the preparation of pathogen inactivated fresh-frozen plasma (FFP).

MATERIALS AND METHODS

Plasma collection

Apheresis plasma (approx. 600 ± 25 mL) was collected from volunteer donors by plasmapheresis on an automated plasmapheresis machine (Haemonetics PCS 2, Haemonetics Corp., Braintree, MA; or Autopheresis-C, Baxter Healthcare Corp., Deerfield, IL) with sodium citrate anticoagulant. For the pathogen inactivation studies, apheresis FFP units were stored frozen (not greater than –18°C) before use. For the plasma function studies, apheresis plasma units were transported at room temperature and processed within 8 hours of collection.

PCT disposable sets and UVA illumination device

The PCT disposable set for treatment of plasma (Baxter Healthcare Corp.) consisted of the following sequentially integrated components: 15 mL of 6 mmol per L amotosalen hydrochloride solution in saline packaged inside a PL 2411 plastic container and protected from UVA light; a 1.3-L PL 2410 plastic container for illumination of plasma; a compound adsorption device (CAD) to reduce the concentration of amotosalen and its photoproducts, which consisted of an adsorbent disk composed of a copolymer of polystyrene and divinylbenzene particles fused with an ultrahigh-molecular-weight polyethylene plastic enclosed in an acrylic housing; and three 400-mL PL 269 plastic containers for storage of the treated plasma.

Plasma was treated by passing through each component in a series of steps. In Step 1, the plasma unit was sterile connected to the amotosalen container and the plasma content passed through the amotosalen container into the illumination container. In Step 2, the plasma containing amotosalen was illuminated with UVA light. In Step 3, the illuminated plasma mixture was passed through the CAD by gravity into the storage containers (Step 4; Fig. 1). The residual amotosalen remaining in plasma after the CAD step is 1.5 µmol per L or less on average, which is consistently achieved.

Illumination of plasma was performed in a UV illumination system (Baxter Model R4R4007, Nova Biomedical, Waltham, MA). The device was capable of illuminating 2 units of plasma per processing cycle. The illuminator delivered a 3 J per cm^2 UVA treatment to each plasma unit in approximately 7 to 9 minutes. During illumination plasma units were reciprocally agitated at approximately 70 cycles per minute.

Inactivation procedures and viability assays for pathogens

Before addition of amotosalen, plasma units of approximately 585 mL (unless otherwise specified) were inoculated with the applicable pathogen to a final concentration of approximately 10^6 infectious organisms per mL whenever possible. In all cases, the inoculum volume consisted of not greater than 10 percent of the final plasma volume. Table 1 summarizes the strain and the supplier of each pathogen evaluated in these studies.

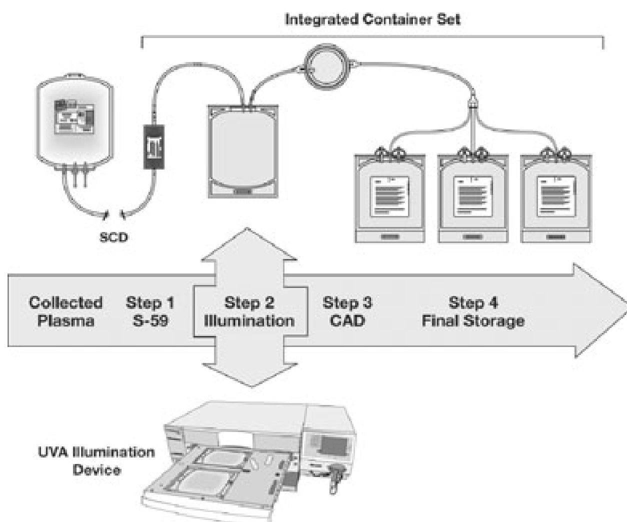

Fig. 1. The PCT system for plasma. The PCT system consists of a UVA illumination device and an integral disposable set. The device can illuminate 2 units of plasma per processing cycle. The disposable set provides a single-use, closed, integrated system for pathogen inactivation treatment of a plasma unit. The integrated disposable set is composed of the following sterile components: an amotosalen (S-59) container, a plastic illumination container, a CAD, and three plastic storage containers. The processing steps are as described under Materials and Methods section. SCD = sterile connection device.

Inoculated plasma units were then treated with 150 μmol per L amotosalen and a 3 J per cm^2 UVA treatment. Samples taken after addition of organism, but before addition of amotosalen, were used to determine the pre-PCT input titer. These samples were serially diluted in assay medium or phosphate-buffered saline before viability measurement. The post-PCT samples were taken immediately after UVA illumination. The CAD step was not performed in the pathogen inactivation studies so that measurement of log reduction was a result of illumination of plasma treated with amotosalen only and not affected by potential pathogen affinity for the CAD. The post-PCT samples were assayed for viable organisms undiluted when possible. In some cases, dilution up to 1:10 was required to prevent toxicity of plasma in the culture system. Table 1 also summarizes the biological assays used for quantifying the number of viable organisms. Detailed assay procedures are as described in the respective references. Pre- and post-PCT titers were quantified with standard assays. The Reed-Muench method was used where end-dilutions were required for quantification.[22] Four replicate experiments (unless otherwise specified) for each organism were performed with four independent units (or pools) of plasma.

The level of inactivation was calculated as log-reduction with the formula

$$\text{Log-reduction} = \text{Log(pre-PCT titer/post-PCT titer)}.$$

TABLE 1. The origin of the test pathogens and established biological methods used to detect and quantify viability

Organism	Strain	Origin (supplier)	Method of detection
HIV-1, cell-free	IIIB	Chronically infected H9 cells (gift from C.V. Hanson)	Microplaque in MT-2 cells[42]
HIV-1, cell-associated	IIIB	Chronically infected H9 cells (gift from C.V. Hanson)	Micro-plaque in MT-2 cells[42]
HTLV-I	2060	California Department of Health Services, Richmond, CA	β-Galactosidase production by infected pA18GBHK-21 cells[43]
HTLV-II	C-19	California Department of Health Services, Richmond, CA	β-Galactosidase production by infected pA18GBHK-21 cells[43]
HBV	MS-2	NIH repository, Bethesda, MD	Infectivity in chimpanzee[44]
HCV	Hutchinson	NIH repository, Bethesda, MD	Infectivity in chimpanzee[45]
DHBV	P-type	Congenitally infected ducks, Hepadnavirus Testing Laboratories, Menlo Park, CA	Infectivity in Legarth-Pekin ducklings[46]
BVDV	NADL	ATCC, Rockville, MD	Plaque in bovine turbinate cells[16]
WNV	3356	Clone lineage I, pFL-WNV (gift from K. Bernard)	Plaque in Vero cells[47]
SARS-CoV	Urbani	CDC, Atlanta, GA	Plaque in Vero-E6 cells[15]
Bluetongue virus	Station	ATCC, Rockville, MD	Plaque in embryonic bovine trachea cells[16]
Human adenovirus	5	Onyx Pharmaceuticals, Inc., Richmond, CA	Plaque on lung carcinoma cells (A549)[16]
S. epidermidis	Septicemia patient	California Department of Health Services, Richmond, CA	Colony formation on Luria Bertani agar[18]
K. pneumoniae	Septicemia patient	California Department of Health Services, Richmond, CA	Colony formation on Luria Bertani agar[18]
Y. enterocolitica	Septicemia patient	California Department of Health Services, Richmond, CA	Colony formation on Luria Bertani agar[18]
T. pallidum	Nichols	University of Washington, Seattle, WA	Infectivity in New Zealand white rabbits[18]
B. burgdorferi	CA4	University of California, Berkeley, CA	Dark field microscopy of cultures in BSK-H medium[18]
P. falciparum	FcB1	Max-Planck-Institute (gift from H. Heidrich)	Infectivity in RBCs[48]
T. cruzi	Tulahuen	University of Washington, Seattle, WA	Infectivity in 3T3 cells[19]
B. microti	C3H/HcN adapted	Wild strain isolated from a field mouse at State University of NY, Stony Brook, NY	Infectivity in C3H/HcN mice[49]

The pre-PCT and post-PCT titers were expressed in scientific notations. The mean level of inactivation and standard deviation (SD) were determined.

PCT of fresh plasma for plasma function studies

The total target volume in the illumination container was 600 ± 25 mL, composed of 585 ± 25 mL of plasma and 15 mL of amotosalen solution. The nominal amotosalen concentration was approximately 150 µmol per L. Each unit of plasma containing amotosalen was illuminated with a 3 J per cm^2 UVA treatment on the R4R4007 illumination device. Plasma samples for evaluation of coagulation factor activity were taken before the addition of amotosalen (baseline, pre-PCT) and after PCT including CAD treatment (post-PCT). Samples were snap-frozen and stored at or below –65°C before testing.

Measurement of in vitro coagulation function

Soluble fibrinogen (Factor [F]I) was measured with a modified Clauss assay in which the clotting time of a diluted plasma sample, after conversion by thrombin into insoluble fibrin, is compared to a standard curve prepared with reference plasma of known fibrinogen concentration. Coagulation factors were assayed with one-stage prothrombin time (PT)-based clotting assays (FII, FV, FVII, FX) or one-stage activated partial thromboplastin time (APTT)-based clotting assays (FVIII, F IX, FXI). The clotting time of a mixture of diluted test plasma sample and plasma deficient in the factor being quantified was compared with a reference curve constructed from the clotting times of five dilutions, ranging from 1:5 to 1:320, of plasma with known activity mixed with deficient plasma. These coagulation tests, as well as the PT and APTT, were performed on an automated coagulation analyzer (MLA Electra 1400C or 1600C, Instrumentation Laboratory Co., Lexington, MA). Reagents included brain thromboplastin (Hemoliance, Instrumentation Laboratory Co.), Platelin L (bioMérieux, Durham, NC), and congenital factor–deficient substrate. The endpoint of all tests was the formation of a clot detected photooptically and measured in seconds. The level of the factor being measured was inversely proportional to the time it takes for a clot to form. Factor assay control plasma (FACT; George King Biomedical, Inc., Overland Park, KS) was used as the reference standard for the coagulation factor assays.

FXIII was measured with the research-use-only FXIII kit (Berichrom, Dade Behring, Marburg, Germany). FXIII, activated by thrombin, releases an activation product that leads to a series of reactions resulting in a decrease in nicotinamide adenine dinucleotide (NADH), detected by monitoring absorbance at 340 nm. The assay was performed on a clot timer (BCT, Dade Behring), and standard human plasma (Dade Behring) was used as the reference standard.

The von Willebrand factor:ristocetin cofactor (VWF:RCo) activity was measured with the BC von Willebrand reagent (Dade Behring). In the assay, lyophilized PLTs are agglutinated by the VWF in the presence of ristocetin, resulting in a decrease in turbidity measured by the change in absorbance on the Behring clot timer. FACT was used as the reference standard.

Protein C (PC) and protein S (PS) were measured with kits (Staclot PC kit and the Staclot PS kit, respectively, both from Diagnostica Stago, Asnieres, France). PC and PS assays were based on the prolongation of the APTT resulting from inactivation of FV and FVIII by activated PC. The activator in the PC assay is an extract of *Agkistrodon c. contortrix* snake venom; the activator in the PS assay is activated PC. The tests were performed on the Behring clot timer. A unicalibrator (Diagnostica Stago) a multiparametric calibrator, was used as the reference standard.

Antithrombin was measured with a kit (Stachrom ATIII, Diagnostica Stago). Plasma containing antithrombin was incubated with a known excess of thrombin. A chromogenic substrate, imidolyzed by the remaining thrombin, was detected photooptically on the MLA Electra 1400C or 1600C coagulation analyzer. FACT was used as the reference standard.

α2-Antiplasmin was quantified with reagents from Diagnostica Stago. In this chromogenic method, plasmin was added in excess to the test plasma, resulting in the formation of antiplasmin-plasmin complexes. The concentration of residual plasmin is measured by its amidolytic activity on a chromogenic substrate measured at 405 nm. α2-Antiplasmin concentration is inversely proportional to the residual plasmin concentration and is determined by color intensity. This analysis was performed by Esoterix Laboratories (Aurora, CO) with an analyzer (STA, Diagnostica Stago).

The mean and SD were determined for each coagulation parameter quantified. The activity of each coagulation parameter remaining after PCT was expressed as percent retention of the pretreatment (baseline) activity. Comparison of the PT and APTT was based on the prolongation of the clotting time after PCT relative to baseline.

RESULTS

PCT inactivation of viruses in plasma

PCT with 150 µmol per L amotosalen and a 3 J per cm^2 UVA treatment inactivated a wide variety of viruses, including enveloped, nonenveloped, DNA, and RNA viruses in plasma (Table 2). Initial viral titers (pre-PCT) of 10^4 to 10^6 infectious viruses per mL were achieved for all viruses except for hepatitis B virus (HBV) and hepatitis C virus (HCV). The highest available titers were used in all

TABLE 2. PCT inactivates enveloped and nonenveloped viruses in plasma at high initial titers*

Viruses	Pre-PCT titer (n = 4)	Post-PCT titer (n = 4)	Log-reduction†
Enveloped			
HIV-1, cell-free	$10^{6.1\pm0.1}$ PFU/mL	$<10^{-0.8\pm0.02}$ PFU/mL	>6.8 ± 0.1
HIV-1, cell-associated	$10^{5.9\pm0.2}$ PFU/mL	$<10^{-0.5\pm0}$ PFU/mL	>6.4 ± 0.2
HTLV-I	$10^{4.0\pm0.2}$ FFU/mL	$\leq10^{-0.5\pm0.8}$ FFU/mL	≥4.5 ± 0.7
HTLV-II	$10^{4.7\pm0.1}$ FFU/mL	$<10^{-1.0\pm0}$ FFU/mL	>5.7 ± 0.1
HBV‡§	$10^{4.5}$ CID_{50}/unit	None detected in 250 mL	>4.5
HCV‡§	$10^{4.5}$ CID_{50}/unit	None detected in 250 mL	>4.5
DHBV	$10^{5.6\pm0.2}$ ID_{50}/mL	$10^{1.2}$-$10^{1.3}$ ID_{50}/mL	4.4-4.5
BVDV	$10^{4.5\pm0.03}$ PFU/mL	$<10^{-1.5\pm0}$ PFU/mL	≥6.0 ± 0.03
WNV	$10^{6.7\pm0.3}$ PFU/mL	$\leq10^{-0.1\pm0.2}$ PFU/mL	≥6.8 ± 0.5
SARS-CoV	$10^{4.0\pm0.1}$ PFU/mL	$\leq10^{-1.5\pm0}$ PFU/mL	≥5.5 ± 0.1
Nonenveloped			
Human adenovirus 5	$10^{5.5\pm0.5}$ PFU/mL	$\leq10^{-1.3\pm0.3}$ PFU/mL	≥6.8 ± 0.4
Bluetongue virus	$10^{4.0\pm0.2}$ PFU/mL	$10^{-1.0\pm0.3}$ PFU/mL	5.1 ± 0.2

* Results are reported as mean ± SD. n = number of replicates done for each virus; PFU/mL = plaque-forming units per milliliter; FFU/mL = foci-forming units per milliliter; ID_{50} = infectious dose necessary for infection of 50 percent of inoculated ducks; CID_{50} = infectious dose necessary for infection of 50 percent of inoculated chimpanzees.

† Log-reduction was calculated as the logarithm of the ratio of the pre-PCT titer to the post-PCT titer.

‡ n = 3.

§ Inactivation was done in 250 mL instead of the 600 mL as specified under Materials and Methods.

cases, whenever possible, to achieve the maximum dynamic range of infectivity.

PCT inactivated a mean of greater than 6.8 logs and greater than 6.4 logs of cell-free and cell-associated human immunodeficiency virus-type 1 (HIV-1), respectively. No biologically active HIV-1 were detected in any of the test samples for four replicate experiments (Table 2). Similarly, human T-cell lymphotropic virus-I (HTLV-I) and HTLV-II were sensitive to PCT and mean log-reductions of at least 4.5 and greater than 5.7, respectively, were obtained.

Inactivation of HBV and HCV by PCT was measured in nonimmune chimpanzees transfused with the entire unit of amotosalen- and UVA-treated plasma that was previously inoculated with $10^{4.5}$ CID_{50} of HBV or HCV (Table 2). Inactivation of each virus was assayed in two chimpanzees. None of the animals in either the HBV or the HCV study showed evidence of viral hepatitis during a 6-month follow-up with serologic, viral nucleic acid, biochemical, or histologic examination of liver biopsies, demonstrating an inactivation of greater than 4.5 logs for both viruses. Further evidence of PCT inactivation of hepadna virus and flaviviridae was demonstrated with, respectively, the model systems duck hepatitis B virus (DHBV) and bovine viral diarrhea virus (BVDV; Table 2). PCT inactivated 4.4 to 4.5 logs of DHBV and at least 6.0 logs of BVDV.

In plasma, PCT inactivated high levels of recently emerging viruses. Mean log-reductions of at least 6.8 and at least 5.5 were achieved for West Nile virus (WNV) and severe acute respiratory syndrome coronavirus (SARS-CoV), respectively (Table 2). PCT inactivated a mean of 5.1 logs of nonenveloped Bluetongue virus and at least 6.8 logs of nonenveloped human adenovirus 5 (Table 2).

PCT inactivation of bacteria in plasma

Gram-positive *Staphylococcus epidermidis* and gram-negative *Klebsiella pneumoniae* and *Yersinia enterocoliticus* were sensitive to PCT. High levels of inactivation were achieved in plasma (Table 3). Initial pre-PCT bacterial levels of $10^{6.6}$ to $10^{6.7}$ colony-forming units (CFU) per mL were achieved with all bacterial species. PCT with 150 μmol per L amotosalen and a 3 J per cm^2 UVA treatment resulted in complete inactivation with no viable bacteria remaining in any of the test plasma samples in all four replicates. Mean log-reductions achieved were greater than 7.3 for *S. epidermidis* and *Y. enterocoliticus* and greater than 7.4 for *K. pneumoniae.*

Spirochetes *Treponema pallidum,* which causes syphilis, and *Borrelia burgdorferi,* which causes Lyme disease, were also sensitive to PCT. No viable organisms were detected after PCT in the test plasma samples in all four replicates, demonstrating mean log reductions of >5.9 and >10.6, respectively.

PCT inactivation of protozoa in plasma

PCT inactivation studies of *Plasmodium falciparum,* the protozoan that causes malaria, *Trypanosoma cruzi,* the pathogen that causes Chagas' disease, and *Babesia microti,* an emerging protozoan causing babesiosis, exhibited mean log reductions of at least 6.9 for *P. falciparum*, greater than 5.0 for *T. cruzi,* and greater than 5.3 for *B. microti* (Table 4). In four replicate experiments, 150 μmol per L amotosalen and a 3 J per cm^2 UVA treatment resulted in no detectable *T. cruzi* and *B. microti* in the test plasma samples.

TABLE 3. PCT inactivates gram-positive and gram-negative bacteria and spirochetes in plasma at high initial titers*

Bacteria	Pre-PCT titer (n = 4)	Post-PCT titer (n = 4)	Log-reduction†
Gram-positive			
S. epidermidis	$10^{6.6\pm0.02}$ CFU/mL	$<10^{-0.7\pm0}$ CFU/mL	>7.3 ± 0.02
Gram-negative			
K. pneumoniae	$10^{6.7\pm0.1}$ CFU/mL	$<10^{-0.7\pm0}$ CFU/mL	>7.4 ± 0.1
Y. enterocolitica	$10^{6.6\pm0.01}$ CFU/mL	$<10^{-0.7\pm0}$ CFU/mL	>7.3 ± 0.1
Spirochetes			
T. pallidum	$10^{5.4\pm0.6}$ ID_{50}/mL	$<10^{-0.5\pm0}$ ID_{50}/mL	>5.9 ± 0.6
B. burgdorferi	$\geq10^{9.9\pm2.3}$ ID_{50}/mL	$<10^{-0.7\pm0}$ ID_{50}/mL	>10.6 ± 2.3

* Results are reported as mean ± SD. n = number of replicates done for each bacterium; ID_{50}/mL = infectious dose necessary for infection of 50 percent of inoculated cells or animal hosts;

† Log-reduction was calculated as the logarithm of the ratio of the pre-PCT titer to the post-PCT titer.

TABLE 4. PCT inactivates protozoa in plasma at high initial titers*

Protozoa	Pre-PCT titer (n = 4)	Post-PCT titer (n = 4)	Log-reduction†
P. falciparum	$10^{5.9\pm0}$ iRBCs/mL	$\leq10^{-1.0\pm0.3}$ iRBCs/mL	≥6.9 ± 0.3
T. cruzi‡	$10^{6.3\pm0.6}$ $TCID_{50}$/mL	$<10^{1.3\pm0}$ $TCID_{50}$/mL	>5.0 ± 0.6
B. microti	$10^{4.9\pm0.4}$ ID_{50}/mL	$<10^{0.5\pm0.02}$ ID_{50}/mL	>5.3 ± 0.4

* Results are reported as mean ± SD. n = number of replicates done for each protozoa; iRBCs/mL = infected red blood cells per milliliter; $TCID_{50}$/mL = infectious dose necessary for infection of 50 percent of inoculated cells; ID_{50}/mL = infectious dose necessary for infection of 50 percent of inoculated mice.

† Log-reduction was calculated as the logarithm of the ratio of the pre-PCT titer to the post-PCT titer.

‡ Inactivation was done in 250 mL of whole-blood derived plasma instead of the 600 mL apheresis plasma as specified under Materials and Methods.

TABLE 5. Maintenance of clotting time and plasma coagulation factor activity after PCT*

Coagulation parameter	Reference range†	Pre-PCT	Post-PCT	Post/pre (% retention)
PT (n = 14)	11.1-13.5	11.2 ± 0.3 sec	11.6 ± 0.3 sec	1.0 ± 0.1 sec‡
APTT (n = 14)	23.0-35.0	26.8 ± 1.4 sec	29.1 ± 1.7 sec	4.3 ± 1.8 sec‡
Fibrinogen (n = 91)	167-379	290 ± 40 mg/dL	209 ± 36 mg/dL	72 ± 5
FII (n = 59)	71-127	96 ± 11 IU/dL	85 ± 11 IU/dL	88 ± 4
FV (n = 91)	77-153	130 ± 23 IU/dL	119 ± 19 IU/dL	92 ± 7
FVII (n = 91)	58-166	123 ± 32 IU/dL	95 ± 20 IU/dL	78 ± 6
FVIII (n = 91)	67-235	157 ± 35 IU/dL	115 ± 28 IU/dL	73 ± 7
F IX (n = 91)	63-143	108 ± 21 IU/dL	88 ± 16 IU/dL	82 ± 4
FX (n = 59)	66-134	100 ± 13 IU/dL	86 ± 11 IU/dL	86 ± 3
FXI (n = 91)	62-142	103 ± 22 IU/dL	87 ± 18 IU/dL	86 ± 5
FXIII (n = 26)	NA	110 ± 11 IU/dL	102 ± 10 IU/dL	93 ± 3
VWF:RCo (n = 12)	NA	114 ± 44 IU/dL	111 ± 41 IU/dL	97 ± 8

* Results are reported as meant ± SD. n = number of replicates (treated plasma units) done for each parameter. (A higher number of replicates was performed for a basic testing panel, with additional parameters included in a subset of studies.) IU/dL = International Units/deciliter.

† The reference range was calculated from the mean ± 2 SD of untreated, conventional plasma.

‡ For PT and PTT, the effect of PCT was calculated by subtracting the pre-PCT values from the post-PCT values.

In vitro coagulation function of plasma after PCT

Evaluation of a comprehensive panel of coagulation parameters showed that PCT conserved coagulation function within levels suitable for transfusion support.[23] On average, the PT and APPT were prolonged after PCT by 1.0 and 4.3 seconds, respectively (Table 5). Fibrinogen, FVII, and FVIII were retained, on average, 72 to 78 percent of the initial pre-PCT activity. All other coagulation parameters retained at least 82 percent of baseline activity, with FV, FXIII, and VWF:RCo retaining at least 92 percent. Antithrombotic proteins (PC, PS, antithrombin) demonstrated a high level of activity retention (Table 6), ranging between 95 and 98 percent of the initial pre-PCT level. α2-Antiplasmin was retained at 80 percent of baseline activity. Coagulation factor and antithrombotic protein activities fell within the reported reference ranges for conventional plasma.[24]

DISCUSSION

These studies showed that PCT with amotosalen and UVA light is effective against a broad range of pathogens in plasma. This finding is not surprising based on the pathogen inactivation efficacy previously demonstrated for PLTs with the same dose of amotosalen and same dose of UVA light.[15-20] The levels of inactivation for representative viruses, bacteria, and protozoa were measured with PCT conditions developed for the commercial application of FFP.

PCT inactivated high levels of enveloped viruses including HIV-1, HBV, and HCV in plasma. Effectiveness against HBV was initially shown by inactivation of DHBV, an established infectivity model for human HBV.[25] A chimpanzee infectivity model was then used to confirm the level of inactivation of HBV in plasma. Effectiveness against HCV was initially shown by inactivation of BVDV, a flavivirus and model for human HCV.[26] Inactivation of HCV was then confirmed with the chimpanzee infectivity model as well. PCT is also effective against other flaviviruses such

TABLE 6. Maintenance of plasma antithrombotic protein activity after PCT*

Protein	Pre-PCT (IU/dL)	Post-PCT (IU/dL)	Post/pre (% retention)
PC (n = 25)	109 ± 15	102 ± 14	95 ± 9
PS (n = 25)	109 ± 12	107 ± 12	98 ± 5
Antithrombin III (n = 26)	94 ± 5	91 ± 6	96 ± 3
α2-Antiplasmin (n = 26)	93 ± 5	75 ± 6	80 ± 4

* Results are reported as mean ± SD. n = number of replicates done for each parameter; IU/dL = International Units/deciliter.

as WNV and, in preliminary studies, dengue virus (data not shown), examples of new pathogens that are of increasing concern to blood centers in North America.

PCT with amotosalen is very effective against cell-associated HIV-1 and against cell-associated HTLV-I and HTLV-II viruses in plasma, demonstrating that amotosalen molecules readily permeate the cell membrane. The ability of the photochemical reagent to permeate the cell membrane as well as nuclear membrane is crucial because some blood-borne viruses exist partially or largely associated with cells. Methylene blue treatment of plasma, for example, is not effective against cell-associated or intracellular viruses.[11] The efficacy of the methylene blue system against intracellular viruses depends on the efficacy of filtration or freeze-thaw to quantitatively disrupt WBCs.[8,27] Recent studies, however, demonstrate that conventional WBC filters fail to eliminate cell-associated cytomegalovirus and HTLV-I from PLT products.[28,29] Thus the methylene blue system is ineffective against these viruses.

The cumulative viral inactivation data in PLTs and plasma would predict that PCT is effective against the majority of enveloped viruses, DNA, or RNA, extracellular or intracellular. Thus PCT has the potential to inactivate new and emerging enveloped viruses and prevent them from entering the blood supply. Indeed, it was found that SARS-CoV was inactivated by PCT in both PLTs and plasma.[15]

Nonenveloped viruses are less common as labile blood component transfusion-transmitted pathogens. Human parvovirus B19, and in very rare cases hepatitis A virus (HAV), however, have been transmitted by component blood transfusion.[30,31] A ubiquitous nonenveloped virus, TT virus, has been reported to be transmitted through the blood supply in Japan, but has yet to be directly linked with a specific disease state.[32]

The efficacy of PCT against nonenveloped viruses in plasma continues to be evaluated and is expected to be similar to the results obtained for PLTs.[16] In these studies, inactivation experiments were only performed with two nonenveloped viruses, bluetongue virus and human adenovirus 5, as examples. Inactivation results comparable to the PLT experiments were obtained. Owing to the low permeability of the capsid, some nonenveloped viruses show little or no inactivation by PCT. The picornaviruses are known to be the most difficult to inactivate by chemical or physical means.[33] The tight capsid structure of picornaviruses such as HAV, polio virus, and encephalomyocarditis virus are thought to exclude even low-molecular-weight compounds such as psoralens from the interior of the virus and PCT is ineffective against these viruses. Human parvovirus B19 and human adenovirus 5, however, were found sensitive to PCT in PLTs.[16,34] The level of inactivation for these nonenveloped viruses can be increased by incubating the spiked blood products with amotosalen before illumination with UVA light.[34]

The methylene blue system also showed variable efficacy against nonenveloped viruses, ranging from ineffective to complete inactivation.[8] Another system for treatment of plasma, the S/D system, is ineffective against nonenveloped viruses. S/D inactivates viruses by disrupting the viral membrane.[10]

PCT is effective for inactivation of gram-positive *S. epidermidis* and gram-negative *K. pneumoniae* and *Yersinia enterocolitica*. *Y. enterocolitica* are cryophilic bacteria that can grow in cold temperatures. High levels of inactivation were achieved for all of these bacteria in plasma. Although transfusion-transmitted bacteremia is not considered a serious problem for transfusion of FFP, it is reassuring to have a pathogen inactivation process that inactivates bacteria as well as viruses. In these studies, inactivation of two spirochetes, *T. pallidum* and *B. burgdorferi*, in plasma was also demonstrated. *T. pallidum*, which causes syphilis, is the only bacteria routinely screened for in blood banks. Under experimental conditions, *B. burgdorferi* has been reported to remain viable in frozen plasma for more than 1 month.[35] PCT has been shown to be effective against these agents and high levels of inactivation were obtained in plasma.

On an international level, other organisms such as *T. cruzi* (Chagas' disease), *P. falciparum* (malaria), and *B. microti* (babesiosis) may be more of a concern than bacteria for frozen plasma because, like viruses, they do not require growth during storage to be a transfusion risk. *P. falciparum* and *B. microti* are intracellular pathogens, as discussed above, and are resistant to methylene blue treatment.[11]

Experiments with plasma are ongoing to measure the level of WBC inactivation by PCT. Based on previous studies with PLTs, it is anticipated that high levels of WBC inactivation could be demonstrated in plasma.[21] Overall, the results presented here show that PCT offers the benefit of a broad spectrum of pathogen inactivation. Neither the methylene blue system nor the S/D system for plasma has shown this broad pathogen inactivation profile.

These studies also confirmed maintenance of adequate plasma coagulation and antithrombotic protein function after PCT. Because FFP is transfused primarily as a replacement for the liver-derived coagulation factors FII, FV, FVII, F IX, FX, and FXI, in vitro studies to evaluate plasma function after processing with PCT were conducted for a panel of coagulation factors, antithrombotic proteins, and clotting time. The proteins most affected by PCT were fibrinogen and FVIII. The changes in coagulation proteins observed in plasma treated with PCT were also associated with slight prolongation in PT and APTT. The slight changes in PT and APTT after PCT, however, were not associated with any adverse clinical observations.[36-39]

Levels of the anticoagulant PC and PS and antithrombin were relatively unaffected by PCT and α2-antiplasmin was conserved by 80 percent. Previous studies on cryoprecipitate prepared from PCT plasma yielded approximately 95 and 88 percent activity retention for fibrinogen and FVIII, respectively, compared to cryoprecipitate prepared from untreated plasma.[40] Furthermore, cryosupernatant prepared from PCT plasma retained adequate levels of critical plasma proteins for plasma exchange therapy in acute thrombocytopenic purpura. The data indicate good preservation of hemostasis control proteins such as PS, α2-antiplasmin, and VWF-cleaving protease activity.[41]

From a blood center perspective, the adoption of PCT would enhance safety of blood products and potentially decrease the number of donors rejected. PCT is easily integrated into the plasma preparation system by its ability to treat individual units of fresh apheresis plasma and small pools (three) of whole blood–derived plasma. For further ease of use, PCT is compatible with the existing plasma collection procedures, FFP processing, and distribution methods in use in blood centers. This PCT system allows rapid illumination and processing of plasma units and utilizes the same illumination device developed for pathogen inactivation of PLT concentrates. Thus, both plasma and PLTs can be treated with a similar processing system and the same illumination device.

In summary, photochemically treated plasma is functionally similar to untreated conventional plasma with the added benefit of pathogen inactivation.

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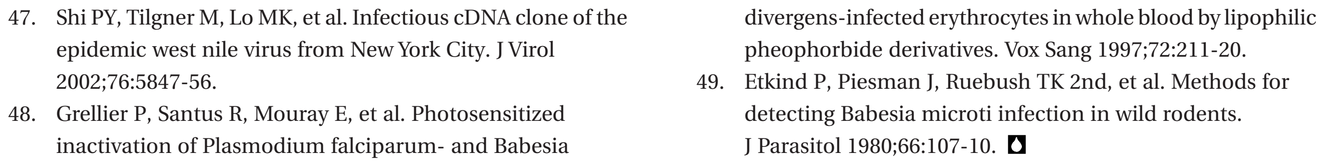

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Wortlaut der Gebrauchsinformation und Fachinformation im Sinne der §§ 11 und 11a AMG

Gebrauchsinformation und Fachinformation

Mit Pathogeninaktivierungsverfahren behandeltes Thrombozytapheresekonzentrat

1. Identifizierung des Arzneimittels

a) Bezeichnung

Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst

b) Stoffgruppe

zelluläre Blutzubereitung

2. Anwendungsgebiete

Die Gabe von Thrombozytenkonzentraten ist indiziert zur Behandlung einer Blutungsneigung, bedingt durch eine schwere Thrombozytopenie infolge thrombozytärer Bildungsstörungen, im Notfall auch bei Umsatzstörungen, jedoch nicht bei einer niedrigen Thrombozytenzahl allein. Damit durch die Zufuhr von Plättchen eine Besserung der thrombozytär bedingten Blutungsneigung zu erwarten ist, sollte vor der Behandlung zunächst deren Ursache abgeklärt werden.

Das „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst" ist auch geeignet zur Anwendung bei gefährdeten Patienten, bei denen das Risiko einer transfusionsassoziierten Graft-Versus-Host-Reaktion vermieden werden soll, wie:

- Frühgeborene (bis zur Vollendung der 37. Schwangerschaftswoche)
- Neugeborene bei Verdacht auf Immundefizienz,
- Neugeborene bei postpartaler Austauschtransfusion*
- Patienten bei allogener Transplantation hämatopoetischer Stammzellen (aus peripherem Blut, Knochenmark oder Nabelschnurblut)
- Patienten 7 – 14 Tage vor autologer Stammzellentnahme
- Patienten bei autologer Stammzelltransplantation (bis ca. drei Monate nach Transplantation)
- Patienten mit schwerem Immundefektsyndrom oder mit AIDS
- Patienten mit M. Hodgkin (alle Stadien)
- Patienten bei Therapie mit Purin-Analoga (z.B. Fludarabin, Cladrabin, Deoxycoformycin)
- Patienten bei Therapie mit Anti-T-Lymphozyten-Antikörpern (z.B. Alemtuzumab, ATG/ALG)
- Patienten bei Hochdosis-Chemotherapie mit oder ohne Ganzkörperbestrahlung, Patienten mit Leukämien, malignen Lymphomen, soliden Tumoren*.

* nicht gesicherte Indikationen

Das „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst" ist ebenso geeignet für Patienten, bei denen eine CMV-Infektion vermieden werden muss, wie:

- Frühgeborene
- Empfänger eines allogenen Stammzellpräparates
- Empfänger mit schweren angeborenen Immundefekten (SCID)
- CMV-negative, HIV-infizierte Patienten
- CMV-negative, schwangere Frauen
- stillende Mütter.

(siehe auch „Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie)")

3. Informationen zur Anwendung

a) Gegenanzeigen

Bekannte Überempfindlichkeit gegen Amotosalen-HCl oder Psoralene. In diesem Fall sollten

Thrombozytenkonzentrate, die mit diesem Pathogeninaktivierungsverfahren behandelt wurden, nicht verwendet werden.
Eine absolute Kontraindikation für Thrombozytentransfusionen gibt es nicht. Bei potentiellen Empfängern eines Stammzelltransplantates (Knochenmark, periphere Stammzellen, Nabelschnurblut), z.B. bei Patienten mit aplastischen Anämien, Leukämien etc., ist die Gabe von Thrombozytenkonzentraten des Transplantatspenders und seiner Blutsverwandten vor der Transplantation unbedingt zu vermeiden.
Relative Kontraindikationen sind u.a. die Anwendung bei

- bekannten Allergien des Empfängers gegen humane Plasmaproteine,
- bekannten Immunthrombozytopenien,
- posttransfusioneller Purpura,
- heparininduzierter Thrombozytopenie,
- kongenitalen Thrombozytenfunktionsstörungen, wie Thrombasthenie Glanzmann oder Bernard-Soulier-Syndrom.

b) Vorsichtsmaßnahmen für die Anwendung

Thrombozytenkonzentrate sind in der Regel AB0-kompatibel über ein Transfusionsgerät mit Standardfilter der Porengröße 170 bis 230 µm zu transfundieren.
Beim Refraktärzustand gegenüber Thrombozytentransfusionen aufgrund einer Alloimmunisierung gegen Antigene des HLA- und ggf. HPA-Systems sind nach Möglichkeit HLA-Klasse-I-kompatible und ggf. HPA-kompatible Thrombozytenkonzentrate zu transfundieren. Die Transfusionsgeschwindigkeit muss dem klinischen Zustand des Patienten angepasst werden.
Bei neonataler Transfusion sollte sorgfältig auf Anzeichen einer Zitratintoxikation geachtet und die Transfusionsgeschwindigkeit dem klinischen Zustand angepasst werden.

c) Wechselwirkungen mit anderen Arzneimitteln, soweit sie die Wirkungsweise des Arzneimittels beeinflussen können und Hauptinkompatibilitäten

Durch Medikamente, die die Thrombozytenfunktion beeinflussen, kann die Wirkung von Thrombozytenkonzentraten vermindert bzw. aufgehoben werden. Wegen der Gefahr von Gerinnselbildungen dürfen kalziumhaltige Lösungen nicht gleichzeitig in demselben Schlauchsystem gegeben werden. Die Beimischung von Medikamenten zum Thrombozytenkonzentrat ist nicht zulässig.

d) Verwendung für besondere Personengruppen

Bei Rh (D) - negativen Kindern und Frauen im gebärfähigen Alter ist wegen der praktisch in allen Thrombozytenkonzentraten vorhandenen Kontamination mit Erythrozyten die Transfusion von Thrombozytenkonzentraten Rh (D)-positiver Spender mit Ausnahme von lebensbe-drohlichen Situationen unbedingt zu vermeiden. Die Transfusion von Thrombozyten-konzentraten Rh (D)-positiver Spender in Rh (D)-negative Patienten lässt sich wegen des Mangels an Rh (D)-negativem Blut nicht immer vermeiden, sollte nach Möglichkeit aber nur in Betracht gezogen werden, wenn es sich um Männer oder um Frauen im nicht gebärfähigen Alter handelt. In solchen Fällen ist stets eine serologische Nachuntersuchung 2 bis 4 Monate nach Transfusion zur Feststellung eventuell gebildeter Anti-D-Antikörper durchzuführen.
Schwangerschaft und Stillzeit: Bei bestimmungsgemäßem Gebrauch bestehen keine Einwände.
Anwendung bei Kindern, Neugeborenen, Föten: Bei bestimmungsgemäßem Gebrauch bestehen keine Einwände.
Anwendung bei Neugeborenen unter Lichttherapie: Neugeborene, die während einer Lichttherapie zur Behandlung von Hyperbilirubinämie Thrombozytentransfusionen benötigen, sollten nur mit Lichttherapiegeräten behandelt werden, die Licht mit einer Wellenlänge größer als 425nm aussenden. Anderenfalls kann es zu einer Wechselwirkung zwischen dem UV-A-Licht und dem Restgehalt des zur Herstellung des „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst" verwendeten Psoralens (Amotosalen) und damit zu einem Erythem kommen. Bei Lichttherapie mit einer Wellenlänge kleiner als 425nm dürfen nur unbehandelte Thrombozytenkonzentrate transfundiert werden.
Stark immunsupprimierten Patienten: Bei bestimmungsgemäßem Gebrauch bestehen keine Einwände.
Auswirkung auf Kraftfahrer und die Bedienung von Maschinen: Nach der Transfusion von Thrombozytenkonzentraten sollte eine Ruhepause von mindestens 1/2 Stunde eingehalten

werden.

e) Warnhinweise
sind nicht angeordnet.

4. Hinweise zur ordnungsgemäßen Anwendung

a) Dosierung

Die Dosierung der Thrombozyten ist abhängig vom klinischen Zustand und der Thrombozytenzahl des Patienten.
Der Thrombozytenbedarf für die initiale Behandlung eines normalgewichtigen Erwachsenen ohne weitere Komplikationen beträgt mindestens 2,5 x 10^{11} Thrombozyten, entsprechend einer Packungseinheit „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst". Eine Überwachung der Therapie, z.B. durch Bestimmung der Thrombozytenzahl oder der Blutungszeit beim Patienten, ist unerlässlich.

b) Art der Anwendung
zur i.v. Infusion

c) Häufigkeit der Verabreichung
nach Indikationsstellung

d) Dauer der Behandlung
nach Indikationsstellung

e) Überdosierung
Eine Gefahr der Überdosierung besteht bei Erwachsenen nicht.

f) Notfallmaßnahmen
Treten Unverträglichkeiten auf, so ist die Transfusion unverzüglich abzubrechen, der Venenzugang jedoch offen zu halten und eine Behandlung, der Schwere der Symptome gemäß, nach den aktuellen Regeln der Notfalltherapie einzuleiten.

5. Nebenwirkungen

- Unverträglichkeitsreaktionen (z.B. urtikarielle Hautreaktionen, posttransfusionelle Purpura und andere anaphylaktoide Reaktionen)
- Unverträglichkeitsreaktionen gegenüber Amotosalen und seinen Photoabbauprodukten (z.B. allergische und andere anaphylaktoide Reaktionen). Immunologische Reaktionen durch Bildung von Neoantigenen sind bisher nicht bekannt.
- Transfusionsassoziierte akute Lungeninsuffizienz (TRALI)
- anaphylaktische Reaktionen bei Empfängern mit angeborenem IgA-Mangel
- Mikrozirkulationsstörungen durch aggregierende Thrombozyten bei massiver Transfusion
- Immunisierung des Empfängers gegen thrombozytäre und nicht-thrombozytäre Antigene
- Obwohl HLA-bedingte Unverträglichkeiten stark verringert sind, können febrile Transfusionsreaktionen in unmittelbarem zeitlichem Zusammenhang mit der Transfusion auftreten.
- Bei Neugeborenen sind bei schneller Transfusion Herz-Kreislaufreaktionen infolge von Citratintoxikationen möglich.
- Durch das zusätzlich angewandte Pathogeninaktivierungsverfahren mit dem „INTERCEPT Blood System" (Amotosalen/UVA) verlieren Viren, Bakterien, Parasiten und Restleukozyten weitgehend ihre Teilungsfähigkeit. Das Risiko einer bakteriellen, viralen oder parasitären Kontamination oder einer transfusionsassoziierten Graft-versus-Host-Erkrankung ist nicht mit allerletzter Sicherheit auszuschließen. Jedoch wird insbesondere die Inaktivierung von umhüllten Viren (HIV, HBV, HCV) und Leukozyten als sicher erachtet. Eine effiziente Inaktivierung von nicht-umhüllten Viren (z.B. HAV und Parvovirus B19) ist nicht gewährleistet. Das Pathogeninaktivierungsverfahren ist gegenüber einem breiten Spektrum von gram-positiven und gram-negativen Bakterien sehr effektiv; jedoch gegenüber einzelnen Bakterien-Spezies (z.B. Pseudomonas aeruginosa) nur eingeschränkt effektiv. Bakterielle Sporen (z.B. von Clostridium perfringens oder Bacillus cereus) lassen sich mit dem hier angewandten Pathogeninaktivierungsverfahren nicht abtöten. Die Belastung mit Pyrogenen wird durch das Pathogeninaktivierungsverfahren nicht unterbunden, daher sind pyrogene Reaktionen (auch schwerwiegende) des Empfängers nicht auszuschließen.

- Bei der Anwendung von aus menschlichem Blut hergestellten Arzneimitteln ist die Übertragung von Infektionskrankheiten durch Übertragung von Erregern - auch bislang unbekannter Natur - nicht völlig auszuschließen. Dies gilt z.B. für Hepatitiden, seltener für das erworbene Immundefektsyndrom (AIDS).
- Im Vereinigten Königreich Großbritannien und Nordirland wurde über Einzelfälle berichtet, in denen bei Empfängern von Transfusionen, deren Spender später an der varianten Creutzfeldt-Jakob Krankheit (vCJK) erkrankten, ebenfalls der „Erreger" (so genannte Prionen) nachgewiesen wurde. Bei der vCJK handelt es sich um eine in Deutschland bislang nicht beobachtete Erkrankung, die durch den Verzehr von bestimmten Nahrungsmitteln aus BSE-kranken Rindern erworben werden kann.

6. Pharmakologische Eigenschaften

Die wirksamen Bestandteile von Thrombozytenkonzentraten sind morphologisch und funktionell intakte Thrombozyten, welche die zellulären Bestandteile des Hämostasesystems darstellen. Die Hämostaseaktivität der funktionell intakten Thrombozyten ist sofort nach der Transfusion gegeben. Die Funktionsfähigkeit und mittlere Überlebenszeit der Thrombozyten nimmt mit der Lagerungsdauer ab. Durch die Leukozytendepletion auf $< 1 \times 10^6$ Leukozyten pro Packungseinheit wird das Risiko einer Immunisierung gegen humane leukozytäre Alloantigene (HLA) und durch das Pathogeninaktivierungsverfahren die Übertragung mitosefähiger immunkompetenter Lymphozyten stark vermindert, somit die Gefahr einer transfusionsassozierten Graft-versus-Host-Reaktion vermieden.

Das Pathogeninaktivierungsverfahren basiert auf der Zugabe von Amotosalen und nachfolgender UVA-Bestrahlung. Trotz der effizienten Entfernung von Amotosalen durch ein Adsorptionsverfahren sind sehr geringe Mengen von Amotosalen (< 2µM) und dessen Photoprodukten D und E (Dimere von Amotosalen) im Thrombozytenkonzentrat nachweisbar. Tierversuche mit einmaliger und wiederholter Verabreichung von Amotosalen, in Dosierungen, die mehr als 100-fach über der klinisch zu erwartenden Exposition von Amotosalen lagen, ergaben keine Hinweise auf ein erhöhtes toxikologisches Risiko für die Anwendung von „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst". Es gibt keine Anzeichen auf Phototoxizität nach intravenöser Anwendung der 40-fachen üblichen klinischen Dosis bei Ratten.

7. Weitere Hinweise

a) Angaben zur Aufbewahrung und Haltbarkeit

Angaben zur Haltbarkeit, besondere Lager- und Aufbewahrungshinweise

- Das Thrombozytenkonzentrat „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst" ist 5 Tage bei 22 ± 2 °C unter gleichmäßiger Agitation haltbar. Nach Ablauf des Verfalldatums darf das Thrombozytenkonzentrat nicht mehr verwendet werden.
- Nach Unterbrechung der o.g. Lagerbedingungen ist das Thrombozytenkonzentrat unverzüglich zu transfundieren.
- Eine durch das Transfusionsbesteck geöffnete Konserve muss unverzüglich verbraucht werden.

b) Optische Prüfung

Unmittelbar vor der Transfusion muss jedes Thrombozytenkonzentrat einer optischen Qualitätsprüfung unterzogen werden, auffällige Thrombozytenkonzentrate (z.B. fehlendes "Swirling-Phänomen", erkennbare Aggregatbildung) dürfen nicht verwendet werden.

c) Zusammensetzung des Fertigarzneimittels

Wirkstoffe (qualitativ und quantitativ)

Human-Thrombozyten aus Apherese

$2{,}5 \times 10^{11}$ bis $6{,}0 \times 10^{11}$ Thrombozyten/Packungseinheit

sonstige Bestandteile:

1 ml „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogeninaktivierung, DRK-Blutspendedienst" enthält:

InterSol	0,53 - 0,68 ml
Plasma der angegebenen Blutgruppe	0,38 - 0,28 ml
Stabilisator ACD-A	0,09 - 0,04 ml

1000 ml Thrombozytenadditivlösung InterSol enthalten:

Natriumcitrat-Dihydrat	3,18 g
Dinatriumphosphat-Anhydrat	3,05 g
Natriumdihydrogenphosphat-Dihydrat	1,05 g
Natriumacetat-Trihydrat	4,42 g
Natriumchlorid	4,52 g
Wasser für Injektionszwecke	ad 1000 ml

1000 ml des Stabilisators ACD-A enthalten:

Citronensäure-Monohydrat	8,0 g
Natriumcitrat-Dihydrat	22,0 g
Glucose-Monohydrat	25,0 g
Wasser für Injektionszwecke	ad 1000 ml

Restgehalt pro Liter:
Amotosalen < 2µM

Restzellzahlen pro Packungseinheit:

Leukozyten	$< 1 \times 10^6$

Restzellzahlen pro ml:

Erythrozyten	$< 4 \times 10^6$

d) Darreichungsform und Inhalt, Behältnis
210 bis 310 ml Suspension im Kunststoffbeutel mit CE-Zertifikat.

Angaben zur Zulassung

e) Zulassungsnummer
PEI.H.03610.01.1

f) Datum der Erteilung der Zulassung
23.02.2009

g) Arzneimittelstatus
Verschreibungspflichtig

8. Sonstige Hinweise

Maßnahmen zur Reduktion des Übertragungsrisikos von Infektionserregern:
Da bei der Anwendung aus menschlichem Blut hergestellten Arzneimitteln die Übertragung von Infektionskrankheiten nicht völlig auszuschließen ist, werden Maßnahmen getroffen, um das Risiko einer Übertragung von infektiösem Material zu minimieren: Für die Herstellung von „Thrombozytenkonzentrat / Apherese, behandelt zur Pathogen-inaktivierung, DRK-Blutspendedienst" werden ausschließlich Spenden gesunder Spender verwendet, die mit negativem Ergebnis getestet wurden auf Humanes Immundefizienz Virus (Anti-HIV-1/2-Ak, HIV-1 Genom), Hepatitis-B Virus (HBsAg, Anti-HBc-Ak), Hepatitis-C Virus (Anti-HCV-Ak, HCV-Genom) und Treponema pallidum (Anti-Treponema pallidum-Ak). Im Hinblick auf eine mögliche Hepatitis-B-Infektion werden bei Erstspendern nur Blutspenden verwendet, die Anti-HBc negativ getestet sind. Bei Blutspendern, die vor dem 01.02.2006 als Mehrfachspender Blut gespendet haben, werden nur Blutspenden verwendet, die Anti-HBc negativ sind, oder bei positivem Anti-HBc Status einen Anti-HBs Wert ≥ 100 IU/ml aufweisen. Darüber hinaus kann durch die Leukozytendepletion das Risiko einer Übertragung von leukozytenassoziierten Viren (HTLV-I/II, CMV, EBV u.a.) und Bakterien (Yersinia enterocolitica) entscheidend vermindert werden. Durch die zusätzliche Pathogeninaktivierung mittels „INTERCEPT Blood System" (Amotosalen/UVA) kann von einer weiteren Reduktion des Übertragungsrisikos von Infektionserregern (Viren, Bakterien und Parasiten) ausgegangen werden.

Qualitätssicherung:
Für die Transfusion von Thrombozytenkonzentraten sind von den Einrichtungen der Krankenversorgung Maßnahmen im Rahmen der Qualitätssicherung nach § 15 des Transfusionsgesetzes zu ergreifen. Dazu gehören u.a. detaillierte Anweisungen sowohl für die Indikationsstellung und Dosierung abhängig von Thrombozytenanzahl und -abfall bei unterschiedlichen Grunderkrankungen, Vorsorgemaßnahmen zur Erhaltung der Unversehrtheit der Konserve vor der Transfusion und Anweisungen zur Nachuntersuchung der Patienten für die Feststellung des Transfusionserfolges, eventuell gebildeter Antikörper und zu ergreifender Prophylaxe. Die Entscheidungskriterien für die Transfusion von Thrombozyten bei primären und

sekundären Knochenmarkinsuffizienzen, aplastischer Anämie oder Myelodysplasie, disseminierter intravasaler Gerinnung, Patienten mit angeborenen Thrombozytopathien/-penien, Autoimmunthrombozytopenien, fötaler bzw. Alloimmunthrombozytopenie, für die Auswahl der Präparate und deren Dosierung sowie die Überwachung der Anwendung sind im Rahmen einer patientenbezogenen Qualitätssicherung durch die transfusionsverantwortlichen Personen festzulegen.

Besondere Vorsichtsmaßnahmen für die Beseitigung:

Die ordnungsgemäße Entsorgung von angebrochenen bzw. nicht mehr verwendbaren Präparaten ist entsprechend den Vorgaben der Einrichtung der Krankenversorgung sicherzustellen.

Die jeweils aktuellen "Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie)" sowie gegebenenfalls ergänzende Veröffentlichungen der Bundesärztekammer und des Paul-Ehrlich-Instituts sind zu berücksichtigen.

9. Datum der letzten Überarbeitung

27.02.2009

Pharmazeutischer Unternehmer / Inhaber der Zulassung:

DRK-Blutspendedienst Baden-Württemberg - Hessen gemeinnützige GmbH
Friedrich-Ebert-Str. 107, 68167 Mannheim, Telefon (0621) 37060

Hersteller, der das Fertigarzneimittel für das Inverkehrbringen freigegeben hat:

DRK-Blutspendedienst Baden-Württemberg – Hessen gemeinnützige GmbH
Institut Mannheim, Friedrich-Ebert-Straße 107, 68167 Mannheim

DRK-Blutspendedienst Baden-Württemberg – Hessen gemeinnützige GmbH
Institut Frankfurt, Sandhofstraße 1, 60528 Frankfurt / Main

DRK-Blutspendedienst Baden-Württemberg – Hessen gemeinnützige GmbH
Institut Ulm, Helmholtzstraße 10, 89081 Ulm

文献15

Department of Biological Standardisation
OMCL Network & Healthcare (DBO)

Pathogen Reduction Technologies for Blood Components for Transfusion: Updated Table March 2008

COUNCIL OF EUROPE
European Committee (Partial Agreement) on blood transfusion (CD-P-TS)

Study coordinators: Miguel LOZANO[1], Jean-Pierre CAZENAVE[2], Marie-Emmanuelle BEHR-GROSS[3]
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Pathogen Reduction Technologies for Blood Components for Transfusion: Updated Table March 2008

Country	FFP				Platelets	Remarks
	Quarantine	Solvent Detergent	Methylen Blue	Intercept	Intercept	
Australia	np	np	np	np	np	Pathogen reduction technologies remain under review
Austria	23,100 units	50,100 units	0%	np	np	
Belgium	np	small percentage	96% minus a small percentage	4% Routine use in 2 centers. On-going evaluation in 1 center	17 % Routine use or on-going evaluation at all centers	
Bosnia & Herzegovina	np	np	np	np	np	Pathogen reduction technologies remain under review
Bulgaria	np	np	np	np	np	
Canada	some	some	np	np	np	Pathogen reduction technologies remain under review
Croatia	Only plasma for fractionation	np	np	np	np	
Czech Republic	100% (about 51,000 liters)	np.	np	np	Only one very small clinical trial	
Finland	np	100% (about 60,000 units per year)	np	np	np	
France	161,597 units	130,504 units	Quarantine plasma to be replaced by methylene blue as of September 2008	5% (about 20,000 units). Use in routine in 1 regional center)	Routine use (100%) in 4 regional centers. On-going evaluation in 3 regional centers	Mirasol clinical trial phase III for platelets completed, under evaluation
Georgia	np	np	np	np	np	
Germany	Around 90 %	Around 10%	Licensed for 2 establishments*	np	Licensed for 1 center *	* % not yet available
Hungary	70%	np	np	np	np	
Iceland	np	np	np	np	np	
Ireland	np	About 100%	np	np	On-going evaluation in 1 center	
Italy	About 15%	About 20%	About 10%	Routine use in 2 centers. Evaluation in 1	Routine use in 8 centers. Evaluation in 1 center	Mirasol evaluation in 3 centers
Malta	85%	np	np	np	np	
Moldova	np	np	np	np	np	

Country	FFP				Platelets	Remarks
	Quarantine	Solvent Detergent	Methylen Blue	Intercept	Intercept	
Montenegro	np	np	np	np	np	
Netherlands	100% *	np	np	np	On-going evaluation by Sanquin	*6 months quarantine
New Zealand	np	np	np	np	np	
Norway	np	100%	np	np	Routine use in 3 centers	
Poland	80% -100%*	np	np	np	np	* 4 months quarantine
Portugal	30%	70 % About 65,000 units	np	np	np	
Romania	Some	np	np	np	np	
Serbia	np	np	np	np	np	
Slovak Republic	16,702 units (23% of plasma for transfusion)	np	np	np	np	
Slovenia	80%	np	np	np	70% of PC as of April 2008	
Spain	36%	np	64%	np	Routine use in 2 centers. Validation on-going in 2 centers	
Sweden	np	Few units	np	np	Routine use in 2 centers. Evaluation in 1 center	
Switzerland	80%	20%	Np	np	On-going evaluation in 1 center	-
United Kingdom	np	All TTP patients	4% (imported plasma for patients below 16 years *)	np	np	* as vCJD precaution
USA	Selected only (FFP WNV source season plasma)	np	np	np	Phase III clinical trials completed	

- FFP=fresh frozen plasma, np=not performed, PC=Platelet concentrate, TTP= Thrombotic Thrombocytopenic Purpura, WNV=West Nile Virus

Techniques de réduction des pathogènes dans les composants sanguins destinés à la transfusion - mise à jour mars 2008

Pays	PFC				Plaquettes	Observations
	Quarantaine	Solvant-détergent	Bleu de méthylène	Intercept	Intercept	
Allemagne	Env. 90 %	Env. 10%	Enregistré dans 2 établissements*	nr	Enregistré dans 1 centre*	*% pas encore connu
Australie	nr	nr	nr	nr	nr	Techniques de réduction des pathogènes en cours d'étude
Autriche	23 100	50 100	0%	nr	nr	
Belgique	nr	Faible pourcentage	96% moins faible pourcentage	4 % Utilisation en routine dans 2 centres. Evaluation en cours dans 1 centre	17 % Utilisation en routine ou évaluation en cours dans tous les centres	
Bosnie-Herzégovine	nr	nr	nr	nr	nr	Techniques de réduction des pathogènes en cours d'étude
Bulgarie	nr	nr	nr	nr	nr	
Canada	Certaines unités	Certaines unités	nr	nr	nr	Techniques de réduction des pathogènes en cours d'étude
Croatie	Plasma pour fractionnement uniquement	nr	nr	nr	nr	
Espagne	36%	nr	64%	nr	Utilisation en routine dans 2 centres. Validation en cours dans 2 centres	
Finlande	nr	100% (env. 60 000 unités par an)	nr	nr	nr	
France	161 597 unités	130 504 unités	Remplacement quarantaine par bleu de méthylène à partir de septembre 2008	5% soit env. 20 000 unités. Utilisation en routine dans 1 centre régional	Utilisation en routine (100%) dans 4 centres régionaux. Evaluation en cours dans 3 centres régionaux	Phase III d'essai clinique Mirasol pour les plaquettes terminée et en cours d'évaluation
Géorgie	nr	nr	nr	nr	nr	
Hongrie	70%	nr	nr	nr	nr	
Irlande	nr	Env. 100%	nr	nr	Evaluation en cours dans 1 centre	
Islande	nr	nr	nr	nr	nr	
Italie	Env. 15%	Env. 20%	Env. 10%	Utilisation en routine dans 2 centres. Evaluation en cours dans 1 centre	Utilisation en routine dans 8 centres. Evaluation en cours dans 1 centre	Mirasol en cours d'évaluation dans 3 centres
Malte	85%	nr	nr	nr	nr	
Moldova	nr	nr	nr	nr	nr	

Pays	PFC				Plaquettes	Observations
	Quarantaine	Solvant-détergent	Bleu de méthylène	Intercept	Intercept	
Monténégro	nr	nr	nr	nr	nr	
Norvège	nr	100%	nr	nr	Utilisation en routine dans 3 centres	
Nouvelle-Zélande	nr	nr	nr	nr	nr	
Pays-Bas	100%*	nr	nr	nr	Evaluation en cours par Sanquin	*Quarantaine de 6 mois
Pologne	80% - 100%*	nr	nr	nr	nr	*Quarantaine de 4 mois
Portugal	30%	70 % (env. 65 000 unités)	nr	nr	nr	
République slovaque	16 702 unités (23% du plasma pour transfusion)	nr	nr	nr	nr	
République tchèque	100% (env. 51 000 litres)	nr	nr	nr	Une étude clinique à très petite échelle uniquement	
Roumanie	Certaines unités	nr	nr	nr	nr	
Royaume-Uni	nr	Tous les patients PTT	4% (plasma importé pour les patients de moins de 16 ans*)	nr	nr	* à titre de précaution contre le vMCJ
Serbie	nr	nr	nr	nr	nr	
Slovénie	80%	nr	nr	nr	Traitement de 70% des CP à partir d'avril 2008	
Suède	nr	petit nombre d'unités	nr	nr	Utilisation en routine dans 2 centres. Evaluation en cours dans 1 centre	
Suisse	80%	20%	nr	nr	Évaluation en cours dans 1 centre	
USA	Sur sélection uniquement (PFC à risque VNO saisonnier)	nr	nr	nr	Essais de phase III en cours	

- CP = concentrés plaquettaires – Env. = environ - nr = non réalisé - PFC = Plasma frais congelé – PTT= Purpura thrombotique thrombocytopénique, VNO = virus du Nil occidental – vMCJ = variant de la maladie de Creutzfeldt-Jakob

CERUS

文献16

February, 2009

Dear Valued Customer,

We have recently been informed of the early conclusion of INTERCEPT data collection for the HOVON 82 “TriPlate” study. We would like to take this opportunity to share with you the information currently provided to us by HOVON and Sanquin, to review information regarding the safety and efficacy of INTERCEPT Platelets, and to outline the steps we will take to clarify the results of this trial and ensure that you receive updated information as it becomes available.

The HOVON 82 study is a three-arm clinical trial comparing (a) platelets collected in 100% plasma, (b) platelets collected in reduced plasma (35%) with Intersol platelet additive solution, and (c) platelets collected in reduced plasma with Intersol and treated with the INTERCEPT Blood System. Platelet components are stored for up to 7 days and all are treated with gamma irradiation when ordered by treating physicians. The study was designed to include approximately 300 patients, resulting in approximately 100 patients for each study arm. The primary endpoint is 1-hour CCI, and secondary endpoints include 24-hour CCI and measures of hemostatic efficacy and safety. The study has been conducted by the HOVON foundation of Dutch oncology centers, in the role of principal investigator, with Sanquin supplying blood products. The study was initiated in 2007.

Cerus was not involved in the design, conduct or data analysis for the HOVON 82 study. Periodically, we have received informal reports that the study was ongoing and that the study was projected to finish in late 2008 or early 2009. Recently, we were informed that enrollment had been halted in the INTERCEPT test arm due to observation of lower CCI data. Cerus has not received data or analyses related to INTERCEPT or the other treatment conditions, and we understand from the study organizers that their analysis of the data is ongoing.

Apart from the HOVON 82 study, the efficacy and safety of INTERCEPT Platelets have been evaluated in eight Phase III/IV clinical studies and an ongoing haemovigilance program documenting over 30,000 transfusions to more than 5000 patients. In comparison to untreated platelets, some studies have observed reduced CI and/or CCI values for INTERCEPT Platelets. However, the studies have not shown clinically significant differences in hemostatic efficacy, and the data supported our 2002 CE mark approval confirming that INTERCEPT Platelets are not clinically different from conventional platelets. Furthermore, the INTERCEPT Platelet clinical dossier has been reviewed and approved by both Afssaps and the Paul Ehrlich Institute.

Our hemovigilance data have documented INTERCEPT Platelet safety in a broad patient population, and longitudinal analyses by long-term users in Belgium and France before and after INTERCEPT implementation have not revealed increased use of platelet components to patients receiving pathogen inactivated platelets.

We are confident that any results emerging from the HOVON data analysis will not change the safety and efficacy conclusions from the existing body of INTERCEPT studies. A summary of these studies is attached for your convenience.

We take the safety and efficacy of our products very seriously. We are in contact with the HOVON study investigators, and are attempting to gain access to the trial data. As soon as results are available to us, and in cooperation with HOVON, we will provide you with updated information.

Thank you for choosing INTERCEPT as your platelet pathogen inactivation treatment. Please feel free to contact us at any time with your questions on this or any other matter.

With kind regards,

Cerus Europe BV

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Platelet Clinical Trials

Buffy-Coat Platelets (euroSPRITE Phase III Clinical Trial) page 3

Apheresis Platelets (SPRINT) page 4

Pediatric Study (Gent, Belgium) page 5

Open-Label Transfusion Study - Germany page 6

Open-Label Transfusion Study - Switzerland page 7

INTERCEPT Haemovigilance Program

Summary to Date - Platelets page 8

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Buffy-Coat Platelets (EuroSprite Phase III Clinical Trial)

Trial Size:	103 patients (52 INTERCEPT + 51 control)
Primary Endpoint:	1-hour CI, 1-hour CCI
Study Sites:	Rotterdam, Netherlands Stockholm, Sweden Bristol, UK Strasbourg, France

This was a randomized, controlled, double-blinded clinical trial with 103 patients to evaluate the efficacy and safety of pooled buffy-coat platelets treated with the INTERCEPT Blood System. The majority (90 out of 103) of patients had a primary diagnosis of hematological diseases. Each patient received either untreated reference or INTERCEPT treated platelets for up to an 8-week period of transfusion support followed by a 4 week surveillance period. Efficacy data on platelet CI/CCI and safety data using assessments of clinical hemostasis based on WHO bleeding scores are reviewed in the following summary. Full results of the clinical trial have been reported (D van Rhenen et al. *Blood* 2003;101(6):2426-33).

The mean 1-hour CI for the INTERCEPT group (27 x 10^9/L) was significantly less than the mean 1-hour CI for the reference group (36 x 10^9/L, p=0.03). Similarly, the mean 24-hour CI for the INTERCEPT group (16.4 x 10^9/L) was also significantly less than the mean 24-hour CI for the reference group (24.7 x 10^9/L, p=0.004). However the mean INTERCEPT platelet dose was 3.89 ± 1.0 x 10^{11} per transfusion compared to 4.32 ± 1.2 x10^{11} per transfusion for the reference group (p≤0.001). When the 1-hour CI was corrected for body size and platelet dose using the CCI, there was no significant difference between INTERCEPT (13.1 x 10^3) and reference (14.9 x 10^3) groups. However, there was a significant difference in the 24-hour CCI between INTERCEPT (7.4 x 10^3) and reference (10.6 x 10^3) groups. When all transfusions (INTERCEPT = 390; reference = 256) were analyzed for 1- and 24-hour platelet count increments using longitudinal regression analysis with multiple covariates, INTERCEPT platelets and reference platelets demonstrated comparable 1-hour CI (p=0.53) and 24-hour CI (p=0.19) for equal platelet doses.

Despite the observed differences in the 1-hour CI, 24-hour CI, and 24-hour CCI, INTERCEPT platelets demonstrated similar hemostatic efficacy to reference platelets. Before platelet transfusion, 71% of patients in the INTERCEPT group and 63% patients in the reference group had at least one episode of bleeding. After platelet transfusion the incidence of bleeding events was lower and similar in both groups (54% INTERCEPT and 49% reference; p=0.62).

CONCLUSIONS: The results of this trial show that equal doses of INTERCEPT buffy coat platelets provided similar 1- and 24-hour post-transfusion platelet CI, and patients treated with INTERCEPT buffy coat platelets exhibited adverse event profiles similar to those who received reference platelets.

Apheresis Platelets (SPRINT Phase III Clinical Trial)

Trial Size:	645 patients (318 INTERCEPT + 327 control)
Primary Endpoint:	Proportion of patients with Grade 2 bleeding (WHO criteria)
Study Sites:	12 blood centers in the United States

This was a randomized, controlled, double-blinded clinical trial with 645 patients to evaluate the efficacy and safety of apheresis platelets treated with the INTERCEPT Blood System. The majority (565 out of 645) of patients had a primary diagnosis of hematological diseases and 75% of patients in each group underwent hematopoietic stem cell transplant (HSCT). Each patient received either untreated reference or INTERCEPT treated platelets for up to a 4-week period of transfusion support followed by a one week surveillance period. Efficacy data on the proportion of patients with WHO Grade 2 bleeding and platelet CI/CCI are summarized in the following sections. Full clinical trial results have been published previously (J McCullough et al. *Blood* 2004;104(5):1534-41, S Murphy et al. *Transfusion* 2006;46(1):24-33, S Murphy et al. *Transfusion* 2006;46(1):24-33).

SPRINT was a powered non-inferiority trial to detect a small difference in Grade 2 bleeding in patients. The primary endpoint of the trial was met despite significant differences in the surrogate efficacy endpoints. INTERCEPT platelets were not inferior to reference platelets in maintaining hemostasis in severely thrombocytopenic patients for up to 28 days. The proportion of patients with Grade 2 bleeding was 58.5% for INTERCEPT group compared to 57.5% for reference group. The trial was not highly powered to demonstrate small differences in more severe bleeding of WHO Grades 3 or 4. These grades of bleeding were comparable between treatment groups (4.1% INTERCEPT group compared to 6.1% Reference group).

The mean 1-hour and 24-hour CI for the INTERCEPT group (21.4×10^9/L and 13.2×10^9/L) was significantly less ($p<0.001$) than the mean 1-hour and 24-hour CI for the Reference group (34.1×10^9/L and 21.5×10^9/L). Similarly, the mean 1-hour and 24-hour CCI for the INTERCEPT group (11.1×10^3 and 6.7×10^3) was significantly less ($p<0.001$) than the mean 1-hour and 24-hour CCI for the Reference group (16.0×10^3 and 10.1×10^3). Similar to the buffy-coat clinical trial, this finding primarily reflected the lower mean platelet dose of 3.7×10^{11} per transfusion in the INTERCEPT group compared to 4.0×10^{11} per transfusion in the Reference group ($p<0.001$).

The time to onset of Grade 2 bleeding after beginning the study was not significantly different between INTERCEPT patients and reference patients ($p=0.78$). However, Grade 2 bleeding occurred on a mean of 3.2 days in the INTERCEPT group as compared with 2.5 days in the reference group ($p=0.02$). This finding again reflected the differences in the mean platelet dose per transfusion in the two groups. More INTERCEPT patients received platelet doses containing less than 3.0×10^{11} (n=190) than reference patients (n=118, $p<0.01$). Comparison of patients receiving comparable platelet doses showed no significant differences between INTERCEPT and reference groups for bleeding or number of platelet or RBC transfusions; despite observation that the CI in response to INTERCEPT platelets (and transfusion intervals) were statistically significantly greater for the reference group. The lower CI values for INTERCEPT platelets suggested that some platelet injury may occur during the INTERCEPT process. This injury does not appear to result in a detectable increase in bleeding, however (S Murphy et al. *Transfusion* 2006;46(1):24-33).

CONCLUSIONS: These data support the conclusion that INTERCEPT platelets may be used according to standard transfusion guidelines whenever platelet transfusions are required. INTERCEPT platelets appear to be safe and effective in management of thrombocytopenic patients.

Pediatric Study

Trial Size:	83 patients (all INTERCEPT)
Primary Endpoint:	Frequency of acute transfusion reactions
Study Site:	Gent, Belgium

Following the CE mark registration, an investigator study of INTERCEPT Platelets in the pediatric population in routine clinical setting has been conducted in one center in Belgium (I Van Haute et al. *Vox Sang* 2006;91(s3):177).

The investigators transfused 500 INTERCEPT platelet components prepared by the buffy coat method to 83 pediatric patients with predominately hematology-oncology diagnoses. The platelet concentrates were prepared by pooling 5 whole blood-derived buffy coats. The pooled platelets were leukoreduced by filtration followed by INTERCEPT treatment for pathogen and leukocyte inactivation. The INTERCEPT Platelet concentrates were not gamma irradiated or tested for CMV. Platelet transfusions were ordered according to hospital guidelines and patients managed according to hospital clinical practice. Eligible pediatric patients were thrombocytopenic, expected to develop thrombocytopenia, diagnosed with a condition associated with thrombocytopenia or receiving therapy that will result in severe thrombocytopenia. Patients were mainly in oncology. Safety and efficacy, assessed by registration of adverse reactions < 24h after transfusion and calculation of corrected count increment [CCI] < 1.5hr post transfusion, were monitored.

Of the 500 transfusions, seven acute transfusion reactions in 6 patients have been noted. No transfusion reactions were of clinical severity greater than grade 1, including fever, urticaria, skin rash, nausea, vomiting, and abdominal pain. Bacterial cultures on INTERCEPT Platelet concentrates were negative. Efficacy was assessed for 493 of the 500 transfused INTERCEPT platelet concentrates. Transfusion episodes per patient was a mean of 6 (range 1 to 49). The number of platelets transfused per unit was a mean of 3.1×10^{11}. Mean platelet 1-hour CCI per transfusion episode was 12,300 (SD 9,450).

CONCLUSIONS: Transfusion of pediatric patients with INTERCEPT platelets provided therapeutic count increments. No unexpected transfusion reactions were attributed specifically to the use of INTERCEPT Platelets.

Open-Label Transfusion Study - Germany

Trial Size:	52 patients (all INTERCEPT)
Primary Endpoint:	Frequency of acute platelet transfusion reactions
Study Site:	University Lüebeck, Germany

This was an observational, single arm, open label study (P Schlenke et al. 2007 *Vox Sang* 2007;93:(s1):171). INTERCEPT platelets were transfused into thrombocytopenic patients according to standard local practices. The primary endpoint was the frequency of overall acute platelet transfusion reactions. INTERCEPT Blood System for platelets was used in place of gamma irradiation for prevention of TA-GVHD. Fifty-two patients were enrolled (54% male/ 46% female) with a median age of 57.5 yrs (range 22 to 78 yrs). All patients had hematological malignancy as primary disease with the largest proportion 40.4% (21/52) having a diagnosis of Acute Myelogenous Leukemia (AML). Most patients had received chemotherapy without stem cell transplant in 65.4% (34/52) and while 32.7% (17/52) patients had received autologous stem cell transplant with chemotherapy and/or radiotherapy.

A total of 560 INTERCEPT platelet components were administered with the mean number of transfusions per patient being 11.0 (median = 6, range 1-71). For 553 reported transfusions, 10 acute transfusion reactions were associated with 9 transfusions (ATR, 1.6%). All ATR were of low Grade 1 severity. Mean 24- hour CI and CCI were 10.9 $x10^9$/L and 6.6 $x10^3$, respectively.

CONCLUSIONS: No bleeding complications were attributable to the INTERCEPT platelets. This study confirmed the safety and efficacy of INTERCEPT platelets for support of thrombocytopenia.

Open-Label Transfusion Study - Switzerland

Trial Size:	46 patients (all INTERCEPT)
Primary Endpoint:	Frequency of acute platelet transfusion reactions
Study Site:	University Hospital Basel, Switzerland

This was an observational, single arm, open label study (L Infanti et al. *Vox Sang* 2008; 95(s1):289). INTERCEPT platelets were transfused into thrombocytopenic patients according to standard local practices. The primary endpoint was the frequency of overall acute transfusion reactions (ATR). INTERCEPT Blood System for platelets was used in place of gamma irradiation for prevention of TA-GVHD.

A total of 551 INTERCEPT platelet components were administered to 46 patients (61% male/39% female), median age 52.8 yrs (range 22 to 80 yrs). The majority (38 patients, 82.6%) of the patients had hematological malignancy as primary disease receiving chemotherapy without stem cell transplant (22 patients) or with stem cell transplant (12 allogeneic, 3 autologous). Preliminary analysis showed that the mean number of transfusion per patient was 12.0±12.52 (range 1-58) and the mean 1-hour CCI was $10.12 \pm 8.06 \times 10^3$.

The rate of adverse events reported in this study was low; 97.8% of INTERCEPT platelet transfusions were without reported ATR.

CONCLUSIONS: This observational study found that transfusions with INTERCEPT platelets were well tolerated, in routine use and exhibited a safety profile consistent with that generally observed with conventional platelet components.

Post-Marketing Surveillance – Summary to Date

Transfusions Monitored: 31,225

Study Sites: 21 sites / 11 countries

An active haemovigilance (HV) program was implemented following CE marking of INTERCEPT Blood System for platelets. This is an open label observational surveillance program to characterize and extend the safety profile of transfusing platelet components treated with the INTERCEPT process in a routine use setting and to document any unexpected adverse events that were not reported in early clinical trials and in patient populations that were not studied before. Please refer to haemovigilance publications for detailed analysis (Osselaer et al. *Vox Sang* 2008:94;(4):315-323, Osselaer et al. *Transfusion* 2008;48(6):1061-71, Osselaer et al. *Vox Sang* 2008; 95(s1):284, Osselaer et al. *Transfusion* 2009:in press, Cazenave et al. *Vox Sang* 2008;95(s1):302-6).

INTERCEPT Haemovigilance Program

Study	Transfusions	Countries	Sites	Locations
HV1	5,106	4	5	Mont Godinne, Belgium Pescara, Italy Bergen, Norway Trondheim, Norway Madrid, Spain
HV2	7,437	3	7	Brugge, Belgium Erasme, Belgium Mont Godinne, Belgium Rennes, France Strasbourg, France St Etienne, France Valladolid, Spain
HV3 (open)	~4,800*	8	11	Luebeck, Germany La Reunion, France Rennes, France St Etienne, France Reykjavik, Iceland Bergen, Norway Lisbon, Portugal Barcelona, Spain Madrid, Spain Uppsala, Sweden Basel, Switzerland
EFS Alsace	13,241	1	1	Strasbourg, France
Pediatric	641	2	2	Vienna, Austria Gent, Belgium

* Including 1,950 transfusions in La Reunion during CHKV epidemic and 489 transfusions in pediatric patients

To date approximately 200,000 doses of INERCEPT platelets have been administered in patients and over 30,000 transfusions have been documented in the HV program. Data are summarized in three large interim analyses:

INTERIM ANALYSIS #1

One of the interim analyses was a meta-analysis of 16,631 transfusions of INTERCEPT platelet components to 3,274 patients (1,973 (60.3%) males/ 1297 (39.7%) females) with a mean age of 57 years (range <1 to 96 years) (Osselaer et al. *Vox Sang* 2008:94;(4):315-323, Osselaer et al. *Transfusion* 2008;48(6):1061-71, Rasongles et al. *Vox Sang* 2008;95(s1):15). Half of the recipients were hematology/oncology patients (1,643, or 50.2%) many of whom received hematopoietic stem cell transplants s (n=307).

Transfusions associated with "related" (possibly related, probably related, or related) adverse events following INTERCEPT platelet transfusion were infrequent (110/16,631 n=0.66%). Eighty-two pts (2.5%) experienced at least one related adverse event following one or more INTERCEPT transfusion. Most reactions were of grade 1 severity and were representative of the events expected with conventional platelet transfusion. The most frequently reported signs/symptoms were chills, fever, and urticaria. Eleven SAE's were reported, with one having causal relationship (hypotension possibly related) to INTERCEPT platelet transfusion. No cases of Transfusion Related Acute Lung Injury (TRALI), TA-GVHD, transfusion-related sepsis, or death due to an INTERCEPT transfusion were reported.

CONCLUSIONS: In summary, 99.34% of INTERCEPT platelet administrations were without a related transfusion reaction. Adverse events following INTERCEPT platelet transfusions classified as related to transfusions were infrequent, mild in severity, and representative of the events expected with platelet transfusion.

INTERIM ANALYSIS #2

The second analysis was based on the routine use experience from EFS-Alsace (Cazenave et al. *Vox Sang* 2008;95(s1):302-6). More than 99% of the patients hospitalized in Alsace that were transfused, according to conventional medical indications, in period 1 (1/1/2003-1/2/2004) with conventional leukoreduced platelet components (100% plasma) or in period 2 (1/9/2006-1/8/2007) with leukoreduced inactivated platelet components (INTERCEPT). Platelet components were prepared either from apheresis or buffy-coats (40/60). The average dose for all platelet components was $4.2 \pm 0.8 \times 10^{11}$. The demography of patients for both periods 1 versus 2 were, respectively, similar in terms of number of patients, age, and gender, and clinical indications: oncohematology (56%/58%), cardiovascular surgery (7%/6%), general medicine and surgery (37%/36%).

The number of platelet components transfused in period 1 was 10,629 (to 2,050 patients); and during period 2, 13,241(to 2,069 patients) INTERCEPT platelet components were transfused. Although processing conditions changed during the two periods, the mean total dose of platelets per patient required in each period remained the same (26.0×10^{11} vs. 27.0×10^{11}). Thus no increase in platelet utilization was observed. During periods 1 and 2, 83.7%/85.2% of patients receiving platelet components were transfused with red blood cell concentrates (RBCC). Mean RBCC transfusions were similar in both periods (14.4 vs. 13.5 units/patient). Responses to platelet transfusion within 48 hours after transfusions were reported for ≥ 99.4% platelet components transfused. The incidence of transfusion reactions per transfusion and per patient decreased with INTERCEPT platelet components (0.53% vs. 0.14% per transfusion, or 2.9% vs. 1.7% per patient). During the two periods, severity and imputation of transfusion reactions was similar and no bacterial sepsis was reported.

CONCLUSIONS: In summary, transfusion of platelet components treated with INTERCEPT to a broad patient population for a spectrum of indications was well tolerated in routine practice. The incidence of adverse events was less than untreated platelet components suspended in plasma. INTERCEPT offers the potential to improve the safety and availability of platelet components for transfusion. Importantly, an increase in the total platelet dose and RBCC transfused to patients in this study was not observed.

INTERIM ANALYSIS #3

The third analysis was based on the routine use experience in the Blood Transfusion Center of Cliniques Universitaires Mont Godinne (Osselaer et al. *Vox Sang* 2008;95(s1):284. Universal routine use of INTERCEPT platelets were initiated in 2003 for transfusion support of patients with thrombocytopenia. The blood component usage and clinical outcome observed for 3 years after adoption of INTERCEPT was compared to those observed during 3 years before INTERCEPT adoption.

The number of patients supported with platelets increased in the period after adoption of INTERCEPT, and approximately 91% required RBCC transfusions in both periods. The distributions (%) of indications for platelet transfusions (Hematology/Cardiovascular Surgery/Medical/Oncology), respectively, were approximately similar in the two periods (Pre: 39/32/22/7 vs. Post: 34/35/22/9). To compensate for loss of platelets due to INTERCEPT processing, approximately 10% more platelets were harvested for INTERCEPT components, resulting in larger average platelet collections (6.3 vs 6.8 x 10^{11}).

Days of platelet transfusion support (14.2 vs. 13.1), number of platelet transfusions per patient (9.9 vs. 10.1), and total platelet dose per patient (41.5 x 10^{11} vs. 42.0 x 10^{11}) did not increase significantly with universal implementation of INTERCEPT. INTERCEPT platelets had no impact on RBCC use.

CONCLUSIONS: In summary, the adoption of INTERCEPT Blood System into routine practice did not affect platelet or RBCC component usage over a 3 year observation period.

OVERALL POST-MARKETING SAFETY EXPERIENCE CONCLUSION: Periodic review of post-marketing surveillance data by Cerus indicates that INTERCEPT Platelet components, transfused in routine practice to a broad patient population, are safe and well tolerated. No unexpected safety issues related to the INTERCEPT Platelet product have been identified.

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HBV isolates, 4 were of nonUS origin (B, C, D, F; all from VBI donors) and 3 were of US origin (A2; 2/3 from VBI donors); A2 is the vaccine genotype. 6/7 were wild type in the pre-S/S region. **Conclusions:** HBV MP NAT yield rates exceed those projected by models and likely reflect the absence of VBI in the models. The high yield suggests the efficacy of HBV MP NAT for donor screening even in the absence of known transmissibility of VBI. The public health aspects of HBV infection in VBI donors require further study.

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P4-020A

Harmful Effects of Red Blood Cell Transfusions: Iron and Inflammation

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Background: Clinical studies suggest that transfusion of packed red blood cells (RBCs) after prolonged storage is associated with increased mortality, infection, and multi-organ failure in hospitalized patients. We hypothesized that iron delivered to the monocyte-macrophage system by rapid clearance of a damaged subpopulation of stored RBCs causes adverse effects and we developed a mouse model to study the mechanism(s) responsible. **Methods:** RBCs from FVB/NJ mice were filter leukoreduced and stored in CPDA-1 for <2 weeks at a hemoglobin of 17.0 g/dL at 4°C, under conditions previously reported to meet FDA 24-hr post-transfusion survival guidelines. Male C57BL/6 mice were transfused with 200 or 400 μL of fresh or stored RBC and sacrificed 2 hr post-transfusion. Plasma cytokine levels were quantified by a multiplex flow cytometric assay, organ iron levels were measured by a wet ashing method, and serum non-transferrin bound iron (NTBI) was determined by a nitrilotriacetic acid-ultrafiltration assay. Acute-phase protein serum amyloid A1 (SAA1)-luciferase transgenic mice were also transfused and bioluminescence was measured for up to 24 hr using the In Vivo Imaging System (Caliper Life Sciences). **Results:** In contrast to fresh RBC transfusions, transfusions with RBCs stored for 2 weeks (i) induced dose-dependent increases in circulating pro-inflammatory cytokines (monocyte chemoattractant protein-1, interleukin (IL)-6, CXCL1, and tumor necrosis factor-α; all $p < 0.05$), (ii) increased serum NTBI levels (undetectable vs. 2.4 ± 0.3 μM; $p < 0.05$), and (iii) led to substantial iron deposition in liver and spleen ($p < 0.05$). Stored RBC transfusions also increased luciferase activity (320-fold) in the hepatic region of SAA1-luciferase reporter mice at 4 hr post-transfusion ($p < 0.05$). To determine if this inflammatory state was caused by the RBCs themselves or by a substance accumulating in the supernatant during storage, normalized doses of washed RBCs, supernatant, or RBC ghosts were transfused; only washed RBC transfusions significantly increased circulating cytokine and NTBI levels ($p < 0.05$). Finally, to examine the role of iron in the inflammatory response, mice were injected with 3 mg of deferoxamine, an iron chelator, just prior to transfusion; this significantly blocked the cytokine response (e.g. reducing IL-6 from 212.8 ± 30.8 pg/mL to 98.4 ± 10.1 pg/mL; $p < 0.05$). In addition, iron chelation inhibited hepatic SAA1 induction by 54% at 4-hr post-transfusion. **Conclusions:** Transfusing mice with older stored RBCs acutely produces serum NTBI and induces a pro-inflammatory response. Iron chelation can decrease the pro-oxidant effects of the iron released following rapid clearance of a damaged subpopulation of stored RBCs, which is responsible, at least in part, for the observed "cytokine storm."

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P5-020A

Clinical Effectiveness and Safety of Pooled, Random Donor Platelet Concentrates, Leucoreduced and Stored up to Seven Days in Either Plasma or Additive Solution with and Without Pathogen Reduction in Hemato-oncological Patients

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Introduction: Extending storage time combined with maintaining or improving the safety of platelet products are the main features in the development of new platelet products. In a multicenter randomised controlled trial we have investigated the transfusion efficacy in hemato-oncological patients of three different buffy-coat derived platelet products: Platelets stored in plasma (plasma-PC), platelets stored in PAS III (Intersol, PAS III-PC) and platelets stored in PAS III treated with psoralen pathogen reduction (Intercept, PR-PAS III-PC). Platelets were stored up to seven days. Here we report on the data of the interim analysis of this study, comparing plasma PC with PR-PAS III-PC. **Methods:** Patients were randomised to receive up to a maximum of five platelet transfusions with either plasma-PC, PAS III-PC or PR-PAS III-PC. Inclusion criteria were: age ≥ 18 years, hemato-oncological disease, expected number of PC transfusions >2 and informed consent. Exclusion criteria were: known or suspected HLA/HPA-immunisation, pregnancy or clinically relevant auto-immune thrombocytopenia. Primary endpoint of the study was 1-hour corrected count increment (CCI). Secondary endpoints were 24-hour CCI, bleeding CTC grade ≥2, transfusion requirement of red blood cell concentrates and PC, PC transfusion interval and adverse transfusion reactions. The study was designed as a non-inferiority study, in which inferiority is defined as a >20% decrease of mean 1-hour CCI. **Results:** The study started March 2007 and at the time of interim analysis, December 2008, there were 199 evaluable patients (plasma-PC n = 68, PAS III-PC n = 64, PR-PAS III-PC n = 67). Based on data from the interim analysis, we report on the PC transfusion data and bleeding complications from the first 135 patients included in the plasma PC arm and the PR-PAS III-PC arm. Transfusions with PR PAS-III PCs (n = 252) resulted in a mean 1-hour CCI of 11.4 ± 5.4 as compared to 17.5 ± 7.1 with plasma PCs (n = 212), resulting in a mean difference of 34.2% (P < 0.0001). The 24-hour CCI was 8.0 ± 5.6 and 13.0 ± 7.9, respectively, resulting in a mean difference of 33.5% (P < 0.0001). 24 patients in the PR PAS-III arm experienced bleeding episodes as compared to 14 in the plasma PC arm (P = 0.045). After reviewing these data, the independent Data Safety Monitoring Board advised us to halt inclusion in the PR-PAS III-PC. **Conclusion:** Although the final analysis of the study still has to be completed, data of the second interim analysis strongly suggest inferiority of platelets stored in PAS III treated with pathogen reduction. As the study has just finished enrolling patients we expect to complete the analysis of all included patients in September 2009.

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P6-020A

Do Multiple Interventions to Reduce Adverse Reactions in Young Female Donors Lower Rates of Reaction? A Preliminary Analysis

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Background: Donor hemovigilance studies have identified donor estimated blood volume (EBV) as one of several risk factors for vasovagal reactions. Pre-donation hydration and practice of applied muscle tension (AMT) exercises have been shown to mitigate the risk of reaction. With the goal of reducing adverse reactions, these interventions were adopted at our blood centers. **Methods:** We report on the rates of mild, pre-faint and loss of consciousness (LOC) reactions in female allogeneic whole blood donors, age 17-22 years, in the same 6-month period in two successive years before and after implementation of interventions consisting of (1) deferring donors younger than 23 years with EBV < 3500 mL, (2) promoting drinking 16 oz of water within 30 minutes before donation, and (3) familiarizing donors with the value of AMT in preventing or limiting reactions. **Results:** In female donors under 23 years of age, there was a 22% decrease in mild reactions (p < 0.0001), 17% decrease in pre-faint reactions (p = 0.054) and 18% decrease in LOC reactions (p = 0.021)—see table. Comparing the rate of mild reactions before and after the adoption of the interventions, we saw a significant decrease even after restricting the analysis to donors with EBV ≥3500 mL, indicating that hydration and AMT may have had an impact. A small number (246) of donors with EBV < 3500 mL donated after the adoption of the interventions. While the change in the reaction rates pre- and post-intervention for donors with EBV < 3500 mL was large, with only 8 of these donors having reactions the number of events pre and post-intervention were not statistically significantly different. **Conclusions:** Hydration, education about AMT and exclusive recruitment of donors with ≥3500 mL of blood appear to have resulted in the desired effect of reducing over all reaction rates, specifically mild and LOC reactions. Among donors with EBV ≥3500 mL there was significant reduction in mild reactions.

Disclosure of Commercial Conflict of Interest

M. Bravo: Nothing to disclose; B. Custer: Nothing to disclose; H. Kamel: Nothing to disclose; P. Tomasulo: Nothing to disclose

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	Pre-intervention	Post-intervention	Relative reduction in rate	p Value
Donations from Female donors < 23 years old (N)	33509	32196		
Mild reactions - Rate/1000 donations	22.9	18.0	22%	<0.001
Pre-faint - Rate/1000 donations	7.5	6.3	17%	0.054
Loss of consciousness - Rate/1000 donations	9.2	7.5	18%	0.021
Donations from female donors < 23 years old with EBV <3500mL (n)	5572	246		
Mild reactions - Rate/1000 donations	32.1	20.3	37%	0.3
Pre-faint - Rate/1000 donations	12.6	0.0	100%	0.077
Loss of consciousness - Rate/1000 donations	13.8	12.2	12%	0.83
Donations from female donors < 23 years old with EBV =/>3500mL (n)	27937	31950		
Mild reactions - Rate/1000 donations	21.1	18.0	15%	0.006
Pre-faint - Rate/1000 donations	6.5	6.3	3%	0.77
Loss of consciousness - Rate/1000 donations	8.3	7.5	9%	0.3

Transfusion-Transmitted Diseases: Retroviruses

S1-010A

Prevalence and Incidence of HIV and HCV Infections Among Allogeneic Donations Since the Introduction of Nucleic Acid Testing in the United States

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Background: Nucleic acid testing (NAT) for HIV and HCV was introduced for screening of blood donations in the US in 1999. This study analyzed temporal trends in prevalence and incidence of these two infections since the introduction of NAT. **Methods:** Data on allogeneic donations between 1999 and 2008 were analyzed. All donations were tested for antibodies and viral RNA for HIV (anti-HIV and HIV RNA) and HCV (anti-HCV and HCV RNA) as well as other markers. Serologic and NAT reactivity were confirmed using additional tests. A confirmed serologic or NAT yield donation was a confirmed infection. Prevalence is the number of confirmed infections over total number of donations tested while incidence is the number of new infections (cases) among repeat donors (RP) over total number of person-years (py) observed. Incidence for first-time donors (FT) was derived by multiplying that among RP by the overall risk ratio of NAT yield rates between FT and RP donors (2.51 for HIV and 3.47 for HCV). Incidence for all donors was the weighted average based on percent of donations from FT and RP donors. Residual risk (RR) was determined using the window period model. **Results:** Prevalence rates (/105), incidence rates (/105 py) and RR (/106 donations) of HIV (anti-HIV or HIV RNA) and HCV (anti-HCV or HCV RNA) are listed in the Table. There was a decrease in prevalence by 22% for HIV and 55% for HCV between 1999 and 2008 (in bold). The incidence of HIV and HCV among repeat donors increased in 2007-2008 compared to 1999-2000 (in bold) although the overall levels remained low at 2.73 or 4.28/105 py respectively in 2007-2008 (in bold), with a RR estimate of 0.68/106 (1:1,467,000) or 0.87/106 (1:1,152,000) (in bold) by assuming an infectious window period of 9.1 or 7.4 days, respectively. **Conclusion:** The prevalence of HCV infections among allogeneic donations decreased significantly since the introduction of NAT. The identified increase in HIV and HCV incidence in 2007-2008 warrants continuous monitoring and investigation.

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仮訳

血漿または血小板用添加液（病原体不活化処理および未処理）中で 7 日間保管した、ランダム供血者由来白血球除去プール血小板濃厚液の血液腫瘍疾患患者における臨床的有効性および安全性

緒言：血小板製剤の安全性を維持し、あるいは向上させた上で、保存期間を延長することが新規血小板製剤開発の主な目的である。多施設共同ランダム化比較試験において、血液腫瘍患者を対象として、3 種類のバフィーコート由来血小板製剤［血漿保存血小板（血漿 PC）、PAS III 保存血小板（Intersol、PAS III-PC）、ソラレン病原体不活化処理済み PASII 保存血小板（Intercept、PR PAS III-PC）］の輸血の有効性を検討した。血小板は最大 7 日間保管した。本稿では、血漿 PC と PR PAS III-PC との比較を行った中間解析データについて報告する。

方法：血漿 PC、PAS III-PC、PR PAS III-PC のいずれかの血小板を最大 5 回まで輸血する試験に、患者をランダムに割り付けた。選択基準は以下のとおり：年齢 18 歳以上、血液腫瘍疾患、予想 PC 輸血回数 2 回以上、同意取得。除外基準は以下のとおり：抗 HLA/HPA 抗体陽性の確定もしくは疑い、妊婦、臨床的に重大な自己免疫性血小板減少症。本試験の主要エンドポイントは、輸血後 1 時間の補正血小板増加数（1-hr CCI）とした。二次エンドポイントは、輸血後 24 時間の CCI、CTC グレード 2 の出血、赤血球・PC 輸血の必要量、PC 輸血間隔、輸血副作用とした。本試験は非劣性試験としてデザインし、1-hr CCI 平均値の 20%を超える減少を劣性と定義した。

結果：2007 年 3 月に試験を開始し、2008 年 12 月の中間解析時において評価可能な患者は 199 例であった（血漿 PC n=68、PAS III-PC n=64、PR PAS III-PC n=67）。中間解析データに基づき、血漿 PC 群、PR PAS III-PC 群に割り当てられた最初の 135 例の患者の PC 輸血データおよび出血性合併症に関して報告する。PR PAS III-PC の輸血（n＝252）後の 1-hr CCI 平均値は 11 .4±5.4 であり、血漿 PC（n＝212）の 17.5±7.1 と比較して、平均値の差は 34.2%（P ＜ 0.0001）であった。24-hr CCI は、それぞれ 8.0 ±5.6、13.0±7.9 であり、平均値の差は 33.5%（P＜0.0001）との結果であった。PR PAS III-PC 群の 24 例が出血事象を発現したが、血漿 PC 群では 14 例であった（P＝0.045）。以上のデータの検討後、独立した Data Safety Monitoring 委員会は、PR PAS III-PC 群への登録中止を勧告した。

結論：本試験の最終解析は完了していないが、2 回目の中間解析データにより、病原体低減化処理 PASIII 保存血小板の劣性が強く示された。本試験は患者の登録が終了したばかりであり、2009 年 9 月までには登録患者全員の解析が終了する予定である。

※HOVON：1985 年に設立されたオランダを中心とする血液がん研究グループ。このグループの主導で、これまでに 100 以上の臨床試験が実施されている。

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IN VITRO CELL QUALITY OF PLATELETS TREATED WITH MIRASOL∩ PRT AT THE BEGINNING OF STORAGE AND GAMMA-IRRADIATED AT DIFFERENT TIMES DURING STORAGE

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Background: Mirasol pathogen reduction technology (PRT) treatment targets nucleic acids and inactivates leukocytes and a wide range of bacteria, viruses and parasites that may be present in blood products. This process involves the addition of riboflavin in combination with UV light. Data from prior clinical studies showed that patients who received Mirasol treated platelets had comparable hemostasis and transfusion requirements to patients receiving untreated control platelets. In addition, no adverse reactions or safety concerns after transfusion to patients were observed. Leukocytes in blood products have been shown to lead to a number of donor anti-recipient responses such as transfusion-associated graft versus host disease (TA-GVHD). Current approaches to inhibit such responses include leukoreduction and exposure of blood components to gamma-irradiation. Although Mirasol treatment by itself has been shown to effectively prevent TA-GVHD, blood centers or transfusion services may want to have the option to also perform gamma-irradiation on platelet units treated with Mirasol.

Aim: The objective of this study was to evaluate the in vitro cell quality of platelets after Mirasol PRT treatment plus gamma-irradiation.

Methods: Single donor platelets suspended in plasma were collected on the Trima apheresis platform. Units were Mirasol treated after collection followed by gamma-irradiation on day 1, 4 or 5 of storage. A panel of in-vitro cell quality parameters was analyzed at day 5 of storage. Data was obtained from two different study sites: the site in the US compared gamma-irradiation on day 4 to day 5 and the site in Spain compared gamma-irradiation on day1 to day 4. Toxicology assessment of samples after treatment was performed at the US site.

Results: The cell concentration of collected products was between $1400–1600 \times 10^6$/mL in 250–300 mL of plasma. The addition of riboflavin (35 + 5 ml) decreased the cell concentration of Mirasol treated products from 1549 ± 88 to $1229 \pm 77 \times 10^6$/mL. As previously described, the Mirasol PRT process induces an increase in platelet glycolysis as represented by increases in glucose consumption and lactate production. The glucose concentration on day 5 in treated products compared to untreated products was 7.22 + 1.74 vs. 7.98 + 1.23 mmol/10^{12} cells. The same trend was observed for the lactate concentration on day 5 in treated products compared to untreated products, which was 9.36 + 1.38 vs. 5.34 + 0.64 mmol/10^{12} cells. The higher metabolic rate triggered by the Mirasol process was also reflected in increased P-selectin expression on day 5 (52.9 + 9.9 vs. 28.2 + 11.0%). Values for pH (22°C) on day 5 were decreased in treated products (7.07 + 0.15 vs. 7.45 + 0.09). Day 5 in vitro cell quality of Mirasol treated products gamma-irradiated on day1 and day 4 or day 4 and 5 of storage showed no statistically significant difference between the time points of gamma-irradiation. At each site, all in vitro cell quality parameters analyzed were comparable, independent of the day of irradiation ($p > 0.05$). Toxicology assessment of Mirasol treated platelets gamma-irradiated on day 5 of storage showed no geno- or acute toxicity or neoantigenicity in treated platelets. The concentration of riboflavin or its photoproducts (2′-Ketoriboflaivn, 4′-Ketoriboflavin, Formylmethylflavin and Lumichrome) was not significantly affected by gamma-irradiation.

Conclusion: As observed in earlier studies the Mirasol PRT treatment process increases platelet cell metabolism. Nevertheless in vitro cell quality parameters remain within acceptable ranges for clinical use. Mirasol treatment plus gamma-irradiation on day 1, 4 or 5 of storage does not alter the *in vitro* cell quality of platelets, making gamma-irradiation at any time point throughout storage feasible.

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THE MIRASOL EVALUATION PROGRAM: USE OF MIRASOL PATHOGEN REDUCTION TECHNOLOGY FOR PLATELETS IN ROUTINE CLINICAL PRACTICE

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Background: MIRASOL pathogen reduction technology (PRT) treatment inactivates leukocytes and a wide range of bacteria, viruses and parasites in blood products. The system involves the addition of riboflavin in combination with UV light. Platelet products are treated in 100% plasma and can be stored in plasma or additive solution. The system is currently CE-marked for plasma and platelets and has been implemented in several European blood centers.

Aims: Evaluation of the Mirasol system for platelets and disposables in routine use.

Methods: The Mirasol PRT System for Platelets and Plasma has been evaluated under routine use conditions in blood centers throughout Europe. Data on ease of use of the process and in vitro cell quality was collected. Follow-up on patients transfused with Mirasol treated platelets was performed. The Mirasol process was evaluated for ease of use by a rating scale from 1 (excellent) to 4 (poor); *in vitro* evaluation included cell counts, swirl, pO2, pCO2, pH, glucose and lactate con-

Table 1. *Most units were transfused prior to day 5 (for abstract P-395).

	Pre-illumination (approx. n=111)	Post-illumination (approx. n=108)	Day 5 (approx. n=34*)
pO_2 (mmHg)	98 +/- 41	75 +/- 39	67 +/- 27
pCO_2 (mmHg)	55 +/- 20	47 +/- 16	23 +/- 8
pH (22°C)	7.3 +/- 0.2	7.2 +/- 0.2	6.9 +/- 0.3
Glucose concentration (mmol)	18 +/- 3	16 +/- 3	9 +/- 3
Lactate concentration (mmol)	4 +/- 3	4 +/- 2	13 +/- 3
Yield ($x10^{11}$)	3.52 +/- 0.8	3.53 +/- 0.7	3.54 +/- 0.5

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centrations. All sites were contacted and actively solicited to report any adverse reaction related to the use of Mirasol treated platelets.
Results: A total of 11 centers in Europe participate in the program. The evaluation program includes assessment of Mirasol treated platelets and Mirasol treated plasma. Currently 166 platelet products collected on Trima, Amicus, Haemonetics and Orbisac have been treated and evaluated; 110 treated products have been transfused. Overall evaluation of the ease of use of the process was rated as excellent. In vitro cell quality results from 7 centers and 111 products have been reported and are summarized in the table below. Swirl was rated positive in all products using either a numeric rating (3 to 0) or as presence/absence of swirl. No platelet loss following treatment and storage was reported. All products met European guidelines in terms of platelet content and for sample pH. No adverse reactions were reported after transfusion of the platelets to patients at any of the participating sites. Evaluation of the Mirasol system for plasma is ongoing.
Conclusions: The results presented here are consistent with previous data obtained from in vitro and in vivo studies, and demonstrate that Mirasol treated platelets are safe can be incorporated effectively in the routine blood bank and transfusion setting.

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SUCCESSFUL TREATMENT OF A POST-PARTUM BLEEDING WITH THE VIRUS INACTIVATED FRESH FROZEN PLASMA SAFE: A CASE REPORT

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Background: Disseminated Intravascular Coagulation (DIC) is a disorder characterized by both acute generalized, widespread activation of coagulation, which results in thrombotic complications due to the intravascular formation of fibrin, and diffuse hemorrhages, associated to deficit of platelets and factors of the coagulation (Factors V, VIII, X, XIII, fibrinogen, prothrombin, anti-trombin, C Protein). The DIC may occur in a variety of disorders among which: sepsis, severe infections, malignancies, obstetric or vascular disorders, and severe toxic or immunological reactions. Nevertheless, the obstetric pathology represents the main causes of DIC and between these the post-partum hemorrhagic shock constitutes an obstetric and transfusion emergency. However, clinical and laboratory diagnosis of the syndrome remains difficult, as there is no single laboratory test, sensitive and specific enough, to allow a definitive diagnosis of DIC.
Aims: In the present work we present the case of an hemorrhagic shock developed in a nullipara at 39° week of gestation, complicated by a DIC, after an emergency caesarean section.
Methods: A 33-year-old nullipara was hospitalized by our Gynecology and Obstetrics Division in the 2008, in order to be submitted to caesarean section. The patient was treated in the last two months by acetil-salicylic acid because of an unknown proneness to thrombosis. The patient was submitted to an emergency caesarean and developed after 4 hours by the birth a vaginal laceration and uterine atony, accompanied by postpartum hemorrage. The clinical study showed that the patient appeared pale, with rapid decrease of the blood-pressure values (70/40 mmHg), heart rate of 110 bpm, diuresis $<$500cc and high breath rate. The laboratory analyses show an Hb of 7.2 mg/dL and Hct of 22%. The patient was therefore submitted to the transfusion of 3 unities of red cell concentrates buffy coat removed (RCC). The laboratory analyses showed after 2 hours the following results: progressive reduction of the platelets (PLT) $<$80.000/µl, fibrinogen $<$ 200 mg/dL, very high values of D-Dimer, INR $>$2.
Results: These results lead to a DIC post-partum diagnosis and the patient was treated by virus inactivated fresh frozen plasma safe. In particular, it was transfused 3 unity/die until the normalization of the coagulations parameters that it happened in turn of 3 days. The screening of the coagulation and the virology analyses performed after 1 month showed a complete remission and a general state of health of the patient.
Conclusions: Numerous studies have shown that after a DIC diagnosis the therapeutic results depend from the timeliness of the recognition of the disease symptoms and from the capacity to make an early diagnosis, in order to identify the cases at risk and to establish a therapy. The data of the our work show that the fresh-frozen plasma safe, remains still today, although the recent use of factors recombinant activated factor VII (FVIIa), a therapeutic treatment of high efficacy and safety in the control of the bleeding post-partum DIC correlated.

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THE INTRODUCTION OF A NOVEL PRION PROTEIN (PRPSC) REMOVAL TECHNOLOGY FOR THE PHARMACEUTICALLY LICENSED PLASMA (OCTAPLAS®)

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Background: Four possible transmissions of variant Creutzfeldt-Jakob Disease (vCJD) through non-leukocyte depleted red blood cell concentrates in the UK have made prion diseases a matter of concern in today's blood therapy. All manufacturers of plasma-derived biopharmaceuticals are requested to perform appropriate prion safety evaluations of their product portfolio, and an improved safeguarding in terms of risk for prion transmission is promoted when possible from a technological point of view and feasible in terms of appropriateness and quality outcome.
Aims: The aim of our studies was to evaluate the incorporation of a novel prion protein removal technology into the manufacturing process of Octaplas®, a solvent/detergent (S/D) treated human plasma. The PrPSc removal was achieved by a chromatographic step, utilising an affinity ligand selected for prion protein binding that was developed by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., USA).
Methods: A validated downscale model of the adapted manufacturing process was used as basis for all investigational studies. Exogenous spike materials derived from brains of hamsters infected with hamster-adapted scrapie 263 K were used to investigate the PrPSc binding capacity of the resin. Standard Western blot assays were used for the detection and determination of PrPSc levels in the various samples.
Results: Our studies demonstrate that PrPSc binds rapidly and with a very high affinity to the novel prion protein-affinity-resin. Based on the amount of PrPSc captured, as determined by Western blotting of both the product fractions and resin, a very high and robust binding capacity in the order of 6 log10 ID50 bound per ml resin was demonstrated in this particular cell-free Octaplas® matrix.
Summary/Conclusions: The incorporation of this new chromatographic technology to remove pathogenic prions, potentially present in plasma, during Octaplas® manufacturing has been shown to be both technologically possible and feasible. The robust, reproducible PrPSc binding demonstrated by the PRDT affinity resin will further improve the safety margin of Octaplas® in terms of prion diseases such as variant Creutzfeldt-Jakob disease.

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Emerging infectious disease agents and their potential threat to transfusion safety

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BACKGROUND: Emerging infections have been identified as a continuing threat to human health. Many such infections are known to be transmissible by blood transfusion, while others have properties indicating this potential. There has been no comprehensive review of such infectious agents and their threat to transfusion recipient safety to date.

STUDY DESIGN AND METHODS: The members of AABB's Transfusion Transmitted Diseases Committee reviewed a large number of information sources in order to identify infectious agents with actual or potential risk of transfusion transmission now or in the future in the US or Canada; with few exceptions, these agents do not have available interventions to reduce the risk of such transmission. Using a group discussion and writing process, key characteristics of each agent were identified, researched, recorded and documented in standardized format. A group process was used to prioritize each agent on the basis of scientific/epidemiologic data and a subjective assessment of public perception and/or concern expressed by regulatory agencies.

RESULTS: Sixty-eight infectious agents were identified and are described in detail in a single Supplement to **TRANSFUSION**. Key information will also be provided in web-based form and updated as necessary. The highest priorities were assigned to *Babesia* species, Dengue virus, and vCJD.

CONCLUSION: The information is expected to support the needs of clinicians and transfusion medicine experts in the recognition and management of emerging infections among blood donors and blood recipients.

INTRODUCTION

The concept of emerging infectious disease (EID) has developed over the last 2 decades, as it became apparent that full control of infectious disease had not been achieved. From 1997 data, the World Health Organization (WHO) estimated that infectious diseases were responsible for about 33% of all deaths worldwide primarily in the developing world, and these diseases remain one of the principal challenges to human survival (WHO The World Health Report 1998: Life in the 21st Century. A vision for all. World Health Organization, Geneva, 1998; http://www.who.int/whr/1998/en/index.html). Emerging infections are defined as those whose incidence in humans has increased within the past 2 decades or threatens to increase in the near future.[1] Emergence may be due to evolution of an existing organism, to the spread of a new agent, to the recognition of an infection that has been present in the population but has gone undetected, or to the realization that an established disease has an infectious origin. Emergence also may be used to describe the reappearance of a known infection after a decline in incidence.[1] The first emerging infection to have a major effect on blood safety was human immunodeficiency virus (HIV), the agent responsible for acquired immunodeficiency syndrome (AIDS), and the lessons learned from

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that epidemic have sensitized us to the need to be alert to those emerging infections that can impact blood safety in the future.

Any infection with an asymptomatic blood-borne phase has the potential for transmission by transfusion, whether the infectious phase is prolonged, as is the case for hepatitis B virus (HBV) or HIV, or short, as in the case of West Nile virus (WNV) or dengue virus (DENV). Other characteristics that are necessary for transmission by transfusion are the survival/persistence of the infectious agent in collected blood or components, and its ability to cause infection by the intravenous route. Transfusion transmission will be of little relevance unless the agent also causes identifiable disease in the recipient. The frequency with which an infection is transmitted to blood recipients depends directly upon the length of the asymptomatic blood-borne period, how often blood is donated during this period, and the immune status of the recipient population. A number of factors relating both to the infectious agent and to the genetic and immunologic makeup of the recipient also will determine the frequency and severity of the disease resulting from the infection.

Concern about transfusion-transmitted infections seems to be driven by two sets of factors. First is the public health impact of the infection, characterized by its frequency and the severity of the outcomes, and by the risk of secondary transmissions that can be determined in more or less quantitative terms. Second is the public reaction to the disease, which appears to be driven, in some examples, more by emotional aspects and is not readily quantitated. The public response may be disproportionate to the severity of the infection. However, both aspects must be considered when responding to the threat of transfusion-transmitted infections. Priority setting in response to potential and emerging transfusion-transmitted infections must evaluate both the public health aspects, driven by scientific data, and the public response to, or perception of, the agents and their risk.

Many mechanisms lead to the emergence of infectious diseases.[2] Most dramatic is the appearance of a completely new human infection. This most often reflects a circumstance in which a zoonotic infection crosses over into the human population. A classic example is HIV/AIDS, which is thought to have occurred as a result of cross-species transmission of simian immunodeficiency viruses from monkeys to great apes and then to humans in Africa.[3] The original transmission perhaps occurred as a result of preparation of bushmeat derived from apes for human consumption. Such species crossings may be accompanied, or facilitated, by genetic changes in the infectious agent.[2,4] In the case of HIV, the subsequent transmission of the virus resulted from a variety of human behaviors involving sexual and injection drug use networks, travel, and blood-borne transmissions. A second example of such a species jump was severe acute respiratory syndrome (SARS), a disease caused by an animal coronavirus previously unrecognized in humans and again probably transmitted from exotic mammals used as a food source.[5] Another cause for emergence is the expansion of existing infections into a larger geographic region and/or a greater proportion of a susceptible population, often brought about by ecological and/or behavioral changes or by population movement. A striking example of this has been the emergence of WNV in the Americas.[6,7] It is unclear how the virus, which is primarily a bird-mosquito pathogen for which humans become an unintentional host, initially entered the United States (US), but its subsequent spread across the continent and into the Caribbean and Latin America has been extraordinarily rapid and complete. Other examples of potentially transfusion-transmissible agents that are expanding geographically include DENV and chikungunya viruses (CHIKV), *Plasmodium* species (malaria), *Babesia* species, and *Trypanosoma cruzi* (the agent of Chagas disease).[8-12] Another source of apparent emergence is the new recognition of existing human agents, often as a result of pathogen discovery techniques. Examples of this include human herpesvirus-8 (HHV-8), the nonpathogenic GB viruses (GBV-C, initially termed hepatitis G virus or HGV),[13] and Torque teno viruses (TTV/SEN-V).[14] Some infections emerge (or re-emerge) as a result of the breakdown of public health measures for previously controlled infections including the failure of initially effective vaccines, antibiotics, or vector control programs; examples are the re-emergence of malaria in areas of prior control and the geographical spread of DENV and CHIKV. Finally, otherwise benign infections may become serious pathogens in the face of modern medical treatments, particularly those involving immunosuppression. This group includes agents like cytomegalovirus (CMV) and other herpesviruses, human parvovirus B19 (B19V), and *Babesia* species.

Several factors that can contribute to the emergence of an infection frequently work together. Environmental change (often as a result of human interventions) is a major source, but changes in living conditions also may have a significant impact. Urbanization, particularly in the developing world, can lead to very crowded conditions with limited hygiene. Social disruption and conflict also have been associated with numerous outbreaks. Of particular relevance to transfusion medicine is travel, which has been a major factor in the spread of emerging infection (consider, for example, HIV, *Plasmodium* species, *T. cruzi*, DENV, and CHIKV).[3] Sometimes the spread of an emerging agent is a result of the importation of an animal host (for example, the introduction of monkeypox into the US), a vector such as a mosquito, or even a food. Mobile reservoirs (e.g., birds) can transport pathogens from one region to another over long distances (for example, WNV or the highly pathogenic H5N1 influenza A virus). In the

context of transfusion, while donor travel may not initiate or expand an epidemic, it can result in the transmission of an exotic, foreign agent to a recipient.

There have been a number of articles and reviews on the subject of emerging infections and their impact, or potential impact, on transfusion-associated illnesses,[9,10,15] but there does not appear to be any systematic review identifying a wide range of such agents and their key properties. Neither is there any comprehensive guidance to transfusion service and clinical staff on how best to recognize and manage emerging transfusion-transmitted infections. In this context, it is important to note that while decisions and recommendations about the overall management of blood safety are likely to be made at the institutional level (i.e., regulatory agency, blood system, or professional organization), individual practitioners also have specific responsibilities. A blood center or transfusion service physician may need to decide whether to accept or defer and how to counsel a donor with a history of disease, infection, or exposure, while a caregiver may be faced with the challenge of diagnosing, recognizing the link to blood, and reporting a suspected posttransfusion infection.

The intent of this Supplement is to provide a set of tools identifying, describing, and prioritizing those EID agents that have an actual or potential risk of transmission by transfusion and for which there is no currently implemented intervention. Of necessity, this list of emerging agents is not, and can never be, exhaustive due to the nature of EID agents, but it does reflect the consensus opinion of a group of experts. The major part of the Supplement consists of a set of 68 Fact Sheets, each of which provides referenced, systematic information about a single agent. (See Appendix 2.) Included is standard background information about each agent, along with an assessment of those characteristics specifically related to transfusion transmission. Although it is not intended that the Fact Sheets should provide specific recommendations about donor or patient management, consensus opinions about prudent approaches to a number of issues (such as donor deferral periods) are included wherever possible based on facts that are currently inferred or known. Additionally, an attempt has been made to rank the agents according to the consensus opinion about their anticipated impact upon blood safety. Such a ranking should not be regarded as definitive, and another group of experts may come to different conclusions. However, it may serve to focus attention on agents that merit more immediate attention in the development of plans for future interventions and might serve to focus the attention of the clinician on possible starting points for the diagnosis of an unfamiliar infection potentially associated with transfusion. It is critical to remember, however, that the very essence of emerging infections is that their evolution and manifestations are inherently unpredictable.

Ideally, there should be systems in place to deal with emerging infections, not only generally, but also specifically in the context of transfusion safety. Primarily, this is the responsibility of agencies that are charged with the maintenance of public health, the management of the blood supply, and its regulation. As it is unlikely that the first occurrence of an emerging infection will be seen in a transfused recipient, it is important that there be a system of assessing the threat and risk of emerging infections for their potential impact on blood safety and availability. This requires a process for evaluating each emerging infection for its transmissibility by this route and for estimating the severity and potential extent of the threat. The risk assessment should help to define the need for, and urgency of, development and implementation of interventions to reduce the risk of transmission of the agent. Such interventions, if implemented, must then be evaluated for efficacy and modified as appropriate.

There is no simple formula for recognizing that a transfusion-transmitted infection has occurred, particularly in the case of a rare or unusual disease agent. Nevertheless, many such events have been recognized by astute clinicians. Knowledge of the potential for transmission of an emerging infection can be valuable and very likely contributed to the relatively early recognition of transfusion transmission of WNV.[16,17] Unusual posttransfusion events with a suspected infectious origin should be brought to the attention of experts in infectious diseases and public health agencies for assistance in identification and follow-up. Investigation of illness occurring a few days or more after transfusion can diagnose infections using serologic or molecular evidence of infectious agents in posttransfusion samples. However, such detection is by no means definitive. A pretransfusion patient sample is extremely helpful if available, as this will reveal whether an infection predated the transfusion. Clinicians may not realize that type and crossmatch samples and diagnostic blood specimens may be available for up to 2 weeks following collection before they are discarded by the laboratory. Finally, recall and further testing of associated donors can tell us whether one or more of them was the likely source of the infection. Ideally, if the responsible agent can be isolated from both donor and recipient, molecular analyses, such as nucleic acid sequencing, can assist in identifying or excluding the same agent from the two sources. When a connection is made, testing of co-component recipients can further confirm transmission from a single donor via multiple blood components.

There are significant problems in recognizing that infections with very long incubation periods may have been transmitted by transfusion; this was illustrated by HIV/AIDS, which did not result in well-defined illness until long after the infection occurred. This delayed early recognition of transfusion-transmitted HIV and concealed

the magnitude of the infectious donor and infected recipient populations. Proactive approaches to transfusion transmission of EIDs, especially those with lengthy incubation periods, include the serologic or molecular evaluation of appropriate donor-recipient sample repositories, or engaging in active surveillance such as that used in the United Kingdom (UK) to identify the transmission of variant Creutzfeldt-Jakob disease (vCJD) by transfusion.[18,19] However, donor-recipient repositories require a large investment to create and maintain and have other limitations such as adequacy of sample size to detect rare events, the timing of collection of the retained samples (which may predate the agent's emergence), and the geography of collected samples which may be outside of the affected area.[20] Hemovigilance programs, while valuable for other reasons, are unlikely to contribute substantially to the identification of newly emerging post-transfusion infections since they are generally designed to identify well-defined acute outcomes.

An important component of preparedness is the establishment of close relationships between blood establishments, regulatory authorities, public health agencies, the medical community, and industry. This was exemplified by the rapid and effective response in the US to the emergence of WNV and the subsequent recognition of its transfusion transmissibility. Appropriate donor screening tests were rapidly developed and deployed within less than a year of the confirmation of the threat to blood safety.[7,21,22]

Although it is reasonable to consider plans for the management of an emerging transfusion-transmitted infection, it is not clear when, whether, or how a response to a potential threat should be triggered. Ideally, given the availability of suitable resources, studies to assess the actual extent and nature of the risk conveyed by high-priority agents would be undertaken. Assessment of the prevalence and incidence of the infection in the donor population and of the nature and dynamics of emergence would provide valuable information, as would investigation of the infectivity of the agent and of potential interventions. These activities may be time-consuming and inappropriate in the face of an explosive outbreak, although they may offer a foundation for decision making in the face of a less dramatic emergence, as was done for *T. cruzi* antibody blood donation screening in the US.[23,24]

The question of when to take specific actions to prevent or mitigate transfusion transmission of an EID agent is beyond the scope of this Supplement. However, the information provided should contribute materially to the factual background necessary to make such decisions and help establish some guiding principles for decision making. Interventions may be based upon questioning prospective donors about their medical risk or exposure histories (although neither the sensitivity nor the specificity of this approach have proven adequate historically as a standalone strategy, with the exception of the dramatic reduction in HIV transfusion transmission in the early 1980s in San Francisco following targeted questioning[25]) and upon the use of laboratory tests, or a combination of these approaches. The availability of a specific donor screening test, however, should make it possible to eliminate or at least refine donor questions.[26] Finally, there is hope that pathogen-reduction methods will provide a generalized, proactive intervention to eliminate or reduce the risk of transmission of emerging infections by transfusion; however, such methods are not yet available for all blood components and their success and safety have not undergone extensive validation in the real world.

Usually interventions are not implemented until it is clear that transfusion transmission has occurred, although in some cases the potential outcome of transmission is considered to be so severe that regulators require action even in the absence of documented transmission as was the case for vCJD (a decision that was based on, and cited in retrospect to justify, a rather stringent form of the precautionary principle[27,28]). Regulators have considered, and some have even implemented, precautions to reduce the risk of transmission of the nonpathogenic simian foamy virus (SFV) out of fear that infection occurring in a human might be accompanied by a mutation conferring disease-causing capability to the virus.[29]

The precautionary principle is often cited when decisions about interventions to reduce the risk of transfusion-transmitted infections are discussed. It is suggested that in the absence of any specific information about the efficacy of an intervention, implementation is appropriate as long as it does no harm. Commentary on the precautionary principle suggests that it should not be invoked without some evaluation to assure that the measure is not extreme and does not exceed other measures taken in similar circumstances.[30] The hope is that information in this Supplement will contribute to informed decision making and to appropriate and well-reasoned application of "precautionism."

FRAMEWORK FOR ASSESSMENT

The Supplement is a project undertaken by the AABB Transfusion-Transmitted Diseases (TTD) Committee. The TTD provides consultation on all facets of transfusion and transplantation-related diseases and associated donor management and laboratory testing. It consists of volunteer members with expertise in various areas of infectious disease and blood center operations, and includes liaisons from the Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), the US Department of Defense (DOD), the

American Society of Hematology, and the Association of Public Health Laboratories.

The TTD committee undertook this project to collate, in a series of Fact Sheets, information concerning EID agents posing demonstrated or potential risk to the safety of transfusion or transplant recipients (Appendix 2). Specifically, the task was to review and prioritize the status of current and EID agents that could be transfusion transmitted with potential adverse outcomes for recipients. Creation of such a thorough review document was judged to be useful for overall policy development and strategic planning. In this document, most of the emphasis is placed on transfusion-transmitted disease agents; however, it should be noted that many issues relevant to agents transmitted by transfusion will overlap with agents that are transplant transmitted. Due to the potential for blood-borne transmission of agents that are transplant transmitted, several agents for which organ transmission has been documented, even in the absence of transfusion transmission, have been included (e.g., rabies virus and lymphocytic choriomeningitis virus).[31,32]

The AABB TTD committee was aware of previous efforts to develop tools for communicating the threat of EID agents entering the blood supply and was able to obtain current versions of Héma-Québec internal EID charts (G. Delage, vice president, Medical Affairs) and information from the US DOD; materials being developed by the US Public Health Service were requested but not made available. Findings of meetings such as the CDC conference on tick-borne diseases[33] and FDA conferences on parvovirus B19, malaria, and donor behavioral risks were scrutinized:

- www.fda.gov/downloads/BiologicsBloodVaccines NewsEvents/WorkshopsMeetingsConferences/ TranscriptsMinutes/UCM055339.pdf
- www.fda.gov/downloads/BiologicsBloodVaccines NewsEvents/WorkshopsMeetingsConferences/ TranscriptsMinutes/UCM054429.pdf
- www.fda.gov/downloads/BiologicsBloodVaccines NewsEvents/WorkshopsMeetingsConferences/ TranscriptsMinutes/UCM054430.pdf

Although the original intent was to prepare a series of consolidated matrices, it was apparent that the amount of information collected exceeded the format of a simple matrix and instead the document evolved into multipage Fact Sheets for each specific agent.

In determining which specific EID agents should be included in this review, the following factors were considered (agents for which the FDA requires donor testing have been excluded from this publication):

- The agent must infect or pose a potential risk to humans.
- With good documentation, the agent must be transmissible by transfusion or by organ/tissue transplantation or such transmission must be biologically plausible (i.e., the agent is present in plasma or associated with blood cells or donor tissue during a time when the donor is asymptomatic or has a biological basis to suggest the possibility to replicate in blood, tissue, or organs).
- The agent must have the possibility of being introduced into the blood supply during an epidemic, following an act of bioterrorism, or inadvertently during its emergence.
- The agent must lack a current intervention strategy that is widespread and known to be effective.

Many of these agents are considered pandemic threats or to have epidemic potential outside the US and Canada, but the primary focus for this Supplement was the presence, or threat, of the agent emerging in the US and Canada.

Development of the Fact Sheets was an iterative process. First a list of potential agents that met the proposed definitions for inclusion was developed and the format for presenting the information was devised. Both naturally occurring agents and agents that could be considered bioterrorism threats were included. The preliminary draft of each Fact Sheet was prepared by an individual TTD member. Each Fact Sheet was then subjected to broad discussion and underwent several additional review cycles by various groups within the TTD. Articles in conventional and esoteric publications were scrutinized to document blood-borne capability. This review included intensive fact checks and standardization of format, culminating in a final review by the entire TTD committee.

Each Fact Sheet contains data published primarily in peer-reviewed journals or texts that are relevant to the agent and its potential to be transfusion transmitted, and when available also includes data relevant to transplant-transmitted agents. They include general background and epidemiologic information, as well as information specific to blood donation. In lieu of a comprehensive bibliography, a suggested reading list is provided for each agent that includes the most relevant citations on the agent's capacity for transfusion transmission, and the source articles documenting transfusion-transmission events. The list of suggested reading includes one or more recent review articles or book chapters that will familiarize the reader with the agent's general biologic and epidemiologic characteristics.

Pertinent consensus categories that were selected by the TTD core leadership for the Fact Sheets were designed to provide the necessary background for each agent as well as other data that were deemed relevant for a discussion of their threat to recipient safety. These include:

Disease Agent:
Disease Agent Characteristics:
Disease Name:
Priority Level:
- Scientific/Epidemiologic evidence regarding blood safety:
- Public perception and/or regulatory concern regarding blood safety:
- Public concern regarding disease agent:

Background:
Common Human Exposure Routes:
Likelihood of Secondary Transmission:
At-Risk Populations:
Vector and Reservoir Involved:
Blood Phase:
Survival/Persistence in Blood Products:
Transmission by Blood Transfusion:
Cases/Frequency in Population:
Incubation Period:
Likelihood of Clinical Disease:
Primary Disease Symptoms:
Severity of Clinical Disease:
Mortality:
Chronic Carriage:
Treatment Available/Efficacious:
Agent-Specific Screening Question(s):
Laboratory Test(s) Available:
Currently Recommended Donor Deferral Period:
Impact on Blood Availability:
- Agent-specific screening question(s):
- Laboratory test(s) available:

Impact on Blood Safety:
- Agent-specific screening question(s):
- Laboratory test(s) available:

Leukoreduction Efficacy:
Pathogen Reduction Efficacy for Plasma Derivatives:
Other Prevention Measures:
Suggested Reading:

Documentation of transfusion transmission and its clinical outcomes was a key component of this project. When documentation was lacking, agents were evaluated for their *potential* for transfusion transmission. For the former, the task was relatively straightforward. For the latter, it was much more difficult because of a paucity of data and difficulties in proving the negative proposition that transfusion is not implicated in transmission. The occurrence of an asymptomatic blood phase and its duration (whether a long-term chronic carrier state or shorter-term acute infection) were the critical elements used to estimate this “risk” of transmission. Available evidence about the persistence or survival of an infectious agent during blood component manufacture and storage was examined where possible. Treatment availability and its effectiveness were reviewed.

Many sources were used to judge the plausibility of blood transmission. Obviously, peer-reviewed publications and textbooks are of great importance and have been cited in the suggested reading, but for several of the judgments, the necessary data have not appeared in those venues. Other reputable and easily accessible public data sources such as the ProMed electronic mailing list server and websites like those of the US CDC and the WHO were used as appropriate:

- http://www.promedmail.org/pls/otn/f?p=2400:1000
- http://www.cdc.gov/
- http://www.who.int/en

An estimate of the level of concern about the health threat from each agent was undertaken to suggest thresholds for intervention(s) to reduce or prevent transmission by transfusion. This was an unavoidably subjective process that included a review of the biology and epidemiology of the agents, an estimate of public and regulatory concerns about the agents and an assessment of the availability of sensitive and specific donor screening approaches. The priority assessment also was driven by questions such as an agent’s geographic location/presence, projections of changes in geographic distribution (where it was going), and where and how frequently prospective donors would be at potential risk of exposure. In addition, the existence of surveillance adequate to allow recognition and reporting of putative transfusion transmissions, whether from physicians treating patients or reports to state or local health departments, federal or national public health agencies or blood collection facilities was considered. The deliberation also took into account the incubation period for each agent and a rough estimation of the period of time that would be required to realize that there is a potential public health threat. In so doing, the TTD committee applied the experience and interventions used for prior emergent transfusion-transmitted agents to evaluate the selected EID agents. These included the potential impact of behavioral and testing interventions in addition to pathogen reduction strategies. Pathogen reduction strategies that were analyzed included the impact of leukoreduction, physicochemical methods used in the manufacture of clotting factors, and methods used outside the US or in development in the US for cellular components and transfusable plasma. Possible interventions were evaluated relative to their potential to be effective and their estimated impact on the donor base.

If there is a shortcoming to what is known about the many agents for which blood is a plausible vector, it is our passive surveillance system for transfusion transmission. For example, how often do clinicians seeing DENV in endemic areas and during epidemics obtain the critical history related to transfusion? Are significant rates of transfusion-transmitted DENV obscured during an epidemic of vector-borne infection? The judgments made in

these Fact Sheets should lead to discussions of where and how to enhance surveillance by clinicians, the transfusion medicine community, and public health agencies if we think the threat from an agent rises to the level of gathering more evidence.

A primary use of the Fact Sheets is to present methods to decrease the risk of a given agent in the event of a transfusion-transmitted threat. However, each Fact Sheet can also serve as a medical and technical resource to blood providers including medical directors or other clinicians and blood center or transfusion service staff in the event that donors or transfused patients present with a history of or evidence for infection with one of these agents. Importantly, readers must understand that these Fact Sheets do not represent regulatory guidance, but instead serve as an indicator of what is known, and as such can be used as a starting point to develop policy.

The agents included in this Supplement have been chosen based on what we know now and the current judgment of the members of the TTD committee. During the time it has taken to complete this work, additional Fact Sheets already have been incorporated into the document and multiple revisions have been made to the original Fact Sheets. As new information is accumulated, the TTD will edit the Fact Sheets. Thus, additions and deletions to the Supplement are to be expected and encouraged; a 3-year cycle is planned. Readers are encouraged to challenge the assessments, provide data, and suggest edits. Much of the value of the Supplement will be realized in the future if its audience sees it as a living document. Mechanisms to receive public review/comments will be communicated through AABB publication channels; those comments may result in revisions of Fact Sheets prior to regularly scheduled revisions, depending on the nature of the comment. The Fact Sheets published in this Supplement will be posted on the AABB website. Modification of the Fact Sheets or new additions to the Supplement will be posted on the AABB website.

PRIORITIZATION OF SPECIFIC AGENTS

The prioritization effort is intended to suggest where intellect and resources should be spent in planning for the future. This is an especially important message for developers of blood donation screening tests or pathogen reduction methods since the lead time for research and development and clinical trials to bring products to the market place is generally many years.

Each agent was assigned a priority risk level under three different categories: scientific/epidemiologic evidence regarding blood safety, public perception and/or regulatory concern regarding blood safety, and public concern regarding the disease agent.

Scientific/epidemiologic risk assessment was based on a review of available data. Factors taken into consideration included proof of transfusion transmission; prevalence of the agent in an asymptomatic population that might donate blood, blood components, or in some cases organs and tissues; the possibility of an outbreak resulting in increased incidence over a specific time frame; the attack rate and severity of disease caused by the agent; and the availability of effective prevention strategies or treatment. If there was no published or other credible documentation that an agent had been transmitted by transfusion to humans, it could be assigned a risk of theoretical or absent. Theoretical was used if transmission was biologically plausible because the agent had a blood phase during asymptomatic infection. Absent was used if this possibility was judged to be remote or if the agent was not associated with any known human disease.

Classification of the public perception and/or regulatory concern for each agent's risk was an inherently more subjective process. Regulatory concern was judged primarily on whether such agents had been discussed in public forums such as the FDA's Blood Products Advisory Committee (BPAC) or as part of other regulatory deliberations (e.g., simian foamy virus, monkeypox, and variants of HIV, HBV or HTLV). The category of public concern about an agent was assessed by reviewing whether the agent had been discussed in the popular press in relationship to transfusion and whether queries to the AABB or major blood providers had occurred.

Each category received an assessment of risk of high, moderate, low, very low, or absent. As previously discussed, the scientific/epidemiologic risk category also included a classification of theoretical. When risk could not be precisely classified, an agent was assigned an intermediate categorization or a range (e.g., from low to very low) depending on where in the US/Canada, or outside the US/Canada, concern exists.

The overall prioritization of risk was a synthesis of the ratings in each category based on consensus of the group with greater emphasis given to ratings in the first two categories: scientific/epidemiologic risk and public and/or regulatory concern regarding blood safety. Categories of agents deemed to be of high or moderate priority are indicated by color: red, orange, and yellow as indicated below. The agents that have not been given a priority status at this time were placed in a white category for the purpose of the Tables in this Supplement; most of these agents remain on the watch list of potential threats, while some have been assessed to have very little possibility of causing disease in transfusion recipients.

Red. Agents with low to high scientific/epidemiologic evidence of risk regarding blood safety with the potential for severe clinical outcomes. This priority also may be influenced by the committee's estimate of the risk of emergence of these agents in the US and Canada as well as public and/or regulatory concern.

Orange. Agents with sufficient scientific/epidemiologic evidence of risk in regard to blood safety that might support their elevation to a higher priority in the future.

Yellow. Agents with absent to low scientific/epidemiologic evidence of risk regarding blood safety for which there is public and/or regulatory concern.

White. Agents that were evaluated but no higher priority appears warranted at this time. This category represents a watch list, subject to modification as circumstances change.

Appendix 1, Table A1 provides a complete listing of all agents by group (i.e., prions, viruses, rickettsiae, bacteria, protozoa, and nematodes); Tables A2 through A11 provide the priority scores for each agent by group. A complete assignment of the prioritized agents is provided in Tables A12 through A14. Those agents associated with documented cases of transfusion transmissions are listed in Table A15. Agents that are vector-borne are listed in Table A16. The Fact Sheets are included as Appendix 2.

Agents classified in the red, orange, and yellow categories are as follows:

- Red category agents (highest priority): human variant Creutzfeldt-Jakob disease, dengue viruses, and *Babesia* species (Table A12).
- Orange category agents: Chikungunya virus, St Louis encephalitis virus, *Leishmania* species, *Plasmodium* species, and *T. cruzi* (Table A13).
- Yellow category agents: chronic wasting disease prions, human herpesvirus 8, HIV variants, human parvovirus B19, influenza A virus subtype H5N1, simian foamy virus, *Borrelia burgdorferi*, and hepatitis A virus (Table A14).

Red category agents

Human variant Creutzfeldt-Jakob disease (vCJD)

The assignment of the risk of transfusion transmission of vCJD in the US is based on scientific/epidemiologic evidence of transfusion transmissibility and was influenced by several opposing factors. In favor of a higher risk were: 1) data from the UK indicating that if a donor is incubating vCJD, there appears to be a risk of transfusion transmission[18] and potentially a risk to hemophiliacs who received UK-derived plasma products prior to the implementation of interventions to decrease BSE that were put into effect in 1996 (vCJD abnormal prion protein found in a patient with hemophilia at postmortem, Health Protection Agency, CJD Section, London, UK, http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733818681?p=1225960597236) and 2) the rapid mortality associated with clinical disease and the lack of effective treatment.[34] In favor of a lower risk were: 1) the presumed very low to absent rate of carriers of the agent in the general US population and 2) the possibility of an even lower carrier rate in the US blood donor population due to travel deferrals based on time spent in the UK and Europe. These resulted in a priority rating of low for the scientific/epidemiologic risk category. However, based on high public concern about this agent in the UK and other areas of the world that has influenced public perception in the US about “mad cow disease,” considerable FDA attention to the issue of vCJD transfusion transmission, and the very difficult donor counseling issues and potential for large numbers of donor deferrals attendant on implementation of a test for vCJD, this agent was assigned a high rating with regard to public concern about blood safety. With all factors taken into account, this led to the assignment of the red priority category for vCJD.

Dengue viruses (DENV)

The assignment of the risk of transfusion transmission of DENV in the US is based on scientific/epidemiologic evidence of transfusion transmissibility,[35,36] and was influenced by several opposing factors. In favor of a higher risk were: 1) the common occurrence of asymptomatic infections and occurrence of viremia during the asymptomatic period; 2) the demonstration of relatively high rates of virus-specific RNA detection in studies of blood donors from endemic areas;[37,38] 3) the occurrence of epidemics that affect a relatively high percentage of the population at any one time; 4) the presence of competent mosquito vectors in large parts of the US; 5) the demonstration of high seroprevalence rates in US populations on the Texas-Mexico border;[39] and 6) the occurrence of localized epidemics in Hawaii and Texas and in Puerto Rico from where collected blood may be imported into the continental US. In favor of a lower risk were: 1) the low incidence of autochthonous transmission in the US and 2) travel deferral for visits to malarial endemic locations that extensively overlap with DENV-endemic areas that should defer returning donors with asymptomatic DENV infection. A consideration of all of these factors led the committee to assign a value of low for scientific/epidemiologic risk in regard to blood safety. However, in non-US DENV-endemic areas, this assignment would be moderate to high based on the prevalence of the agent. Public concern for blood safety was judged to be very low to absent in the US but moderate to high in some dengue-endemic areas. Overall, these considerations led to the classification of DENV as red priority category agents.

Babesia species

Based on the large number of transfusion-transmitted cases reported and the perception there is gross underreporting,[40,41] *Babesia* was assigned a risk rating of moderate to high on scientific/epidemiologic grounds regarding blood safety. Pertaining to public concern, the

agent was assigned a very low rating nationally but a moderate rating in areas known to be endemic for the agent (i.e., states in the Northeast and upper Midwest). Regulatory concern is evidenced by sponsorship of a workshop on transfusion-associated babesiosis in September 2008 by the FDA (http://www.fda.gov/downloads/BiologicsBloodVaccines/NewsEvents WorkshopsConferences/TranscriptsMinutes/UCM051501.pdf). Overall, the increasing recognition of transfusion-transmitted cases, the severe outcomes that can occur in immunocompromised and asplenic transfusion recipients, and the lack of effective interventions to prevent transfusion transmission led to the classification of this agent in the red priority category.

Orange category agents

Chikungunya virus (CHIKV)

Due to the lack of any proven transfusion-transmitted cases, CHIKV was assigned a scientific/epidemiologic risk rating of theoretical in regard to blood safety. However, several scientific and epidemiologic factors were judged to contribute to the potential for an increased risk from this agent in the future. These include the rapid re-emergence of the infection in the Indian Ocean and parts of Africa and Asia since 2005,[42] the geographical spread of the virus by travelers returning from endemic areas to nonendemic regions, the presence of appropriate mosquito vectors in various geographic locations including the US, the increased vector efficiency of newly emerged strains, and the presence of asymptomatic viremia in infected individuals.[43,44]

St Louis encephalitis virus (SLEV)

Due to the lack of any proven transfusion-transmitted cases, SLEV was assigned a scientific/epidemiologic risk rating of theoretical regarding blood safety. However, due to its close phylogenetic relationship and similar epidemiology to WNV, and the possibility for large outbreaks of St Louis encephalitis in the US, this agent was judged to have the potential to be a blood safety concern in the future.

Leishmania species

These agents were assigned a scientific/epidemiologic risk rating of low in regard to blood safety. This risk rating was influenced by several opposing factors. In favor of a higher risk were: 1) transfusion transmission documented in at least three cases (and perhaps as many as 10) in which the transfused recipients were either infants or immunocompromised patients;[45] 2) the propensity for chronic carriage of the agent; and 3) the presence of asymptomatic parasitemia. In favor of a lower risk were: 1) lack of any documented transfusion transmission in the US or Canada and 2) potentially effective prevention methods (specific geographical deferral for time spent in Iraq or travel to other areas where leishmaniasis and malaria are both endemic, and the widespread use of leukoreduction in North America). Public concern regarding blood safety was judged to be low based on discussions at BPAC meetings. Because these agents have been demonstrated to be transmissible by transfusion and because they can potentially be introduced into the US by returning military personnel (as well as by other travelers), it was judged that *Leishmania* species could be of increasing significance to blood safety in the future.

Plasmodium species

Malaria is a major infectious disease associated with transfusion in many emerging and developing countries. In contrast, this risk is much lower in developed countries due to lack of endemicity of the agent. The *Plasmodium* species were assigned a scientific/epidemiologic risk rating of low in the US and in most nonendemic countries in regard to blood safety due to their low prevalence coupled with the effective use of donor deferrals due to travel, residence, or having had malaria. However, the risk may be moderate to high in some nonendemic countries based on the demographics and travel patterns of their donor population. Similarly, public concern regarding both blood safety and transmissibility by other routes is likely to vary between endemic and nonendemic regions. In the US, the level of public concern was judged to be moderate. Several factors contribute to the possibility that malaria may increase as a transfusion risk. These include the re-emergence of the disease in nonendemic geographic regions due to immigration and travel, an increase in sporadic cases of “airport malaria,” the occurrence of autochthonous transmission in nonendemic countries when plasmodia are introduced by immigrants (or rarely travelers), the possibility that global climate change could result in an expanded range of vectors, and the lack of a screening assay to interdict donors for whom risk is not recognized.

Trypanosoma cruzi

T. cruzi is included even though an FDA-licensed test for blood donor screening has been available since December 2006. The assignment of a blood safety scientific/epidemiologic risk rating for this agent was influenced by the implementation of blood donor screening in 2007 by the majority of US blood centers. Based on evidence of transfusion transmission in Central and South America, documentation of transfusion-transmitted cases in North America prior to donor screening, and the risk mitigation achieved by donor screening, this agent was assigned a scientific/epidemiologic risk rating of low regarding blood safety. When assessing public concern for blood safety, *T. cruzi* was assigned a rating of moderate based

on public and regulatory attention given to the introduction of blood donor screening. Although the risk of transmission of this agent should be decreasing in the US, uncertainties persist regarding autochthonous transmission and the sensitivity of the donor screening assay. After 2 years of universal donor screening by most blood collection facilities in the US, testing strategies will likely be modified to a selective strategy, based upon at least one-time testing of every donor. The committee felt that this agent should be assigned an overall priority of orange, at least for the interval required for data relating to the efficacy of donor screening and the number of previous transfusion transmissions (as assessed by lookback investigations) to be collected and analyzed.

Yellow category agents

Chronic wasting disease (CWD)

This prion agent has never been detected in humans and no transfusion transmission has occurred, leading to a blood safety scientific/epidemiologic risk rating of theoretical. However, because of public awareness of another prion agent, vCJD, which is associated with mad cow disease and lethal human infection, it was judged that there was low to moderate public concern about the possibility that the CWD prion agent also might cross the species barrier.[46-48] Public concern specific to blood safety, however, was judged to be very low. Because of new scientific and public focus on this agent and its associated disease in deer and elk in the US and Canada, the limited amount of research that has been done to date, the potential for this prion agent to behave in a manner similar to vCJD, and the extensive opportunities for donor exposure attendant to the popularity of hunting, public health agencies (i.e., CDC) and regulatory agencies responsible for blood safety have expressed some concern even in the absence of any substantiating data. As a result, this agent was assigned an overall priority rating of yellow.

Human herpesvirus 8 (HHV-8)

Although HHV-8 transfusion transmission has been proven in Africa,[49] a material risk of such transmission in the US has not been convincingly established.[50] The US risk should be considerably lower than that in Africa based on several factors: the frequency of HHV-8 in the blood donor population is lower in the US, the storage age of blood components is longer, and leukoreduction is commonly, though not universally, employed. Despite transfusion transmission, clinical disease has not been documented to have resulted from such transmission. Taken together, these factors resulted in a scientific/epidemiologic risk rating of low in regard to blood safety. Public concern for blood safety also was judged to be low. This agent was assigned an overall priority rating of yellow based on FDA concerns regarding potential transfusion transmission. These concerns would increase if donor deferral criteria were to be modified for males who have sex with males, due to the relatively high prevalence of infection in this population.

HIV variants

Due to the lack of any proven transfusion-transmitted cases, these agents were assigned a scientific/epidemiologic risk rating of theoretical regarding blood safety. Although the wild-type agent (HIV-1, Group M) is transfusion transmitted, transmission of HIV variants has not been documented. Assuming HIV variants are transmissible by transfusion, the risk should be minimal in the US due to low local prevalence, cross-reactivity of HIV screening tests, and use of questions for malaria exposure that would temporarily exclude donors who traveled to areas in Africa where HIV variation occurs at a high rate. Since HIV variants may result in AIDS, the public concern in regard to blood safety was judged to be low to moderate. In addition, the FDA has, appropriately, continued to be concerned about the ability of blood screening tests to detect all HIV variants and the ability of donor history questions to screen out all potentially at-risk donors. For these reasons, HIV variants have been assigned a priority rating of yellow.

Human parvovirus B19 (B19V)

Transfusion transmission of B19V from blood components has been proven, with at least four cases documented in the literature. However, the frequency of transmission has not been determined or estimated through established mathematical models. These factors resulted in the assignment of a scientific/epidemiologic risk rating of very low to low regarding blood safety. Public concern regarding blood safety risk was judged to be very low in the US with the exception of some concern among specific patient groups (i.e., patients with hemophilia, those with chronic anemia such as sickle cell disease or thalassemia, bone marrow transplant recipients, and other immunocompromised individuals). Based on historical transmissions of B19V to recipients of Factor VIII, there has been ongoing scientific and regulatory concern about the safety of plasma derivatives, leading many manufacturers and regulatory authorities to require B19V DNA qualification testing of incoming plasma and release testing of manufactured lots. When such B19V DNA testing is applied to recovered plasma, the issue of how to manage associated remaining in-date components from B19V DNA-positive donors has been a subject of much debate. For these reasons, this agent has been assigned a priority rating of yellow.

Influenza A virus, subtype H5N1

Due to the lack of any proven transfusion-transmitted cases, this highly pathogenic avian influenza agent was assigned a blood safety scientific/epidemiologic risk rating of theoretical. Furthermore, based on the biology of known influenza viruses and of H5N1, it was concluded that transfusion transmission was unlikely to occur.[51] However, because H5N1 has been discussed as an agent that could lead to a worldwide influenza pandemic, public concern about community transmission of this agent was judged to be high, whereas concern related specifically to blood safety was judged to be very low. The high profile of this agent in the public health sector, especially with regard to pandemic planning, led to assigning a priority rating of yellow.

Simian foamy virus (SFV)

Although SFV transfusion transmission has been documented in experiments in nonhuman primates, transmission by transfusion has not been demonstrated in humans. In addition, this agent has not been shown to cause any human disease. For these reasons, this agent was assigned a scientific/epidemiologic risk rating of theoretical with regard to blood safety. Public concern about this agent was judged to be absent. In contrast, some level of concern has been demonstrated by regulatory agencies. This concern relates to the theoretical possibility that clinical disease does occur but has not yet been recognized and/or that mutated strains of this agent may eventually show increased pathogenicity in humans.[2,4] In the US, SFV has been discussed at meetings of the BPAC without concern expressed by stakeholder groups or other members of the public (http://www.fda.gov/ohrms/dockets/ac/04/transcripts/2004-4074t1.htm). In contrast, Health Canada, the Regulatory agency in Canada (http://www.hc-sc.gc.ca/dhp-mps/brgtherap/activit/fs-fi/fact_simian_foamy_virus_spumeux_simien_feuillet-eng.php), requires a permanent deferral for potential blood donors whose employment involves contact with monkeys or their body fluids.[29] For these reasons, this agent has been assigned a priority rating of yellow.

Borrelia burgdorferi

Due to the lack of any proven transfusion-transmitted cases, this agent was assigned a scientific/epidemiologic risk rating of theoretical regarding blood safety. If transfusion-transmission does occur, it is likely to be rare. Conversely, given that this agent has been recognized and studied for many years, public concern about the general transmissibility of this agent and its resulting disease manifestations (i.e., Lyme disease) was judged to be moderate, whereas public concern regarding blood safety was judged to be very low. Based primarily on the public apprehension of Lyme disease in areas of the country with a higher prevalence of this disease, the agent has been assigned a priority rating of yellow.

Hepatitis A virus (HAV)

HAV transmission through blood is uncommon, but well documented, and can lead to secondary cases especially when transmission occurs among infants in neonatal intensive care units. Viremia often precedes the development of symptoms by 7-14 days. The presumed rarity of transfusion-associated cases is due to several factors that include a short viremic phase, low concentration of virus in the blood, absence of a carrier state, neutralization of the virus by specific antibody in other components concurrently administered, routine immunization in populations with high HAV incidence, and increasing prevalence of immunity in recipients with age. HAV remains in the yellow category based on the fact that the scientific/epidemiologic evidence regarding blood safety is low but public concern is low to moderate, especially during a community outbreak of the disease. In the US, risk of transmission during a common source epidemic is mitigated by the addition of a specific question to the donor questionnaire regarding exposure that leads to a 120-day deferral postexposure.

Selected specific white category agents

In addition to the previously categorized agents, several agents on the watch list (i.e., white category agents) merit further discussion, either because of recently changing information (e.g., hepatitis E virus [HEV] and *Anaplasma phagocytophilum*) or because of concerns about the potential use of the agent in a bioterrorist attack. Some of these latter agents are discussed in more detail in the section on bioterrorism.

Hepatitis E virus (HEV)

A small number of cases of HEV transmission by blood transfusion has been documented[52] both in areas classically viewed as endemic for human infection as well as in developed, industrialized countries.[53] The sporadic (nontransfusion) cases in humans observed in the US have been mostly imported from endemic areas.[53] This has led to assignment of a scientific/epidemiologic risk rating of very low for this agent. The impact of this disease currently remains very low in the US and public concern is absent for blood safety and clinical disease. Nevertheless, the potential for human infection in the US remains a possibility given that an HEV reservoir exists in pigs. The existence of this reservoir, combined with several cases of transfusion transmission in other developed countries, indicates that HEV may increase in priority.

Anaplasma phagocytophilum
There have been two published case reports of transfusion-transmitted infection in the US (one as an abstract[54] and one by the CDC in Morbidity and Mortality Weekly Report[55]). Due to this low number of reported transfusion-transmitted cases, the scientific/epidemiologic risk assigned was very low. However, it is likely that more cases have occurred and have not been recognized or reported. Given the high seroprevalence rates in donors in some geographic locations, the demonstrated survival of the organism in refrigerated red cells, documented transfusion transmission in animal models, and an unknown period of asymptomatic bacteremia, it is possible that an increasing number of transfusion-transmitted cases will be recognized if surveillance is adequate.

CATEGORIZATION INTERSECTION: SCIENCE VERSUS THE PUBLIC'S PERCEPTION

As noted, categorization of agents on the scientific/epidemiologic scale was based on data that appeared in the scientific literature. In contrast, the public perception categorizations were developed by a more subjective process. Agents may have low scientific priorities but may be high on the public's "radar" screen if the agents are perceived as being those that might lead to significant human disease. As a means of showing the various conflicts or agreements in priorities, agents having red, orange, or yellow priority levels were plotted by factor-analytic representation[56] (Fig. 1) where the x-axis is the scientific/epidemiologic scale that ranges from a theoretical transfusion-transmission risk to higher levels of proof of transmission and/or disease severity and incidence. In contrast, the y-axis is public concern (i.e., *perception* of risk) ranging from absent to high that occurs when there is the perceived potential for dread, catastrophic or fatal consequences often accompanied by social stigma (e.g., HIV/AIDS) with lack of control over the outcome. Risk communication and risk management science have shown that the higher the perceived risk, the more people want to see the risk reduced, and the more they want to see strict regulation employed to achieve the desired reduction in risk. Risk management efforts are destined to fail unless they are structured using such a two-way process (expert data synthesis, opinion, and public perception).[56] The lesson that we have learned since HIV emerged as a transfusion-transmitted agent is that what we do to protect the safety of the blood supply is dependent on both science and the expectations of the communities that we serve.

CATEGORY A AGENTS OF BIOTERRORISM: BLOOD SAFETY IMPLICATIONS AND ACTIONS IN THE EVENT OF AN ATTACK

Blood establishments need to be appropriately prepared in the event of a bioterrorist event. Key concerns are the management of the existing and future blood supply once an attack has been identified. This requires knowledge of the potential impact of agents of bioterrorism on blood safety and availability. Different actions may need to be taken depending upon the magnitude of the attack and the agent(s) involved.

The CDC has classified several agents that might be used for bioterrorism. Those considered to be of the gravest concern are classified as Category A (http://www.bt.cdc.gov/agent/agentlist-category.asp#a), and include the agents of anthrax, botulism, plague, smallpox, tularemia, and viral hemorrhagic fevers. These agents, exclusive of botulism, are listed in Appendix 1, Table A17. The characteristics and potential actions relative to these agents are summarized in Appendix 1,

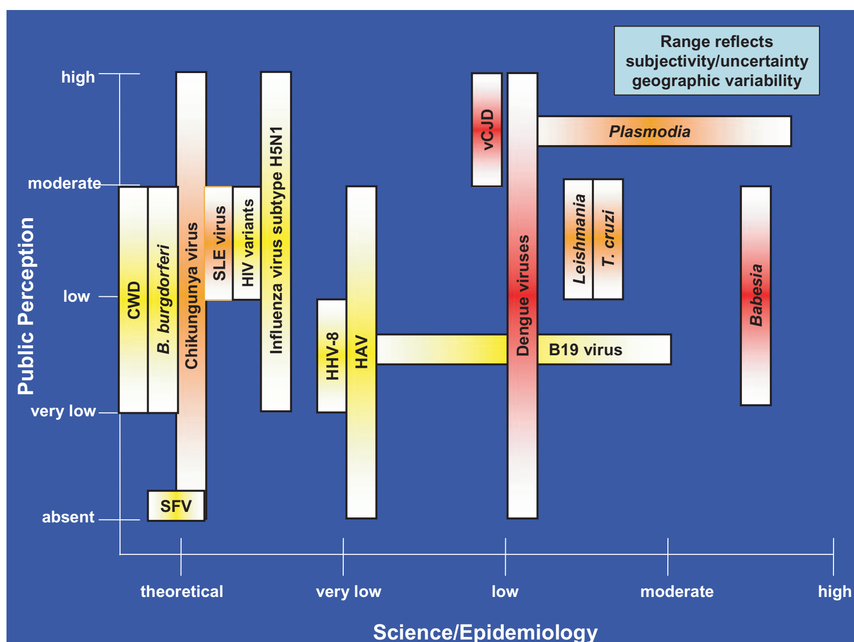

Fig. 1. EID agent priority matrix, where the y-axis represents the priority based on public perception ranging from absent to high and the x-axis represents the priority based on science and epidemiology ranging from theoretical to high (see text).

Table A18. These high-priority agents include organisms that pose a risk to national security because they:

- Can be easily disseminated.
- Can be transmitted from person to person.
- Result in high morbidity or mortality rates and have the potential for major public health impact.
- Might cause public panic and social disruption.
- Require special action for public health preparedness.

The items that require specific consideration for blood organizations in relation to a bioterrorist attack are:

- The risk of infectivity of blood components if an exposed individual gives blood prior to the appearance of symptoms.
- The risk of contamination of collected blood as a result of direct or indirect deposition of the agent on blood containers (also putting staff at risk).
- The impact of a bioterrorist attack on the availability of donors and facility personnel.
- The impact of the attack upon the need for blood components.
- The impact of regulatory actions taken in response to an attack.

A major attack may compromise the safety of the blood supply to such an extent that blood collection operations would have to be shut down in the area impacted by the attack. In addition, it may be necessary to quarantine in-date products, at least until the nature, extent, and likely date of the attack is known. However, an attack of lesser magnitude (for example, the anthrax attacks in 2001) will probably require management of each donor and product according to individual circumstances.

The CDC bioterrorism page (http://www.bt.cdc.gov/agent/agentlist.asp) is a useful resource providing descriptive information on all agents and ways to manage suspected attacks or exposures. There are also links to other sites covering issues including reporting and cleanup. A series of articles in the *Journal of American Medical Association* provides very useful information and the specific reference is noted in each subtitle below. The key characteristics of the agents listed in Table A18 are outlined.

Anthrax[57]

Anthrax is caused by the gram-positive, spore-forming bacterium, *Bacillus anthracis*. Three forms of disease are recognized: inhalation anthrax resulting from respiratory exposure (most likely to occur in the event of a deliberate attack); gastrointestinal anthrax resulting from the consumption of contaminated food; and cutaneous anthrax manifesting as skin lesions, most often on the hand. It has also become apparent that individuals may be colonized but exhibit no symptoms. Inhalation and gastrointestinal anthrax have incubation periods of 1-7 days, and if untreated, fatality rates of 97% and 25-60%, respectively. Cutaneous anthrax has an incubation period of 1-12 days and an untreated fatality rate of 20%. Anthrax is not transmitted from person to person. While *B. anthracis* is frequently present in the blood of ill patients, the FDA reports that bacteremia is thought to be extremely unlikely in asymptomatic individuals. Consequently, anthrax transmission by blood transfusion is not believed to occur provided blood is collected only from healthy donors with normal temperatures.

A bioterrorism attack would most likely occur through distribution of anthrax spores by the aerosol route and/or distribution of the spores in powdered form. Spores are resistant to sterilization by chemicals or heat and restoring a contaminated area to a safe condition is difficult and time-consuming. In the event of known contamination of a blood establishment, it would be necessary to evacuate staff and donors and to close the establishment pending remediation.

In 2001, following the anthrax attacks, the FDA issued Guidance for Industry entitled: "Recommendations for assessment of donor suitability and blood and blood product safety in cases of possible exposure to anthrax" (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm076711.htm). In this document, the FDA did not recommend any changes to standard donor screening and blood collection practices to identify or otherwise query donors who may have been exposed to anthrax. (Note that the document was prepared after a rather limited exposure and that regulatory advice might change in the face of a massive attack.) The FDA did recommend deferral for donors with diagnosed anthrax, suspected skin lesions, or known colonization until they have completed a full course of antibiotic treatment and have been shown to be free of the bacteria. Donors with skin lesions suspected to be anthrax should be deferred until the lesion is later shown not to be anthrax or until the lesion is shown to be anthrax and the individual completes a full course of treatment and the infection is considered to be resolved.

In the event that a donor reports postdonation information of anthrax, in-date blood components from prior donations should be quarantined and retrieved promptly; this should date back to the time of exposure or 60 days prior to onset of illness, whichever is shortest. The FDA also recommends recipient notification and that medical directors should determine an appropriate course of action in the event of postdonation illness among donors suspected of having been exposed. Should direct contamination of components with anthrax spores occur, blood products would have to be destroyed.

Botulism[58]

Botulism results from exposure to a toxin produced by the bacterium *Clostridium botulinum*. The toxin is extraordinarily potent and a lethal human dose is thought to be 0.09-0.15 μg intravenously, 0.70-0.90 μg by inhalation and 70 μg orally. Natural forms of botulism are foodborne, wound, and intestinal, but all have similar clinical outcomes, namely an acute, afebrile descending flaccid paralysis that may lead to death from paralysis of the muscles of respiration. The incubation period depends on the quantity of preformed toxin to which an individual is exposed and, in the case of foodborne exposure, may be from 2 hours to 10 days, but is usually 18-36 hours. A bioterrorist attack is likely to occur as a result of distribution of the toxin by the aerosol route or perhaps by deliberate contamination of food. An attack via the water supply is technically not feasible.

There is no possibility of person-to-person transmission of *C. botulinum*. It would seem very unlikely, if not impossible, that a lethal dose could be transmitted by blood transfusion, as such transmission would require that the donor would have to have a lethal dose of preformed toxin in the blood. Thus, no Fact Sheet was developed for *C. botulinum*. Donor deferral and quarantine and retrieval of components will not be needed unless an attack targeted a blood establishment for contamination of products.

Plague[59]

Plague is caused by the gram-negative bacterium, *Yersinia pestis*. There are two major forms of disease: bubonic, usually acquired by the bites of fleas that have fed on bacteremic rodents; and pneumonic, resulting from aerosol (respiratory) exposure. Primary or secondary septicemic plague also may occur. Pneumonic plague is the form most likely to occur as a result of a bioterrorist attack, although intentional transmission using infected fleas has occurred. The overall mortality of bubonic plague is about 15%, but 50-60% of cases may die in the absence of treatment. Untreated pneumonic disease is usually fatal. Pneumonic disease may be transmitted from person to person through respiratory droplets. The incubation period is 1-7 days or 1-4 days for primary pneumonic plague. A bioterrorist attack would manifest itself as an outbreak of rapidly fatal respiratory illness 1-6 days after the attack; secondary cases would occur. Affected individuals may have been bacteremic briefly at some time prior to the appearance of illness; therefore, their donated blood must be presumed to be infectious. Quarantine and retrieval of blood collected up to 10 days prior to the recognition of the outbreak would probably be prudent in the event of a large outbreak. Presenting donors exposed to known cases should be deferred (probably for 2 weeks or until completion of a prophylactic course of antibiotics). Staff or donors with respiratory symptoms should be referred for medical evaluation and treatment. In the event of a major regional outbreak, it may be necessary to suspend blood collection in that region until the outbreak is terminated. Significant residual contamination of facilities is unlikely, as the bacterium is very sensitive to environmental inactivation.

Smallpox[60]

Smallpox is caused by a large DNA virus known as the variola virus. Unlike other potential bioweapons, smallpox has been eradicated in nature. The disease spreads rapidly from person to person by droplet and aerosol routes and by direct contact: contaminated surfaces such as bedding and clothing also may spread the infection. Individuals are most infectious from the time that the rash appears until 7-10 days thereafter. The usual incubation period is 12-14 days (with a 7-17 day range), and there is asymptomatic viremia starting about 3-4 days after exposure. The mortality is up to 30% and there is no known effective treatment. A biological attack would likely involve aerosol release that would be recognized as a cluster of cases. Each case may infect 10-20 others and isolation of cases would be required. Although there is no reported case of transmission of smallpox by transfusion, this remains a possibility. Therefore, robust interventions would be required for blood establishments, including deferral of cases and case-contacts and quarantine and recovery of blood components collected up to 21 days prior to the first case. Second wave cases would complicate the situation. There also would be obvious concern about staff exposure and it may be necessary to cease all operations until the extent of the outbreak is determined. Environmental contamination from the attack itself will not be a serious issue as the virus is not expected to persist in the environment for more than 2 days. It is likely that an attack would result in the implementation of widespread vaccination programs. The FDA has provided guidance on donor management in the context of a smallpox vaccine program (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/ucm075115.htm). It should be noted that this guidance requires donor deferral and such a deferral may have a significant impact upon product availability (particularly for platelets). An attack with smallpox is likely to be extremely challenging to the blood system.

Tularemia[61]

Tularemia is caused by a small, gram-negative bacterium, *Francisella tularensis*. This organism infects a number of wild animals naturally and may be found as an environmental contaminant. Humans may be infected via the aerosol or droplet route, accidental inoculation, the bite of

fleas or other arthropods, or even via food or water. The agent is highly infectious, with as few as 10 organisms causing disease. However, person-to-person transmission does not occur. It is likely that a bioterrorism attack would be accomplished via an aerosol release. This would result in an outbreak of acute, febrile illness with respiratory symptoms. The incubation period is 3-5 days, with a range of 1-14 days. Although subjects may be incapacitated in a matter of days, the untreated disease may be prolonged and can last weeks to months. Untreated inhalation tularemia may have a mortality rate of 30-60%. Bacteremia does occur and deferral of cases and individuals thought to be at risk of primary exposure would be necessary, pending successful completion of therapy or of antibiotic prophylaxis. Additionally, quarantine and recovery of products collected from the time of the presumed attack until its recognition would be advisable. Although the organism is relatively hardy, simple surface decontamination with 10% bleach would be adequate and residual contamination from the attack is not anticipated to be a problem.

Viral hemorrhagic fevers[62]

This is a complex area as about eight different viruses belonging to four different groups are thought to be potential threats for a bioterrorist attack. However, there are some general shared characteristics. All are enveloped RNA viruses and all tend to cause a similar illness. Person-to-person spread by direct contact with infected blood and body fluids is a primary route of transmission, especially among those providing direct care for infected persons. Viruses that might be used in this context include Ebola, Marburg, Lassa, New World arenaviruses, Rift Valley fever, yellow fever, Omsk hemorrhagic fever, Crimean-Congo hemorrhagic fever, and Kyasanur Forest disease viruses. A deliberate attack would most likely involve an aerosol release of virus. The result would be an outbreak of undifferentiated febrile illness 2-21 days later. Clinical manifestations could include rash, hemorrhagic manifestations, and shock. Case fatality rates would depend upon the agent used, but vary from a low of 0.5% (Omsk hemorrhagic fever) to a high of 90% (Ebola). Diagnosis would not be easy and it might take time before the cause of the outbreak was established. Depending on the agent used, considerable caution may have to be used in patient care. Some, but not all, of the viruses are somewhat responsive to selected antiviral drugs. These viruses are present in the blood, but little is known about viremia in the presymptomatic phases. Quarantine and recovery of products from infected, and possibly exposed, donors collected during the incubation period would have to be undertaken. If the involved virus were not identified, the presumed maximum incubation period would have to be used; this could be 4 weeks or more. Secondary cases would be most likely to occur among caregivers with direct contact with symptomatic cases; consequently, it may be necessary to defer such individuals from donation. The viruses are not expected to survive very long in the environment, so there should be no significant facility clean-up issues for blood establishments. Finally, it should be noted that the clinical effects of these agents may result in a need for blood components, and especially platelets, to correct the bleeding.

PATHOGEN REDUCTION AS A SAFETY STRATEGY FOR EID

Introduction

This Supplement presents detailed information on selected EID agents, including their biology, potential threat to recipient safety, and existing interventions. Historically, the transfusion medicine community's response to EID agents has been to add new donor deferral criteria and/or new screening tests. EIDs will continue to appear, and continuous addition of deferrals and screening tests may not be sustainable. Furthermore, this approach involves waiting for disease emergence, identification of the agent, understanding of epidemiological risk factors of infection to craft donor deferrals, and then development and implementation of an assay. During this interval before introduction of the assay, morbidity and mortality may accumulate. This reactive strategy of donor qualification via questions and tests will continue unless more broad-reaching interventions are developed and implemented. Pathogen reduction (PR) offers a proactive strategy to address these threats; if a PR technology offers a broad spectrum of inactivation, there is a high likelihood that it will inactivate the new agent, thereby preventing infections and perhaps obviating the need for the introduction of new donor deferral criteria and new screening tests. PR should also be of benefit in those situations where an assay fails to detect an infectious agent due to a low level of antigen/antibody/nucleic acid in the test sample during the "window period" when the analyte has not reached its detection threshold. Finally, PR may interdict agents with very long incubation periods or unrecognized pathogenicity for which the association with transfusion may be obscure.

The intent of the following section is to provide an overview of what is known regarding PR systems that have progressed to clinical trials or have been implemented in other countries. It provides a brief review of each PR method by blood component including: the company name and the name of the technology, the company's website (as a source of additional unpublished data), a description of how the process would be used, the agent's mechanism of action, a composite table of published inactivation results, and a brief summary of the clinical performance and regulatory status. There is also brief commentary on limitations of PR, including

the long and complex regulatory cycle (especially in the US and Canada), reductions in product efficacy or toxicology associated with PR technologies, and lastly, agents that might be resistant either inherently or due to high concentrations of the agent in the product. This review does not address costs associated with the use of these technologies.

PLATELET PATHOGEN REDUCTION SYSTEMS

There are three technologies for PR in platelets, none of which is licensed for clinical use in the US or Canada:

- Cerus Corporation INTERCEPT Blood System™ (http://www.cerus.com) Concord, CA, USA;
- CaridianBCT Biotechnologies Mirasol® PRT (http://www.caridianbct.com/) Lakewood, CO, USA; and
- MacoPharma's Theraflex© UV. (http://www.macopharma.com/) Tourcoing, France.

Cerus Corporation INTERCEPT Blood System for platelets

Platelets suspended in approximately 65% additive solution (InterSol, Fenwal, Inc., Lake Zurich, IL) and 35% plasma are treated by adding amotosalen, a psoralen compound, to the platelets and delivering 3 Joules (J)/cm^2 ultraviolet A light (320-400 nm) in approximately 5 minutes. This results in irreversible cross-linking of nucleic acids. Unreacted amotosalen and photoproducts are adsorbed during incubation of illuminated platelets in a separate container containing the Compound Adsorption Device (CAD) for at least 4 hours and up to 16 hours; the platelets are transferred to a final container for storage and transfusion.[63] The process is shown in Fig. 2.

CaridianBCT Biotechnologies Mirasol for platelets

Platelets suspended in 100% plasma are treated by adding riboflavin (the normal nutrient, vitamin B2) to the platelets, then delivering 6.2 J/cm^2 ultraviolet (UV) light (265-370 nm) in approximately 10 minutes. This results in irreversible photo-oxidative damage to nucleic acid. After illumination, the product is ready for transfusion without further processing.[64,66] The process is shown in Fig. 3A. Alternatively, CaridianBCT Biotechnologies is developing a process in which a hyperconcentrated platelet with low plasma content can be collected and photochemically treated; after treatment, platelet additive solution (PAS) is then added to yield a platelet stored in 65% PAS and 35% plasma (Fig. 3B) (Raymond Goodrich, pers. comm., 2009).

MacoPharma Theraflex UV for platelets

Platelets suspended in approximately 65% Storage Solution for Platelets (SSP, including magnesium and potassium, MacoPharma, Tourcoing, France) and 35% plasma are loosely placed on a quartz plate in bags to produce a thin platelet layer (approximately 4-5 mm), then treated

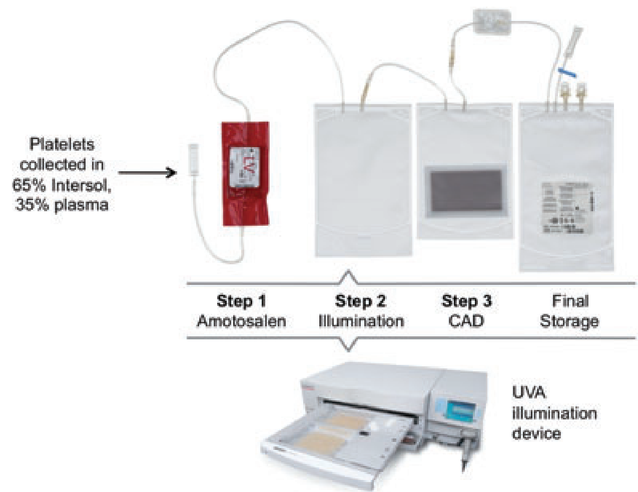

Fig. 2. Cerus Corporation INTERCEPT Blood System for platelets.

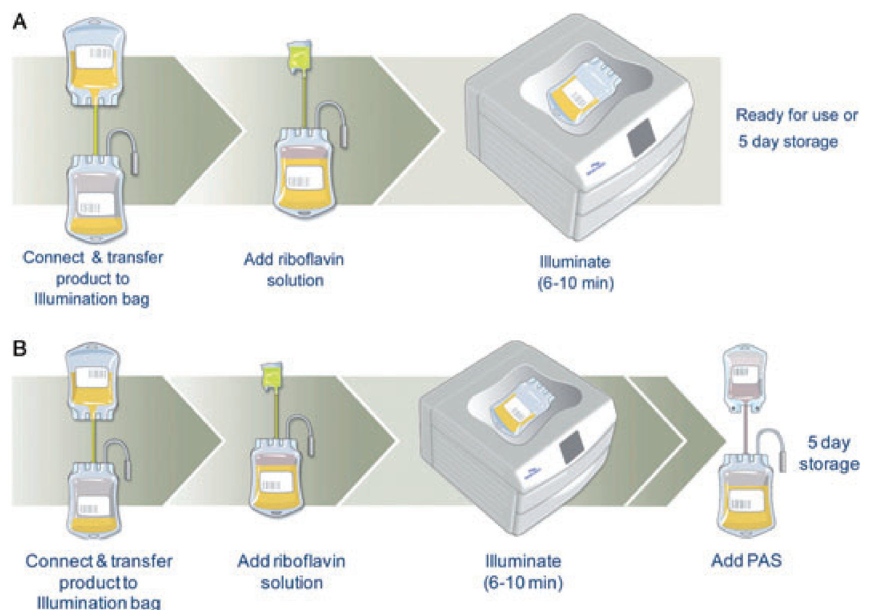

Fig. 3. CaridianBCT Biotechnologies Mirasol for platelets. (A) Platelets stored in 100% plasma. (B) Platelets stored in PAS.

by exposure to 0.3 J/cm^2 monochromatic wavelength UV light (254 nm) with intense agitation (approximately 100 cycles/min) for about 1 minute.[65] No photoactive compound is required; nucleic acid damage presumably occurs due to cyclobutyl ring formation. After illumination, the platelets are transferred into a storage container and are ready for transfusion with no further processing. The process is shown in Fig. 4.

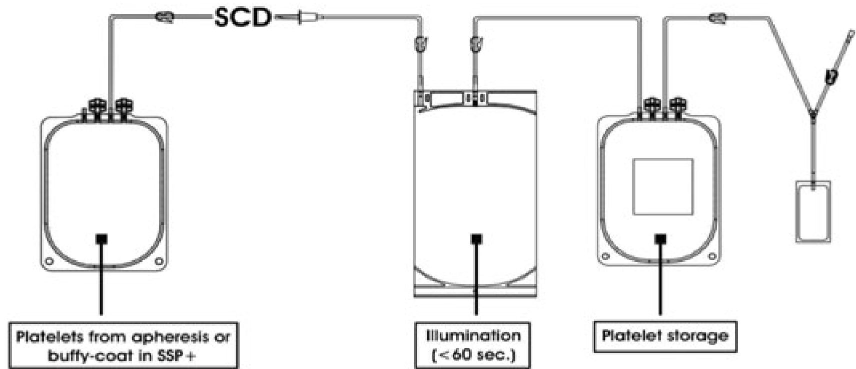

Fig. 4. MacoPharma Theraflex UV for platelets.

Pathogen reduction in platelets

Data that have been published in peer-reviewed journals are provided in Tables 1 through 4. Data from abstracts or unpublished data available from studies conducted by the manufacturers are included as personal communications from the companies' representatives. Cerus (INTERCEPT) has demonstrated >6.4 log activity for CHIKV in platelets (Lily Lin, pers. comm., 2009). CaridianBCT Biotechnologies (Mirasol) has demonstrated inactivation of the following agents: **Viruses**: Sindbis virus (3.2 logs); influenza A virus (>5.3 logs); infectious bovine rhinotracheitis virus (2.1 logs); HAV (2 logs); HBV (>4 logs by PCR); bovine enterovirus (3.0 logs); pseudorabies virus (2.5 logs); encephalomyocarditis virus (3.2 logs); CHIKV (2-3 logs); **Bacteria/Yeast**: *Acinetobacter baumannii* (1.8 logs); *Klebsiella oxytoca* (1 log); *K. pneumoniae* (2.8 logs); *Streptococcus mitis* (3.7 logs); *S. pyogenes* (2.3 logs); *Yersinia enterocolitica* (3.3 logs); *Candida albicans* (1.8 logs); and **Protozoa**: *Plasmodium falciparum* (>3.0 logs); and *Babesia microti* (>5.0 logs) (Raymond Goodrich, pers. comm., 2009). MacoPharma Theraflex UV process for platelets using only UVC and agitation has been presented at meetings held by the AABB, ISBT, and German Society for Transfusion Medicine and Immunohaematology and has shown inactivation against the following viruses: WNV (>5 logs); Sindbis virus (5.6 logs); encephalomyocarditis virus and porcine parvovirus (5-6 logs); vesicular stomatitis virus (VSV) (>6 logs); Suid herpesvirus 1 (3.7 logs); HIV-1 (1.4 logs) (Frank Tolksdorf and Stefan Reichenberg, pers. comm., 2009).

TABLE 1. Log_{10} reduction of known transfusion-transmitted viruses in platelets

Virus	INTERCEPT[63]	Mirasol[64,66]
Enveloped		
HIV-1		
• Cell-free	>6.2	5.9 (includes cell-associated)
• Cell-associated	>6.1	See above
• Proviral	To limit of detection	4.5
HBV	>5.5 CID_{50}*	
HCV	>4.5 CID_{50}	
HTLV-I, cell-associated	4.7	
HTLV-II, cell-associated	5.1	
CMV, cell-associated	>5.9	
WNV	>6.0[67]	5.2
Nonenveloped		
B19V	4-5.5[68]	
HAV	0†	

* Chimp Infectious Dose where 50% of the animals become infected.
† No reduction was observed (Lily Lin, pers comm., 2009).

TABLE 2. Log_{10} reduction of model viruses in platelets

Virus	Model for	INTERCEPT[63]	Mirasol[64,66]
Enveloped			
Vesicular stomatitis	Enveloped viruses	>5.8	>6.3
Influenza A (H5N1)	Influenza viruses	≥5.9[69]	
Duck HBV	HBV	>6.2 ID_{50}*	
Bovine viral diarrhea	HCV	>6.0	
SARS		>6.2[70]	
Vaccinia		>5.2	
Nonenveloped			
Bluetongue	Nonenveloped viruses	6.1-6.4	
Calicivirus	Nonenveloped viruses	1.7-2.4	
Simian adenovirus 15	Nonenveloped viruses	0.7-2.3	
Human adenovirus 5	Nonenveloped viruses	>5.7	
Porcine parvovirus	Nonenveloped viruses	0†	≥5.0

* Infectious dose where 50% of the ducks are infected.
† No reduction was observed.

PLASMA PATHOGEN REDUCTION SYSTEMS

The same three companies have processes for PR in plasma. Cerus and CaridianBCT Biotechnologies use the same photoactive substance and process as employed in

TABLE 3. Log_{10} reduction of bacteria and spirochetes in platelets

	INTERCEPT[71]	Mirasol[66]	Theraflex UV[65]
Gram Negative			
Escherichia coli	>6.4	>4.4	≥4.0
Serratia marcescens	>6.7	4.0	≥5.0
Klebsiella pneumoniae	>5.6		4.8
Pseudomonas aeruginosa	4.5	>4.5, >4.7*	≥4.9
Salmonella choleraesuis	>6.2		
Yersinia enterocolitica	>5.9		
Enterobacter cloacae	5.9		≥4.3
Gram Positive			
Staphylococcus epidermidis	>6.6	4.2	4.8
S. aureus	6.6	3.6	≥4.8
S. aureus MRSA Strain		4.8	
Streptococcus pyogenes	>6.8		
Listeria monocytogenes	>6.3		
Corynebacterium minutissimum	>6.3		
Bacillus cereus (includes spores)	3.6		
B. cereus (vegetative)	>5.5	1.9†	4.3
B. cereus (isolated from donated blood)		2.7	
Bifidobacterium adolescentis	>6.0		
Propionibacterium acnes	>6.2		4.5
Lactobacillus species	>6.4		
Clostridium perfringens (vegetative form)	>6.5		≥4.7
Spirochetes			
Treponema pallidum	≥6.8 to ≤7.0		
Borrelia burgdorferi	>6.8		

* ATCC 43088 and ATCC 27853, respectively.
† ATCC 7064.

TABLE 4. Log_{10} reduction of protozoa/rickettsia in platelets

	INTERCEPT	Mirasol
Plasmodium falciparum	≥6.0[72]	
Trypanosoma cruzi	>5.3[73,74]	6.0[75]
Leishmania mexicana	>5.0[76]	
Leishmania major, strain Jish	>4.3[76]	>5.0[77]
Babesia microti	>5.3[72]	
Orientia tsutsugamushi		>5.0[78]

their respective platelet PR system outlined above with some minor modifications. MacoPharma (Theraflex© MB-plasma) has developed a photochemical process that incorporates methylene blue (MB) and visible light. None is approved for clinical use in the US or Canada. These processes are shown in Figs. 5 through 7, respectively.

Octapharma (Lachen, Switzerland; http://www.octapharma.com) developed a PR process for pooled plasma units intended for large-scale manufacturing of what is commonly known as solvent/detergent (S/D) plasma (Octaplas).[79,80] A slightly different S/D plasma product manufactured by Vitex was licensed in the US but is no longer marketed.[79,81,82]

Cerus Corporation INTERCEPT Blood System for plasma

The INTERCEPT system for plasma is similar to their platelet system. After addition of amotosalen to the plasma and illumination, the plasma flows through a Compound Adsorption Device (CAD) to remove unreacted amotosalen and photoproducts. The process is shown in Fig. 5.

CaridianBCT Biotechnologies Mirasol plasma system

CaridianBCT Biotechnologies plasma system requires a transfer of the plasma into a final freezing/storage container after illumination. This process is shown in Fig. 6.

MacoPharma Theraflex MB-plasma system

MacoPharma's Theraflex MB requires 1 µM MB and 180 J/cm² illumination dose using low-pressure sodium lights with a peak wavelength of 590 nm for inactivation. Since MB cannot permeate leukocytes, the Theraflex MB disposable is offered in two formats: if the plasma has already been membrane filtered, the set does not include a leukoreduction filter. Alternatively, if the plasma has not been leukoreduced, the disposable incorporates a leukoreduction filter. Both configurations include a filter for removal of MB and photoproducts.[84] This process is shown in Fig. 7.

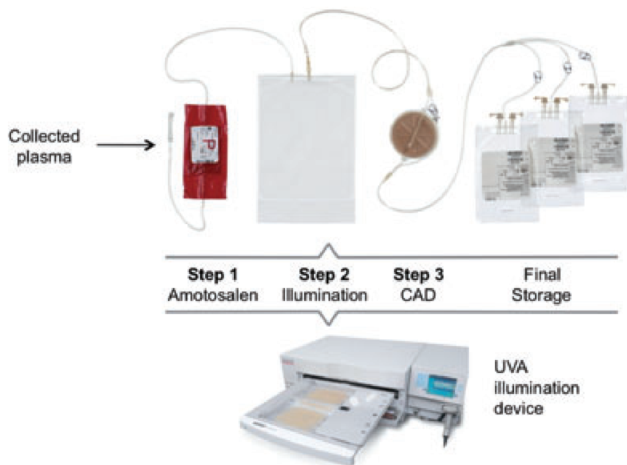

Fig. 5. Cerus Corporation INTERCEPT Blood System for plasma.[83]

Octapharma octaplas® plasma system

S/D treatment of plasma is performed by adding 1% (w/w) tri(n-butyl) phosphate (TNBP) and 1% (w/w) octoxynol-9

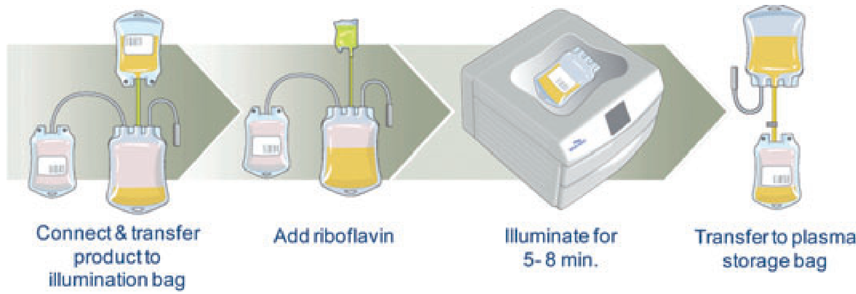

Fig. 6. CaridianBCT Biotechnologies Mirasol plasma system.

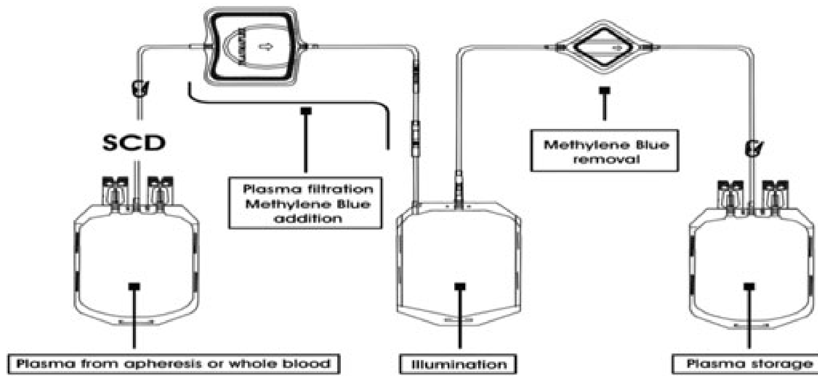

Fig. 7. MacoPharma Theraflex MB-plasma system.

TABLE 5. Log_{10} pathogen reduction for known or potential transfusion-transmitted viruses in plasma

Virus	INTERCEPT[83]	Theraflex MB[85,86]	Octaplas[81,82]
Enveloped			
HIV-1			
• Cell-free	>6.8	≥5.45	≥7.2
• Cell-associated	>6.4		
HBV	>4.5 CID_{50}*		≥6.0 CID_{50}
HCV	>4.5 CID_{50}		≥5.0 CID_{50}
HTLV-I, cell-associated	≥4.5		
HTLV-II, cell-associated	>5.7		
CMV		≥4.08	
WNV	≥6.8	≥5.78	
CHIKV	≥7.6		
Nonenveloped			
B19V	1.8		
HAV	0†		

* Chimp Infectious Dose where 50% of the animals become infected.
† No reduction was observed.

(Triton X-100) to plasma and incubating for 4 hours at 30°C. The plasma is then subjected to oil extraction and phase separation to remove the TNBP; solid phase extraction to remove the Triton X-100; sterile filtration and aseptic filling into plastic containers; and fast freezing at ≤–60°C, followed by storage at ≤–30°C.[80] It should be noted that S/D treatment of plasma is not intended as a PR method for nonenveloped viruses. S/D plasma is manufactured by pooling approximately 600-1500 source or recovered plasma donations, respectively; it is possible that transmission of an agent may occur if that agent is resistant to the S/D inactivation process or if neutralizing antibody is not present at a sufficient titer to neutralize the challenging agent.

Pathogen reduction in plasma

Published results for pathogen reduction in plasma are provided in Tables 5 through 8. Data from abstracts or unpublished data available from studies conducted by the manufacturers are included as personal communications from the companies' representatives. Cerus (INTERCEPT) has demonstrated inactivation of >5.5 logs of *O. tsutsugamushi* in an animal model. CaridianBCT Biotechnologies (Mirasol) has evaluated inactivation of a variety of transfusion-transmitted and model viruses (enveloped and nonenveloped) and parasites. The inactivation spectrum in plasma is the same as Mirasol platelets, since the platelets are suspended in 100% plasma and plasma actually constitutes the bulk of the media in which the inactivation process is being performed (Raymond Goodrich, pers. comm., 2009). Octapharma has conducted additional recent viral inactivation studies that have shown: HIV-1 (>6.3 logs); VSV (≥7.5 logs); Sindbis (>5.4 logs); pseudorabies virus (>6.3 logs); and Herpes simplex virus-2 (>6.1 logs). Since the product is cell free, risk from cell-associated viruses is also reduced. Further studies have demonstrated a substantial immune neutralization capacity in plasma pools for the following viruses: B19V (10.8 logs); HAV (≥10.0 logs); HEV (≥9.4 logs); Coxsackievirus B6 (≥8.6 logs); HSV-1 (>11.1 logs) and poliovirus type 1 (≥10.9 logs). (Tor-Einar Svae and Marc Maltas, pers. comm., 2009); Immune neutralization occurs when an antibody specific for an infectious agent binds to that agent and renders it noninfectious, but such neutralization cannot always be relied on because of the unknown prevalence of specific antibodies in the donor population.

RED BLOOD CELL PATHOGEN REDUCTION SYSTEMS

CaridianBCT Biotechnologies is using a photochemical process for treating whole blood which incorporates

TABLE 6. Log_{10} reduction of model viruses in plasma

Virus	Model for	INTERCEPT[83]	Theraflex MB[85,86]	Octaplas[81,82]
Enveloped				
Vesicular stomatitis	Enveloped viruses		>4.9	≥8
Influenza A (H5N1)	Influenza A viruses	>5.7	≥4.4	
Duck HBV	HBV	4.4-4.5	≥6	≥7.3 ID_{50}
Bovine viral diarrhea	HCV	≥6.0	≥5.44	≥6.1
Sindbis	HCV			≥6.9
Pseudorabies virus	CMV, HSV		≥5.48	≥7
Infectious bronchitis virus	Coronaviruses		4.9	
Herpes simplex virus-2	Enveloped viruses			>6.1
SARS		≥5.5		
Nonenveloped				
Bluetongue	Nonenveloped viruses	5.1		
Calicivirus	Nonenveloped viruses		≥3.9	
Human adenovirus 5	Nonenveloped viruses	≥6.8	≥5.33	

TABLE 7. Log_{10} reduction of bacteria and spirochetes in plasma

	INTERCEPT[83]
Gram Negative	
Klebsiella pneumoniae	≥7.4
Yersinia enterocolitica	>7.3
Anaplasma phagocytophilum (HGA) agent	>4.2
Gram Positive	
Staphylococcus epidermidis	>7.3
Spirochetes	
Treponema pallidum	>5.9
Borrelia burgdorferi	>10.6

TABLE 8. Log_{10} reduction of protozoa in plasma

	INTERCEPT[83]	Theraflex MB
Plasmodium falciparum	≥6.9	
Trypanosoma cruzi	>5.0	>4.9 to >5.8[87]
Babesia microti	>5.3	

riboflavin and UV light that is similar to their Mirasol process for platelets and plasma. This product is currently being tested in clinical trials in the US under an FDA-approved investigational device exemption (IDE) (Raymond Goodrich, pers. comm., 2009).

Cerus Corporation is designing a process for RBCs that uses a chemical cross-linker, specific for nucleic acid. Cerus' product entered a Phase III trial several years ago in the US, but issues with neoantigen formation brought that trial to a halt. A redesign of the process has eliminated immunogenicity in laboratory and animal studies. A Phase I trial in healthy volunteers is underway (Lily Lin, pers. comm., 2009).

CLINICAL TRIALS/CLINICAL EXPERIENCE

Platelet clinical trials

Cerus Corporation INTERCEPT platelets have undergone examination in at least 4 clinical trials in patients requiring platelet transfusion support. In the US, Cerus also conducted radiolabel recovery and survival studies in healthy volunteers, including a study in which treated platelets were also gamma irradiated.[88] Results from these studies showed a 15-20% decrease in radiolabel recovery and survival studies in INTERCEPT platelets compared to control platelets. One of the patient trials was a bleeding time study. The bleeding time correction and time to next transfusion were not statistically different between the groups, despite a lower corrected count increment (CCI) in the INTERCEPT group.[89]

INTERCEPT platelets were evaluated in thrombocytopenic patients in a two-arm, double-blind clinical Phase III-like trial in Europe using buffy coat platelets (*euroSPRITE*).[90] One hundred three (103) subjects were enrolled in this trial, 52 patients received 311 test transfusions, and 51 patients received 256 control transfusions. Patients who received test platelets showed no statistically significant differences in their CCI compared to controls at 1 hour; however, the CCIs at 24 hours did become significantly different. There was an insignificant decrease in time between transfusions (3.0 vs. 3.4 days, test vs. control, respectively). Clinical hemostasis, hemorrhagic, and aggregate adverse events were similar between groups. Cerus also conducted a smaller pilot study in Europe that showed that 7-day INTERCEPT platelets were well tolerated and prevented bleeding. Noninferiority in terms of 1-hour CCI could not be demonstrated with a prespecified noninferiority margin of 2200.[91]

In the US, Cerus conducted a large (n = 645) Phase III double-blind, two-arm trial (INTERCEPT vs. untreated platelets) in thrombocytopenic patients using apheresis platelets (*SPRINT*). Clinical efficacy as measured by incidence of WHO bleeding Grade 2, 3, and 4 was not different between the two groups. However, 1-hour CCI, days to the next platelet transfusion, and number of platelet transfusions were statistically different, all favoring the control group.[92] This finding reflected a difference in the mean platelet dose per transfusion in the two

groups; the INTERCEPT group patients received a higher proportion of doses $<3.0 \times 10^{11}$. There was a statistically significant reduction in transfusion reactions in those patients receiving the INTERCEPT platelets compared to the control (3 vs. 4.1%). However, there was an increase in three specific pulmonary events in the INTERCEPT group: acute respiratory distress syndrome (ARDS), pneumonitis not otherwise specified, and pleuritic chest pain. Reanalysis of this apparent increase of pulmonary adverse reactions with INTERCEPT transfusions by both the study investigators and independent experts blinded to patient treatment did not confirm this difference. The original observations were attributed to inconsistent reporting of ARDS from SPRINT study sites and characteristics of the classification system used.[93,94]

CaridianBCT Biotechnologies Mirasol Platelets have been tested in three clinical trials. The company studied in vivo recovery and survival of platelets in normal volunteers and found an approximate 25% decrease in recovery and survival in the Mirasol group.[95] Results of a Phase III-like clinical trial at six blood establishments and six hospitals in France were reported at the 2008 AABB Annual Meeting.[96] In this study, efficacy data were analyzed on 80 subjects who received Mirasol-treated (test) or control apheresis platelets. Patients who received test platelets showed a statistically significant ($p < 0.002$) lower 1-hour CCI compared to control platelets. However, the CCIs were not statistically different at 24 hours. There was also a statistically shorter time between transfusions during the period of the first eight transfusions in the test group compared to the control; this difference was not observed beyond the eighth transfusion. The number of platelet transfusions per patient, total platelet dose, platelet transfusion per day of support, percent refractory patients, the number of red cells per patient, the number of WHO Grade 2, 3 and 4 bleeding events, and serious adverse events were not statistically different between the groups ($p > 0.05$). No neoantigenicity was observed.

MacoPharma has completed preclinical studies of its Theraflex UV process; it is currently under evaluation in Phase I studies.

Platelet clinical experience

To date, over 200,000 Cerus INTERCEPT platelet doses have been transfused in routine clinical use. An ongoing postmarketing observational hemovigilance program has been established to monitor the safety profile of INTERCEPT platelets. Two reports representing over 12,500 transfusions of INTERCEPT platelets to 2051 patients in 11 European centers in five countries from October 2003 to January 2007 demonstrated good clinical tolerance and a safety profile similar to untreated platelets. No episodes of transfusion-related acute lung injury or transfusion-associated graft-versus-host disease (TA-GVHD) were reported. Furthermore, the use of 65% additive solution to replace plasma in this platelet PR system may have contributed to a lower rate of acute transfusion reactions.[97,98] In a study completed in 2006 at the Blood Transfusion Center, Mont Godinne, Belgium, blood component usage was evaluated in two 3-year blocks, one before implementation of INTERCEPT and one after, in 688 and 795 patients, respectively. Primary diagnoses were similar in the two study groups: hematology (approximately 40%), oncology (approximately 6%), cardiovascular surgery (approximately 30%), and other surgery and general medical services (approximately 20%). Red cell and platelet usage was statistically unchanged before and after implementation of INTERCEPT.[99]

In a separate study, the routine use experience from two periods in EFS-Alsace, France was compared, one before implementation of platelet additive solution and INTERCEPT and one after, in 2050 and 2069 patients, respectively. In both periods, patients were transfused according to conventional medical indications. The results show that transfusion of INTERCEPT platelets to a broad patient population for a spectrum of indications was well tolerated. The incidence of adverse events was less than untreated platelet components suspended in plasma. No increase in the total platelet dose and RBCs transfused to patients was observed.[100]

Two additional clinical studies were conducted per country specific requirements: one in Luebeck, Germany[101] and the other in Basel, Switzerland.[102] Physicians ordered INTERCEPT platelets according to standard clinical practice. However, INTERCEPT platelets were used in place of gamma irradiation for prevention of TA-GVHD. In the Luebeck study, 560 INTERCEPT transfusions were administered to 52 patients with hematological malignancies. In the Basel study, 551 INTERCEPT platelet components were administered to 46 patients of whom 38 were hematology-oncology patients. The results of these studies show low rates of overall acute platelet transfusion reactions. No bleeding complications were attributable to the INTERCEPT platelets. Results of an investigator study involving 500 INTERCEPT platelet transfusions in 83 pediatric hematology-oncology patients in a routine clinical setting were reported.[103] This study showed that transfusion of pediatric patients with INTERCEPT platelets was well tolerated and provided therapeutic count increments.

As part of CaridianBCT Biotechnologies Mirasol Evaluation Program, several hundred Mirasol-treated platelet products have been transfused in routine use. No Mirasol-related adverse reactions have been reported at any of the participating blood centers or hospital sites. This program has been expanded considerably in 2009 and is targeting several thousand transfusions which will

be monitored for adverse event reporting (Raymond Goodrich, pers. comm., 2009).

Plasma clinical trials

INTERCEPT plasma has been evaluated in clinical studies in the US. In their first human clinical study, subjects donated plasma with half of the plasma treated with the INTERCEPT system and half prepared as standard fresh frozen plasma (FFP). Subjects then received warfarin over 4 days to lower the Factor VII levels. On day 4, subjects received either their own standard FFP or INTERCEPT-FFP. After 2 weeks, subjects underwent an identical protocol and received the other type of FFP. Factor VII kinetics were the same in subjects after either INTERCEPT FFP or standard FFP.[104] The efficacy and safety of INTERCEPT plasma in patients with congenital coagulation factor deficiencies was evaluated in a single-arm open-label clinical trial. The results of this 34-patient trial with deficiencies of coagulation factors I (fibrinogen), II, V, VII, X, XI, and XIII demonstrated that INTERCEPT plasma provided coagulation factor recovery and pharmacokinetics comparable to conventional plasma, with prothrombin time (PT) and activated partial thromboplastin time (aPTT) responses sufficient for adequate hemostasis.[105] Furthermore, in a randomized, double-blind clinical trial in 121 patents with acquired coagulopathy, Mintz et al. demonstrated that INTERCEPT-FFP supported hemostasis similar to conventional FFP, with no differences in the use of blood components, clinical hemostasis, or safety.[106] In a Phase III trial, Cerus evaluated the safety and effectiveness of INTERCEPT-FFP compared to standard FFP in a small group of patients with thrombotic thrombocytopenic purpura (TTP). Remission was achieved in 14 of 17 (82%) patients receiving INTERCEPT-FFP, and 16 of 18 (89%) patients receiving standard FFP. Time to remission, relapse rates, time to relapse, total volume, and number of FFP units exchanged were not significantly different between both groups. No antibodies to amotosalen were detected.[107] A hemovigilance program similar to that established for INTERCEPT platelets is ongoing to document and monitor the safety of INTERCEPT plasma transfusion.[108]

Methylene blue processes similar to MacoPharma Theraflex MB have been used in Europe for over 10 years, with over 4 million units transfused in various clinical settings. However, there have been no large controlled, randomized clinical trials comparing MB-FFP to standard FFP. Most patient studies have been small and/or used laboratory rather than clinical endpoints.

CaridianBCT Biotechnologies has evaluated their Mirasol-treated plasma in several *in vitro* studies and it has been demonstrated to meet the 14th Edition, Council of Europe Guidelines for protein content for standard FFP (Raymond Goodrich, pers. comm., 2009).

Octapharma octaplas has been evaluated in several uncontrolled, observational trials in liver transplant, cardiac surgery, and TTP patients, with no differences noted between S/D plasma and FFP. There have been a few randomized, controlled clinical trials in patients with severe coagulopathy or undergoing cardiopulmonary bypass surgery, with no clinical differences noted. Whether these studies were sufficiently powered to see any differences is debatable.

Plasma clinical experience

Over 6 million units of Octapharma octaplas have been transfused, and it has been accepted as therapeutically equivalent to standard FFP. No postmarketing hemovigilance trials have been published.

MacoPharma Theraflex MB has been registered or is in routine use in 20 countries, including Germany, Switzerland, Spain, Greece, Italy, France, Belgium, and the UK. In spite of laboratory observations demonstrating some loss of coagulation factors, clinical reports have been generally satisfactory. Postmarketing hemovigilance studies from Greece and Spain evaluating 8500 units and 88,000 units, respectively, have been reported, with no adverse events associated with MB-FFP and satisfactory clinical outcomes.[109,110] Castrillo et al.[111] also reported a 5-year experience with MB plasma with no adverse reactions observed. MB-FFP in TTP patients has been evaluated in several small studies, comparing MB-FFP to untreated FFP and its effectiveness is a subject of debate. Two studies in Spain, one retrospective and one prospective, demonstrated that there was a lower remission rate and higher volume of FFP required for treating TTP when using MB-FFP compared to control (untreated) FFP.[112,113] It has been hypothesized that this is due to decreased levels of ADAMTS13, the enzyme generally thought to be deficient or inhibited in these patients; however, MB treatment does not affect the activity of ADAMS13.[114,115]

Limitations of pathogen reduction

Agents with intrinsic resistance to PR processes include prions, some nonenveloped viruses such as HAV, and bacterial spores. Furthermore, extraordinarily high-titer viruses like B19V or HBV may not be inactivated below an infectious dose. Therefore, surveillance for the emergence of new agents will remain critical even after the introduction of PR.

Since blood components currently carry very low risks of infectious agent transmission, any manipulation to further mitigate known risks will be difficult to justify if it introduces any material new risk to transfusion recipients. Critical questions have been raised about

short- and long-term safety of PR systems to transfusion recipients, to blood center and hospital staff who may be exposed to them, and to the environment in which they are manufactured, used, and in which they are disposed.

Potential toxicologic effects of the candidate PR systems have been examined in many dimensions. These include acute, subacute, and chronic toxicity, blood component incompatibility, genotoxicity, carcinogenicity, and impact on reproduction and development.

In general, the residual levels of active PR ingredients in fully processed blood components are below the limit of detection in available direct toxicity assays due to robust removal steps included in the processes. Also, water-soluble molecules are rapidly excreted with no accumulation in fat. There are, however, diverse reaction products derived from the active agents and establishing large safety margins for these products is difficult due to dose per volume constraints (John Chapman, pers. comm., 2009).

Compatibility with cellular elements and plasma proteins is critical. Neoantigenicity with sensitization to treated components is one aspect. Another is the impact on the recovery, survival, and function of blood elements. It is important to understand whether or not PR will increase transfusion requirements related to loss of therapeutic product, or if processing disturbs the delicate balance in physiologic pathways like coagulation.

All PR methods in development for cellular blood products rely on interactions with microbial nucleic acids, raising the specter of carcinogenicity and mutagenicity, and of adverse impacts on reproduction and development. The study of these potential effects is very difficult, expensive, and time-consuming. While in vitro and animal studies can be completed with reasonable speed and economy and are reassuring when negative, the clinical events of interest are expected to be quite rare and may have very long latent periods until recognition. This raises barriers on the path toward regulatory approvals. Special, more vulnerable populations may need additional focus in clinical studies. These include pregnant and fertile women, the fetus, newborns, and growing children, those requiring massive acute transfusions, and patients who receive chronic transfusion support. There needs to be a level of comfort that those with impaired kidney and liver function are not at elevated risk of adverse events.

Although the companies have been authorized to market their products in many countries (Table 9), adoption and routine use of these technologies has been relatively limited. Blood centers, hospitals, and transfusion services will continually evaluate the cost versus benefit of each of these technologies; at the present time, the most efficient and effective process(es) is unknown.

PATHOGEN REDUCTION AND ITS POTENTIAL FOR EID AGENTS

The potential utility of PR can be appreciated by reviewing the list of 16 agents assigned red, orange, or yellow priority in this exercise. The data cited, either for specific agents or relevant models, suggest that only the vCJD prion of the red agents, and the CWD prion, human parvovirus B19, and HAV among the others, will likely escape inactivation from clinically relevant titers in platelets and plasma with application of the systems being brought forward. The data available for RBCs are not adequate to instill great confidence at this point, but early data certainly support concentrated research efforts. While some see the absence of a single process applicable to all components as a barrier to the use of PR, just the prospect of controlling bacterial contamination of platelets, the most common serious infection associated with contemporary blood transfusion in the developed world, is an example of its potential power. Pathogen reduction would be a useful intervention to reduce transfusion transmission of the agents responsible for babesiosis, Chagas disease, and malaria. Babesiosis from RBC transfusion is widely understood to be more prevalent than published reports have suggested, and antibody to *T. cruzi* testing has been adopted nearly universally in the US while emerging evidence demonstrates that *T. cruzi* transmission may be less common than had been anticipated when decisions were made to pursue donor testing. Deferral for minimal risk of transfusion-transmitted malaria remains a source of serious donor loss. A rational approach to testing for these three parasites may be selective screening strategies, but less than universal screening is a strong disincentive for test builders to bring a donor screening assay through the rigorous and costly regulatory approval process; thus, market forces may prevent or delay test development. Potentially, PR bypasses this “one agent-one test” approach while protecting both recipients and the donor supply. Similarly, if the next agent to emerge as a serious threat is an enveloped virus like HBV, HIV, HCV, and WNV, it is probable that the approaches in development will be robust, prospectively obviating the need for deferrals and testing. It is also anticipated that a thorough review of the currently used donor screening questions and tests will be required to assess their continued need once robust pathogen reduction methods are available. Similarly, well-controlled postmarketing studies will be needed to determine if adverse outcomes occur as a result of widespread use of PR and the impact of PR on transfusion-transmitted disease.

CONFLICT OF INTEREST

Peyton S. Metzel is an employee of Fenwal, Inc. Fenwal, Inc. manufactures InterSol platelet additive solution and

TABLE 9. Development/regulatory status

	Platelets			FFP			
Regulating agency or development status	INTERCEPT	Mirasol	Theraflex UV	INTERCEPT	Mirasol	Theraflex MB	Octaplas
Development Status, US	Phase III Completed	Phase I Completed		Phase III Completed			Phase III Completed
Development Status, Europe	Completed	Completed	Phase I	Completed	Completed	Completed	Completed
US FDA							
Canadian MOH						✓	
CE Mark or MAA*	CE	CE	CE	CE	CE	CE	MAA
For sale In:							
• Argentina						✓	✓
• Australia						✓	
• Austria							✓
• Belgium	✓	✓		✓	✓	✓	✓
• Brazil						✓	✓
• Canada							✓
• Croatia							✓
• Czech Republic	✓			✓			✓
• Finland	✓	✓		✓	✓		✓
• France	✓			✓		✓	✓†
• Germany	✓					✓	✓
• Greece	✓			✓		✓	
• Gulf Central Committee							✓
• Hungary	✓			✓			✓
• Iceland	✓	✓			✓		✓
• Ireland	✓						✓
• Italy	✓	✓		✓	✓	✓	✓
• Kazakhstan		✓			✓	✓	
• Kuwait	✓	✓			✓		✓
• Luxembourg		✓			✓		✓
• Malaysia	✓			✓		✓	
• Mexico							✓
• Netherlands							✓
• New Zealand							✓
• Norway	✓	✓		✓	✓		✓
• Oman							✓
• Poland	✓	✓		✓	✓	✓	
• Portugal	✓	✓		✓	✓		✓
• Romania							✓
• Russia	✓			✓		✓	
• Saudi Arabia		✓			✓		✓
• Singapore	✓					✓	
• Slovakia	✓	✓		✓	✓		✓
• Slovenia	✓			✓			✓
• Spain	✓	✓		✓	✓	✓	
• Sweden	✓	✓		✓	✓		✓
• Switzerland							✓
• Thailand	✓			✓	✓		
• Turkey	✓						
• UAE		✓					✓
• UK						✓	✓
• Vietnam	✓						
• Yemen							✓

* Council of Europe approval or Marketing Authorization Approval. A CE Mark alone is not adequate regulatory authority to market a device in many countries in Europe including the UK, France, and Germany.
† SD-plasma available, similar to Octaplas.

is the contract disposables manufacturer for Cerus Corporation INTERCEPT platelets and plasma. No other conflicts of interest were declared.

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Efficacy of apheresis platelets treated with riboflavin and ultraviolet light for pathogen reduction

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BACKGROUND: Pathogen reduction technologies for platelet (PLT) components offer a means to address continued viral transmission risks and imperfect bacterial detection systems. The efficacy of apheresis PLTs treated with riboflavin (vitamin B2) plus ultraviolet (UV) light (Mirasol, Navigant Biotechnologies) was investigated in a single-blind, crossover study in comparison to untreated PLTs.

STUDY DESIGN AND METHODS: Normal subjects (n = 24) donated PLTs by apheresis on two occasions at least 2 weeks apart. Units were randomized to control or test arms, the latter receiving the addition of 28 mL of 500 µmol per L B2 and exposure to 6.2 J per mL UV light. PLTs were stored for 5 days with biochemical and hematologic analyses performed before and after illumination on Day 0 and at the end of storage. An aliquot of each unit was radiolabeled and returned to determine recovery and survival.

RESULTS: The PLT content of treated units was maintained from Day 0 ($4.1 \times 10^{11} \pm 0.4 \times 10^{11}$) to Day 5 ($4.0 \times 10^{11} \pm 0.4 \times 10^{11}$). Treatment with B2 plus UV light was associated with an increase in lactate production with concomitant increases in glucose consumption. pH (control, 7.38 ± 0.07; test, 7.02 ± 0.10) was well maintained throughout storage. Recovery of treated PLTs (50.0 ± 18.9%) was reduced from that of control PLTs (66.5 ± 13.4%); survival was similarly shortened (104 ± 26 hr vs. 142 ± 26 h; $p < 0.001$).

CONCLUSIONS: PLTs treated with B2 plus UV light demonstrate some alterations in in vitro measures but retain in vitro and in vivo capabilities similar to pathogen-reduced and licensed PLT components that have been shown to have useful clinical applicability. The recovery, survival, and metabolic properties of Mirasol PLTs should provide sufficient hemostatic support in thrombocytopenia to justify patient clinical trials.

Advances in donor screening and testing have reduced the risks of transmission of human immunodeficiency virus and hepatitis C virus by more than 10,000-fold in the past two decades.[1] Diversion and detection methods have been introduced to reduce the risks of bacterial contamination in platelets (PLTs).[2] Despite these efforts, risks of viral and bacterial contamination remain. Furthermore, various parasitic risks have yet to be addressed successfully, including those of Chagas disease, and the evolution or introduction of new viruses into the blood supply may cause significant morbidity and mortality before their recognition can lead to the development and implementation of effective testing.[3] Therefore, important health benefits may accrue with the introduction of pathogen reduction technologies (PRTs) in blood components.

Many recipients of PLT transfusions are especially vulnerable to the presence of contaminating pathogens. Their immunosuppressed and/or neutropenic states may blunt effective response to a pathogen. Although some recipients of PLT transfusions may succumb to their

ABBREVIATIONS: B2 = riboflavin (vitamin B2); PRT(s) = pathogen reduction technology (-ies).

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underlying disorder, the success of more effective treatments for malignancies over the past several decades is eliminated if the patient dies because of a transfusion-transmitted infection. Thus, the means to reduce the infectivity and pathogenic potential of contaminating organisms in PLT components remains an important goal.

The success of a proposed PRT may be judged in terms of safety and efficacy.[4] The treatment must not, in itself, create more risk than it is removing, and it must reduce the presence of viable or infective pathogens to a clinically useful degree. Similarly, the transfusible component must be well tolerated and retain a sufficient proportion of its efficacy (viability and thrombogenicity in the case of PLTs) to achieve the clinical aims of the transfusion.

Riboflavin (vitamin B2) has been shown to interact with genomic material and irreversibly, covalently combine with RNA and DNA to preclude transcription and translation of pathogens' genetic material on exposure to ultraviolet (UV) light and/or to cause breakage of the chromosomal strand.[5-8] The process has been shown to be effective against viruses, bacteria, and parasites with sufficient reductions in infectivity (generally ≥5 log) to provide a useful safeguard for transfusion recipients.[9-14] The low toxicologic concern regarding use of a vitamin to achieve this task has been bolstered by embryofetal, subchronic, and high-dose toxicology studies; pharmacokinetic analysis; neoantigenicity, cytotoxicity, and hemocompatibility experiments; chromosome aberration and Ames tests; and a mouse micronucleus mutation assay, all of which failed to demonstrate results suggestive of human toxicity.[15-20] In vitro analyses of treated, plasma-suspended PLTs indicated good retention of biochemical and functional variables through 5 days of storage following treatment.[21,22]

To move investigation of the effect of treating PLTs with B2 and UV light to the next stage, we conducted a crossover study in blinded normal subjects to determine the in vivo efficacy of treated versus control (untreated) PLTs after 5 days of storage via autologous recovery and survival studies of radiolabeled PLTs.

MATERIALS AND METHODS

The study received the approval of local institutional review boards at each participating location and was conducted under an investigational device exemption from the FDA. Twenty-nine normal, healthy adult subjects (20 men and 9 women, all without reproductive potential) who met all FDA and AABB[23] criteria for PLT donation pertaining to the health of the donor were recruited into the study at two locations (15 at one site; 14 at the other) without regard to previously obtained recovery and survival results. Written informed consent was obtained from subjects. Five subjects did not complete the study owing to inadvertent duplicate illumination of the PLT component, intercurrent illness (influenza), venipuncture difficulties, psychiatric hospitalization (owing to an undisclosed existing condition), and incomplete sample collection; a total of 24 paired data sets (17 men, 7 women) were thus generated.

Subjects donated 1 unit of leukoreduced apheresis PLTs on an automated blood collection system (Trima Accel, Gambro BCT, Lakewood, CO) on two occasions at least 2 weeks apart with a target of 1640×10^3 PLTs per µL in 270 mL of plasma–acid citrate dextrose (ACD)-A. PLTs were placed in ELP bags composed of polyvinyl chloride plasticized with *N*-butyryl tri-*n*-hexyl citrate.[24] Units were held undisturbed immediately after collection for 2 to 3 hours to allow dissociation of any PLT aggregates. The PLT content of each unit was verified to be in the target range of 1400×10^3 to 1880×10^3 PLTs per µL 2 to 4 hours after collection; if necessary, the concentration was reduced to within the allowed range with some of the 150 mL of plasma that had been collected at the time of the plateletpheresis. The protocol allowed for a repeat collection if any unit failed to meet the content criterion or other problems developed that precluded successful analysis and return of the unit.

Units were designated to undergo treatment with B2 plus UV light (Mirasol, Navigant Biotechnologies, Lakewood, CO; treated arm) or not (control arm) according to a statistical package randomization scheme provided by the sponsor. (If subjects were withdrawn from the study, they were replaced by subjects who assumed their place in the randomization table.) At 2 to 4 hours after collection, a volume of 250 ± 5 mL (256 g) of PLTs was gravimetrically transferred from the ELP collection bags to the PLT illumination-storage bag to be either Mirasol-treated or stored without further processing (control). B2 solution (500 µmol/L, 28 ± 1 mL) was added to the test unit through a sterile barrier filter with a syringe. After thorough mixing, a 3- to 5-mL sample was withdrawn for in vitro assessments. The unit was then secured in a prototype illumination device and exposed to 5.0 J per cm^2 of UV light (corresponding to 6.2 J/mL delivered to the PLT unit), a process that took 8 to 10 minutes in the temperature-controlled environment of the illuminator (illuminating wavelengths, 265-370 nm). After the process, a 3- to 5-mL postillumination sample was obtained from the test unit for in vitro assessments. One 3- to 5-mL sample was obtained from the control product for in vitro assessments. The units (control and treated) were stored in the same PLT illumination-storage bag under normal blood banking conditions of 22 ± 2°C with horizontal agitation for 5 days. On Day 3 or Day 4, a 3- to 5-mL sample was taken for bacterial culture to document sterility.[25] At the end of the 5-day storage period, a 3- to 5-mL sample was withdrawn from the products for in vitro assessments and radiolabeling. (All sampling was conducted with a sterile

connecting device. All unit handling and processing was performed at each test site.)

PLTs were labeled with standard techniques before return into the original donor with standard techniques.[26,27] A 10-mL aliquot was removed from the unit, and the PLTs were concentrated by centrifugation. Approximately 100 μCi of the radiolabel (^{111}In-oxine, Medi-Physics, Arlington Heights, IL) were added to a suspension of the PLTs for a 20-minute room temperature incubation. The PLTs were then washed with a mixture of plasma and ACD-saline. By use of a dose calibrator, 10 to 20 μCi of the labeled PLTs was taken up into a syringe for return into the subject. (The remainder was used for standards and quality control procedures.) Samples were taken from the contralateral arm within the first 3 hours after return as well as daily for 1 week (for determination of the proportion of injected label recoverable) and again on Day 10 (to allow for correction of activity associated with red blood cells). ^{111}In emissions were counted at 176 to 190 keV. Recovery was calculated from the injected radioactivity (corrected for preinjection radiolabel elution[27]), a formulaic projection of the subject's blood volume,[27] and back-extrapolation to the time of injection with the multiple-hit model on a standardized computer program[28,29] and verified by a separate calculation with a validated computer program (SAS Institute, Inc., Cary, NC). Survival was recorded as the mean residual life span determined by the multiple-hit model.

In vitro analyses were conducted on Day 0 (before treatment in the test units) and on the last day of storage. PLT concentration was determined from a CBC assessment with a hematology analyzer (Advia 120, Bayer Diagnostics, Tarrytown, NY; or 9110-plus Baker Instruments, Allentown, PA). For this purpose, a sample was transferred to an ethylenediaminetetraacetate tube, which was rotated at 22 ± 2°C until the sample was analyzed. The lactate and glucose concentrations were determined on an automated lactate-glucose analyzer (either the ABL-705, Radiometer-America, Westlake, OH; or the Hitachi 917, Hitachi High-Technologies, Tokyo, Japan). The rates of lactate production and glucose consumption during the 5 days of storage were calculated with a concentration normalized to the initial PLT concentration. Unit pH was analyzed on an automated blood gas analyzer (ABL-705, Radiometer-America; and Model 855, Bayer Diagnostics) at 37°C and converted to $pH_{22°C}$ algebraically. O_2 and CO_2 partial pressures were analyzed on an automated blood gas analyzer (ABL-705, Radiometer-America; or Model 248 or 855, Bayer Diagnostics). Expression of CD62 or GMP-140 (P-selectin) was determined by flow cytometry within 14 days after fixation in 1 percent paraformaldehyde per the method recommended by the Biomedical Excellence for Safer Transfusion (BEST)[30] based on fluorescence intensity. PLT morphology was characterized with a visual swirl scoring technique with a three-tiered scoring system: positive swirl (inhomogeneity readily visible throughout the entire bag, 3), intermediate swirl (some inhomogeneity visible, 1), and negative swirl (turbid, 0).

Results are expressed as means ± 1 standard deviation (SD). Statistical analysis was conducted comparing control versus treated units with a t test statistic with a p value of less than 0.05 used to reject the null hypothesis of no difference. Paired analyses were applied wherever appropriate.

RESULTS

All subjects participating in the study had no adverse events related to their participation. All PLT returns were well tolerated. All unit cultures were negative. Ten repeat apheresis procedures were performed in nine subjects. Five repeat apheresis collections were required in the experimental arm and four were required in the control arm owing to factors such as intercurrent illness precluding return and handling of units in manners not consistent with the study design. One apheresis procedure had to be aborted because of infiltration.

The PLT concentrations were 10 percent lower in the treated units reflecting the dilution caused by the addition of the B2 but total PLT counts remained unchanged (Fig. 1). In addition, there was no difference in PLT count between before treatment to the end of storage in the treated units.

Treated units demonstrated accelerated glycolysis with lower glucose concentration at the end of storage, higher lactate concentration, and increased rates of consumption of glucose and production of lactate (Figs. 2A and 2B). There was, however, residual glucose in all units at the end of the storage period, and the pH value, although lower in treated units, remained above 6.8 in all units (Fig. 2E). Blood gas measurements showed a decrease in pO_2 and pCO_2 during the storage period

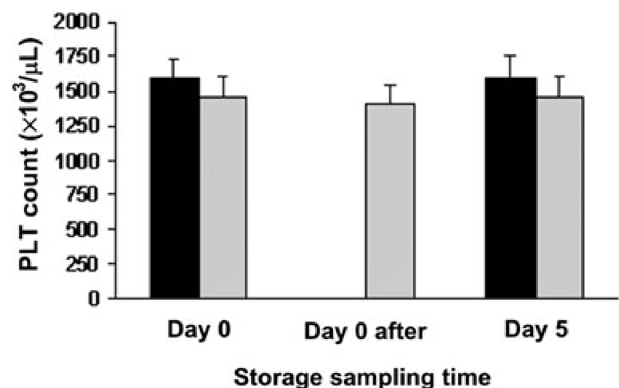

Fig. 1. PLT concentration. Results of testing before and after treatment (Day 0) and at end of storage (Day 5). (■) Mean of control units; (□) treated units. Vertical bars represent 1 SD.

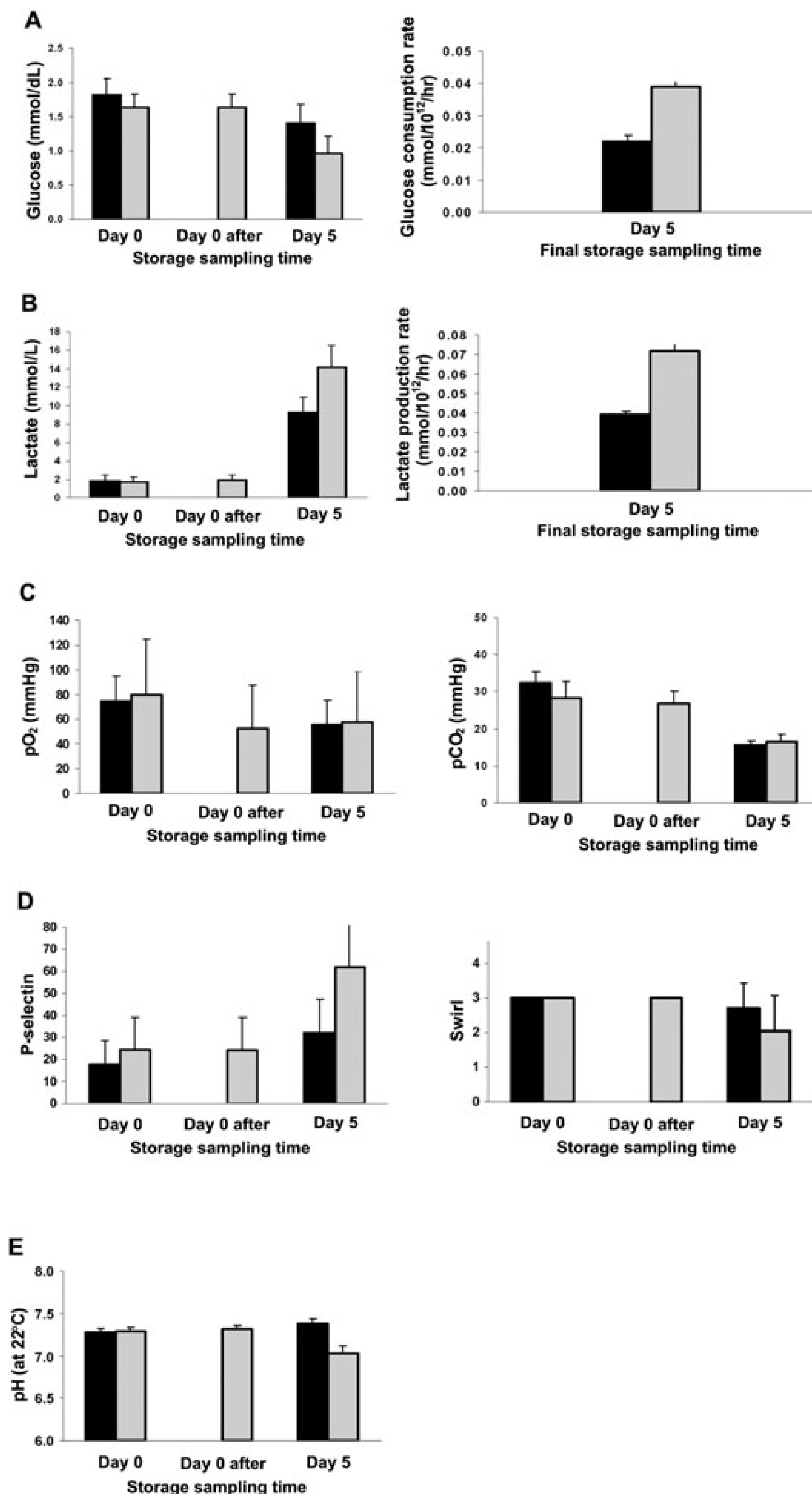

Fig. 2. Results of testing before and after treatment (Day 0) and at end of storage (Day 5). (■) Mean of control units; (□) treated units. Vertical bars represent 1 SD. (A) Glucose concentration and consumption rate. (B) Lactate concentration and consumption rate. (C) Oxygen and carbon dioxide partial pressures. (D) P-selectin expression and swirling. (E) Unit acidity.

(Fig. 2C). The Day 0 and Day 5 results were not different between control and treated units' pO_2; pCO_2 was significantly lower in test units before treatment but higher at the end of storage. P-selectin expression increased throughout storage in both groups of units and was higher on Day 5 in test than control units (Fig. 2D). The assessment of swirling was not different at the beginning of storage but was lower on Day 5 in treated units. The differences in in vitro measures between control and treated units at the end of the storage period (as shown in the figures) were all significant except for pO_2 and pCO_2.

Radiolabel was taken well by both test and control PLTs on Day 5 (Table 1). The recovery of radiolabeled PLTs from two subjects' control units exceeded biologic possibility (i.e., recovery >100%) for reasons that could not be determined. These control units' in vivo results were excluded from further analysis, but the treated units' results were included in (unpaired) analyses. The mean recovery and survival of treated PLTs were reduced when compared with the recovery and survival of the control units (Table 1).

Possible relationships between in vitro measures and in vivo PLT recovery were analyzed by means of linear regression across both types of units (Table 2). The best correlations with in vivo recovery were seen for the rate of glucose consumption ($p = 0.0000$), lactate concentration ($p = 0.0000$), rate of lactate production ($p = 0.0001$), pH ($p = 0.0002$), glucose concentration ($p = 0.0031$), and P-selectin ($p = 0.0078$). The in vitro variable that correlated most closely with survival was Day 5 pH ($p = 0.0021$). With stepwise multiple regression, recovery and survival were each fitted against all in vitro variables. It should be noted that there are strong correlations between many of the in vitro variables themselves, which has a confounding effect on obtaining a definitive model. In the case of recovery, the final regression model obtained by the stepwise regression method contained only the rate of glucose consumption. In the case of survival, pH was the only variable main-

tained in the model. When these analyses were repeated by also including treatment (control vs. treated) in the model, only the rate of glucose consumption remained as a significant predictor of recovery. In the model for survival, only treatment was significant.

DISCUSSION

This randomized crossover study in normal subjects documented that treatment of leukoreduced apheresis PLTs with B2 plus UV light to effect pathogen reduction causes a reduction in radiolabeled autologous recovery and survival but retention of sufficient in vivo efficacy to predict clinical utility. The in vitro analyses indicated that the treated PLTs were undergoing glycolysis at an accelerated rate compared to untreated controls. This metabolic substrate, however, was not exhausted during a 5-day storage period, and the acidity of the treated units, although greater than for control units, did not exceed the capabilities of the PLTs to maintain functional metabolism. One may speculate that a "hypermetabolic state" may be induced by the treatment, but the magnitude of this does not preclude continued viability of the cells. The effect of the treatment process was also seen through increased P-selectin expression and reduced swirling at the end of the storage period in comparison with untreated control units. Importantly, the PLT concentrations, although lowered in the test units by the addition of 28 mL of solution containing the B2, were well maintained through treatment and storage. Thus, although all systems that utilize UV light treatment as part of PRT appear to have negative effects on PLT physiology,[31] the Mirasol system appears to have less of an effect on the number of PLTs available remaining in the unit through the treatment process.

The reductions in recovery and survival can be assessed in several different ways. Because the laboratories participating in this study use the same radiolabeling procedure and have participated jointly in other studies, the results of this study can be usefully compared. The recoveries and survivals reported here for the control units were very similar to those reported in a study on (untreated) leukoreduced PLTs after 5 days of storage (recovery, 63.0 ± 11.2%; survival, 161 ± 38 hr)[32] giving confidence that the variability introduced into such analyses over time and over different subjects is minimal when a standardized protocol is used. Furthermore, the recovery of untreated PLTs stored for 7 days (53.9 ± 13.8%), which was adequate for FDA acceptance of approval for 7-day storage on Trima PLTs, was very close to that observed with treated PLTs stored for 5 days in this trial (50.0 ± 18.9%). (The survival of treated PLTs at 5 days of storage, 104 ± 26 hr, was shorter than that of untreated PLTs at 7 days, 134 ± 45 hr, $p < 0.05$.)

Both of these proposed modifications to PLT systems (extended storage to reduce outdating and pathogen reduction treatment to reduce infectious risks) offer useful advantages but obviously carry with them certain downsides, including reduced efficacy. A trade-off is inevitable in such situations, and value judgments will need to be made to determine whether a reduction of in vivo efficacy is mitigated by reducing the risk of certain pathogens. It is, however, encouraging to note that Mirasol PLTs at 5 days of storage appear similar to untreated PLTs at 7 days of storage, an approach that has been shown to have clinical utility.[25] Because comparison of a new approach to PLT handling (such as PRT) to a previously licensed method may lead to "creeping inferiority" via repetitive comparisons,

TABLE 1. Results of radiolabeling studies*

	Control	Treated	p value
Radiolabel uptake efficiency (%)	64.2 ± 17.2	59.5 ± 21.2	>0.05
Recovery (%)	66.5 ± 13.4	50.0 ± 18.9	<0.05
Survival (multiple hit, hr)	142 ± 26	104 ± 26	<0.05

* Results of Day 5 radiolabeling with ^{111}In and autologous return. Results shown are 22 observations from the control cycle and 24 from the treated cycle.

TABLE 2. Correlation via linear fit of Day 5 in vitro measures with recovery and survival

	Correlation with recovery			Correlation with survival		
Measure	Correlation coefficient (r)	F value	p value	Correlation coefficient (r)	F value	p value
PLT concentration	0.3235	5.1422	0.0283	0.1651	1.2327	0.2729
Glucose concentration	0.4269	9.8054	0.0031	0.3514	6.1989	0.0166
Glucose consumption rate	–0.5981	24.5106	0.0000	–0.3505	6.1619	0.0169
Lactate concentration	–0.5836	22.7236	0.0000	–0.3564	6.4036	0.0150
Lactate production rate	–0.5608	20.1895	0.0001	–0.3652	6.7707	0.0126
$pH_{22°C}$	0.5263	16.8536	0.0002	0.4426	10.7206	0.0021
pO_2	–0.2061	1.9527	0.1693	–0.0725	0.2325	0.6320
pCO_2	–0.0829	0.3047	0.5838	–0.0272	0.0325	0.8578
P-selectin expression	–0.3875	7.7734	0.0078	–0.1767	1.4179	0.2401
Swirling	0.1275	0.7270	0.3985	0.2704	3.4710	0.0691

an alternative approach to evaluating in vivo efficacy has been suggested wherein the recovery and survival of PLTs handled in the new system are compared to fresh PLTs from the same subject.[33] This proposal, which requires the "test" PLTs to demonstrate at least two-thirds the recovery and one-half the survival of fresh PLTs in the same subject, has been successfully validated as providing a realistic and reproducible benchmark.[34,35] The current study was designed before the proposal for comparison with fresh PLTs. Use of a standardized protocol for preparation of the fresh PLTs yields recoveries in the range of 60 to 65 percent and survivals approximating 200 hours.[26,36] Applying the target proportions, one would expect that acceptable performance would fall into the range of 40 to 44 percent recovery and approximately 100 hours of survival. The Mirasol-treated PLTs prepared in this study would thus appear to meet the new standard, but a direct comparison with noninferiority statistical comparison would, of course, be needed to verify this.[37]

A similar study has recently been published with amotosalen and UV light to effect PRT in PLTs.[38] A direct comparison of in vivo results would suggest that Mirasol-treated PLTs offered improved recovery and survival, although technical differences cannot be excluded as the source of this.

This study has documented the effects of applying a PRT system for PLTs that has a low potential for toxicity. The system yields PLTs that clearly show the effect of the treatment, in terms of altered biochemical analyses and reduced recovery and survival, but that retain sufficient viability and functionality to predict their clinical utility. This study therefore would suggest that clinical trials to investigate the effectiveness of Mirasol PLTs in thrombocytopenic patients are warranted.

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Assessment of the genotoxic potential of riboflavin and lumiflavin

A. Effect of metabolic enzymes

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Summary

The mutagenic potential of riboflavin and its photodegradation product lumiflavin was evaluated using the *umu* test, SOS chromotest and Ames Salmonella assay. Both riboflavin and lumiflavin by themselves were found to be non-mutagenic. On treatment with rat liver microsomal enzymes (S9) or caecal cell-free extract (CCE), lumiflavin acquired mutagenicity, while the status of riboflavin remained unaffected. Activation of lumiflavin by metabolic enzymes was found to result in an alteration of its spectral characteristics.

Riboflavin, a vitamin, has found widespread application in food products both as a nutrient and as a colouring agent (Counsell et al., 1981). Unlike many other food colourants it has commanded unquestioned favour from regulatory agencies all over the world (Horwitt, 1972; FAO, 1981), since earlier toxicological studies have failed to reveal any deleterious effects (Purchase et al., 1978; Haveland-Smith, 1981). However, evidence obtained by many other investigators has indicated that riboflavin when exposed to light could produce mutagenic (Griffin et al., 1981; Bradley and Sharkey, 1977; Pathak and Carbonare, 1988) as well as cytotoxic effects (Lee, 1969; Misra et al., 1987, 1990) in eukaryotic cells. Synergistic effects of riboflavin with light have also been shown to bring about alterations in DNA and in individual nucleotides in vitro (Uehara, 1966; Speck et al., 1976; Korycka-Dahl and Richardson, 1980; Alvi et al., 1984). These studies clearly indicated the toxic potential of riboflavin. However, the molecular mechanisms associated with the interaction of riboflavin or its photodegradation product lumiflavin with living cells are poorly understood.

In the present report an attempt has been made to assess the mutagenic potential of riboflavin as well as its photodegradation product lumiflavin using three short-term tests, namely the *umu* test, SOS chromotest and Ames/Salmonella assay.

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Materials and methods

Riboflavin (7,8-dimethyl-10-(D-ribityl) isoalloxazine) was a kind gift from Glindia Ltd. (Bombay, India). Lumiflavin (7,8,10-trimethyl isoalloxazine), 4-nitroquinoline-*N*-oxide (4NQO), 9-aminoacridine (9AA), mitomycin C (Mit C), nalidixic acid, benzo[*a*]pyrene (Bap), rutin, nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (G-6-P), L-histidine · HCl, biotin, *p*-nitrophenyl phosphate (PNPP), sodium dodecyl sulfate (SDS) and *O*-nitrophenyl-α-D-galactopyranoside (ONPG) were purchased from Sigma Chemical Company (St. Louis, MO, USA), 4-dithio-DL-threitol (DTT) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) were acquired from Fluka AG (Germany). Aroclor 1254 was obtained from Monsanto (St. Louis, MO, USA). Nutrient broth, tryptone and yeast extract were acquired from Difco Laboratories (Detroit, MI, USA). All other chemicals and solvents were of AR grade, the latter were distilled before use. The purity of riboflavin and lumiflavin was ascertained by TLC using silica gel with two different solvent systems, viz. pyridine–glacial acetic acid–water (10 : 1 : 40, v/v) and *n*-butanol–acetic acid–water (40 : 10 : 50, v/v).

Salmonella typhimurium TA100, TA98 and TA97a were kindly provided by Prof. Bruce N. Ames (University of California, Berkeley, CA, USA). *Salmonella typhimurium* TA1535/psk 1002 was obtained from Dr. Yoshimitsu Oda (Osaka Prefectural Institute of Public Health, Nakamachi-1, Japan) while *Escherichia coli* PQ37 was acquired from Dr. Maurice Hofnung (Institut Pasteur, Paris, France). Characteristics of the strains were confirmed and frozen permanents prepared according to the published protocols (Maron and Ames, 1983; Quillardet and Hofnung, 1985). Working stock cultures of Ames strains were maintained on nutrient agar slants and those of other strains on Luria agar slants and were stored at 0–4°C until use.

Preparation of liver S9 fraction

Male Wistar rats weighing 200–250 g were injected intraperitoneally (500 mg/kg body weight) with Aroclor 1254 (200 mg/ml DMSO). On the fourth day, feed was removed and on the following day the animals were killed by cervical dislocation. Livers were removed under sterile conditions into ice-cold 0.15 M KCl and S9 fraction was prepared according to the method of Maron and Ames (1983). The tissue was homogenised in 0.15 M KCl using a Potter Elvehjem glass homogeniser with 5 up and down strokes of a teflon pestle (25% homogenate). The homogenate was then centrifuged at $9000 \times g$ for 10 min and the supernatant (S9 fraction) was decanted and was dispensed in 2-ml aliquots in sterile plastic tubes which were frozen quickly in liquid nitrogen and stored at −80°C until use. The S9 mixture was prepared freshly before use (Ames et al., 1975) by incorporating 4 mM NADP, 8 mM $MgCl_2$ and 33 mM KCl to a 1 : 10 diluted fraction. Dilution for SOS chromotest was carried out with Luria broth instead of a buffer. The mixture was sterilised using a 0.45-μm Millipore filter. The metabolic activity of S9 was ascertained using the Ames/Salmonella assay (Ames et al., 1975). After activation benzo[*a*]pyrene exhibited 900 additional revertants.

Caecal cell-free extract

Caecal cell-free extract (CCE) was prepared following the procedure of Brown and Dietrich (1979). Male Wistar rats weighing about 200–250 g were killed by cervical dislocation and the caecum was removed. The caecal contents were suspended in cold Krebs-Ringer phosphate buffer, pH 7, containing 2.5 mg/ml DTT for the Ames/Salmonella assay or *umu* test or in Luria broth for the SOS chromotest. The suspensions were homogenised in a Potter Elvehjem homogeniser and sonicated for 5 min (Sonics and Materials Inc.). The sonicated extract was centrifuged at $13{,}000 \times g$ for 15 min and the supernatant was filtered through a Millipore filter (0.45 μm) and stored at −80°C until use. CCE-activated rutin showed 200 revertants/100 mg/plate with *Salmonella typhimurium* TA98.

Mutagenicity assays

(1) Umu test. The general screening of mutagenicity of riboflavin and lumiflavin was carried out using the *umu* test (Oda et al., 1985). The system consisted of 0–100 μg/ml of test com-

pounds, 2.5 ml of exponentially growing cells (OD_{600} adjusted to 0.25–0.3) and 33 mM phosphate buffer, pH 7.4. The mixture was incubated at 37°C for 180 min and was centrifuged at 3020 $\times g$ for 10 min. The pellet was washed 3 times with 0.1 M phosphate buffer, pH 7.4, resuspended in 3 ml of the same buffer and OD_{600} was determined. 0.2 ml of this suspension was added to 1.8 ml of Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl and 1 mM $MgSO_4$, pH 7) followed by 50 μl SDS (0.1%) and 10 μl chloroform. After mixing it thoroughly, 0.2 ml ONPG solution (4 mg/ml in 0.1 M phosphate buffer, pH 7) was added to the reaction mixture and incubated at 28°C for 20 min. The reaction was stopped by the addition of 1 ml of 1 M Na_2CO_3 and the absorbances at 420 nm and 550 nm were determined. The activity of β-galactosidase was calculated according to Miller (1972). The effect of metabolic enzymes on riboflavin and lumiflavin was assessed by incorporating 0.5 ml of S9 or CCE in the original incubation mixture.

(2) SOS chromotest. The assay was performed using the procedure of Quillardet and Hofnung (1985) with minor modifications. 0.5 ml of an 18-h-old culture was diluted to 5 ml with Luria broth (1% Bacto tryptone, 0.5% yeast extract, 1% sodium chloride and 0.002% ampicillin) and incubated with shaking at 37°C for 3 h; the optical density at 600 nm was adjusted to 0.25–0.3 (2×10^5 cells/ml). The cell suspension was diluted to 10 ml with Luria broth and 0.6 ml of the diluted cell suspension was incubated with 0–100 μg/ml of test compounds at 37°C for 120 min and centrifuged at 3020 × g for 10 min. The pellet was washed 3 times with 0.1 M phosphate buffer, pH 7.4, resuspended in 0.6 ml of the same buffer and activities of alkaline phosphatase and β-galactosidase were determined. The enzyme activities were calculated according to the formula of Quillardet and Hofnung (1985). The effect of metabolic enzymes on riboflavin and lumiflavin was assessed by incorporating 5 ml of S9 or CCE to 5 ml of cells in place of Luria broth.

Ames / Salmonella assay. The liquid preincubation procedure was adopted for determining the mutagenicity of riboflavin and lumifavin (Maron and Ames, 1983). The incubation mixture consisting of 0.2 ml of a 16–18-h-old culture of tester strains, *Salmonella typhimurium* (TA100, TA98 and TA97a), 0–100 μg/ml test compounds and 0.1 ml PBS, pH 7.4, was incubated at 37°C for 30 min and centrifuged at 3020 $\times g$ for 10 min. The pellet was resuspended in 0.4 ml of 0.1 M PBS, pH 7.4. To this suspension 2 ml molten soft agar was added, mixed rapidly and spread immediately on preset minimal agar plates. The activation potential of the metabolic enzymes on riboflavin and lumiflavin was assessed by repeating the above assay in the presence of 0.1 ml S9 or CCE.

Effect of metabolic enzymes on riboflavin and lumiflavin

Aliquots of riboflavin or lumiflavin (160 μg/ml) were incubated with 3 ml S9 or CCE at 37°C for 60 min. After incubation lumiflavin was extracted with 3 ml chloroform, the solvent was evaporated off under nitrogen, the dried residue was redissolved in methanol and analysed by spectral scan and TLC. Riboflavin was extracted with amyl alcohol and quantitated by spectral scanning and TLC in the same solvent.

Protein

The protein contents of the S9 fraction and CCE were assessed using Lowry's method with bovine serum albumin as the standard (Lowry et al., 1951).

Results and discussion

Data on the mutagenicity of riboflavin and lumiflavin evaluated with the *umu* test, SOS chromotest and Ames/Salmonella assay are presented in Tables 1, 2 and 3 respectively. It was observed that riboflavin did not demonstrate any mutagenic response, before and after exposure to metabolic enzymes. These results are in agreement with the earlier reports on the non-genotoxicity of riboflavin by Purchase et al. (1978), Haveland-Smith (1981) and Combes and Haveland-Smith (1981).

In contrast to this, lumiflavin after activation by S9 and CCE showed significant mutagenicity in the *umu* test, SOS chromotest and Ames/

TABLE 1

ASSESSMENT OF GENOTOXIC POTENTIAL WITH THE *UMU* TEST

Compound (μg/ml)	β-Galactosidase activity[a] (Units/OD_{600})		
	1	2	3
0	97.00 ± 4.00	136.50 ± 4.10	137.66 ± 1.88
Riboflavin			
25	98.00 ± 1.40	144.13 ± 3.05	136.13 ± 4.07
50	101.10 ± 1.82	144.06 ± 3.61	142.80 ± 4.38
100	94.28 ± 3.30	134.23 ± 3.65	138.66 ± 5.92
Lumiflavin			
25	142.66 ± 4.26	153.31 ± 2.25	183.06 ± 5.92
50	130.20 ± 3.93	230.10 ± 6.68	236.18 ± 2.19
100	129.33 ± 7.05	267.43 ± 8.09	247.80 ± 2.76

The values represent mean ± SD of 4 independent experiments.

[a] Activity of β-galactosidase is calculated as units = $A_{420} \times 1000$/time.

1, Without S9 or CCE; 2, with S9; 3, with CCE.

Salmonella assay (Tables 1, 2 and 3). Lumiflavin per se was non-mutagenic in any of the test systems. However, it showed mutagenic response after activation by metabolic enzymes. In the presence of S9 or CCE, lumiflavin elicited a 2-fold increase in β-galactosidase activity in the *umu* test as well as in the SOS chromotest. S9-mediated activity of lumiflavin to an extent of 1.9, 2.9 and 4.8 times was also observed in *Salmonella typhimurium* TA100, TA98 and TA97a respectively in the Ames/Salmonella assay (Table 3). Lumiflavin after exposure to CCE showed mutagenic response in strain TA97a by increasing the histidine revertants to 3 times the spontaneous revertants while the other two strains remained unaffected. To our knowledge this is the first report on the genotoxic potential of a photodegradation product of riboflavin. Many compounds such as polyaromatics, aromatic amines, acridines and azo compounds are known to bring about frameshift mutations in the Ames test with and without metabolic activation (von der Hude et al., 1988). Ohta et al. have shown that the SOS function-inducing activity of 2-aminoanthracene increased markedly in the presence of S9 mix. The significance of metabolic enzymes in bringing about mutation in the Ames test is also highlighted by Prival and Mitchell (1982). Although the precise mechanism underlying lumiflavin-induced mutagenicity is not clear at present, the investigations of Kuratomi and Kobayashi (1977) with isolated DNA molecules indicated an interaction of lumiflavin with polynucleotides, especially with poly(G). They have suggested that these interactions could be ascribed to the possible intercalations of flavins with the DNA bases.

TABLE 2

ASSESSMENT OF GENOTOXIC POTENTIAL WITH THE SOS CHROMOTEST

Compound (μg/ml)	β-Galactosidase[a] units			Alkaline phosphatase[a] units			Ratio β-gal/AP			Induction factor[b]		
	1	2	3	1	2	3	1	2	3	1	2	3
0	1.49 ± 0.55	2.10 ± 0.52	1.81 ± 0.27	10.50 ± 1.00	11.00 ± 3.60	10.90 ± 1.80	0.14	0.19	0.16	1.00	1.00	1.00
Riboflavin												
25	1.66 ± 0.57	2.00 ± 0.50	2.10 ± 0.45	10.40 ± 0.50	10.30 ± 4.20	11.20 ± 2.50	0.15	0.19	0.18	1.00	1.00	1.10
50	2.10 ± 0.45	1.80 ± 0.52	1.80 ± 0.26	10.80 ± 1.30	10.10 ± 4.20	10.70 ± 3.70	0.19	0.17	0.16	1.30	0.89	1.00
100	2.20 ± 0.57	1.75 ± 0.25	1.60 ± 0.23	10.70 ± 2.8	10.70 ± 1.40	11.20 ± 2.40	0.20	0.16	0.14	1.40	0.84	0.87
Lumiflavin												
25	1.56 ± 0.40	3.67 ± 0.58	3.90 ± 0.36	11.40 ± 4.40	10.90 ± 2.80	10.30 ± 0.98	0.14	0.33	0.28	1.00	1.73	1.75
50	1.60 ± 0.36	4.00 ± 1.00	3.26 ± 0.25	10.60 ± 0.65	10.40 ± 1.60	10.50 ± 0.50	0.15	0.38	0.31	1.00	2.00	1.93
100	1.80 ± 0.34	4.20 ± 0.26	3.60 ± 0.36	11.30 ± 2.20	10.40 ± 1.20	11.00 ± 0.85	0.15	0.40	0.32	1.00	2.10	2.00

Values represent mean ± SD of 4 independent experiments.

[a] Activities of β-galactosidase and alkaline phosphatase are calculated as units = $A_{420} \times 1000$/time.

[b] Induction factor = R at tested concentration/R at concentration zero.

1, Without S9 or CCE; 2, with S9; 3, with CCE.

TABLE 3

ASSESSMENT OF GENOTOXIC POTENTIAL WITH THE AMES/SALMONELLA ASSAY

Compound (μg/ml)	Number of revertants								
	TA100			TA98			TA97a		
	1	2	3	1	2	3	1	2	3
0	104.40 ± 15.60	85.66 ± 21.30	68.30 ± 13.50	26.22 ± 4.10	21.80 ± 5.30	23.00 ± 5.70	105.30 ± 12.41	120.10 ± 31.19	119.20 ± 20.80
Riboflavin									
25	73.37 ± 12.80	81.00 ± 22.50	78.16 ± 32.50	18.25 ± 6.60	25.40 ± 3.90	19.50 ± 5.50	101.00 ± 10.80	99.12 ± 12.50	105.12 ± 19.70
50	79.70 ± 17.10	77.12 ± 19.85	72.00 ± 23.50	17.25 ± 4.10	21.66 ± 7.90	23.20 ± 3.80	111.50 ± 28.40	143.60 ± 27.40	126.80 ± 24.70
100	85.50 ± 16.80	67.40 ± 23.19	87.80 ± 14.30	23.00 ± 7.30	22.50 ± 5.0	24.10 ± 3.90	88.00 ± 5.70	127.25 ± 15.40	106.80 ± 10.22
Lumiflavin									
25	95.25 ± 32.20	139.10 ± 33.20	71.50 ± 0.70	28.60 ± 3.70	35.10 ± 4.50	25.40 ± 3.80	186.00 ± 20.21	197.80 ± 29.73	280.40 ± 46.70
50	92.33 ± 12.61	146.33 ± 24.33	97.60 ± 19.90	20.85 ± 4.37	48.33 ± 7.80	24.00 ± 3.39	177.10 ± 28.41	488.18 ± 108.70	295.40 ± 61.80
100	60.28 ± 12.20	163.37 ± 26.40	66.60 ± 15.20	25.50 ± 5.80	63.40 ± 12.80	22.40 ± 2.30	172.90 ± 16.20	583.12 ± 69.90	360.00 ± 49.50

Values represent mean ± SD of 6 independent experiments.
1, Without S9 or CCE; 2, with S9; 3, with CCE.

TABLE 4

TLC OF RIBOFLAVIN AND LUMIFLAVIN AFTER EXPOSURE TO THE MAMMALIAN METABOLIC ENZYMES S9 OR CCE

Sample	Rf
Riboflavin	0.71
Lumiflavin	0.63
Rb + S9	0.71
Rb + CCE	0.72
Lm + S9	0.62
Lm + CCE	0.63

Riboflavin and lumiflavin were separated by TLC using the solvent system pyridine–glacial acetic acid–water (20:2:80, v/v).

Such intercalations can lead to alteration in the reading frame of DNA culminating in frameshift mutations (Auerbach, 1976). Our results do indicate a positive mutagenic response (Table 3) in tester strains TA98 and TA97a which detect frameshift mutations. Observation with tester strain TA100 points to yet another mode of DNA damage by lumiflavin, namely base-pair substitution. Different mechanisms such as replacement of bases can lead to base-pair substitution (Stanier, 1976).

The treatment with the metabolic enzymes S9 and CCE did not alter Rf values of riboflavin (0.72) and lumiflavin (0.63) (Table 4). No spectral change was observed with riboflavin, before or after the treatment with metabolic enzymes (Fig. 1). The absorption maximum of lumiflavin at 221 nm, however, was found to shift to 232 nm (Fig. 2) and to 229 nm respectively on exposure to S9 and CCE (Fig. 3).

It is interesting to note that lumiflavin expresses mutagenicity only after its activation by metabolic enzymes. Enzymes of S9 and CCE are known to activate a variety of chemicals by epoxidation, oxidation, reduction, hydroxylation, acetylation and by conjugation reactions (Bartsch et al., 1982). The near identical spectral characteristics (Fig. 3) and Rf values (Table 4) observed for riboflavin and lumiflavin before and after the treatment with metabolic enzymes suggest that these compounds did not undergo major structural alterations during activation. However, the

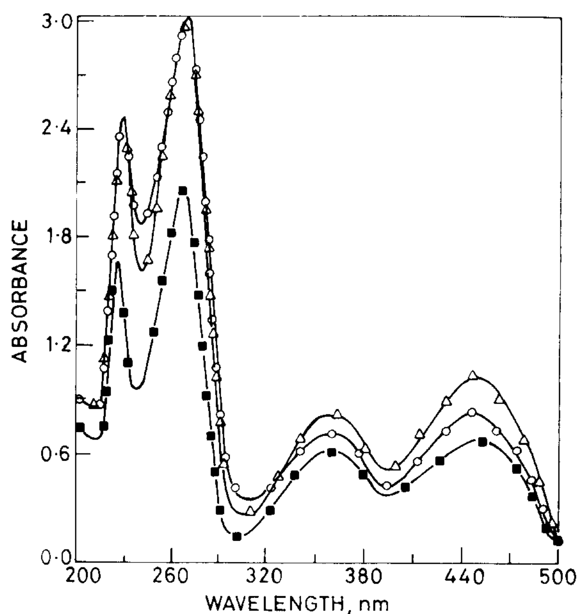

Fig. 1. Effect of S9 and CCE on the spectral characteristics of riboflavin. ■, Riboflavin; △, riboflavin + S9; ○, riboflavin + CCE. Details regarding the treatment of riboflavin with S9 and CCE and the extraction of the products are given in Materials and methods.

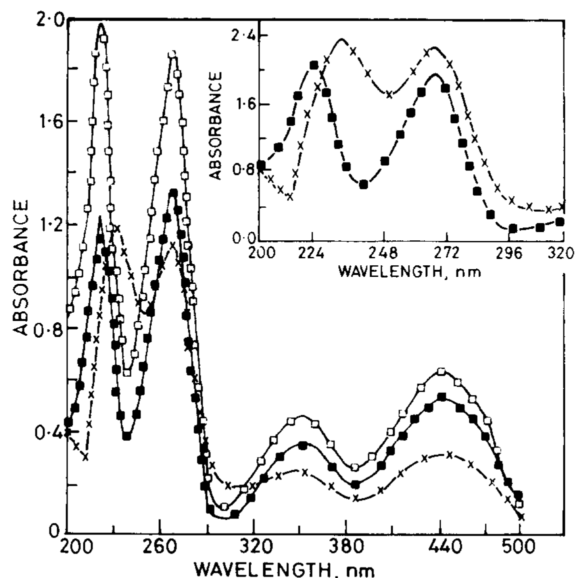

Fig. 2. Effect of S9 on the spectral characteristics of lumiflavin. ■, Chloroform extract of lumiflavin; □, standard lumiflavin; ×, lumiflavin + S9. Details regarding the treatment of lumiflavin with S9 and the extraction of the products are given in Materials and methods. The spectral pattern at 200–320 nm is expanded in the inset.

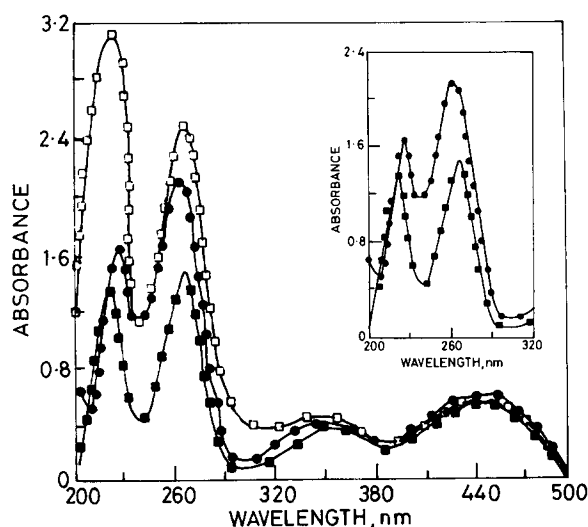

Fig. 3. Effect of CCE on the spectral characteristics of lumiflavin. ■, Chloroform extract of lumiflavin; □, standard lumiflavin; ●, lumiflavin + CCE. Details regarding the treatment of lumiflavin with CCE and the extraction of the products are given in Materials and methods. The spectral pattern at 200–320 nm is expanded in the inset.

minor (10 nm) shift of peak in the case of lumiflavin indicated that the changes may not be extensive (Figs. 2, 3 and 4). Other modifications such as tautomerisation or the production of reactive oxygen species which has the potential to induce mutagenicity may also be involved (Winston and Cederbaum, 1983; Albano et al., 1988).

The foregoing discussion clearly indicated that lumiflavin, a photodegradation product of riboflavin, can induce mutagenicity. This is particularly significant because many naturally occurring food products contain riboflavin which has the potential to produce lumiflavin-like compounds (Holmstrom, 1964). Findings in our own laboratory have shown that exposure of riboflavin to sunlight in aqueous model systems as well as in natural food products like milk can lead to its conversion to form lumiflavin.

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Technical Note

Separation, Identification and Quantification of Riboflavin and its Photoproducts in Blood Products using High-performance Liquid Chromatography with Fluorescence Detection: A Method to Support Pathogen Reduction Technology¶

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ABSTRACT

A medical device using riboflavin (RB) and light is being developed for the reduction of pathogens in platelet concentrates (MIRASOL™ pathogen reduction technology [PRT]). A high-performance liquid chromatography (HPLC) method for the quantification of RB and its main photoproduct, lumichrome (LC) in blood components has been developed and validated. In addition, the same method has been used to identify and quantify the presence of additional photoproducts–catabolites of RB. Levels of these agents before and after treatment as well as endogenous levels present in normal donor blood are reported using this analytical technique. The method allows for quantitative and qualitative analysis of RB and LC in blood components using HPLC-fluorescence detection, a Zorbax® SB-CN (stable bond cyano) column and a methanol–water mobile phase. Quantitation and qualitative analysis of additional photoproducts of RB was also performed, but the method has not been validated for these other components. The method described has passed an 8 day validation and has been found to be adequate for its intended use. The range of the method for RB is 0.016–1.500 μ*M* and for LC is 0.060–1.500 μ*M*. The method detection limit for RB is 0.0006 μ*M* and for LC is 0.012 μ*M*. The acceptance criteria for repeatability were met; the relative standard deviation for RB was 0.64% and for LC was 0.76%. The acceptance criteria for bias were met with a 97% average recovery for RB and a 102% recovery for LC. Samples were centrifuged and diluted 1:50 with 0.9% saline before analysis. No protein precipitation or extraction was required. A mass balance of approximately 93.4–94.4% was achieved after exposure of products to UV light in the intended pathogen reduction treatment method. The method permitted the identification of photoproducts in blood that were both naturally occurring and produced after photolysis of blood samples treated with the PRT process. The identity of these photoproducts has been established using HPLC Tandem Mass Spectrometry (MS/MS) and UV spectroscopic methods and has been correlated with known metabolites and catabolites of RB. HPLC with fluorescence detection using a reverse phase cyano-column allows for accurate separation, identification and quantification of both RB and LC in blood products without the need for solvent extraction or protein precipitation. Additional photoproducts could also be identified and quantified using this method. The presence of these agents in normal, untreated blood suggests that their presence in blood is ubiquitous.

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Abbreviations: FAD, flavin adenine dinucleotide; FMF, formylmethyl flavin; FMN, flavin mononucleotide; HIV, human immunodeficiency virus; ^{1}H NMR, proton nuclear magnetic resonance; HPLC, high-performance liquid chromatography; HPLC-MS/MS, high-performance liquid chromatography-Tandem Mass Spectrometry 2KF, 2′ keto-flavin; 4KF, 4′ keto-flavin; LC, lumichrome; PRT, pathogen reduction technology; RB, riboflavin; TCA, trichloroacetic acid; UV-VIS, UV–visible.

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INTRODUCTION

The collection, separation and transfusion of red blood cells, platelets, whole plasma and fractionated plasma components are mainstays of our health care system. Each of these elements is essential for the preservation of life and the treatment of disease. Despite years of effort, suitable substitutes have yet to be developed.

In the early 1980s, it became clear to the medical community that these life-sustaining and essential therapeutic elements were transmitting life-threatening diseases (1). The primary causative agents of these diseases were identified as the human immunodeficiency virus (HIV) and hepatitis C virus. Because of the delay in development of suitable detection systems, these agents passed undetected into the blood supply. The fact that these potentially fatal diseases were transmitted by blood, and the lack of suitable blood substitutes, posed a tremendous challenge for members of the medical community. The resulting concerns

raised by these events triggered a surge of research into methods to purify, reduce or eliminate infectious agents in the world's blood supply.

We are developing a medical device for the reduction of pathogens in platelet concentrates. The technology uses light and the photosensitizer riboflavin (RB) (vitamin B_2). The extent of our interest in RB as a pathogen reduction agent for blood stems from over 70 years of research literature that details vitamin B_2's chemistry, toxicology and the *in vitro* and *in vivo* function in sensitizing the photochemistry of nucleic acids (2–4). Interest in the chemistry of this compound was based on its involvement in metabolic and nutritional functions as well as its behavior in individuals subjected to phototherapy (5–8). Before this research, RB had never been evaluated as a possible sensitizer for use in the *ex vivo* photochemical treatment of blood products. The presence of RB in our diets (9–11), the extensive knowledge of its photochemical properties and its well-behaved toxicological profile makes it an ideal candidate for a blood additive in this application (12,13). Previous studies have demonstrated the ability of this process to inactivate viruses including Human Immunodeficiency Virus (HIV), Porcine Parvovirus (PPV) and West Nile Virus (WNV) and bacteria including *S. epidermidis* and *E. coli* in platelet products while maintaining normal cell quality parameters suitable for transfusion (14).

One of the concerns about using any chemical agent as an additive to blood components for use in pathogen reduction technology (PRT) applications arises from the potential introduction of new chemical agents into the blood supply. These chemical agents may, on their own accord, raise concerns regarding increased toxicological risks that could, in certain circumstances, outweigh the risks associated with potential disease transmission of the blood component. The toxicological profile of RB is very well known and characterized. However, after exposure of RB to light, photochemical reactions can lead to the degradation of the molecule, yielding several photoproducts (15). These photoproducts result primarily from the decomposition of the ribityl side chain in the parent molecule (16,17). Several of these intermediates and breakdown products have been isolated and characterized (16–19).

Exposure of RB in aqueous solution to light leads to rapid photobleaching, measured at the absorption maximum of 447 nm (20). At alkaline pH, lumiflavin is a major breakdown product of RB (20). Under neutral and acidic conditions (the pH at which the pathogen reduction process is performed), lumichrome (LC) is the main breakdown product. Several intermediate by-products produced through metabolic or photochemical degradation of RB have also been identified. These include the 2′ keto-flavin (2KF) and 4′ keto-flavin (4KF) and formylmethyl flavin (FMF) (16). The isolation and characterization of these agents has been hindered by both the low levels present in isolated samples and the low sensitivity of methods of isolation and quantification available to the research community for study of these agents.

The ability to identify these agents in blood components as they naturally occur and the further ability to quantify or characterize them in samples before and after PRT treatment would permit direct examination of the potential impact of PRT on blood component chemistry and toxicology. Such studies, although not definitive, could be used to examine potential consequences in this regard in the blood transfusion setting. Hence, in support of clinical trials and toxicology studies involving this treatment regimen, we have developed a novel high-performance liquid chromatography (HPLC) method for the separation, identification and quantification of RB, LC and other photoproducts of RB in apheresis platelets both before and after PRT treatment. Typically, the measurement of flavins in blood components involves trichloroacetic acid (TCA) precipitation of proteins (21–34). This evolved from the earlier Warburg and Christian methods and was used with high salt (usually ammonium sulfate) both to dissociate flavocoenzymes (flavin mononucleotide [FMN] and flavin adenine dinucleotide [FAD]) from flavoenzymes and more recently to inactivate enzymes that could hydrolyze the flavocoenzymes or even catabolize the released RB. Subsequent extraction and concentration of the flavins was done with phenol or benzyl alcohol.

Table 1. Solvent gradient program parameters for HPLC method

Time (min)	Methanol (%)	Water (%)
0.0	23.0	77.0
8.0	27.8	72.2
16.0	60.0	40.0
17.0	100.0	0.0
18.0	100.0	0.0
19.0	23.0	77.0
25.0	23.0	77.0

In this study we describe a sensitive and robust method for the accurate determination of both RB and LC in blood components without the need for protein precipitation or extraction. We also discuss the findings from direct examination of blood components before and after addition of RB and exposure to light as is proposed in the blood sterilization treatment protocol. These results are presented in terms of potential toxicological and medical implications for this blood safety initiative.

MATERIALS AND METHODS

Chemicals. HPLC grade methanol was purchased from Fisher Scientific (Pittsburgh, PA). RB and LC were purchased from Sigma–Aldrich (St. Louis, MO). RB was used without further purification. LC was purified as described below. Reagent grade water was prepared using a Barnstead E-Pure water purification unit (Dubuque, IA). Saline (0.9%) was purchased from B. Braun Medical Inc. (Irvine, CA).

HPLC apparatus and chromatographic conditions. The HPLC system consisted of an Agilent 1100 equipped with a quaternary pump (model G1311A), an autosampler (model G1329A), an autosampler thermostat (model G1329A), a thermostatted column compartment (model G1316A), a fluorescence detector (model G1314A) and a fraction collector (model G1364A). The fluorescence detector flow cell volume was 8 μL. The HPLC column used was a Zorbax® 80Å SB-CN, 4.6 × 250 mm, 5 micron. The precolumn was a Zorbax® 80Å SB-CN 4.6 × 12.5 mm, 5 micron. The nominal backpressure was 96 bar at the beginning of the run. The fluorescence detector settings were changed during each run. Initial fluorescence detector settings were maximized for detection of RB followed by maximum sensitivity for LC. The settings were: excitation 268 nm, emission 525 nm for the first 14.5 min, followed by excitation 260 nm, emission 470 nm. The small change in baseline upon wavelength change did not significantly affect the integration. A manual injector program was set up using four wash vials (reagent grade water) to wash the needle between runs and prevent carryover of RB and LC into the next run. The left- and right-column temperatures were set to 50°C. The autosampler temperature was set to 5°C. Flow rate was 1.0 mL/min. The injection volume was 10 μL. Run time was 25 min.

Reagent grade water and methanol were used as the mobile phase and a gradient program was set up (Table 1) to separate RB and LC from the other photoproducts. A six-point calibration curve was used with RB and LC concentrations ranging from 250 to 1500 n*M*. Calibration stock

consisted of 50 μ*M* RB and 50 μ*M* LC in 0.9% saline, pH 5.0. All stock sample concentrations were confirmed by spectrophotometric analysis of RB and LC determined from extinction coefficients of the purified compounds. The stock was diluted to the appropriate calibration concentrations with 0.9% saline. An excellent straight-line fit was obtained for both RB and LC ($r^2 \geq 0.999$ in both cases). The precolumn was replaced after every 30–60 samples.

HPLC-MS/MS analysis. Photoproducts of RB were isolated in 10 separate fractions using a fraction collector. Injection volume was maintained at 500 mL. A diode array detector was used during the separation with settings of stored range from 190 to 500 nm, peak width <0.05 min, slit 4 nm.

The following table contains the fraction collection start and stop times:

Fraction number	Start time (min)	End time (min)	Duration (min)
1	3.9	4.3	0.4
2	4.4	4.8	0.4
3	5.2	5.5	0.3
4	5.6	6.0	0.4
5	6.1	6.4	0.3
6	6.6	7.0	0.4
7	8.9	9.3	0.4
8	9.7	10.0	0.3
9	11.6	12.0	0.4
10	13.2	13.4	0.2

Isolated fractions were analyzed using the diode array detector to identify UV maxima in the individual peaks. Samples were then forwarded to Cardinal Health (Raleigh, NC) for HPLC-MS/MS analysis. They were protected from light and shipped at room temperature. For analysis, samples were transferred to dark glass vials at room temperature. Analysis of all samples was performed using a Cohesive Technologies 2300 HPLC system coupled to a Sciex API3000 triple quadrupole mass spectrometer. Extractions of each of the unknown solutions were performed on a Cohesive Cyclone-P 1 × 50 mm extraction column and on either a 250 × 4.6 mm cyano or a 150 × 4.6 mm cyano analytical column for analytical separations. All mass spectrometric analysis was done using electrospray negative ionization. In cases where more signal was needed, samples were concentrated using a Turbo-vap at 30°C with nitrogen gas. The instrument was protected from light during the evaporation. For all injections, the following MS/MS transitions were scanned, based on analysis of standards: RB, 375.1 > 255.1 amu; KF, 373.1 > 241.3 amu; LC, 240.9 > 198.1 amu; FMF, 282.9 > 240.2 amu.

Apheresis platelet preparation. Single donor platelets (a minimum of 270 mL) were collected by an accredited blood bank facility using a Gambro BCT Inc. TRIMA® Automated Blood Component Collection System. The platelet product was held between 2 and 30 h in the TRIMA collection bag before subsequent processing. The bag containing the platelets was connected to the pathogen reduction illumination–storage bag (ELP™) using a Terumo® Sterile Tubing Welder. After sterile connection, 250 ± 5 mL of platelet product was transferred into the illuminator bag, which contained 28 mL of RB solution (500 μ*M*). The transfer tubing was sealed using a Sebra® hand-held, radio frequency tubing sealer. After connection, the two bags were separated and the original collection bag was discarded. Each final platelet product to be treated contained 1260×10^3 to 1690×10^3 platelets/μL suspended in approximately 278 mL of 90% autologous plasma in a 1 liter citrate-plasticized, polyvinyl chloride ELP bag with an illumination surface area of 347 cm^2/side.

Plasma derived from buffy coats. Buffy coat platelets were prepared from whole blood that was stored at room temperature for 16–24 h after collection. The platelets were spun down at 16 100 RCF for 3 min and diluted 1:50 with 0.9% saline before analysis.

The PRT process. A complete description of the procedures used for this process has been described previously (Ruane *et al.*). Briefly, the platelet concentrates were treated separately with 6.2 J/mL of light. The lamp phosphor possesses an output range from 265 to 370 nm. The platelet product was placed in a product chamber where mixing (on a motorized platen) and exposure to light took place. Total illumination ranged from 8 to 10 min. Dedicated fans were used to cool each lamp chamber and product chamber. Product temperatures during illumination ranged from 22°C to 24°C.

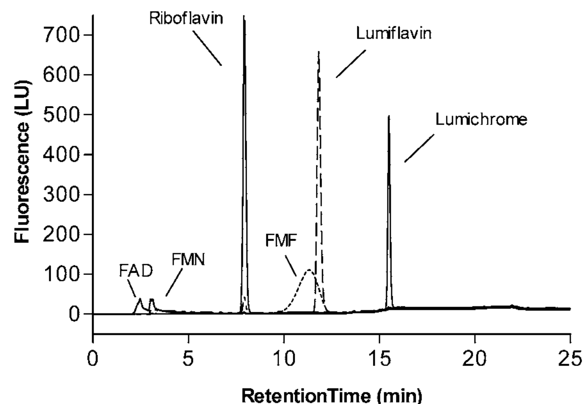

Figure 1. HPLC chromatograms of samples containing various potential interfering agents including lumiflavin, FMN, FMF and FAD.

Purification of LC. The average purity of reagent LC (Aldrich PN 10,321-7) was found to be approximately 68–85% as determined by negative ion mass spectroscopy and HPLC. LC was purified before use as an analytical standard as follows: reagent grade LC (250 mg) was added to a 250 mL amber volumetric flask and diluted to the mark with reagent grade water. The solution was sonicated (VWR® sonicator model 150HT) at 80°C for 2 h and then vacuum filtered while hot, using a 0.45 μm filter. The filter cake, which consisted of purified LC, was dried at 105°C for 2 h (the impurity is soluble in water and LC is relatively insoluble). The LC purity after this process was found to be greater than 99%. LC purity was confirmed using mass spectrometry, proton nuclear magnetic resonance (^{1}H NMR), HPLC and elemental analysis. An API3000 triple quadrupole mass spectrometer using negative ionization mode observed the parent M–1 ion 241 *m/z* concurrent with the LC nominal molecular weight of 242 g/mol in the purified sample. Elemental analysis—found: C 59.04, H 3.81, N 22.95; required: C 59.50, H 4.16, N 23.13. ^{1}H NMR (D_2O), 2.39, s, 3H; 2.41, s, 3H; 7.61, s, 1H; 7.78, s, 1H.

Preparation of FMF reference compound. FMF was synthesized according to the method of Fall and Petering and characterized using NMR and mass spectrometry (35).

Calibration standard preparation. Analytical standards were prepared by diluting a stock solution of 50 μ*M* RB and 50 μ*M* LC in saline. The stock solution was prepared by adding LC (12.1 mg) to a 1 liter amber volumetric flask and adding approximately 950 mL of pH 5.0 saline. The solution was sonicated using a VWR sonicator (model 150HT) at 70–100°C for approximately 2 h until all LC was in solution. RB (18.8 mg) was then added and the solution allowed to cool while stirring overnight. It was then filled to the mark with pH 5.0 saline. Calibration standards were prepared fresh every 24 h.

HPLC validation for quantitative analysis of RB and LC. Validation of the method for RB and LC, the principal photoproduct of RB was carried out during an 8 day period. Both these agents were present in sufficient quantities in blood products to validate the methodology. Other components (FMF, 2KF, 4KF) were isolated by bulk HPLC and characterized by mass spectroscopy and UV–visible (UV-VIS) spectroscopy. Results from these studies were used to determine concentrations of these agents in blood components, but validation of the methodology for quantitative determination of these components was not performed. Linearity of the calibration curve was 0.999 (r^2) or better for all 8 days of validation. For five replicates of both RB and LC at three concentrations (25, 37.5 and 50 μ*M*), the average relative standard deviations were RB = 0.64% and LC = 0.76%. For these 30 samples, the average percent recovery for RB was 97% whereas the average percent recovery for LC was 102%. The limit of quantitation for RB (undiluted) was 16 n*M* and for LC was 60 n*M*. Samples were diluted 1:50 with 0.9% saline pH 5.0 before analysis. The range of the method for RB is 0.016–1.500 μ*M* and for LC 0.06–1.500 μ*M*. A typical analysis involved two replicates of each sample. No loss of peak shape, peak height or retention time was noted for runs using as many as 15 samples (30 samples when replicates are accounted for).

During the 8 day validation, all retention times for RB samples were within 7.8 ± 0.2 min and all retention times for LC samples were within

Table 2. RB and photoproducts characteristics

Compound	Average HPLC retention time (min)	UV_{max} (nm)	MS/MS transitions (amu)
RB	8.4	223, 267, 374, 444	375.1 > 255.1
LC	15.5	218, 260, 352	240.9 > 198.1
2KF	9.1	224, 268, 372	373.1 > 241.3
		446	373.1 > 255.1
4KF	9.9	222, 268, 374	373.1 > 241.3
		446	373.1 > 255.1
FMF	10.8	222, 268, 372, 446	282.9 > 240.2

Table 3. FMF standard *versus* isolated compound

Compound (FMF)	Retention time (min)	UV_{max}	Mass spectra ions(m/z)
Standard reference	10.8	224, 268, 372, 446	283 (parent), 240 (daughter)
Isolated compound	10.8	222, 268, 372, 446	283 (parent), 240 (daughter)

15.4 ± 0.2 min. The average resolution was 10.1 and the average tailing was 0.34, passing the Food and Drug Administration validation requirements of ≥2.0 resolution and ≤2.0 tailing (36).

Reinjection precision analysis was performed to determine whether an analytical run could be restarted in case of instrument failure. Analysis was performed on a calibration curve and on 30 samples, 10 each at three different concentrations. The calibration curve was followed by 30 samples, including blanks and check standards every 10 samples, simulating a routine analysis. After completion of the sequence, the entire sequence was restarted. The difference in measured RB and LC concentrations from the first run and second run was less than ±1.5%.

Interference. Several analytes were run to test for interference including FAD, FMN, lumiflavin and FMF. None of these analytes interfered with the analysis of RB and LC. Results from sample runs with these agents are included in Fig. 1.

RB and photoproduct analysis in platelet concentrates. Ten platelet concentrates spiked with 50 μ*M* RB were treated with 6.2 J/mL light. The concentrations of RB, LC and other photoproducts were measured before and after spiking with RB, after illumination and after 5 days of storage on a Helmer shaker, respectively (22–24°C, shaker speed of 72 ± 5 cpm). All samples were stored in the dark until analysis, whereupon a 4 mL sample was drawn from each platelet product. Each sample was centrifuged at 3000 RCF for 10 min to remove the cellular components. The supernatant was respun at 16 100 RCF for 3 min and the supernatant diluted 1:50 with 0.9% saline. The samples were then placed in amber HPLC vials for analysis. Analysis included characterization of peaks associated with the 2KF, 4KF and FMF as well. Quantification of these latter samples was conducted on the basis of extinction coefficients for the parent molecule and HPLC analysis. Identification of peaks associated with these agents was performed through analysis of isolated fractions by mass spectroscopy–HPLC and UV-VIS absorption characteristics (Table 2). In the case of FMF, direct comparison with a *de novo* synthesized standard was possible (Table 3).

Additional samples taken from normal donors were analyzed for the presence of RB and RB catabolites or photoproducts. These samples were assayed directly without any RB addition to determine the levels of each component present in a natural state in blood components.

RESULTS

RB, LC, 2KF, 4KF and FMF analysis in apheresis platelets

The level of RB and LC and other catabolites of RB in the platelet products after spiking with 50 μ*M* RB are listed in Table 4. The average RB concentration after the addition of 28 mL of 500 μ*M* RB to 250 ± 5 mL platelet product was 48.3 μ*M* (n = 10, Table 4). PRT treatment resulted in 78.9% (n = 6) recovery of the RB originally present after dilution and before illumination. An additional 6.2% of this initial level of RB was recovered in the form of LC, resulting in a total of 85.1% recovery of the initial level of RB. The remaining levels were identified to be composed of FMF, 2KF and 4KF. Separation of these components by bulk HPLC was conducted to isolate purified fractions of each. These samples were examined to obtain mass spectra data and UV-VIS spectra characteristics. Upon storage for 5 days, the amounts of RB and LC both increased by a measurable extent (Fig. 2b,c). A total of 80.8% RB (n = 10), 7.4% LC, 1.4% 2KF, 0.3% 4KF and 3.5% FMF were recovered (n = 10), yielding a total mass balance of 94.4% after 5 days of storage. These results are given in Table 4. The HPLC chromatograms of the PRT-treated platelets before illumination, after illumination and after 5 days of storage are shown in Fig. 2.

Quantitative analysis of endogenous RB and photoproducts–catabolites in plasma

A modified version of the current method was used to measure endogenous concentrations of RB and RB catabolites in plasma. The injection volume was increased from 10 to 100 μL and the dilution with saline was reduced from 1:50 to 1:5. This gave a 100-fold increase in RB detection limits. A six-point calibration curve was run on the same day of analysis using standards ranging from 1 to 50 n*M* (1, 2, 5, 10, 20 and 50 n*M*). Thirty samples of plasma derived from buffy coat platelets were analyzed for RB and its photoproducts–catabolites. The average concentration of RB was 23.9 n*M* (range 8.6–79.6 n*M*) (see Fig. 3). Excellent results were obtained with this adapted HPLC method. Blanks were run before and after the calibration curve and between each of the 30 samples. RB was not detected in any of the blanks. The linear regression of the calibration curve was greater than 0.999. Levels of LC and other photoproducts–catabolites of RB present in these products varied as shown in Table 5. Out of 30 products in total analyzed using this method, all samples demonstrated measurable levels of RB. Only 23 of the 30 products demonstrated measurable levels of LC. A total of three of the 30 had measurable levels of all four major photoproducts. These also corresponded to those samples having the highest concentrations of RB. The average concentrations determined for each of these products as well as the ranges observed are listed in Table 5.

DISCUSSION

The methodology described represents a sensitive HPLC method for the quantification of RB, LC, 2KF, 4KF and FMF in blood components that does not require a protein precipitation or organic solvent extraction step. The method simply removes the cellular components by centrifugation followed by dilution with saline and analysis using a reverse phase cyano-column (Zorbax® 80Å SB-CN, 4.6 × 250 mm, 5 micron). We have found that the low concentration of protein after dilution does not interfere with the assay.

Typically, C18 reversed phase columns are used to analyze RB and related compounds in aqueous solutions (15,18–20,22,24–29,31–34, 36). However, we found that the use of this stationary phase was not satisfactory and separation of photoproducts from RB after photolysis was not feasible. A reverse phase cyano-column was found to be optimal in resolving compounds closely eluting to RB (Fig. 2). Other columns were used and separation was not optimal

Table 4. RB and photoproducts in apheresis platelets*

	PRE (μ*M*)†	POST (μ*M*)‡	POST 5 days (μ*M*)†
RB	48.33 ± 1.13 (47.1–50.4)	38.68 ± 0.68 (38.0–39.9)	39.65 ± 1.22 (37.0–41.3)
% of initial		78.9	82.0
LC	0.23 ± 0.08 (0.1–0.3)	2.90 ± 0.20 (2.5–3.0)	3.60 ± 0.30 (3.2–4.1)
% of initial		6.2	7.5
2KF	N/A	1.27 ± 0.17 (1.10–1.49)	0.71 ± 0.12 (0.59–0.90)
% of initial		2.57	1.44
4KF	N/A	0.53 ± 0.03 (0.48–0.58)	0.16 ± 0.04 (0.11–0.22)
% of initial		1.06%	0.32%
FMF	N/A	1.99 ± 0.24 (1.63–2.32)	1.72 ± 0.22 (1.42–2.07)
% of initial		4.02	3.49
Totals		45.4 μ*M*; 93.4%	45.8 μ*M*; 94.4%

*PLT, apheresis platelet product before spiking with 50 μ*M* RB; PRE, after spiking (50 μ*M* RB); POST, after treatment; POST 5 days, after a further 5 days of storage.
†n = 10.
‡n = 6. Values in parentheses are the range.

(Agilent (Zorbax) Columns: SB-C18, XDB-C18, 300 Extend C18, 300 SB-C8, XDB-C8, SB-C8, 300SB-C3, 300SB-CN, Carbohydrate, SB-Aq, Eclipse AAA, NH2, Diol and GF-250).

The measurement of vitamin B_2 in blood components is not new (15,16,19–24,32). Typically, FAD in the blood is converted to the more stable FMN. Free RB and FMN are then measured after their extraction into organic solvents. These methods also require the acidic (usually TCA) precipitation of blood proteins before analysis. This has one significant drawback. RB and related compounds tend to coprecipitate with the protein making accurate determinations difficult. In most cases, although agents such as FMF, 2KF and 4KF have been previously identified, their low levels in naturally occurring blood products and the low sensitivity inherent in previous methodologies prohibited their routine identification and quantification in blood components. The increased sensitivity of the method described in this study permits this direct analysis.

The method described in this article has been developed to support a PRT known as MIRASOL PRT. The technology involves the inactivation of pathogens in platelet concentrates using 50 μ*M* RB and light. During the course of this study, we measured the amount of RB, LC, FMF, 2KF and 4KF in 10 apheresis platelet products before and after treatment. The platelet concentrates were also stored at 22–24°C on a Helmer shaker for 5 days, whereupon the amounts of each of these agents were reanalyzed. We were able to accurately determine the amount of RB converted to photoproducts during the process. A total of 78.9% RB remained immediately after irradiation. This amount increased upon 5 days of storage to 82.0%, presumably because of photoproducts (not LC) reverting back to RB during this period. The amount of LC also increased upon storage from 6.2% to 7.5%. The total mass balance of both compounds on Day 5 was 89.5%. The remaining 10.5% is made up of other photoproducts that have been positively identified and quantified through mass spectroscopic and other analytical methods. Figure 2 shows an overlay of HPLC chromatograms of RB in a diluted platelet product (1:50), before illumination (a), after illumination (b) with the PRT process and Day 5 after treatment (c). The photoproducts β and γ have been identified as 2KF and 4KF, respectively. Their UV-VIS absorbance spectra (both are nearly identical to that of RB) and mass spectra are consistent with this hypothesis. Results from HPLC, UV-VIS analysis and mass spectra data are depicted in Table 2. FMF (δ) has been positively identified by comparison with an authentic sample. Results from this analysis are depicted in Table 3. Quantification of these photoproducts has been conducted using the extinction coefficients for the parent molecule and are included in Table 4.

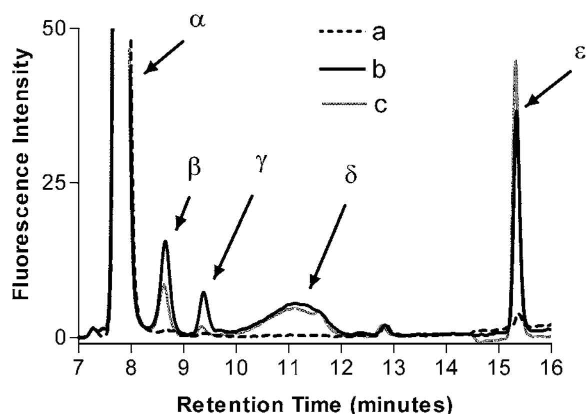

Figure 2. RB (50 μ*M*, diluted 1:50 with 0.9% saline, pH 5.0) in apheresis platelets illuminated 6.2 J/mL with UV. (a) Before illumination. (b) After illumination (RB = 78.9%, LC = 6.2%). (c) After illumination and 5 days storage (RB = 82.0%, LC = 7.5%). α, RB; β, mass spectrum and UV-VIS are consistent with 2KF; γ, mass spectrum and UV are consistent with 4KF; δ, mass spectrum and UV are consistent with FMF; ε, LC.

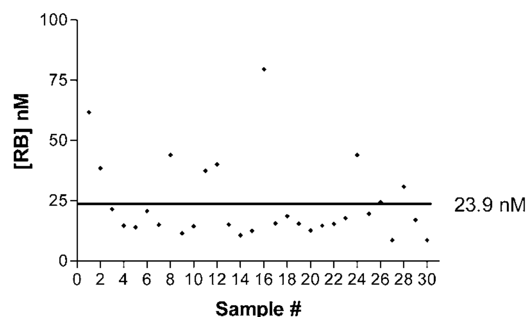

Figure 3. Endogenous concentration of RB in 30 plasma samples derived from buffy coat platelets. Average = 23.9 n*M*.

Table 5. RB and photoproducts in normal, untreated blood

Compound	Concentration (n*M*) average (range)
RB	23.9 (8.6–79.6)
LC	11.4 (0.0–75.3)
2KF	2.0 (0.9–3.4)
4KF	1.2 (0.5–2.4)
FMF	5.0 (1.7–11.5)

Hustad has reported a value of 6.9 n*M* (2.7–42.5 n*M*) for RB concentration in plasma (n = 63) (37). In a later report, Hustad reported the average RB concentration in the plasma of 124 senior citizens as 15.3 n*M* (5.4–28.4 n*M*) (25). Capo-chichi reported in 2000 that the median concentration of RB in 10 infants and 10 adolescents to be 20.9 n*M* (12.7–53.4) and 18.5 n*M* (8.2–57.8), respectively (25). We now report for n = 30, an average value of 23.9 n*M* (8.6–79.6 n*M*) (Fig. 3). The higher mean value observed in this study might be a reflection of the fact that no additional protein precipitation or extraction step was required in this procedure resulting in higher retention of starting levels of RB, or might be due to partial leakage of erythrocytes still present in the buffy coat platelet products.

Photolysis of RB during the MIRASOL process results in the formation of four photoproducts: 2KF, 4KF, FMF and LC. These four photoproducts were found to be present in apheresis platelets that had not undergone any photochemical treatment, although at a much lower concentration (Table 5, Fig. 4). It is also important to note in this context that the concentrations measured in the platelet products will be diluted by a factor of 16- to 20-fold upon infusion of the products into a patient's blood stream, thus lowering the difference between concentrations determined in these products and the levels naturally circulating in blood. The demonstration of the existence of these agents in naturally occurring blood products suggests that the introduction of a RB-based PRT process will not introduce new agents into the blood supply, which are not already present to some extent. The consequence of increased levels of these agents in blood products resulting from this process is being evaluated in separate toxicology studies, which include short-term and long-term exposure to each of the agents described and characterized in this study (38–45). The presence of these agents in our blood, the ubiquitous nature of RB exposure, its presence in our diets and our ability to metabolize it and manage its inherent photochemistry suggests a low risk profile for this product. These features in combination with the pathogen reduction capacity of this system have the potential to greatly enhance the safety of blood products presently offered in routine clinical practice.

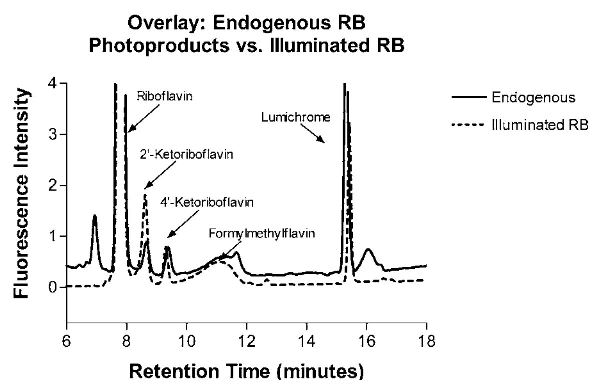

Figure 4. HPLC photochemical profile generated during treatment of 50 μ*M* RB in apheresis platelet concentrates (MIRASOL-treated apheresis platelet concentrates) overlaying the photochemical profile generated by direct measurement of a sample of untreated apheresis platelets.

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文献23

Toxicity Testing of a Novel Riboflavin-Based Technology for Pathogen Reduction and White Blood Cell Inactivation

Heather L. Reddy, Anthony D. Dayan, Joy Cavagnaro, Shayne Gad, Junzhi Li, and Raymond P. Goodrich

The Mirasol PRT System (Gambro BCT, Lakewood, CO) for platelets and plasma uses riboflavin and UV light to reduce pathogens and inactivate white blood cells in donated blood products. An extensive toxicology program, developed in accordance with International Organisation for Standardisation (ISO) 10993 guidelines, was performed for the Mirasol PRT system. Test and control articles for most of the reported studies were treated (test) or untreated (control) blood products. For some studies, pure lumichrome (the major photoproduct of riboflavin) or photolyzed riboflavin solution was used. Systemic toxicity was evaluated with in vivo animal studies in the acute and subchronic settings. Developmental toxicity was evaluated with an in vivo animal study. Genotoxicity and neoantigenicity were evaluated with in vitro and in vivo tests. Hemocompatibility and cytotoxicity were assessed with standard, in vitro assays. The pharmacokinteics, excretion, and tissue distribution of ^{14}C-riboflavin and its photoproducts was evaluated with an in vivo animal study. The possible presence of leachable or extractable compounds (from the disposable set) was evaluated with novel assays for measuring these compounds in blood. No treatment-related toxicity was observed in any of the studies.
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THE COLLECTION, SEPARATION, and transfusion of red blood cells, platelets, plasma, and fractionated plasma components are essential medical practices, often required for the preservation of life and for the treatment of disease. Although the transfusion of these components is a vital therapy, transfusions are still associated with some risk for transmission of disease to the patient. Bacteria, viruses, and parasites are all potential sources of infection that have been transmitted by allogeneic transfusions.[1] Residual donor white blood cells (WBCs) can cause a series of severe immune responses in a transfusion recipient, resulting in the failure of the transfusion or other complications as a result.[2-4] Prion-transmitted diseases are also a recent and growing concern owing to the potential transfusion-mediated transmission of variant Creutzfeldt-Jakob disease (vCJD).[5-8]

A number of safety measures have been taken to reduce the risk of disease transmission. They include increased diligence in preparing the donor's arm before donation and diversion of the first portion of blood from the donor to decrease the risks of bacterial contamination of the blood product.[9] Decreasing the risk of bacteria-related morbidity and mortality in recipients was also the driver for the reduction in storage time for platelets from 7 to 5 days.[10] Additional approaches to prevent disease transmission include the use of questionnaires to screen donors and thereby minimize the risks of collecting blood with viral or other diseases, the use of antibody testing and nucleic acid testing to detect viruses, and the use of culture techniques to detect bacteria.[1,11] γ-Irradiation has been used to reduce the possibility of transfusion-associated graft-vs-host disease (GVHD) in susceptible patient populations.[12] Leukoreduction has also been adopted in some parts of the world to reduce the possibility of prion transmission.[13]

Despite the use of the various approaches, as outlined, transfusion-transmitted infection from agents such as HIV, hepatitis B virus, hepatitis C virus, and bacteria continue.[14-16] In part, these events arise from the inability of available tests to detect agents in blood at low levels, which are nonetheless infectious, or during a latent period. Risks related to transfusion-associated GVHD remain, primarily because γ-irradiation of blood components is not universally used. There are some infectious agents for which screening tests are not routinely used, notably Parvovirus B19; cytomegalovirus; and the causative agents of parasitic diseases such as Chagas disease, malaria, babesiosis, and leishmaniasis.[17-19] The recent entry of West Nile virus (WNV) into the blood supply in North America is an example of the emergence of a new

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Table 1. Reported Toxicity Findings After Riboflavin Administration

Study type	Species	Route	Results	Ref
Acute	Mouse	SC	1200 mg/kg: slight reduction in body temperature 2400 mg/kg: neuromuscular effects and mortalities	27
Acute	Mouse	IP	LD_{50} = 340 mg/kg LD_0 = 750 mg/kg	28,29
Acute	Rat	IP	LD_{50} = 560 mg/kg	27
Acute	Rat	SC	LD_0 > 5000 mg/kg	27
Acute	Rat	Oral	LD_{50} > 10000 mg/kg	27
Acute	Mouse	IV	LD_{50} > 50 mg/kg	30
Acute	Dog	Oral	NOAEL of 2000 mg/kg	27
Subacute 4 d	Mouse	SC	2/6 Mortalities at 1000 mg × kg^{-1} × d^{-1}	28
Subchronic 4-6 wk	Rat	Oral	Increased liver enzymes at 2.0 mg × kg^{-1} × d^{-1}	31
Chronic 22 mo	Rat	Oral	Mild liver cell hypertrophy at 1.5 mg × kg^{-1} × d^{-1}	32
Chronic 140 d	Rat	Oral	50 mg × animal × d^{-1}, no adverse effects	27
Chronic 5 mo	Dog	Oral	25 mg × kg^{-1} × d^{-1}, no adverse effects	27
Developmental	Rat	Oral	During pregnancy and lactation NOAEL of 40 mg × kg^{-1} × d^{-1}	33
Reproduction	Rat	Oral	3 Generations, NOAEL of 50 mg × animal × d^{-1}	27
Carcinogenicity	Rat	Oral	1.5 mg × kg^{-1} × d^{-1} for 22 mo, no evidence of carcinogenicity	32
Micronucleus	Mouse	IP	Four doses at 1000 mg/kg, negative	28
Micronucleus	Mouse	Perioral	100 mg/kg, negative	34
Micronucleus (oral mucosa)	Human	Perioral	200 mg × kg^{-1} × wk^{-1} for 1 y, negative	35
Clastogenesis	CHL	Preincubation	Negative	36
Gene mutation	*E coli*	Preincubation	Negative	37
Ames test *S typhimurium*	TA1535, TA1537, TA1538, TA98, TA100 ± S9 activation	Preincubation	10 μmol/plate, negative results	38
Ames test *S typhimurium*	TA100, TA102, TA98, TA97A; ± S9 activation, cecal cell-free extract	Preincubation	25-100 μg/mL, negative results	39
Pharmacokinetics	Human	IV	11.6 mg/subject in bolus (242 mL) C_{max} = 1209 nmol/L Disposition $t_{1/2}$ = 0.1006 h Renal clearance $t_{1/2}$ = 0.7385 h	40

Abbreviations: SC, subcutaneous; IP, intraperitoneal; LD_{50}, lethal dose at which 50% of the test population survives; LD_0, lethal dose at which none of the test population survives; NOAEL, no observable adverse effect level; CHL, Chinese hamster lunch fibroblast cell line; C_{max}, maximum concentration in the bloodstream; $t_{1/2}$, time to reduction in concentration by 50%.

agent for which testing was not readily available. Since the appearance of WNV in the United States, several cases of transfusion-related WNV infections have occurred.[20] The low levels of WNV present in blood during a subject's subclinical viremia makes its detection difficult even with the most sensitive of the available nucleic acid testing technologies.[21]

Pathogen reduction via a chemical or photochemical process has also been used as a blood safety measure. Methylene blue–treated and solvent/detergent-treated plasmas have been used as transfusion products, such as fresh frozen plasma (FFP), and in the manufacture of fractionated and purified blood-derived products such as factor VIII concentrate. These treatments are effective against enveloped viruses and yield only slight reductions in protein quality.[22,23] Recently, a technology using a psoralen compound and UV light has been developed for the reduction of pathogens as well as the inactivation of leukocytes in platelets and in plasma.[24-26]

Similarly, the Mirasol PRT System (Gambro, BCT, Lakewood, CO) was developed to provide reduction of a wide variety of pathogens and inactivation of leukocytes. This device uses UV light and riboflavin to reduce pathogens. Riboflavin was chosen as a photochemical sensitizer because of its well-documented safety profile, as evidenced by

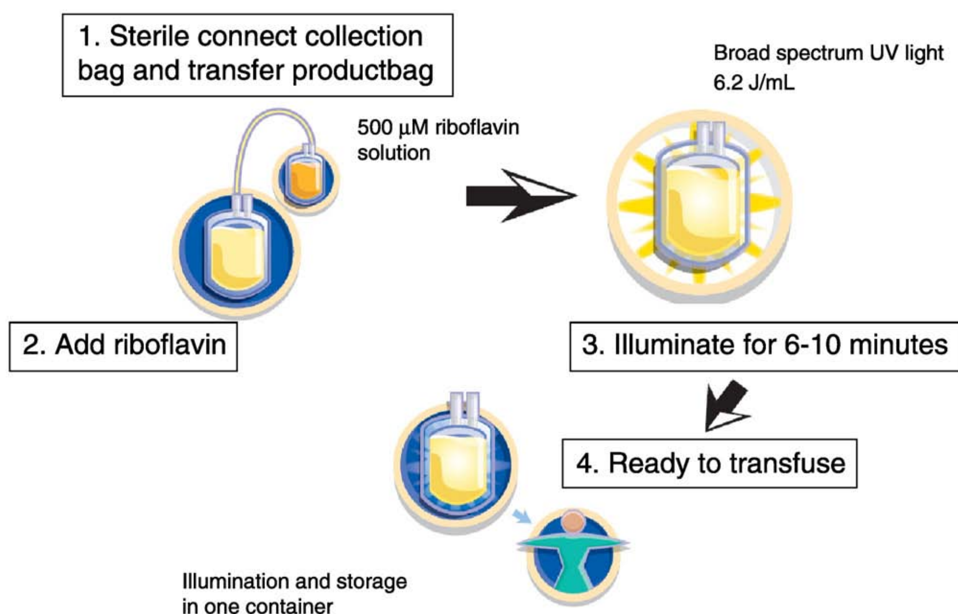

Fig. 1. Diagram of the steps involved with the use of the Mirasol PRT System. Platelets or plasma product in an illumination/storage bag is combined with riboflavin solution to achieve approximately 50 μmol/L and then exposed to 6.2 J/mL UV light. The treated product is then ready for clinical use or blood bank storage. Riboflavin remains in the treated blood product, no removal device is used.

the reports referenced in Table 1. It is an essential nutrient for all living organisms, including humans. The novel nature of this pathogen reduction treatment (PRT) required comprehensive toxicological evaluation to prove that use of this technology to treat blood components would not introduce new hazards. The strategy, nature, and results of these toxicological studies are the subject of this review.

MIRASOL PRT SYSTEM—DESCRIPTION

The Mirasol PRT System consists of an illuminator, the illumination/storage bag, and riboflavin solution (Fig 1). The Illumination/Storage bag is made of biocompatible materials and is adapted from the container used to collect and store platelet products obtained with the Trima automated blood collection device (Gambro, BCT). The sterile riboflavin solution contains riboflavin (500 μmol/L) in a 0.9% sodium chloride (NaCl) solution with pH adjusted with hydrochloric acid to be in the range of 4.0 to 5.0. A volume of 35 ± 5 mL of this solution is added to a blood component product to produce a final concentration of approximately 50 μmol/L. The illuminator delivers the necessary dose of UV light (6.2 J/mL) to the contents of the illumination bag based on product volume (derived from weight) and measured flux rate. Robustness studies demonstrated that the system is suitable for use with initial platelet or plasma product volumes ranging from 170 to 365 mL and platelet product cell concentrations ranging from 1100 to 2100 × 10^3/mL.

The Mirasol PRT System reduces the infectivity of pathogens by 3 combined mechanisms. The first is the direct damage of nucleic acids of pathogens with the UV light source used in this system. The second is the damage of pathogen nucleic acids, proteins, and membranes by reactive oxygen species generated when riboflavin absorbs light and interacts with dissolved oxygen in solution. The Mirasol process was designed to minimize the contributions of this mechanism because of its nonspecific nature. The third is the damage of pathogen nucleic acid by the interaction of riboflavin with nucleic acids. Exposure of riboflavin to light when it is associated with or in proximity to nucleic acids leads to a Type I photochemical reaction that can induce oxidation of guanine residues and strand breaks. The result of this chemistry is irreversible damage to the nucleic acid.[41]

MIRASOL PRT PHOTOCHEMISTRY

Photolysis of riboflavin during treatment results in the formation of 4 major, quantifiable photoproducts: 2′-ketoriboflavin, 4′-ketoriboflavin, formylmethylflavin, and lumichrome. Several minor components are present in negligible quantities and

Table 2. Concentration of Riboflavin and Photoproducts in Treated and Untreated Platelets[42]

	Mirasol PRT platelets* (n = 30)			Untreated platelets (n = 30)
	Pretreatment† (μmol/L)	Posttreatment‡ (μmol/L)	Day 5, treated§ (μmol/L)	nmol/L
Riboflavin	48.33 ± 1.13‖ (47.1-50.4)¶	38.68 ± 0.68 (38.0-39.9)	39.65 ± 1.22 (37.0-41.3)	23.9# (8.6-79.6)
Lumichrome	0.23 ± 0.08 (0.1-0.3)	2.90 ± 0.30 (2.5-3.0)	3.60 ± 0.30 (3.2-4.1)	11.4 (0.0-75.3)
2-Ketoflavin	ND	1.27 ± 0.17 (1.10-1.49)	0.71 ± 0.12 (0.59-0.90)	2.0 (0.9-3.4)
4-Ketoflavin	ND	0.53 ± 0.03 (0.48-0.58)	0.16 ± 0.04 (0.11-0.22)	1.2 (0.5-2.4)
Formylmethylflavin	ND	1.99 ± 0.24 (1.63-2.32)	1.72 ± 0.22 (1.42-2.07)	5.0 (1.7-11.5)

Abbreviation: ND, not detected.

* All studies for treated products were performed with and without γ-irradiation. No significant difference (Student *t* test, $P < .05$) was observed.

† Platelet product with Mirasol riboflavin solution, before UV light exposure.

‡ Platelet product with Mirasol riboflavin solution, immediately after UV light exposure.

§ Platelet product with Mirasol riboflavin solution and UV light exposure, stored for 5 days at room temperature with agitation.

‖ Mean ± 1 SD.

¶ Range of measured concentrations.

\# Mean.

represent less than 1% of the total photoproduct peak areas. These minor components have been identified as isomers of the major components by analysis using high-pressure liquid chromatography followed by 2 stages of mass spectroscopy. Each of these 4 major photoproducts is a normal metabolite of riboflavin, and each has been detected in untreated apheresis platelets (Table 2), although at much lower concentrations than observed in Mirasol PRT–treated platelets.[42] The concentrations measured in Mirasol PRT–treated blood products are diluted by a factor of 16- to 20-fold upon infusion of the products into a patient's blood stream (based on 250-300 mL infused into a subject of 5600 mL blood volume on average), thus reducing the concentrations toward the levels naturally circulating in the bloodstream. The existence of these photoproducts in freshly donated, nonilluminated blood products shows that a riboflavin-based PRT system will not introduce new chemicals into the blood supply.

These photoproducts have been examined in earlier studies of riboflavin photolysis in various solutions. After exposure to light, decreased reactivity of the solutions was demonstrated.[43-45] The results clearly show that conversion of riboflavin from its native form into the photoproducts leads to decreased reactivity of the solution owing to decreases in the extinction coefficients of the resulting solution (photobleaching).

MIRASOL PRT SYSTEM—EFFICACY

Pathogen reduction with the Mirasol PRT System has been tested with a wide variety of enveloped and nonenveloped viruses as well as gram-negative and gram-positive bacteria.[46] Prevention of GVHD due to WBC inactivation by the Mirasol PRT System has been studied both in vitro and in vivo.[47,48] Parasite reduction studies have been conducted in collaboration with the Walter Reed Army Institute of Research and are the subject of separate publications.[49,50] The results of these studies with bacteria, viruses, parasites, and WBCs demonstrate broad efficacy and capacity for the process in the reduction of pathogenic agents in blood components.

Blood components treated with riboflavin and UV light exhibit satisfactory quality, as assessed by in vitro measurements.[46,51,52] Initial studies on protein quality after treatment demonstrated adequate retention of coagulation factors, coagulation inhibitors, and immunoglobulins, all at levels required for therapeutic efficacy.[53,54] Some platelet quality indices were reduced in treated products in comparison with untreated controls but remained within acceptable transfusion limits.[46,51,52] These studies were repeated after γ-irradiation of the products and showed no significant additional effect (C. Hardwick and R. Goodrich, unpublished data, 2003). Despite the differences in in vitro measurements, Mirasol PRT–treated platelets exhibited normal function in models of adhesion, aggregation, and thrombus formation.[55]

Data from a clinical trial in healthy human subjects illustrated that Mirasol PRT–treated platelets exhibited decreased recovery and survival time in comparison with untreated platelets. Nevertheless, recovery values and cell quality indices for Mirasol PRT–treated platelets remained well

within the acceptable range for clinical utility of platelet products currently licensed and in routine use.[56,57] Additional studies involving evaluation of the efficacy of treated platelet products in the clinical setting with thrombocytopenic patients are in progress.

THERAPEUTIC USE AND TOXICITY OF RIBOFLAVIN IN HUMANS

Riboflavin was chosen as the photosensitizing agent for this pathogen reduction process because it can be photoactivated to damage pathogens; it has a substantial history of safe clinical use; and it is both a naturally occurring vitamin (B_2) and an essential dietary nutrient. The latter features afforded it the advantage of a well-known and well-characterized safety profile on which to base its evaluation in transfusion medicine. The metabolic role of riboflavin has been well characterized, principally as a source of the essential coenzymes flavin adenine mono- and dinucleotides. The recommended daily intake of riboflavin is about 1.3 mg/d for the average adult[58-60] and up to 1.6 mg/d for the lactating woman. Riboflavin is administered in medical practice by the oral, intramuscular, and intravenous (IV) routes to treat deficiency states, and it is widely taken orally as a dietary supplement. Recommended therapeutic doses by any route are up to 30 mg/d for an adult,[61] but there are records of people taking up to 200 mg/d or more orally for 6 months with no adverse effect.[59,62]

Riboflavin is very widely used as a permitted food coloring in the United States, where it has "generally regarded as safe" status,[63] and in Europe, where it has been approved by the Scientific Committee on Food.[64] The Joint Food Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives has reviewed its use in food and it has been given a value for acceptable daily intake (ADI) of 0 to 0.5 $mg \cdot kg^{-1} \cdot d^{-1}$, which is a maximum ADI of 35 mg/d for a subject of average weight (70 kg).[58]

Riboflavin is given to newborn babies undergoing phototherapy for neonatal jaundice.[65,66] Administration of supplemental riboflavin to these vulnerable patients has become standard practice because the treatment-accelerated clearance of bilirubin via phototherapy could also make them at least temporarily deficient in riboflavin owing to overlap of the light absorption spectra of riboflavin and bilirubin.[66-69] To avoid the risk of the potential riboflavin photochemistry-induced deficit, riboflavin (3 mg/kg perioral) is conventionally given to infants during the phototreatment regimen. Concern was once expressed about the toxic potential of riboflavin with direct phototherapy of newborns, based on theoretical consideration of its possible effect on DNA in the presence of light and on the results of experiments in simplified in vitro systems,[70,71] but there have been no reports of adverse effects in the clinical setting. This includes results from large retrospective analyses, which directly evaluated potential tumorigenic consequences in more than 50 000 infants undergoing the therapy for more than 10 years after phototherapy.[70] This type of therapy continues in wide clinical use today.[72]

There are a few claims of uncertain quality in individual subjects of "sensitization" and possible enhancement of epileptiform seizures after several months of high daily doses of riboflavin (eg, >5 $mg \cdot kg^{-1} \cdot d^{-1}$ for >6 months), but those reports remain unsubstantiated.[61,62] In no other reports of high oral and parenteral doses of riboflavin in humans have toxic effects been described,[73-78] probably because of its physiologic nature and the rapid excretion of an excessive dose.[40]

There is an abundance of references on the toxicity of riboflavin administered by various routes. These data support its use in total parenteral nutrition regimens. Table 1 summarizes representative published toxicity data for oral, subcutaneous, perioral, intraperitoneal, and IV exposures to riboflavin.

Although the safety of riboflavin has been extensively studied, there were no reports that directly supported its use in the Mirasol PRT System. Therefore, Navigant Biotechnologies, Lakewood, Colo, conducted a comprehensive preclinical safety evaluation program in support of the Mirasol PRT System, designed to investigate all potential sources of concern. The work was done in compliance with International Organisation for Standardisation (ISO) 10993.[79]

METHODS

Test Article Definition

To establish the safety of Mirasol PRT–treated blood products, in vivo animal toxicity studies were performed with treated platelets (and in some instances with treated plasma), as well as with pure lumichrome, and with photolyzed riboflavin in

Table 3. Studies of Systemic Toxicity

	Acute toxicity			Subchronic toxicity
	Mirasol PRT–treated rat platelets	Mirasol PRT–treated dog platelets	Photolyzed riboflavin solution	Mirasol PRT–treated dog plasma
No. of animals/group	10 Male rats 10 Female rats	4 Male dogs 4 Female dogs	10 Male rats	6 Male dogs; 2 in recovery 6 Female dogs; 2 in recovery
Duration of study	14 d; dosing on d 1	14 d; dosing on day 1	9 d; dosing on day 1	13 wk with 4 wk for recovery group; dosing daily, 6 d/wk
Dose route	IV injection via tail vein	IV infusion via catheter	IV injection via tail vein	IV infusion via catheter
Group 1				
Dose material	Untreated rat platelets	Untreated dog platelets	Sterile 0.9% NaCl	Untreated dog plasma
Dose frequency	Three 2.0-mL injections, 2 h apart	Three 40-mL infusions, 2 h apart	Three 2.0-mL injections, 2 h apart	Daily 40-mL injections via catheter
Group 2				
Dose material	Mirasol PRT–treated rat platelets	Mirasol PRT–treated dog platelets	Photolyzed riboflavin solution	Mirasol PRT–treated dog plasma
Dose frequency	Three 2.0-mL injections, 2 h apart	Three 40-mL infusions, 2 h apart	Three 2.0-mL injections, 2 h apart	Daily 40-mL injections via catheter
Group 3				
Dose material	Mirasol PRT–treated, γ-irradiated rat platelets	Mirasol PRT–treated γ-irradiated dog platelets	Photolyzed riboflavin solution	NA
Dose frequency	2.0-mL injections	40-mL infusions	2.0-mL injections	
Group 4				
Dose material	Mirasol PRT–treated, γ-irradiated rat platelets	Mirasol PRT–treated γ-irradiated dog platelets	NA	NA
Dose frequency	Three 2.0-mL injections, 2 h apart	Three 40-mL infusions, 2 h apart		

Abbreviation: NA, not applicable.

the absence of platelets and plasma. The Mirasol PRT System parameters used to prepare test articles were always within the range verified for clinical use of the device.

Wherever possible, Mirasol PRT–treated platelets in autologous plasma were used as the test article so that any toxicity from the individual components and any interaction between them (storage bag, riboflavin, light, blood product) would be detected. The doses used in the in vivo studies were designed to maximize exposure while minimizing volume-associated effects and discomfort to the animals. In some cases, pure compounds were used to yield higher doses of the compounds of interest in a study (such as lumichrome and photolyzed riboflavin). In certain animal studies, such as embryo-fetal development in rats and subchronic repeated-dose test in dogs, the volume of animal platelets required to prepare test and control articles for the study would have been very large and would have been required over many weeks. To obtain a consistent test article in as humane a fashion as possible for those studies, species-specific plasma was used rather than platelets. The photochemistry of riboflavin yields equivalent photoproduct profiles in plasma products and in platelet products (which consist mainly of plasma). The absence of platelets eliminates the possibility of detecting toxic alterations to the platelet surface; however, that issue was addressed in the neoantigenicity and ^{14}C-riboflavin binding studies. Therefore, when necessary and appropriate, plasma was regarded as an appropriate substitute for platelets and was used as the test article. Treatment of plasma with the Mirasol PRT System is identical to the treatment of platelets.

Sample Size Determination, Statistics, and Data Analysis

Sample size determination for studies was based on historical experience or published requirements where appropriate. In all cases, data were maintained in validated databases allowing archiving and analysis of data. All studies, assays, and data analysis were performed at accredited contract testing laboratories in accordance with Good Laboratory Practice (GLP) requirements, except

where noted. Comparisons of data between study groups were analyzed using Student *t* test, Wilcoxon distributions and other suitable analyses as appropriate for the particular study data being analyzed.

Systemic Toxicity Studies

Acute toxicity. Two acute toxicity studies, 1 in rats and 1 in dogs, evaluated the acute toxicity of Mirasol PRT–treated, species-specific platelet products, with or without γ-irradiation. In those studies, both males and females were used, to reveal any sex-dependent effects. In addition, an acute toxicity study of photolyzed riboflavin in saline was performed to test the toxicity of higher doses of riboflavin and its photoproducts than are attainable in a blood product. The dose volume used in all rat studies (2.0 mL) corresponded to a range of doses per body weight (mean dose of 9.0 ± 0.7 mL/kg for males and 11.5 ± 1.3 mL/kg for females in the study of treated rat platelets and 9.4 ± 0.3 mL/kg for the male rats in the study of photolyzed riboflavin). The dose volume used in the acute dog studies (40 mL) yielded mean doses per body weight of 3.9 ± 0.3 mL/kg for males and 5.1 ± 0.4 mL/kg for females. Table 3 provides a summary of the number of animals and of the dose frequency, route, and material for the acute toxicity studies. Each study was performed in accordance with ISO 10993[80,81] and International Conference on Harmonization guidelines.[82] These studies evaluated any immediate, systemic toxicity due to administration of Mirasol PRT–treated platelet products by observations of mortality, clinical signs of toxicity, hematology, clinical chemistry, food consumption, general behavior, and weight gain. Complete necropsies were conducted for all animals, and macroscopic observations were made. In the study performed with dogs, electrocardiographic examinations were conducted before randomization, at the conclusion of dosing, and before necropsy. The studies provided observations of effects on major physiologic systems, such as functioning of the nervous, cardiovascular, and respiratory systems, to exclude any pharmacologic effects of test article administration.

Subchronic toxicity. The repeated dose study for subchronic toxicity was done in the dog to support more prolonged and repeated administration of Mirasol PRT–treated platelets to patients, some of whom may require intermittent transfusions over several weeks. Parameters evaluated in the repeated dose toxicity study included morbidity and mortality, outward signs of toxicity (behavioral and physical examinations), clinical chemistry of blood and urine, hematologic indices, body weights and food consumption, and pathologic and histopathologic evaluation of tissues. Test and control article administration is described in Table 3. The daily dose (40 mL) was equivalent to the human dose; the range of doses relative to body weight was 4.7 ± 0.3 mL/kg in the first week of dosing and 4.1 ± 0.3 mL/kg in the last week of dosing for the males and 5.6 ± 0.5 mL/kg in the first week of dosing and 4.8 ± 0.4 mL/kg in the last week of dosing for the females. On days 0 and 86 of the study, blood samples were removed from the animals to measure riboflavin and lumichrome and thereby assess any accumulation of riboflavin or photoproduct during the course of this experiment. Two animals per sex per group were observed for a 4-week recovery period after the 13-week dosing regimen. At the end of the terminal and recovery periods, complete necropsies were performed. Organ weights were recorded, and select tissues were microscopically examined for the non–recovery group animals.

Reproductive Toxicity Study

Embryo-fetal development in rats exposed to Mirasol PRT–treated products. The developmental toxicity potential of rat plasma treated with the Mirasol PRT System was evaluated in female Sprague-Dawley rats (mean gestation interval = 22 days). Time-mated, pregnant animals in the test and control groups (n = 25 for control, n = 24 for test group) were given IV PRT-treated or untreated products, respectively, at a dose of 2.0 mL per animal (7.7 ± 0.4 mL/kg on the first day of dosing, 6.0 ± 0.3 mL/kg on the last) on gestation days 6 through 17. The animals were observed over the course of the study for clinical signs, body weight, and food consumption. On gestation day 20, the animals were necropsied, and the ovaries and uterus were investigated for the total number of implantations, total number of corpora lutea, early and late resorptions, and viable and nonviable fetuses. Individual body weights and sex of the fetuses were noted. Gravid uterine weights were recorded, and adjusted body weight changes were calculated. All fetuses were given an external examination and processed for visceral or skeletal examination, and malformations and developmental variations were recorded.

Table 4. Mammalian Erythrocyte Micronucleus Test

Dose (20 mL/kg)	No. of mice/sex dosed	No. of mice/sex used for bone marrow collection after dose administration	
		24 h	48 h
Vehicle control: 0.9% NaCl for injection	10	5	5
Negative control: untreated human platelets	10	5	5
Test article: Mirasol PRT–treated platelets			
Low dose (diluted in saline 1:3)	5	5	0
Middle dose (diluted in saline 1:1)	5	5	0
High dose (undiluted)	10	5	5
Positive control: cyclophosphamide (50 mg/kg)	5	5	0

Genotoxicity Studies

Three different tests were performed to evaluate the genotoxicity of Mirasol PRT–treated human platelet products: an in vitro test for gene mutations in bacteria, an in vitro test for clastogenicity in mammalian cells, and an in vivo bone marrow micronucleus test.

Bacterial reverse mutation assay (Ames test). Mirasol PRT–treated, day 5 platelets (with and without γ-irradiation) and control, untreated day 5 platelets were tested using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2 uvrA in the presence and absence of rat-liver derived S9, prepared by the contract research organization. The assay was performed in 2 phases, using the plate incorporation method and normal saline as the diluent. The first phase established the dose range for the confirmatory mutagenicity assay and provided a preliminary mutagenicity evaluation. In the first phase, the dose levels tested were 0.33, 1.0, 3.3, 10, 33, 100, 333, and 1000 μL per plate. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article with dose levels of 3.3, 10, 33, 100, 333, and 1000 μL per plate.[83,84]

The in vitro test for gene mutations in bacteria (Ames test) was also performed with lumichrome, the major product of riboflavin photolysis. The in vitro mutagenicity of lumichrome was evaluated in the dark to test its mutagenic potential in the absence of light activation. The maximum concentration tested was at the limit of solubility of lumichrome.

Chromosomal aberration in Chinese hamster ovary cells. Mirasol PRT–treated platelet products and control, untreated platelet products were tested to assess potential clastogenicity (chromosome breakage) in vitro with the mammalian chromosome aberration test. The assay was performed using Chinese hamster ovary (CHO) cells.[85-87] Based on the findings from the preliminary toxicity assay, the doses chosen for the chromosome aberration assay using the test article (Mirasol PRT–treated platelets) and negative control (untreated platelets) ranged from 0.005 to 0.1 mL/mL for all 3 exposure groups. The test and control articles were soluble in saline at a concentration of 1 mL/mL, the maximum concentration used. Both test and control article were tested in the absence and the presence of an Aroclor-induced S9 activation system.

The CHO cells were exposed to test or control article for 4 and 20 hours in the nonactivated test system and for 4 hours in the S9 activated test system. All cells were harvested 20 hours after treatment initiation. Selection of doses for microscopic analysis was based on toxicity of the test article (the lowest dose with at least 50% reduction in cell growth and 2 lower doses) in all harvests.

Mammalian erythrocyte micronucleus test. Mirasol PRT–treated platelets and control, untreated platelets were tested to assess clastogenicity in vivo with the mammalian erythrocyte micronucleus test.[88-90] An increase in the frequency of micronucleated polychromatic erythrocytes is expected in the bone marrow of animals exposed to genotoxic compounds. The assay was performed in 2 phases. In the pilot toxicity study, neat test article was administered intraperitoneally to 5 male and 5 female mice at a volume of 20 mL/kg (the highest optimal single-dose volume). No mortality was observed during the course of the study. All mice were observed for clinical signs of toxicity after dose administration and daily for 3 days afterward. Body weights were recorded before dose administration, 1 day after, and 3 days after dose administration. Results from the pilot study were

used to assess toxicity of the test article and to set dose levels for the definitive study.

In the definitive micronucleus study, 1 intraperitoneal injection was administered to the male and female mice in each group. The animals were observed for clinical signs of toxicity after dose administration. Table 4 describes the study groups, the number of animals per group, and test or control articles given to each group, as well as the sacrifice times. Immediately after sacrifice, bone marrow was aspirated from the femurs into a syringe. Two slides of bone marrow suspension were prepared and fixed for each mouse. The slides were analyzed for the presence of micronuclei in bone marrow cells, polychromatic erythrocytes, and normochromatic erythrocytes.

Neoantigenicity Studies

The potential for Mirasol PRT–treated platelets to exhibit neoantigenicity (the production of new antigenic determinants due to the treatment) was evaluated with 2 in vitro and 1in vivo test. Because the photoproducts associated with the Mirasol PRT System are the same as metabolites, catabolites, and photoproducts of riboflavin normally present in humans under routine conditions, there was no reason to consider that they might themselves affect the immune system, and there were no indications of any such effect in the toxicity tests.

^{14}C-riboflavin binding evaluation. The potential for neoantigen formation was first assessed in vitro by measuring the binding of radiolabeled riboflavin to platelets and plasma proteins after Mirasol PRT treatment and comparing the results with those obtained with untreated controls. This method assesses binding of a radiolabeled compound to proteins and cellular membranes. The presence of covalently bound compounds on cellular membranes or proteins indicates that chemical modification has occurred. This modification could be seen as foreign in the bloodstream and result in an immune response. Immune responses to antibiotics, bound to the surface of red blood cells, have been observed.[91] Quinacrine mustard, which binds to proteins and blood cells, also results in the production of antibodies.[92-94] If Mirasol PRT treatment were to lead to chemical modification of blood constituents in vitro, the in vivo generation of an immune response might be possible. Prior studies have identified the potential for binding of riboflavin to albumin.[95] Reports of additional studies, carried out in nonphysiologic conditions, including the use of artificial media preparations, anaerobic conditions, and nonphysiologic pH values, claimed to demonstrate the formation of riboflavin-protein adducts via tryptophan residues in the protein.[95] This work also extended further to show the formation of possible antibodies against such complexes.[97] Other studies with human plasma samples were unable to reproduce these results and instead showed riboflavin interactions to occur primarily with immunoglobulins, which act as natural carriers for the compound in vivo and are a potential source of claims for antibody generation.[98,99] The studies of ^{14}C-riboflavin binding before and after photolysis were thus undertaken to assess the potential for adduct formation under the specific conditions used for Mirasol treatment of blood products.

To determine the distribution of riboflavin and its photoproducts after Mirasol PRT treatment, a known amount of radiolabeled riboflavin (^{14}C) was added to unlabeled riboflavin solution, and the resulting solution was used to treat platelet products from 6 different donors with the Mirasol PRT System. Preillumination samples from each platelet product were used as controls, and test articles were postillumination samples. Cell concentrations, protein-associated radioactivity, and protein concentrations were determined for all samples. The method was sensitive enough to determine differences of less than 1% in binding of ^{14}C-labeled compound, at micromolar concentrations per milligram of protein, if such effects were occurring. Given prior reports of the levels and extent of photochemical modification of proteins under specific conditions of treatment, if such modifications were occurring with Mirasol treatment, this method would be able to detect it.[96,97]

Evaluation of nonimmunologic alterations. To assess the potential for nonimmunologic alterations to cells that could subsequently induce an immunologic response, another test, the Capture-P assay, (Immucor, Inc, Norcross, GA) was used to evaluate the binding of immunoglobulin G to Mirasol PRT–treated platelets, and to untreated platelets, after exposure to allogeneic and autologous plasma. Platelet products, and the associated plasma, from 2 different donors were used to provide the test and control articles for this study. Platelets were tested with the Capture-P assay after 1 and 5 days of

storage. This study was non-GLP but was well-documented, performed in a respected clinical laboratory, and included appropriate controls.

Neoantigenicity evaluated with the Ouchterlony assay. The formation of new antigens was evaluated in vivo by comparing the immune response between animals given control (untreated) human platelets and animals given Mirasol PRT–treated human platelets. Rabbits were immunized with control article or with 1 of 2 test articles (Mirasol PRT–treated, day 5 human platelets, with or without γ-irradiation). The immunizations occurred on days 0, 21, and 42 of the in-life portion of the study. On day 49, sera from the immunized animals in the test and control groups were collected and tested for antibody production. Antibodies were collected and used in the Ouchterlony assay to incubate with either control or test article. Lines of immunoprecipitation were examined to determine reactions of identity, partial identity, or nonidentity. Partial identity immunoprecipitation lines would indicate neoantigenicity if the test article contained new antigens arising as a result of the treatment. Sensitivity of the assay was evaluated with chicken albumin and serum from rabbits immunized with that compound. The chicken albumin could be detected by weak precipitin lines at 0.15 μg/40 μg plasma protein and at 0.15 μg/200 μg plasma protein, with antibody present at 10, 15, 20, or 25 μg per well in the assay plate.

Cytotoxicity Studies

Agar diffusion test. Mirasol PRT–treated human platelets and control, untreated human platelets were tested to assess cytotoxicity with the agar diffusion assay. The indicator cells for this assay were mouse fibroblast L929 cells, which are classically used for cytotoxicity studies because they demonstrate sensitivity to leachable cytotoxic compounds.[100]

Cultures of L929 cells were used in the assay after replacement of the liquid medium with a serum-supplemented medium/agar mixture that is stained with a vital dye, neutral red. The culture was protected from light for the duration of the assay to prevent cell damage elicited by photoactivation of the stain. The test article and control article were applied directly to a filter paper disc (surface area 100 mm^2) at a volume of 100 mL and placed on the surface of the agar. All plates were incubated for 48 hours, at 37°C ± 1°C, in a humidified atmosphere containing 5% ± 1% carbon dioxide. The negative control article (negative control plastic) and the positive control article (natural rubber) were prepared and exposed similarly to the test and control articles. The extent of decolorization was evaluated at times 0, 24, and 48 hours.

Minimal essential medium elution study. The minimal essential medium elution study[100] was conducted to evaluate the potential cytotoxicity of Mirasol PRT–treated human platelets. The test was designed for the evaluation of test article extracts with assessment of biologic reactivity of a mammalian cell culture (L929). Aliquots of test article (Mirasol PRT–treated human platelets) and control article (untreated human platelets) were centrifuged, after which the resulting platelet pellets were mixed with the extraction medium (minimal essential medium) and incubated at 37°C ± 1°C for 24 ± 2 hours. After extraction, the medium was removed and applied to an L929 cell culture. The L929 cultures were incubated for 48 hours, at 37°C ± 1°C, and then evaluated for the response of the cell monolayer. Biologic reactivity (cellular degeneration and malformation) was rated on a scale of 0 to 4.

In addition, the primary product of riboflavin photolysis, lumichrome, was tested for in vitro cytotoxicity by direct exposure to the indicator. The lumichrome solution, at the maximum possible lumichrome concentration, was exposed to the indicator cells.

Hemocompatibility Studies

Hemolysis test. Mirasol PRT–treated human platelets and control, untreated human platelets were assessed for hemolytic activity in direct contact with human blood.[101-103] Fresh, whole human blood (type-matched to be compatible with test and control articles) was collected into EDTA-coated Vacutainer tubes. The positive control article solution was obtained by adding 10 mL of sterile water for injection per vial. The test and control article solutions were obtained by adding 5 g of material to 10 mL of sterile 0.9% NaCl. All vials were incubated in a 37°C ± 2°C water bath for 30 minutes, followed by addition of 0.2 mL of diluted human blood and incubation in a 37°C ± 2°C water bath for 60 minutes. After incubation, the vials were centrifuged for 5 minutes at approximately 1500*g*, and the absorbance of each supernatant was determined against an NaCl blank

Table 5. Summary of Samples for Pharmacokinetic Study

Group no.	Urine*	Feces*	Cage rinse	Cage wash and wipe	Blood†	Tissues‡
1 (Intact)	Predose, 0-12 h, 12-24 h, 24-h intervals to 144 h	Predose, 24-h intervals to 144 h	24-h intervals to 120 h	144 h	144 h	144 h
2 (Intact)	NA	NA	NA	NA	1 h, 8 h, 24 h, 48 h, 96 h	1 h, 8 h, 24 h, 48 h, 96 h

* Urine and feces collected on wet ice. Urine assayed directly by liquid scintillation chromatography (LSC). Remaining urine saved for analysis of metabolites.

† Blood collected from all animals in groups 1 and 2 predose and at designated time points. Prep and assay plasma by LSC. Remaining plasma saved for analysis of metabolites.

‡ Tissues collected include the following: group 1—residual carcass, pelt (hair and skin with underlying fat layer), and tail skin; group 2—small intestine, large intestine, spleen, kidneys, liver, lymphatics (lymph nodes, includes splenic, inguinal, and popliteal), and femur bone marrow (right and left).

at 540 to 545 nm. Test and control articles were tested in triplicate.

Platelet function studies. Platelet function and the thrombogenicity of Mirasol PRT–treated platelets were directly evaluated in a series of non-GLP experiments. The hemostatic activity and thrombogenicity of treated platelets were measured in an ex vivo model in which Mirasol PRT–treated human platelets were mixed with whole blood previously depleted of platelets. The details of the experimental system are described by Perez-Pujol et al.[55] In brief, 8 Trima apheresis platelet collections were performed to collect more than 340 mL at a concentration between 1180 and 2160 × 10^6 cells/mL. Half of the product volume was treated with the Mirasol PRT System. The other half was used as the paired control. Treated and control platelets were stored in a platelet storage incubator at 22°C ± 2°C for 5 days. Samples were tested using flow cytometric and perfusion methods on days 0, 3, and 5 of storage.[55]

The flow cytometric analysis used commercially available monoclonal antibodies, tagged with fluorescein or phycoerythrin, to detect glycoprotein (GP) IIb-IIIa, GPIb, GPIV, P-selectin, the 53-kd lysosomal membrane protein, and coagulation factor V. The exposure of aminophospholipids on the outer leaflet of the platelet membrane was also quantified using annexin V. The binding of fibrinogen and von Willebrand factor was detected with the corresponding polyclonal antibodies, and nonspecific membrane immunofluorescence was evaluated.

Blood for perfusions was obtained from healthy volunteers and depleted of platelets and leukocytes by filtration. Samples taken from the test and control articles at different days were incorporated into the thrombocytopenic blood at volumes aimed to increase the platelet count in the perfusate to 150 × 10^9/L with a final volume of 20 mL. Perfusions were performed at 37°C in annular chambers with enzymatically denuded New Zealand rabbit aorta segments. Blood was recirculated through the chamber for 10 minutes at a shear rate of 800 s^{-1}. At the end of the perfusion, the segments were rinsed with 20 mL of PBS, removed from the chamber, and sliced off and washed with a fixing buffer. Platelets interacting with the subendothelium were evaluated and classified as either adhesion, contact platelets that are attached but not spread and groups of platelets that form aggregates of less than 5 μm in height, or thrombus, platelets that form aggregates of 5 μm or more in height.

Coagulation proteins and complement pathway. Mirasol PRT–treated human platelets and control, untreated human platelets were stored at 22°C for 5 days, after which samples were removed and centrifuged to isolate the plasma fraction. The plasma fraction was analyzed with function-based assays for key protein components of each hemostatic pathway—the contact pathway, the thrombin pathway, the fibrinolytic pathway, the complement pathway, and the coagulation pathway. Factor XI (FXI), factor XII (FXII), high-molecular-weight kininogen (HMWK), prekallikrein (PK), and activated factor XII (FXIIa) were assessed as components of the contact pathway. Prothrombin fragments 1 and 2 (F1+2), thrombin-antithrombin complex, and D-dimer were assessed as components of the thrombin pathway. Plasminogen (PLG), α_2-antiplasmin (A2A), and PLG

Table 6. Summary of the Results of the Systemic and the Reproductive Toxicity Studies

Test	Findings
Systemic toxicity	
Acute toxicity of photolyzed riboflavin in rats	• No test-article–related changes in mortality, clinical signs, body weight, food consumption, clinical pathology, organ weights, or macroscopic pathology evaluations were observed.
Acute toxicity of Mirasol PRT–treated platelets in rats*	• No test-article–related mortality, clinical observations, body weight changes, food consumption changes, or macroscopic changes were observed.
Acute toxicity of Mirasol PRT–treated platelets in dogs*	• No test-article–related mortality, clinical observations, body weight changes, food consumption changes, or macroscopic changes were observed.
Subchronic toxicity of Mirasol PRT–treated plasma in dogs	• No toxicologically significant findings were observed in clinical, physical, electrocardiographic, or ophthalmascopic examinations. • Macroscopic observations did not yield any test-article–related effects in dogs of either sex. • Test-article–related findings for decreased body weight, increased eosinophils, increased chloride, and decrease thymus weights with associated microscopic findings were noted; however, these findings were minor and were not considered to be toxicologically significant.
Reproductive toxicity	
Embryo-fetal development in rats exposed to Mirasol PRT–treated plasma	• No test-article–related clinical signs of toxicity were observed during treatment period, no dam had all fetuses resorbed, and no deaths occurred during study. • Treated group exhibited significant increase in gestation body weight on gestation day 18, and body weight changes were significantly increased on gestation day intervals 12-15, 6-18, and 0-20. Increase correlated with significantly increased food consumption in treated-group animals. • No significant differences were observed in fetal weight (g), gravid uterine weight, adjusted weight change, postimplantation loss, nonviable fetuses/, litter size, early and late resorptions, mean no. of corpora lutea/litter, no. of implantation sites, preimplantation loss, no. of viable fetuses/letter, and sex ratio (% males/litter). • No external or test-article–related visceral abnormalities were reported in any of fetuses examined. No significant difference in skeletal malformation and variations were observed in control and treated groups.

*Indicates that study was done with and without γ-irradiation of treated blood products.

activator inhibitor type 1(PAI-1) were assessed as components of the fibrinolytic pathway. CH50/ classical pathway, C3a, C5a, and C1 esterase inhibitor were assessed as components of the complement pathway, and antithrombin III, protein C (PC), and protein S (PS) were assessed as components of the coagulation pathway. Mean values for test and control articles were compared using Student *t* test. In addition, assays for chemotaxis (response to C5a) and assays of myeloperoxidase release from cytochalasin B–treated human polymorphonuclear leukocytes (response to C3a and C5a) were used to further assess physiologic relevance of any changes after treatment.

Pharmacokinetics of Photolyzed ^{14}C-Riboflavin in Rats

The in vivo pharmacokinetics and elimination of riboflavin and its photoproducts in rat plasma treated with the Mirasol PRT System were also studied. Sterile-filtered rat plasma was mixed with 500 μmol/L ^{14}C-riboflavin, treated by the Mirasol PRT System, and administered to male CD rats by IV injection in a single dose. The study consisted of 2 treatment groups of male Crl: CD(SD) IGS BR

Table 7. Summary of the Results for the Mammalian Erythrocyte Micronucleus Study

Test	Findings
Test for clastogenicity in mammalian cells (in vivo; mouse bone marrow micronucleus test)	• No mortality occurred at any dose level during course of micronucleus study. • Clinical signs, noted on days after dose administration, included lethargy and piloerection in males and females dosed with untreated platelets and in all animals dosed with test article (neat and diluted). In addition, hunched position was noticed in animals at middle (1:1 dilution) and high (neat) dose. All other mice treated with vehicle or positive control articles appeared normal during course of study. • No appreciable reduction in ratio of polychromatic erythrocytes to total erythrocytes was observed in groups treated with negative control or test article, relative to vehicle control groups. This suggests that negative control and test article did not inhibit erythropoiesis. No significant increase in incidence of micronucleated polychromatic erythrocytes in negative control or test-article–treated groups relative to respective vehicle control groups was observed, regardless of sex, dose, or bone marrow collection time.

rats. A treated group of 3 rats (group 1) and a treated group of 15 rats (group 2) each received the radiolabeled test article at a target radiolabeled riboflavin dose level of 0.48 mg/kg with approximately 555,000 Bq per animal (mean actual dose levels of approximately 0.38 mg/kg and 547,600 Bq per animal). The dose volume for both groups was approximately 4 mL per animal.

All animals were observed twice daily for morbidity, mortality, injury, and the availability of food and water. Urine and feces were collected from group 1 animals predosing and at intervals through 144 hours postdosing. In addition, whole blood was collected, and plasma was prepared, predosing and at the end of the experiment. The tissues collected for postmortem study were residual carcass, pelt, and tail skin, as well as small intestine, large intestine, spleen, kidneys, liver, lymphatics, and right and left femur bone marrow. The concentration of radioactivity in whole blood, plasma, urine, feces, tissues, and cage residues was determined, and analyses of the kinetics of ^{14}C-labeled compound excretion were performed. The plasma and tissue concentrations obtained were for radioactivity associated with riboflavin and its photoproducts, rather than for individual compounds. Table 5 summarizes the samples analyzed for each group. The pharmacokinetic parameters of riboflavin and photoproduct in plasma, small intestine, large intestine, spleen, kidneys, liver, lymphatic, and femur bone marrow tissues were determined from mean concentration-time data in the test animal. Appropriate validated software was used to calculate these parameters.

Measurement of Leachable and Extractable Compounds in Mirasol PRT–Treated Products

In addition to standard medical device testing for plastic components, several novel methods were applied to evaluate the levels of leachable and extractable compounds in human blood products after treatment with the Mirasol PRT System. This non-GLP analysis was performed to identify and quantify compounds that might migrate directly into the platelet product as a result of treatment with the Mirasol PRT System. Untreated and Mirasol PRT–treated products were analyzed by gas chromatography and mass spectrometry for volatile and semivolatile organic compounds, by inductively coupled plasma-atomic emission spectroscopy (ICP) for metals, and by Fourier transform infrared spectroscopy (FTIR) for polymeric materials.

RESULTS

In all studies of toxicity, γ-irradiated Mirasol PRT–treated products yielded the same results as non–γ-irradiated, Mirasol PRT–treated products.

Systemic Toxicity

No toxicologically significant findings were observed in any of the studies of acute toxicity (Table 6). No toxicologically significant findings were observed in the repeated-dose toxicity study

Table 8. Clotting Factor Levels in Treated and Untreated, Control Platelet Preparations After 5 Days of Storage

	Reference range[104]	Control (n = 6)	Treated* (n = 6)	P
FXI (IU/mL)	0.42-1.44	0.86 ± 0.13	0.59 ± 0.12	<.05
FXII (IU/mL)	0.40-1.52	1.40 ± 2.0	1.07 ± 0.23	<.05
PK (IU/mL)	0.65-1.35†	1.11 ± 0.06	0.73 ± 0.10	<.05
HMWK (IU/mL)	0.65-1.35†	0.78 ± 0.12	0.68 ± 0.29	NS‡
Antithrombin III (IU/mL)	0.72-1.45	0.82 ± 0.01	0.84 ± 0.11	NS
PC (IU/mL)	0.58-1.64	1.02 ± 0.19	0.75 ± 0.8	<.05
PS (IU/mL)	0.56-1.68	0.16 ± 0.08 (3 of 6 @ <0.11)	0.13 ± 0.03 (4 of 6 @ <0.11)	NS
PAI-1 (IU/mL)	0-31†	3.6 ± 1.3	17 ± 4	<.05
PLG (IU/mL)	0.68-1.44	1.04 ± 0.10	0.88 ± 0.08	<.05
A2A (IU/mL)	0.72-1.32	0.88 ± 0.05	0.82 ± 0.07	<.05
D-D (ng/mL)	0-255†	5/6 <110, 1 at 139	<110	NA§
FXIIa (ng/mL)	0-3.6†	1.5 ± 0.2	4.8 ± 0.9	<.05
F1 + 2 (nmol/L)	0.4-1.8†	0.5 ± 0.0	1.0 ± 0.1	<.05
TAT (ng/mL)	0.0-5.0†	3 of 6 <0.6; 0.7 ± 0.1	1.1 ± 0.4	NA
C3a (ng/mL)	0-940†	5510 ± 552	16 594 ± 4939	<.05
C5a (ng/mL)	4.7-9.5†	8 ± 1	16 ± 3	<.05
C1 esterase Inhibitor (mg/mL)	8.0-19.5†	11 ± 2	7 ± 1	<.05
CH50/classic pathway (U/mL)	176-282†	281 ± 56	21 ± 18	<.05

Abbreviations: PC, protein C; PS, protein S; A2A, alpha-2-plasmin; D-D, D-dimer; TAT, thrombin-antithrombin complexes.

* Values reported for plasma from Mirasol PRT–treated platelets are corrected for the dilution due to the addition of riboflavin.

† Reference range specific to laboratory or assay kit.

‡ $P > .05$.

§ Most data below detection limit of assay.

(Table 6). In the repeated-dose toxicity study, the levels of riboflavin and lumichrome in blood samples from animals receiving Mirasol PRT–treated products were below the limits of quantification, as were the levels in blood samples from animals receiving untreated plasma. These results were consistent with the observed rapid clearance of riboflavin after IV administration, both in the literature[40] and in the pharmacokinetic study with ^{14}C-riboflavin in Mirasol PRT–treated products.

Developmental Toxicity and Genotoxicity

No developmental toxicity was observed in the embryo-fetal development study. All fetuses were examined for malformations and developmental variations (Table 6). No mutagenicity was observed in the Ames test (with and without S9 metabolic activating fraction) for treated or control human platelets, or for lumichrome. The in vitro and in vivo tests for clastogenicity in mammalian cells (chromosomal aberration in cultured CHO cells and micronucleus test in mouse bone marrow cells, respectively) were also performed with Mirasol PRT–treated products. Human platelets treated with the Mirasol PRT System gave negative results in all genotoxicity experiments. Detailed results of the in vivo genotoxicity experiment are summarized in Table 7.

Neoantigenicity and Cytotoxicity

Results of studies using ^{14}C-labeled riboflavin and exposure of platelets and plasma to UV light did not demonstrate any detectable binding of riboflavin or its photoproducts to platelets or to

Table 9. Clotting Factor Levels in Mirasol PRT–Treated FFP

		Mirasol FFP	
	Reference range[104]	Treated*, assayed postillumination (n = 27)	Treated*, stored for 1 y (n = 27)
Fibrinogen (mg/dL)	145-385	227 ± 60	216 ± 32
FVIIIa activity (IU/mL)	0.52-1.55	0.6 ± 0.2	0.9 ± 0.2
FII activity (IU/mL)	0.65-1.54	0.8 ± 0.1	0.8 ± 0.1
FV activity (IU/mL)	0.54-1.45	0.7 ± 0.1	0.6 ± 0.1
FVII activity (IU/mL)	0.62-1.65	0.9 ± 0.2	0.6 ± 0.1
FIX activity (IU/mL)	0.45-1.48	0.8 ± 0.2	0.7 ± 0.1
FX activity (IU/mL)	0.68-1.48	0.8 ± 0.1	0.8 ± 0.1
FXI activity (IU/mL)	0.42-1.44	0.8 ± 0.2	0.6 ± 0.1
FXII activity (IU/mL)	0.40-1.52	1.0 ± 0.3	0.8 ± 0.1
Total protein (g/L)	48-64	57 ± 2	53 ± 3
Antithrombin III (IU/mL)	0.72-1.45	0.8 ± 0.1	1.0 ± 0.1
PC (IU/mL)	0.58-1.64	1.1 ± 0.2	0.9 ± 0.2
PS (IU/mL)	0.56-1.68	0.8 ± 0.1	1.0 ± 2
PLG (IU/mL)	0.68-1.44	0.8 ± 0.2	0.9 ± 0.2
A2A (IU/mL)	0.72-1.32	1.0 ± 0.2	0.9 ± 0.1
HMWK (IU/mL)	0.65-1.35	0.8 ± 0.2	0.7 ± 0.2

* Values reported for Mirasol PRT–treated plasma are corrected for the dilution due to the addition of riboflavin.

Table 10. Complement Test Results in Treated and Untreated, Control Platelet Preparations

Sample	Chemotaxis assay	Enzyme release assay
Reference range	Positive control = 60.4 Negative control = 16.8	Positive control = 1.13 Negative control = 0.32
Control (n = 6)	22.8 ± 1.8	0.41 ± 0.03
Treated (n = 6)	24.2 ± 5.0	0.45 ± 0.14
P	NS	NS

NOTE: NS, $P > .05$.

plasma proteins. No evidence of neoantigenicity was observed with the Ouchterlony assay, indicating that no new antigens were formed during treatment with the Mirasol PRT System. Treatment with the Mirasol PRT System did not result in greater immunoglobulin G binding than what was observed in comparison with untreated controls, when assessed with the Capture-P assay. In the tests of lumichrome cytotoxicity, and of the cytotoxicity of Mirasol PRT–treated products, no cytotoxicity was observed.

Hemocompatibility

In tests of hemocompatibility, no hemolysis was observed. In functional assessments, when mixed with thrombocytopenic whole blood, the function of Mirasol PRT–treated platelets was well preserved, in comparison with controls.[55] Treated platelets displayed no evidence of hyperactivation or hypercoagulability. In evaluation of plasma removed from platelet products stored for 5 days, statistically significant changes in PRT-treated samples were observed in FXI, FXII, PK, PC, PS, PAI-1, PLG, A2A, FXIIa, and F1+2. The activities of FXII, PK, PC, PAI-1, PLG, and F1+2 were within the reference range. The activities of the remaining proteins (FXI, PS, A2A, HMWK, and FXIIa) were outside the reference range, but the changes were not deemed to be clinically relevant compared with the levels in normal, untreated blood (Table 8). Similar studies on platelets after γ-irradiation yielded similar findings. Because analysis of plasma protein levels in platelet products stored at room temperature for extended periods shows degradation in protein quality due to storage alone, Mirasol PRT–treated FFP was assessed for factor activity. Results are presented in Table 9 for samples held for 8 hours at room temperature after collection and before treatment with Mirasol PRT to simulate worst-case conditions for routine treatment of plasma products. Samples were then treated and stored frozen until analysis of factors in standard coagulation assays. Results for all samples analyzed remained within historical reference ranges based on untreated, historical, and concurrent controls. Results for Mirasol PRT–treated FFP are representative of values obtained for plasma from Mirasol PRT–treated platelets immediately posttreatment.

The plasma from Mirasol PRT–treated human products contained significantly more C3a desarg

Table 11. Results of Pharmacokinetic Study and Test for Leachables and Extractables

Test	Findings
Pharmacokinetics of photolyzed ^{14}C-riboflavin in Mirasol PRT–treated rat plasma	Approximately 95% of total radioactivity administered to animals was eliminated within approximately 260-275 h postadministration Based upon observed areas under the curve, tissues exhibiting highest to lowest overall exposure were liver > kidneys > large intestine > small intestine > spleen > femur bone marrow > lymphatics Observed mean $t_{1/2}$ estimates determined from radioactivity excretion rate data in urine (55.3 h) and determined from radioactivity excretion rate data in feces (55.1 h) were in excellent agreement with $t_{1/2}$ estimate (52.1 h) for plasma
Assessment of leachable and extractable compounds using standard medical device biocompatibility testing	No significant differences before and after exposure to treatment conditions
Assessment of leachable and extractable compounds in Mirasol PRT–treated human platelets and untreated human platelets	No polymeric material was detected by FTIR in either treated or untreated platelet extracts All metals detected in treated-platelet extracts were present in untreated-platelet extracts in similar amounts.

Abbreviation: FTIR, Fourier transform infrared spectroscopy.

and C5a desarg (2.7- and 1.8-fold higher, respectively) and significantly lower values for C1 esterase (1.8-fold lower) and CH50 (14-fold lower) than in plasma from untreated controls (Table 8). The desarg forms of C3a and C5a are biologically inactive forms of the anaphylatoxins produced in the common pathway of complement activation. The increase in these inactive forms in treated products and decrease in the active component, CH50 (total hemolytic complement), are consistent. Little to no complement-derived activity was observed in plasma from either Mirasol PRT–treated platelets or untreated controls, and there were no significant differences between treated and control results (Table 10). Similar studies performed on products after γ-irradiation yielded identical results (data not shown).

Pharmacokinetics of Photolyzed ^{14}C-Riboflavin in Rats

After a single IV administration of Mirasol-treated plasma containing photolyzed ^{14}C-riboflavin, the radioactivity was well distributed from the whole blood to tissues selected for assay within the first hour postdose. Most of the excreted urinary radioactivity was recovered by 12 hours postdose, and more than half of all radioactivity was excreted in urine. Blood levels of radioactivity declined rapidly post-dose, as expected from studies of riboflavin metabolism and excretion in humans.[40] Measurements of the radioactivity associated with the ^{14}C-riboflavin–treated plasma indicated rapid initial apparent distribution (and/or clearance) from the systemic circulation that appeared to be complete within the first 8 to 48 hours postdose. Table 11 contains a summary of the key study results.

Leachables and Extractables

The leachables and extractables analyses detected no polymeric material in either test or control platelet products (Table 11). The Mirasol illumination/storage bag does not contain the plasticizer di(2-ethylhexyl)pthalate (DEHP), and testing verified that this plasticizer was not present in treated and stored products. No toxicologically relevant concentrations of metals were found. These results correlate with those from the biocompatibility testing of the Mirasol illumination/storage bag elements—all elements are biocompatible.

DISCUSSION

The design of the Mirasol PRT device, the strategy of the investigative work, and the specific experiments that were conducted to evaluate toxicity were based first on scientific considerations and then on the need to comply with regulatory requirements. Consideration was also given to minimizing the need for animal experimentation as far as permitted by the information required and the need for reliable scientific experiments. The system design and the experimental program were intended to answer potential scientific and medical concerns and to permit a comprehensive safety assessment of the Mirasol PRT System. The design of the preclinical safety studies was also intended to conform to specific regulatory requirements in different countries, adapted to the nature of this innovative device. The official guidelines used for the study design included ISO 10993[79] for medical devices and the general toxicity and pharmacokinetic guidelines for drugs of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.[82,105] The nonclinical toxicity program was based throughout on studying preparations that had undergone Mirasol PRT treatment, a process shown to be effective in reducing levels of several types of infectious agents.[46,49,50,106,107]

The testing of treated blood products has examined the potential for toxicity resulting from the entire process (from addition of riboflavin and controlled illumination in the Mirasol illumination bag) and has examined the potential for harm to the blood products themselves. Assessments included effects on the cells and plasma proteins, the toxicity of riboflavin itself and its photoproducts, and the effects on other system components comprising the medical grade plastic connectors and bags used in this process. The results obtained have been compared with extensive literature on the effects of riboflavin and its photoproducts in animals and humans arising from its medical use as a vitamin and more widely as a food colorant or additive. The methods used have been those recommended by current official guidelines interpreted according to the special nature and properties of platelets and plasma and designed to provide as much information as possible from focused in vitro and in vivo studies. The difference in size between human patients and in vivo animal

test systems has restricted application of the standard toxicological strategy of administering a range of doses far exceeding the human dose to reveal possible toxic hazards. In studying Mirasol PRT–treated blood products, the largest volumes or doses have been given consistent with maintaining the welfare of any animals used and to separate effects due to toxicities of the components from toxicities due to volume overload alone.

The results of the in vivo and in vitro toxicity tests have reproduced previously reported findings (Table 1) for riboflavin and its photoproducts in animals and humans under relevant conditions, despite the special circumstances of the presence of blood cells and proteins and the use of various medical-grade plastic connectors and storage containers. There has been no indication, even after repeated administration of the maximum achievable exposure to Mirasol PRT–treated products (6 times per week for 13 weeks in the subchronic test in the dog), of any untoward effect on the functions of the major physiologic systems or in producing any target organ toxicity. Furthermore, a variety of special tests have shown that no detectable new antigens are present on Mirasol PRT System–treated cells or proteins and that such preparations did not stimulate antibody formation. The wide range of toxicity tests has also excluded any genotoxic risk and any toxic effect on pregnant animals and on embryo-fetal development. Local tolerance at the site of IV infusion has been shown to be good. The treated platelet preparations have not shown any cytotoxicity, nor did they leach unwanted substances from the containers and connectors used in the Mirasol system.

These results indicate the lack of specific toxicity of Mirasol PRT–treated products, and because of the experiments done, they give a strong indication that the same medical acceptability is likely also to apply to both human platelets and to human plasma treated in the same way. The absence of toxicity was considered likely given the extensive prior experience in humans and the laboratory experience of the safety of the chemicals involved (riboflavin, lumichrome, and the other riboflavin photoproducts), but it has been very important to confirm that prediction and to extend it to include the special cells and plasma proteins also involved in the Mirasol System. To this should be added the evidence of retention of acceptable platelet function and survival after treatment, which has been the subject of additional peer-reviewed publications.[46,51,55,57]

Thus, there is evidence of the safety of this process in a broad range of laboratory tests from which a qualitative extrapolation of safety in patients can be made. Quantitative prediction of safety, or at least of the margin of safety represented by the difference between the relative exposures of man and animals and the observed "no effect level" in the laboratory, must be more cautious, but there are relevant data on which to base some realistic predictions.

The likely exposure of humans to riboflavin and its photoproducts is 0.077 mg/kg for each unit of product transfused. Likely exposure of recipients in a clinical setting was calculated on the assumption of a mean recipient weight of 70 kg, nominal riboflavin solution concentration of 500 μmol/L, mean riboflavin photoconversion of 18%, and nominal riboflavin solution volume of 35 mL. This exposure level can be compared with the lethal dose at which 50% of the population survives reported for IV riboflavin in mice (50-100 mg/kg)[30] yielding a safety factor of at least 50/0.077 = 649. However, this safety margin calculation is somewhat illusory because there is no reliable indication from the clinical literature, or from appropriate animal experiments, of the toxic dose of riboflavin in man, and so, the upper bound of the "no toxic effect level" remains unknown. It is reasonable only to conclude that there is no reason to predict toxicity in man due to parenteral exposure to riboflavin and its photoproducts in Mirasol PRT–treated products until much higher exposures are reached than would ever be feasible from platelet or plasma transfusions. For similar reasons, comparison of exposure from treated platelets with the ADI for riboflavin of 0 to 0.54 mg $\times$ kg^{-1} $\times$ d^{-1} is no less misleading because the ADI refers to life-long, daily exposure to the substance in the diet and is not an appropriate comparator for a short-term parenteral exposure even if the latter is repeated up to several times per week, at least for a few months.

In these circumstances, which are not unusual for physiologically essential substances, the best that the toxicologist can offer is the prediction that riboflavin and its photoproducts have low experimental toxicity in vivo. Published work suggests that administration of the agent in the extreme may result in renal damage due to crystalluria as the most prominent toxic effect in rodents. This work is

derived from studies in which animals were given very large IV doses, and the harmful effect is very probably a consequence of the limited aqueous solubility of riboflavin. This is far removed from any circumstance associated with administration of Mirasol PRT–treated products in the transfusion setting. There is no realistic indication that chemical toxicity will be a limiting factor in any likely course of treatment with Mirasol PRT–treated products.

Results from all genotoxicity studies with the Mirasol PRT System have been negative. This has included evaluation of all photoproducts and photolyzed platelets and plasma at the maximum tolerable or maximum soluble limits for these assays. The absence of any indication of potential tumorigenic effects in the subchronic and other tests, the lack of genotoxicity, the chemical nature of riboflavin and its photoproducts, and the lack of suspicion arising from the physiologic role and human experience of this vitamin obviated the need for further carcinogenicity testing. Prior carcinogenicity testing of riboflavin in historical studies (Table 1) is consistent with these findings and also supportive of the lack of carcinogenic potential of this compound.

The Mirasol PRT System has been evaluated in an extensive preclinical safety evaluation program. The historical literature available on riboflavin and its photoproducts as well as the results of this work support its safety profile in man. The results obtained from the studies conducted and reported here are consistent with these data and indicate that the risk of toxicity due to the use of this system in the transfusion setting should be low.

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文献25

自己宣言のための
CEマーキング適合対策実務
ガイドブック

2005年3月

日本貿易振興機構

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はじめに

ニューアプローチ指令とは

EU 統合の一環として、産業界の技術上の統一を図るために、1985 年 5 月に「技術的な整合と規格へのニューアプローチ」が決議された。ニューアプローチ指令に規定された製品については、基本的要求事項に適合していなければならない。また、この要求事項に適合している製品の EU 域内での出荷、流通のためには、CE マーキングが貼付されている必要がある。

現時点では、以下のニューアプローチ指令が出されている。

（1）低電圧機器	（2）単純圧力容器
（3）玩具	（4）建設資材
（5）EMC	（6）機械
（7）個人保護具	（8）非自動重量計
（9）埋込式能動型医療機器	（10）ガス燃焼機器
（11）熱水ボイラ	（12）民生用爆薬
（13）医療機器	（14）爆発危険場所における機器とシステム
（15）レジャー用船艇	（16）エレベータ
（17）冷蔵機器	（18）圧力容器
（19）遠隔通信端末機器	（20）体外診断用医療機器
（21）計測器	

ニューアプローチ指令を理解するためには、下記の用語の定義を理解する必要がある。

市場投入およびサービス開始(Placing on the market and putting into service)

EU域内に初めて製品を市場投入（またはサービス開始）することを指す。加盟国は製品が意図された目的に対して適切に設置、維持管理、および使用される限り、製品の市場投入・サービス開始に関わらず、安全、健康、またはその他の公共利益がリスクにさらされることのないように、必要な措置を講じる義務を負う。これには、加盟国側の市場監視義務も伴う。

必須要求事項 (Essential Requirements)

必須要求事項は指令の附則に規定され、指令の目的達成に必要なあらゆる事項を網羅する。市場投入・サービス開始できるのは、必須要求事項に準拠する製品のみである。

- 必須要求事項は公共の利益を保護するために必要な要素を規定する。
- 必須要求事項は強制である。必須要求に準拠した製品のみを市場投入およびサービス開始できる。
- 必須要求事項は、製品のもつリスク要素として評価、適用されなければならない。

自由な移動(Free movement)

ニューアプローチ指令の発効に伴って、EU**内での各指令に関する技術規格は統一され、また指令の要求と整合しない規則(例えば、指定された機関による認可や認証の要求)は撤廃された。つまり、各指令の適用対象となる機器は、該当する全ての指令の要求に従うことによって、どの**EU**加盟国においても流通できる。**

セーフガード条項(Safeguard clause)

CE**マーキング貼付製品が使用され、安全、健康、または公共の利益を損なう可能性がある場合、加盟国は、当該製品の市場投入を禁止または制限するため、あるいは当該製品を市場から撤退させるために適切な措置をすべて講じる。**

適合性評価(Conformity Assessment)

EU**市場に投入される製品は、**CE**マーキングを貼付するための適合性評価手続をとる必要がある。**

ノーティファイドボディ（公認機関）（Notified Bodies**）**

ノーティファイドボディ（公認機関）は、指令が定めた要件を満たしているかどうか、第三者機関として適合性評価を実施する。各加盟国の領域内に設立された機関の中から各加盟国で指定される。製品群によっては、ノーティファイドボディにより試験、認証をしなければならないものもある。

整合規格（Harmonized Standard**）**

整合規格は、欧州委員会とEU**の規格機構の間で合意された一般指針に従って作成された規格である。**

それぞれの指令に対する適合性の推定に用いることのできる整合規格のリストは、欧州官報（Official Journal**）で公表されているので、適合宣言のために整合規格を用いる場合にはそのリストを確認して適切な規格を選択する必要がある。**

移行期間（Transition Period**）**

ニューアプローチ指令は、指令が新たに発令された場合、または指令が改訂される場合、移行期間が設定されている。

移行期間の最終日までは、製造業者は国内制度、または改訂前の指令に従って設計・製造された製品の市場流通ができる。移行期間中は、製造業者がいずれを適用するかを選択できる。移行期間経過後は、適用される指令に準拠した製品のみが、EU**市場投入とサービス開始を許可される。**

1 CE マーキングとは

1.1. CE マーキングの原則 (CE Marking)

- CEマーキングは製造業者に課され、適用される要求事項に製品が適合していることを示すマークである。
- 製造責任者による適合宣言がなされ、CEマーキングを貼付した製品は、
  - 適用される全てのEU条項に準拠している。
  - 適切な適合性評価手続きを完了している。

CEマーキングは、製造業者がCEマーキングを貼付することにより、当該製品が適合していることを示すマーキングである。言いかえれば、CEマーキングが貼付されている製品は、適用される全条項に準拠し、適切な適合性評価手続きを経たことを意味している。CEマーキングの貼付を定める指令は、基本的にニューアプローチ指令によって、CEマーキングの貼付が決められている。

1.2. CE マーキングを貼付する製品

- 特定の指令が別に定める場合を除き、ニューアプローチ指令の対象とされた製品については、CEマーキングは強制であり、市場投入および上市前に、すべての製品に貼付されなければならない。
- 製品が複数の指令の対象であり、それらの指令がすべてCEマーキング貼付を定めている場合、CEマーキングは、当該製品がこれらすべての指令に準拠しているとみなされることを示す。
- CEマーキングを定める指令の対象外製品は、CEマーキングを貼付してはならない。

2 ニューアプローチ指令 (New Approach Directive)

2.1 指令の対象製品

- ニューアプローチ指令は、EU市場で初めて市場投入（またはサービス開始）する予定の製品に適用される。したがって同指令は、加盟国で製造された新製品、ならびに第三国から輸入された使用済み製品・中古品・新製品に適用される。
- 製品の概念は個々のニューアプローチ指令で異なり、製品が指令の範囲内かどうかを検証するのは製造業者の責任である。
- 大幅に変更された中古製品が、改めてEU市場で市場投入・サービス開始する際に、適用される指令の条項に準拠すべき新製品とみなされる場合がある。これは、特に規定されない限りケースバイケースで検討される必要がある。
- 性能、目的、種類を変えずに修理された製品は、ニューアプローチ指令に従った適合性評価の対象ではない。
- 特別または排他的に軍事・政治目的が意図された製品は、特定のニューアプローチ指令の範囲か

ら明示的に除外される。その他の指令に関しては、EC条約第296条に従い、加盟国は軍事目的専用の製品を特定の条件下で指令適用分野から除外できる。

2.2. 複数の指令を同時に適用する必要性

- 複数のニューアプローチ指令が定める必須要求事項は、当該製品に関連する要求の対象となる危険の種類に応じて、相互に重複または補完する場合がある。
- 製品を市場投入および流通開始できるのは、製品が適用されるすべての指令の条項に準拠し、かつ適用されるすべての指令に従って適合性評価が実施された場合のみである。

2.3. 市場投入とサービス開始

- 市場投入とは、EU域内での流通または使用を目的として製品を初めてEU市場で入手可能にする初期の活動である。入手方法が有料・無料のいずれであってもこれにあてはまる。
- サービス開始は、EU域内でユーザーが製品を初めて使用する時点を指す。
- ある製品をEU域内で初めて市場投入およびサービス開始する際、当該製品はニューアプローチ指令に準拠していなければならない。

ニューアプローチ指令は、適用される指令に定められた高水準の安全予防に準拠した製品の、自由な移動を確保するために策定される。したがって、加盟国はかかる製品の市場投入を禁止、制限、または阻害してはならない。

以下の製品は市場投入とはみなされない。

・ 第三国の製造業者からEU域内の正式代表者に移管された製品で、かつ、当該製造業者が当該代表者に製品の指令準拠を確保させている場合
・ 製品が追加処理（組立、梱包、加工、ラベリングなど）のために製造業者に移管された製品
・ 自由に流通する許可を税関から（まだ）受けていない製品、別の税関の手続き下にある製品（輸送、倉庫保管、一時輸入など）、あるいは自由地帯にある製品
・ 第三国に輸出する目的で加盟国内で製造される製品
・ 見本市やデモに展示される製品
・ 製造業者の在庫、またはEU域内で設立された正式代表者の在庫中にあり、かつ、まだ市場で入手可能ではない製品（適用される指令で特に定めた場合を除く）

カタログ中または電子商取引の手段で提示された製品は、それが初めて実際に市場で入手可能になるまではEU市場に投入されたとはみなされない。誤解を招く広告の禁止を目的とする規則・原則を尊重するために、EU市場での流通を意図された非準拠製品には、そのことが明示さなければならない。

製品は、EU市場に投入される際、適用されるニューアプローチ指令ならびに他のEU法に準拠していなければならない。したがって、EU域内で製造された新製品および第三国から輸入された全製品（新品か中古かにかかわらず）は、EU市場で初めて入手可能となるとき、適用される指令の条項に準拠

していなければならない。加盟国は、これを市場監視の枠組みにおいて確保する義務を負う。EU市場にある使用済み製品は、EC条約第28条および第30条に既定された原則に従って自由な移動の対象となる。

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3 製造者責任

3.1. 製造業者とは

ニューアプローチ指令での製造業者とは、自身の利益のために EU 市場への製品投入を意図している製品の設計と製造の責任者である。製造業者は、EU 市場に投入予定の製品が、適用されるニューアプローチ指令の条項に従って設計・製造され、かつ必須要求に照らして適合性評価が行われるようにする義務を負う。製造業者は、最終製品、既製部品、既製構成部品を使用でき、あるいは、これらの業務を外注できる。ただし製造業者は、常に全体管理を維持し、また製品に対する責任能力を有していなければならない。

3.2. 正式代表者（Authorized representative）

製造業者は、任意の個人または法人を、自身の代理となる正式代表者として任命できる。
ニューアプローチ指令の目的から、正式代表者は EU 域内の者を任命しなければならない。
製造業者は、自身の代理である正式代表者の行動に対して一般的責任を維持する。

3.3. 輸入業者／市場投入の責任者

ニューアプローチ指令の定義では、輸入業者（市場投入の責任者）とは、第三国から製品を EU 市場に製品を投入する、EU 域内で設立された任意の個人または法人である。輸入業者は、当該製品の製造業者が EU 域内で設立されておらず、かつ EU 域内に製造業者の正式代表者が存在しない場合、製品に関する必要な情報を市場監視機関に提供できなければならない。

3.4. 製造物責任

製造物責任に関する指令の対象は、個人または私有財産への損害の原因となる、EU 域内で製造されるか EU 域内に輸入されたあらゆる製品である。したがって同指令は、ニューアプローチ指令の範囲内の製品にも適用される。製造物責任に関する指令は、最終製品の原材料と構成部品に加え、あらゆる動産および電力を対象とする。

製造物責任、つまり損害を補償する責任は生産者にある。生産者とは、最終製品の製造業者、または最終製品の構成部品の製造業者、原材料の生産者、あるいは（商標貼付などにより）自身を製造業者として提示する者である。第三国から EU 市場に製品を投入する輸入業者は、すべて製造者責任に関する指令に従い製造業者とみなされる。製造業者が特定できない場合、製品の各供給者は、合理的な期間内に製造業者の身元または製品供給者を負傷者に通知しない限り製造物責任を負う。

製造業者は、欠陥製品が原因で個人（死亡、負傷）および私有財産（私用の物品）に発生した損害を補償しなければならない。指令では 1 件につき 500 ユーロ以下の財産への損害は対象外である。精神的損害(苦痛など)については国内法で対応する。指令は欠陥製品自体の破壊を対象としないので、製造物責任に関する指令の下でその補償義務はない。これは、国内法の権利を侵害しない。

製造物責任に関する指令により、加盟国は、連続事故について金額の上限を設けることができ、設定額の最低は 7 千万ユーロに固定されている。ただし、現在ほとんどの加盟国は上限を設けていない。市場投入から 10 年後、生産者の製造物責任は訴訟中でない限り消滅する。

4　適合性評価とモジュール

自己宣言

指令の要求への適合性は、自己宣言によって示すことができる場合がある。これにより、機器の出荷に先立って認証や認可を得なければならない場合と比較して、製造業者の負担は軽減され、また出荷までの時間の短縮が可能である。

この自己宣言とは、その機器が指令の要求に適合していることを自らの責任で保証する旨を宣言することを指す。従って、自己宣言を行なった製造業者、及びその宣言書に署名した個人は、その宣言に関して大きな責任を持つことになる。

自己宣言ルートでのリスク

- 適合すべき規格の評価の誤り
- 技術構造ファイルの不備
- 現地での安全不適合による裁判などの不測の事態　　　　　　　　など

適合評価が難解なため、ノーティファイドボディによる適合評価や、一部、型式試験を受け、適合証明書を技術構造ファイルに組み込むケースが増えている。

適合性評価について

- 適合性評価はモジュールに細分化される。モジュールは、製品の最大範囲に適用されるいくつかの手続きからなる。
- モジュールは、製品の設計フェーズまたは生産フェーズ、あるいはその両方に関わる。8つの基本モジュール、およびそれら8つの考えられるバリエーションを多様な方法で相互に組み合わせることによって、完全な適合性評価手続きを確立することができる。
- 原則として、設計フェーズおよび生産フェーズ中、製品はモジュールに従った適合性評価の対象である。

- **各ニューアプローチ指令は、考えられる適合性評価手続きの範囲と内容を規定し、それは安全予防の必要な水準を提供するとみなされる。指令はまた、複数の選択肢が用意される場合に、どのような条件下で製造業者が選択できるかの決定基準を設けている。**

製品が指令に適合しているかどうかを証明する方法として、モジュール方式という手法を使う。製造業者は、必要なモジュールを選択し、あるいは組み合わせることによって、いくつかの方法の中から適切な証明方法を選択することができる。ニューアプローチ指令は、どの指令も必ず設計内容の評価と生産システムの評価の両面から構成されている。そのため、どのモジュールを選択するについても、設計面と生産面のいずれもの適合性を証明しなければならない。

基本モジュール

A（生産の内部管理）	社内の設計・生産管理を対象とする。このモジュールではノーティファイドボディによる関与は不要で、自己宣言により、必須要求事項への適合を宣言し、CEマーキング貼付が必要となる。
B（型式試験）	設計フェーズを対象とし、生産フェーズでの評価を規定するモジュールが後に続かなければならない。ノーティファイドボディによりEC型式認証書を取得しなければならない。
C（型式への適合性）	生産フェーズを対象とし、モジュールBの後に続く。モジュールBに従って発行されたEC型式認証書に記載されている型式との適合していることを宣言し、CEマーキングの貼付が必要となる。
D（生産の品質保証）	生産フェーズを対象とし、モジュールBの後に続く。製造業者が設定した生産、最終製品の型式試験、生産、試験のための品質システムの管理・認証が必要。ノーティファイドボディは、ISO 9001規格に準じて評価。
E（製品の品質保証）	生産フェーズを対象とし、モジュールBの後に続く。製造業者が設定した生産、最終製品の型式試験、生産、試験のための品質システムの管理、認証が必要。ノーティファイドボディは、ISO 9001規格に準じて評価。
F（製品の試験）	生産フェーズを対象とし、モジュールBの後に続く。ノーティファイドボディは、モジュールBに従って発行されたEC型式認証書に記載された型式への適合性を管理し、適合証明書を発行する。
G（ユニット試験）	設計フェーズと生産フェーズを対象とする。ノーティファイドボディは各製品を個別に調査し、適合証明書を発行する。
H（全体の品質保証）	設計フェーズと生産フェーズを対象とする。製造業者が設定した設計、製造、最終製品の検査、試験に関する品質システムの承認・管理を行うノーティファイドボディの介入により、ISO 9001から派生する。

モジュールAは、「内部生産管理」と呼ばれる、製品が指令に適合していることを自己評価できるルートである。指令ごとに、準備すべき技術構造ファイルの内容が決まっているため、該当すべき指令を熟読し、技術構造ファイルと適合宣言書を作成し、CEマーキングを貼付することで、対策は完了できる。

しかし、CEマーキング貼付後、製品に問題が生じた時は、適合宣言書の署名をした製造業者に製品に関する責任がかかることになる。万が一、不適合が当局に確認された場合には、製品を速やかに市場から引き上げる、または製造業者が、禁固刑や罰則の対象になることもある。

一部の製品については、ノーティファイドボディによる型式試験が必要とされる場合もあるので、適合評価に不安が残る場合には、指令を熟知しているノーティファイドボディに確認すべきである。

5　主要ニューアプローチ指令概要

5.1　機械指令　(98/37/EC)

連結した部品または構成部材の集合で、少なくともそのうちのひとつが適切な作業装置、制御、動力回路などによって動くもの。つまり、部品の集合体で可動部があるもの。

5.1.1　機械指令の対象外製品例

人力のみを動力とするもの(昇降機を除く)、患者に直接使用される医療機器、遊園地・娯楽施設用に特定された機器、蒸気ボイラー、タンク、圧力容器、原子力用機器、機械を構成する放射能源、火器、危険物の容器、移動のための手段となるもの、船舶、人を運ぶロープウェイ・ケーブルカー、農業・林業用トラクター、車・警察用機器、人を運ぶ昇降機、鉱山の巻上げ機械、劇場のエレベーター、工場現場でのホイスト

また、機械指令　付属書IVに指定された製品以外は、型式試験を受ける必要がないので、適合宣言書と技術文書を作成し、CE マーキングを貼付することで、自己宣言ができる。言い換えれば、付属書Ⅳに記載されている製品群は、ノーティファイドボディによる関与が必要となる。

付属書IV

A.　機械

1.　木材及び類似材料、肉および類似材料の加工用丸のこ（刃がひとつ、または多数のもの）

1.1　固定ベットを持ち、工作物の送り込みを手動又は動力で行い、作業中に刃先部が固定される切断機械

1.2　手動の往復運動をするのこぎりベンチ、またはキャリッジを備え、作業中に刃先部が固定される切断機械

1.3　内蔵した機械的送り装置で加工物を送り、手動で加工物の取り付けや取り外しができる、作業中に刃先部が固定される切断機械

1.4　機械的な工作物贈り装置を持ち、手動で工作物の取り付けや取り外しができる、作業中に刃先部が移動する切断機械

2.　木工用の手動表面削り盤

3.　手動で加工物の取り付けや取り外しができる木工用の自動片面仕上盤

4.　木材及び類似材料、肉及び類似材料の加工用で、対象物を手動で取り付けや取り外しができる、固定式または可動式ベットを持つ帯のこおよび可動キャリッジを持つ帯のこ

5.　1.から 4.までと、7.に記載した種類を組み合わせた、木材及び類似材料用の加工機械

6.　手動式でバイトホルダ付き木工用ほぞとり盤

7.　木材及び類似材料用の手動式垂直スピンドル成型機

8.　木工用携帯式チェーンソー
9.　手動で工作物の取り付けや取り外しができる、プレスブレーキを持つ金属冷間加工用プレス。可動作業部分の工程は 6mm 以上、速度は 30mm/s 以上のもの
10.　手動で取り付けや取り外しができる射出式または圧縮式プラスチック成形機
11.　手動で取り付けや取り外しができる射出式または圧縮式ごむ成形機
12.　次のような地下作業用の機械：
-レール上の機械：機関車および緩急車
-水圧天ばん支持装置
–地下作業用の機械設備に装備する内燃機関
13.　圧縮機能付き家庭用廃棄物収容用の主導積み込みトラック
14.　自在継ぎ手を持ち、セクション 3,4,7 に記載されたガードなどと取り外し可能な伝動軸
15.　昇降用車両
16.　3m を越える垂直高さから落下する危険のある昇降用機械
17.　花火製造用の機械

B　安全部品
1.　特に人を探知し、安全を確保する電気感応装置（非物質的バリア、センサマット、電磁探知機）
2.　両手操作により安全機能を確実にする論理ユニット
3.　9,10,11 に記載した圧搾機を保護する自動可動式の遮蔽物
4.　転倒時保護構造（ROPS）
5.　落下物に対する保護構造（FORPS）

機械指令で適合すべき整合規格には下記の 3 タイプがある。
タイプ A 基本規格：すべての機械に共通に適用できる基礎概念、設計一般原則
（例 ：EN292 機械の安全性／言葉の定義）
タイプ B 一般規格：広範囲な機械に適用できる安全性・安全装置について
（例 ：EN418 非常停止）
B1:　安全に関する特定事項（安全距離、温度など）
B2:　安全関連デバイス（インターロック、ガード、センサーなど）
タイプ C　製品規格：特定の機械に関する詳細な安全要求事項
（例 ：EN201 プラスチック射出成形機）

5.2　低電圧指令 (73/23/EEC)

50～1000V（AC）、75～1500V（DC）の電源で駆動する製品。
電気的要因によって、危険が発生する可能性のある機械について低電圧指令の適合が必要である。
対象：交流　50～1000V　直流　75～1500V で作動する機器

次の安全性を確保できた設計の適合をしなければならない。
-電気機器そのものから生じる電気的危険
-電気機器に対する外部の何らかの影響で生じる電気的危険

電気機器ユーザーが機器の操作方法を正しく理解できるように、機器本体、または添付する警告、注意書きに必要情報を明示しなくてはならない。

5.2.1 低電圧指令の対象外製品例

他の指令や規則の対象となるために対象外とされる製品。例えば、爆発しやすい環境で使用される電気機器、放射線及び医療用の機器、人や荷物用エレベーター、電気メーター、家庭用コンセントやプラグ。電気柵制御機器、船や飛行機・鉄道で使われる特別な機器

5.3 EMC 指令（89/336/EEC, 2004/108/EC）

電磁波を発生し、また外部からの電磁波によって影響をうける製品に関する規制。特に除外されたものを除く、ほとんどの電気/電子機器が対象となる。

この指令は「電磁両立性に関する加盟国の法律の近似化のための欧州議会、並びに欧州閣僚理事会指令」のような意味の長いタイトルを持つが、通常は単にEMC **指令と呼ばれる。**
なお「EMC**」という用語は、電磁両立性**(electromagnetic compatibility) **を意味している。**

5.3.1 EMC 指令の対象外製品

適用対象から除外されるのは、以下製品となる

- **単一の機能ユニット**(single functional unit) **として供給されないもの**
- **エンド・ユーザーによる使用が想定されていないもの**
- **本質的に、他への電磁干渉を生じず、また他からの電磁干渉を受けることがないもの**
- Regulation (EC) No. 1592/2002 **の対象となる航空用製品**
- **無線規則の元でアマチュア無線家が使用する、市販されない無線機器**
- **同一の保護要求をカバーする、より限定的な指令**(R&TTE **指令、自動車**EMC **指令、医療機器指令など**) **の対象となるもの**
- **展示会などでの展示のみを目的とするもの**

装置に組み込むための部品として製造業者に供給されるコンポーネントは、エンド・ユーザーによる使用が想定されていないことから、適用対象外と見做すことができる。しかし、装置に組み込むためのコンポーネントであっても、それがエンド・ユーザー向けに供給される場合(**例えば、小売店で一般消費者向けに販売される、パーソナル・コンピュータ用のマザー・ボードやディスク・ドライブのように**) **には、**EMC **指令の対象となり得る。**

5.3.2　EMC 指令の目的

EMC 指令の最大の目的は、以下の２点である。

電磁干渉の防止

この目的のため、EMC 指令は、(1) 機器が発生する電磁妨害が無線/通信機器やその他の機器の動作を妨害しないこと、及び(2) 意図した環境において許容できない性能低下を生じることなく動作できるように、予期される電磁妨害への耐性を持つこと、の２つを保護要求(protection requirements) として定めている。EMC 指令の対象となる機器を流通させるためには、この保護要求を満足させることが必須となる。

自由な流通の保証

EMC 指令の発効に伴って、EU 内での EMC に関する技術基準は統一され、また各国ごとの独自の EMC 規制 (例えば、指定された機関による認可や認証の要求) は撤廃された。つまり、EMC 指令の適用対象となる機器は、EMC 指令を含む、該当する全ての指令の要求に従うことによって、EU 内のどの国においても流通できる。

5.3.3　EMC 指令の特徴

EMC 指令は次のような特徴を持つ。

広い適用範囲

基本的に市販される電気/電子機器のほとんどは、EMC 指令の対象となる。この点で、日本の電気用品安全法のような、指定された品目のみが対象となる規則とは大きく異なる。

エミッションとイミュニティの双方のカバー

EMC指令の保護要求は機器が他に干渉を与えないこと(エミッション) のみではなく、機器が他からの干渉を受けないこと(イミュニティ) も要求している。日本の電気用品安全法やVCCI 規制、アメリカのFCC 規制のように、ほとんどの機器についてはエミッションのみを規制することが多い中で、これはEMC指令の大きな特徴である。

5.3.4　89/336/EEC と 2004/108/EC

現在、89/336/EEC と2004/108/EC という、２つのEMC 指令が存在している。

89/336/EEC

これは1989 年に発行され、指令92/31/EC、及び93/68/EEC による修正を受けた上で、10 年以上にわたって使用されてきた。この指令は以後2004/108/EC によって置き換えられるが、2009年7 月19日までは、有効である。

2004/108/EC

これは2004 年12 月に発行された改訂版のEMC 指令であり、89/336/EEC は2007 年7 月20 日をもってこの新しい指令で置き換えられる。その後、2009年7 月19 日までは89/336/EEC に従った機器の出荷も認められる移行期間となる。2009 年7 月20日以降は89/336/EEC は完全に使用できなくなり、それ以降に出荷する機器については2004/108/EC に従うことが必須となる。89/336/EEC からの変更は主として適合手続に関するものであり、技術的要求に関する変更はない。

5.3.5　EMC 指令への適合手続

適合手続の選択

EMC 指令の適用対象となることがわかったならば、まず、どちらの指令を適用するか(上記のように、2007年7 月19 日までは89/336/EEC のみが、2009 年7月20 日以降は2004/108/EC のみが適用可能) と、整合規格を全面的に適用するかどうかを選択する。

89/336/EEC ― 規格ルート

これは、指令の保護要求への適合性を主張する根拠とするために、整合規格を全面的に適用する場合に用いる手続きである。多くの場合、機器が所定の整合規格に適合するかどうかの試験をEMC 試験所で行ない、その結果に基づいてその機器が保護要求に適合しているかどうかの判断を行なうことになる。その機器が保護要求に適合していると判断したならば、適合宣言書を作成した上で、機器にCE マーキングを貼付する。なお、規格ルート、TCFルートという呼び方は通称であり、指令ではこのような表現は用いられてはいない。

89/336/EEC ― TCFルート

これは、整合規格を適用しなかった、あるいは部分的に適用した場合に用いる手続きである。この手続きでは、製造業者は指令の保護要求への適合の根拠を示す技術文書 (technical construction file; 技術構成ファイル) を作成し、コンピテント・ボディ(competent body)に技術報告書、もしくは証明書を発行してもらう。技術文書を作成し、コンピテント・ボディからの技術報告書、もしくは証明書を入手したならば、適合宣言書を作成した上で、機器に CE マーキングを貼付する。

2004/108/EC

2004/108/EC においては、製造業者は、EMC に関する評価を実施し、指令の保護要求への適合性を確認することが求められる。しかし、該当する全ての整合規格を正しく適用した場合には、それをもって評価が実施されたものと見做すことが可能である。従って、該当する整合規格に従って試験を行ない、その結果に基づいてその機器が保護要求に適合していると判断したならば、それ以上の評価は不要と考えることができる。これは 89/336/EEC の規格ルートに相当するものであるが、89/336/EECの場合とは異なり、この場合でも技術文書の作成は必須となる。

整合規格を全面的に適用しなかった場合には、EMC に関する評価の実施によって、それが保護要求に適合することを確認することが必要となる。これは 9/336/EECのTCFルートに相当するものであ

るが、89/336/EEC の場合とは異なり、外部の機関の関与は求められていない。上記のいずれかの方法によってその機器が保護要求に適合していると判断したならば、技術文書と適合宣言書を作成した上で、機器に CE マーキングを貼付する。

製造業者は、整合規格を適用したかどうかに関わらず、ノーティファイドボディに意見を求めることが可能である。ノーティファイドボディに意見を求めるかどうかは全く任意であるが、ノーティファイドボディは、EMC 指令に関する知識や経験を持っており、必要に応じてノーティファイドボディに相談することで、不適合の可能性がある部分の指摘などの有益なアドバイスを受けられる。

5.3.6 規格の選択

整合規格を適用する (部分的にであっても) ことを選んだならば、適用する規格を選択することが必要となる。適用する規格は、EMC 指令の元での整合化が欧州官報 (Official Journal)で公表された規格の中から、次のような原則に従って選択する。

その規格が適用可能であるかどうかの判断は、それぞれの規格に明記されている適用範囲(scope) に基づいて行なう。基本的には、適用する規格は機器の意図された用途によって決定する。例えば、コンピュータを内蔵した測定器はあくまでも測定器であり、情報技術機器とはならない。
適用可能な規格が複数ある場合は、その機器により相応しい(通常は、適用範囲がより限定的な)規格を優先する。その機器に適用可能な製品規格や製品群規格がある場合は、一般規格を適用することはできない。製品規格 (product standard) や製品群規格 (product family standard) は特定の種類の製品に適用されるものであり、情報技術機器、AV 機器といった製品群に適用されるものから、無停電電源装置、アーク溶接機といった非常に限定された種類の製品に適用されるものまで、多数の規格がある。一般規格 (generic standard) はある環境で使用される製品に横断的に適用されるものであり、住商業環境向けのもの、及び工業環境向けのものが用意されている。

規格によっては EMC の一部の側面 (例えばエミッションとイミュニティの一方のみ) しかカバーしていない場合がある。このような規格を適用する場合には、必要な全ての側面をカバーするように複数の規格を組み合わせて適用する必要がある。商用電源に接続される機器に対する高調波電流エミッション規格の適用も、この別の例となる。

複合機能機器については、それぞれの機能に対して異なった規格を適用する必要がある場合もある。

整合規格のリストは欧州委員会のニューアプローチに関するサイトで確認でき、その他の情報も手に入れることができる。この整合規格のリストは次ページのように記載されている。

89/336/EEC のTCF ルートを用いる場合、及び2004/108/EC に従って評価を実施する場合には、整合規格以外の規格を使用することも可能である。このような規格の例としては、整合化されていない国際規格や国内規格、あるいは業界規格が考えられる。

その規格によって置き換えられる規格

対応する国際規格

その規格によって置き換えられた規格が適合性の推定を与えなくなる日付(日、月、年)

C 98/8 EN Official Journal of the European Union 23.4.2004

Commission communication in the framework of the implementation of the Council directive 89/336/EEC

(2004/C 98/05)

(Text with EEA relevance)

(Publication of titles and references of harmonised standards under the directive)

整合規格の番号とタイトル

ESO (1)	Reference and title of the standard	Reference document	Reference of the superseded standard	Date of cessation of presumption of conformity of the superseded standard Note 1
Cenelec	EN 50065-1:2001 Signalling on low-voltage electrical installations in the frequency range 3 kHz to 148,5 kHz — Part 1: General requirements, frequency bands and electro-magnetic disturbances		EN 50065-1:1991 +A1:1992 +A2:1995 +A3:1996 Note 2.1	Date expired (1.4.2003)
Cenelec	EN 50065-2-1:2003 Signalling on low-voltage electrical installations in the frequency range 3 kHz to …			
	… immunity for residential, commercial and light-industrial environments	IEC 61000-6-1:1997 (Modified)	EN 50082-1:1997 Note 2.1	1.7.2004
Cenelec	EN 61000-6-2:1999 Electromagnetic compatibility (EMC) — Part 6-2: Generic standards — Immunity for industrial environments	IEC 61000-6-2:1999	EN 50082-2:1995 Note 2.1	Date expired (1.4.2002)
Cenelec	EN 61000-6-2:2001 Electromagnetic compatibility (EMC) — Part 6-2: Generic standards — Immunity for industrial environments	IEC 61000-6-2:1999 (Modified)	EN 61000-6-2:1999 Note 2.1	1.7.2004
Cenelec	EN 61000-6-3:2001 Electromagnetic compatibility (EMC) — Part 6-3: Generic standards — Emission standard for residential, commercial and light-industrial environments	CISPR/IEC 61000-6-3:1996 (Modified)	EN 50081-1:1992 Note 2.1	1.7.2004
Cenelec	EN 61000-6-4:2001 Electromagnetic compatibility (EMC) — Part 6-4: Generic standards — Emission standard for industrial environments	IEC 61000-6-4:1997 (Modified)	EN 50081-2:1993 Note 2.1	1.7.2004

EN 61000-6-2:2001 は IEC 61000-6-2:1999 に対応するが、内容には相違点がある

EN 61000-6-2:1999 は 2004年 7月 1日以降は適合性の推定を与えなくなる

EN 61000-6-2:1999 は EN 61000-6-2:2001 によって置き換えられる

整合規格リスト例 (Offical Journal 2004/C 98/05)

5.3.7　試験の実施

規格を適用する場合、選択した整合規格に従って試験を実施し、その規格の要求を満足するかどうかの判断を行なう。実際の試験は外部の試験所 (EMC **テスト・ラボ**) **に依頼する場合が多いと考えられるが、その場合でも試験条件** (**試験時に接続する周辺機器や動作条件、イミュニティ試験における合否判定の基準など**) **の決定は製造業者が行なう必要があることに注意する。複雑な装置の場合には、試験に先立っての検討や打ち合せを特に慎重に行なうことが必要となる。試験を実施し、合格を示す試験報告書を入手したならば、それを適合の根拠として使用することができる。但し、試験所が発行する試験報告書には、その装置の特定のサンプルをある条件で試験した結果が示されるだけであり、外部の試験所から試験報告書を入手した場合であっても、適合性の判断や適合性の維持の責任は製造業者にある。**

5.4　医療機器指令 (93/42/EEC)

EU **には、医療機器に関する下記三種類の指令がある。**

- **医療機器指令** (93/42/EEC)
- **体外診断医療機器指令** (98/79/EC)
- **埋込型能動医療機器指令** (90/385/EEC)

体外診断医療機器指令 (98/79/EC) **では、診断用医薬品及び関連機器が対象に、また、埋込型能動医療機器指令** (90/385/EEC) **では、ペースメーカー等の埋込型機器がそれぞれ対象になる。**

ここでは、一般の医療機器を対象とした医療機器指令 (93/42/EEC) **について、記述する。この指令は、一般医療機器及びその付属品に適用する。医療機器は器具、機械、用具、材料またはその他の品目であって、単独での使用あるいは組み合わせての使用かを問わず、また適用のために必要なソフトウェアを含む、製造業者が人体への使用を意図した製品で、**

- **疾病の診断、予防、監視、治療又は苦痛緩和**
- **傷害または身体障害の診断、予防、監視、治療又は苦痛緩和**
- **解剖学又は生理学上の検査、代替又は修正**
- **受胎調節**

を目的としたものである。

なお、日本では医療機器として扱われていない福祉用具、健康器具なども この指令の対象となる場合がある。

指令の構成は、条文（Article）と付属書（Annex）とに大別される。条文はCEマーキング貼付の方法について、また付属書には条文を満たすための要領が詳しく記載されている。

条文（Article）	付属書（Annex）
３条：製品の基本要件への適合	付属書１：基本要件（製品として必ず満たさなければならない要求事項）
５条：基本要件への適合を示す整合規格	
８条：保護措置	－
９条：製品のリスクによるクラス分類	付属書９：クラス分類基準
１０条：市場投入後に起こる事故に関する情報	
１１条：製品のリスクの度合いに応じた適合性評価手順及び適合宣言	付属書２－７：適合性評価手順 付属書８：カスタムメード機器、臨床評価用機器
１４条：市場出荷責任者の登録、製造業者の登録事務所及びＥＣ域内責任者	－
１５条：臨床試験	付属書１０：臨床評価
１６条：通知機関	付属書１１：通知機関認定基準
１７条：ＣＥマーキング	付属書１２：ＣＥマーキング

医療機器におけるＣＥマーキングは、当該医療機器指令への適合性を患者、医療従事者、安全管理機関、行政当局に対して示すための表示である。EUにおけるＣＥマーキングは、製品の安全性や有効性を製造業者の責任において自らが適合性の宣言を行うものであり、行政当局等によって承認･認証を行う日本の制度とは大きく異なる。

医療機器は、付属書１（Annex I）に示される基本要件を満足していることが必須となる。この基本要件への適合性は製造業者の責任において立証されなければならない。そのため技術文書（technical documentation file）を作成し、基本要件への適合性を常に客観的証拠として提示できるようにしておく必要がある。この基本要件への適合は、常に最新の技術レベルを反映しなくてはならず、多くの場合、欧州整合規格を用いて立証される。

また、CEマーキングのための適合性評価には幾つかのルートがあり、医療機器のリスクに応じたクラス分類によって選択できるルートが変わる。医療機器指令では人体に対する医療機器の適用（使用）期間、体内への侵襲性、電気的エネルギーの使用、中枢循環器系及び神経系への適用などといった要素を考慮し、最も低リスクのClass I よりClass IIa，Class IIb，そして最もリスクの高いClass IIIの４つクラスに分類される。製造業者は、その責任において、取り扱う医療機器のリスクに応じた管理が求められる。

ただし、医療機器は患者の生命へ直接影響を与える機器であり、リスクが高くなれば全てを製造業者の責任だけに委ねる自己宣言のみでは市場投入に際して懸念がもたれる。したがって、リスクの高い製品は第三者が関与し、チェック機能を持たせることによって安全性や有効性を確実にして行こうとする仕組みになっている。

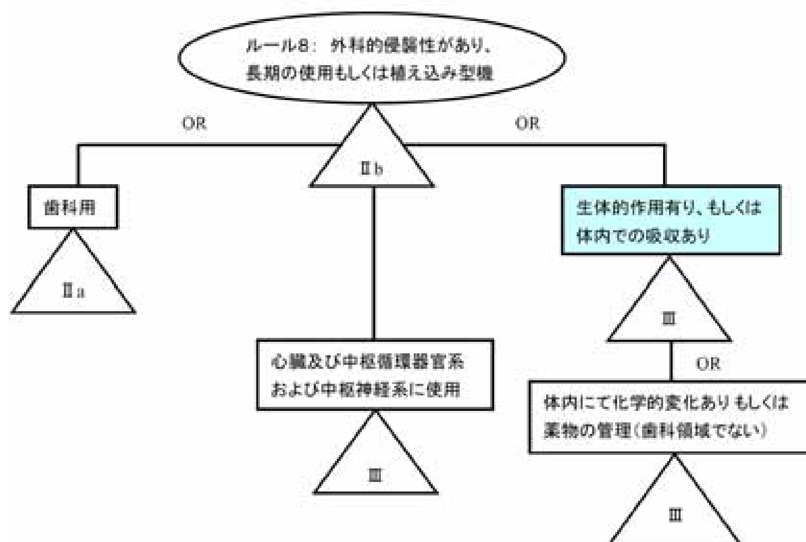
この第三者とは、当局より通知されたノーティファイドボディであり、この機関の関与によって、基本要件への継続的な適合性が監視されることになる。例えばClass I に該当する手術用メスは基本的に製造業者の責任において自己宣言を行うことによってＣＥマーキングを表示して販売することができる一方、それよりリスクの高いClass IIa の補聴器等は、その適合性評価の一部もしくは全過程においてノーティファイドボディの関与が義務付けられている。

5.4.1 クラス分類

ここでは適合性評価ルートの選択基準となるクラス分類のルールについて説明する。医療機器はその製品の特質にあわせて、下記のように大別される。

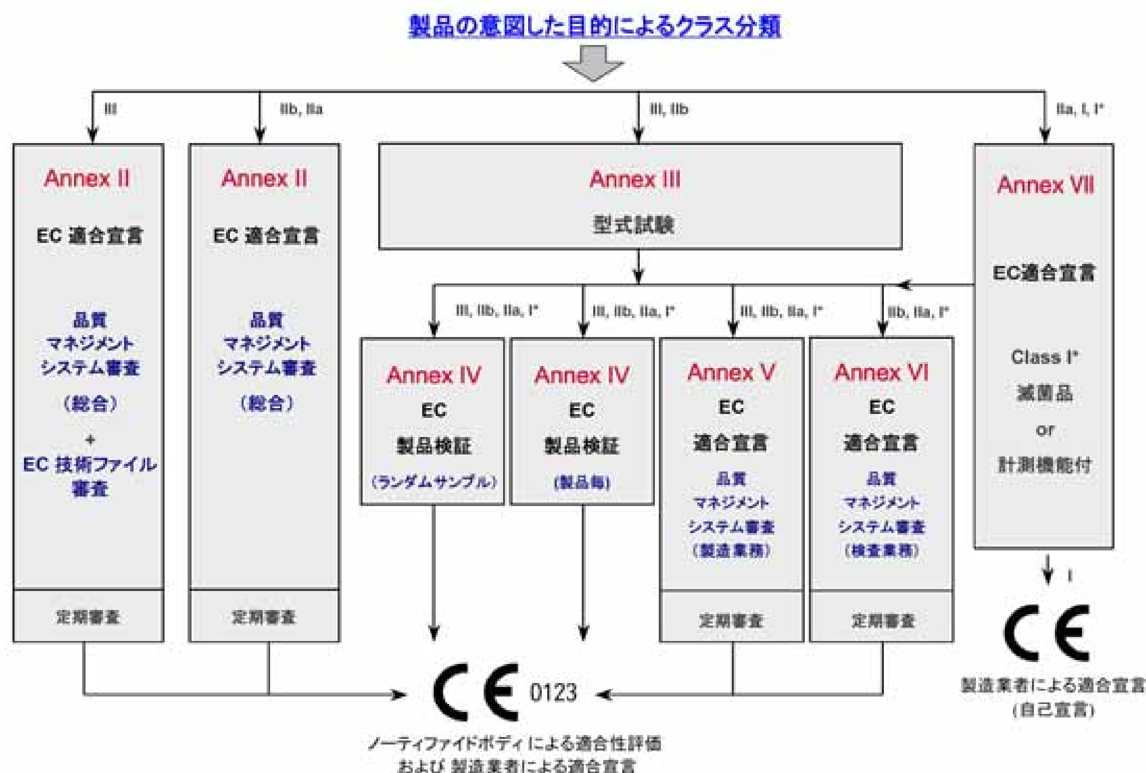
製品特徴	適用ルール
・非侵襲性医療機器 侵襲性：体表もしくは開口部を通じ一部または全部が体内に貫通するような機器	ルール　１：患者との接触がないもしくは皮膚を介しての接触があるのみ ルール　２：血液、体液、体組織およびガスの処理及び保管 ルール　３：血液、体液、その他液体の組成の改善 ルール　４：創傷部に対しての接触（機械的保護、滲出物吸収）
・侵襲性医療機器	ルール　５：体の開口部に対し適用（外科的方法でなく） ルール　６：侵襲性機器で一過的（６０分以下）な使用 ルール　７：侵襲性機器で短期的（６０日以下）な使用 ルール　８：侵襲性機器で長期的（６０日以上）な使用または埋込型機器
・能動医療機器 握力･重力以外の動力源及び 電気的エネルギー源の変換によって作用する機器	ルール　９：エネルギーの変換もしくはエネルギーを使用する治療用機器 ルール１０：診断用機器 ルール１１：薬液の投与／注入 或いは 体内より物質を取り出す機器 ルール１２：その他の能動医療機器
・その他特別ルール	ルール１３：薬剤を伴い補助的に作用する機器 ルール１４：性病の予防を目的とした機器 ルール１５：医療機器の洗浄を目的とした機器 ルール１６：診断用Ｘ線フィルム ルール１７：動物由来の機器 ルール１８：血液バック

実際に外科手術に用いる抜糸を必要としない縫合糸のクラス分類の例をあげる。まず最初に意図した使用目的から上表の大まかな分類より該当するルール分類を見つける。この場合、縫合糸は外科手術により体に対し侵襲的なのでルール５～ルール８中のいずれかに該当する。その中より、抜糸を行わず体内で吸収されるためルール８の外科的侵襲性があり、長期の使用または埋込型機器に該当する。以下の判断経路にて「体内での吸収あり」に該当するため Class III **となる。**



5.4.2　適合性評価ルートの選択

医療機器のリスクに応じたクラス分類によって適合性評価手順が異なる。
適合性評価ルートは付属書II～付属書VII（Annex II－VII）に規定した各モジュールで構成されており、単独または２つの組み合わせによって適用する。またそのルートには柔軟性があり、ひとつのクラスに対し複数の選択肢がある。製造業者の業務内容（設計を行わない、生産数量が少ない等）に応じて選択することが可能となる。



Annex II，V，VI － 品質システムによる適合宣言
医療機器のための品質マネジメントシステムISO13485 に準拠した品質管理体制の確立・維持が該当し、Annex IIは対象範囲として設計から最終検査までの全体を、Annex V は製造と最終検査を、Annex VI は最終検査に特定したもので、何れの場合もノーティファイドボディが適合性の審査を行う。
Annex III － 型式試験による適合宣言
製造業者によって規定された製造工程で製造された代表サンプルについて、欧州整合規格等を用いてノーティファイドボディが実際に評価を行い、製品が基本要件を満たしているか否かを評価する。
Annex IV － 製品検証による適合宣言
製品毎もしくは製品バッチからサンプルした最終製品をノーティファイドボディが試験し、製品毎もしくはロット毎に認証書を発行する。
Annex VII － 適合宣言
比較的リスクの低いClass I ならびにClass IIaに適用されるモジュールで、基本要件への適合性は製造業者の責任で行われる。Class I の場合はノーティファイドボディの関与なしに自己宣言することができる。

例えばClass IIa **の医療機器の場合、適合性評価のルートは**Annex II**（総合品質マネジメントシステム）の適用もしくは**Annex VII**（適合宣言）＋** Annex V**（製造業務品質マネジメントシステム）など計５つあり、製造業者は、その中より最も自らの業務に適した１つを選択する。**

5.4.3 基本要件（必須要求事項）

CE**マーキングの貼付を行う医療機器は、付属書１（**Annex I**）に示される基本要件を満足していることが必須となる。**

Ｉ．全般的要件	１．患者の健康・安全を考慮した設計・製造
	２．設計の方法は一般に認められた技術水準を考慮し、安全原則に適合
	３．意図する性能を達成するための設計、製造及び包装
	４．患者又は使用者の健康や安全を脅かす程に悪影響を与えない設計・製造
	５．輸送及び保管条件下における性能劣化を防止する設計・製造
	６．有効性が起こりうる不具合を上回る設計・製造
II．設計及び組立てに関する要件	７．化学的・物理的及び生物学的特性（例：生体適合性）
	８．感染及び細菌汚染（例：包装、滅菌）
	９．構造及び環境特性（例：他の機器との組み合わせ使用時の安全性）
	１０．測定機能を有する機器（例：規定法定単位の使用）
	１１．放射線に関する防護
	１２．エネルギー源に接続される機器またはエネルギー源を有する機器に関する要件
	１３．製造業者が供給する情報（表示・ラベル・取扱説明書 等）
	１４．臨床データによる実証

上記項目は能動・非能動機器に関わらず、全ての医療機器に適用できるよう制定されているため、医療機器の特性によっては適用できない場合がある。そのような場合には、適用できない理由を明確に記述する。

5.5　　R&TTE 指令　無線及び電気通信端末機器指令 (1999/5/EC)

Radio Equipment and Telecommunications Terminal Equipmentの略語。無線機器と電気通信端末機器の市場への出荷、自由な流通、サービス供与に関する指令。公衆通信回線に接続される通信端末機器だけでなく無線機も対象となる。

該当機器

・周波数帯9kHz-3,000GHzの無線機器

- 通信端末機器
- 部分的に次の機器にも適用される
- 医療機器の通信機能の部品やユニット等
- 車載用通信ユニット

指令の対象外機器

- 商用目的でないアマチュア無線機器
- 海上無線機器
- 音声＆TV受信機
- 民間航空関連機器
- 航空管制関連機器＆システム
- ケーブルと配線

R&TTE指令が適用されるすべての機器は、次の二つの何れかのクラス識別（ECI: Equipment Class Identifier）が行われる。このクラス識別は、機器の使用者が判別できることを保証しなければならない。

Class１：EU全域での使用が可能な機器
例）ISDN機器、PSTN機器、GSM、受信機など
Class２：EU全域での使用が不可で、特定の国での使用に限られる機器
例）TETRA機器、TERAPOL機器、PMR機器、テレメータなど

回線に接続される製品については、モジュールAとして、技術文書ファイル及び適合宣言書によって、製造者の自己宣言が可能。その場合、回線に接続される製品で、自己宣言したものに関しては、CEマーキング貼付のみが必要となり、ノーティファイド・ボディのID番号は不要となる。

無線機には、CEマーキングとノーティファイド・ボディのID番号は必要である。無線機で整合規格が存在すれば、追加の国別認証は不要となるが、ノーティファイドボディの関与は必要となる。警告（アラート）マークはCEマーキングと同サイズで表示しなければならない。

5.6　玩具指令 (93/68/EEC)

14歳未満の子供を対象とした玩具。EN71（物理的および機械的特徴、可燃性、重金属移行　など）に適合している場合は、モジュールAにより自己宣言ができる。EN71に適合しない製品については、モジュールB+Cを適用することが必要である。具体的には、型式試験認証、型式への適合宣言が必要となる。

コレクター収集向け商品、またスポーツ製品などの新たにリスク評価が必要になる製品は、この指令の対象にならない。対象製品によってモジュール分けされない。

6. 技術文書の作成方法

各指令によってばらつきがあるが、技術文書、技術構造ファイル、TCF（Technical Construction Files）などと定義されている。この章では総称して、技術文書とする。

いくつかのニューアプローチ指令は、適用される要求に製品が適合していることを証明する情報を記した、技術文書の作成を製造業者に義務付けている。この技術文書は、品質システム（モジュールD、E、H、およびそのバリエーション）に基づいて指令が適合性評価手続きを規定している場合、品質システム文書の一部となることもある。

技術文書は、指令が別の期間を明示する場合を除き、当該製品の最終製造日から最低10年間保管されなければならない。これは、製造業者またはEU域内に設立された正式代表者の責任である。場合によっては、輸入業者またはEU市場に製品を投入する者がこの責任を負わなければならない。

技術文書の内容は、関連する製品に基づいて指令ごとに定められる。一般に、技術文書には設計、製造、および製品の運用について記載しなければならない。技術文書の内容の詳細は製品の性質によって異なる。また、関連指令の必須要求への製品適合性を証明するうえで、技術的視点から何が必要とされるかによって異なる。あるいは、（整合規格が適用される場合には）規格対象の必須要求を示すことにより関連指令の代わりにそれらの整合規格に適合していることを証明するうえで、技術的視点から何が必要とされるかによっても異なる。

いくつかの指令では、当該手続きが実施される加盟国の公用語、ノーティファイドボディが設立された加盟国の公用語、あるいはノーティファイドボディが許可した言語で技術文書を記述することが要求される。第三者による適切な方法での検証を要求している適合性評価手続きを実施するために、技術文書は常にノーティファイドボディが理解できる言語で記述されなければならない。一部のニューアプローチ指令で、この点が明示されていない場合も同様である。

技術は日進月歩であり、最初に技術文書を作成した時点での技術的要求水準と数年後とでは大きな差異がでる可能性がある。製造業者は常に最新の技術水準を反映し、適合状態を維持することが求められる。

例えば整合規格が改訂された場合、それを反映し基本要件に適合しなければならない。
また、製品のリスクも取り巻く環境によって、変化していくため、リスク分析を含め、定期的に技術文書が現在の状況に対応したものになっているか否かの見直しが必要になる。

6.1 機械指令

技術文書は、当該機械が機械指令の要求事項に合致していることを裏付ける資料である。当然ながら、適合宣言の作成に先立ち作成される。作成に当たっては、EU 圏内のいずれかの国の公用語とする。特に 1 冊のファイルにまとめる必要はなく、万が一当局により要求された場合に、速やかに提出することができればよい。すなわち、各関連部門で書類を管理しておき、必要に応じまとめて提出できれば良い。当局からの要求とは、最悪の場合、事故である。そのときに技術文書は「物言わぬ弁護人」の役割を担うわけであるから、指令などの記述だけにこだわらず、関係者以外でも分かるような記述方法が要求される。機械指令では、以下の事項を技術文書とする旨の記載がある。

- 制御回路図とともに、機械全体の図面
- 計算、試験結果などとともに、機械の健康と安全の必須要求事項への適合を確認するために必要な機械の詳細図
- 以下のリスト:
  - 機械指令の必須要求事項
  - 規格、および機械の設計時に用いられた他の技術仕様書類
- 機械の危険源削除に用いた手法の説明。必要に応じ、試験所の技術レポートや技術文書
- もし、整合規格を用いて適合を宣言するのであれば、製造者もしくは他の試験所が行った試験の結果を示す技術レポート
- マニュアルのコピー

機械全体の図面とは、当該機械を知るための資料で、制御回路の説明とともに、機械の動きを知ることを目的としている。以下に述べる各種資料を正しく理解するための重要な情報となることを考えて作成する。特に安全制御回路図に関しては、一般回路図とは別に作成しその制御内容を分かりやすくする。機械の仕様書などに記載があればそれを流用できる場合もある。

機械の詳細図とは、各部の図面で、必要に応じ荷重計算や開口部と危険部の距離計算などを記して要求事項に適合していることを示す。当然ながら、ブロック図や機能説明図、回路図、プログラミング関連資料なども必要に応じ含まれる。油圧や空圧の回路図などもここに含まれる。また、購入品の関連試料もここに含まれる。それらはカタログの写しであったり、各購入品に対する指令への適合宣言書や第三者認証を示す認証書の写しであったりする。その他、部品リストなども必要となる。

図面類の作成に当たっては、独自の記号や略号を使用することは極力避けなければならない。もし、やむを得ず使用する場合は、その説明を別途行い、誤解を避ける必要がある。
次に、適合を証明するために用いた規格類のリストである。用いた規格はすべてリストアップする。また、規格には含まれないが計算などの根拠となる資料(学術文書など)をリストアップする。
技術文書の第 1 は、リスク評価レポートである。方法や様式は規定されていないので、どの方法を用いても良いが、本レポートには、採用した安全方策の説明や安全と確認するに至った理由などが記述されている必要がある。その他、説明のため必要に応じ、各リスクに対して確認した試験所のレポ

ートなどを用意する。

技術レポートとは、規格に従って適合を確認したことを示すレポートである。該当する規格(ひとつとは限らない)の要求項目に対し、その適合･不適合を確認して作成するが、説明のためいくつかの資料を添付することが多い。マニュアルに関しては、サービスマニュアルのように、自社サービスステーション向けで作成し、時としてユーザーに渡らないようなマニュアルも含む。

全体として技術文書に要求される内容は「自社製品がいかにして要求事項に適合しているか」を説明することである。機械指令の技術文書に関する記述は一般的な事項なので、その字句にこだわった狭義の判断を避け、広義な理解のもとでそろえることが重要である。また、時として、「技術文書はユーザーに提供するものだ」との誤解もあるが、冒頭にも述べたとおり、技術文書は最悪の場合、事故の場合に自社製品の正当性を示す資料となるものであり、ユーザーに提供する性格のものではない。従って､技術文書の作成に当たってはこれらの基本的考え方を忘れないようにすることが重要である。

機械指令の項目には記載されていない事項であるが、設計管理、生産管理、品質管理などは、製品を安全にするために重要な管理項目である。これらの管理体制の上に、上記書類が作成され必要に応じ変更･管理されながら維持される必要がある。設計管理として、初期の開発段階だけではなく、設計変更が行われる際にも、その変更により新たな危険が生じないことが確認されなければならない。それらの確認の資料は新たな技術文書の1ページとして追加されるか変更される必要がある。その変更履歴は管理され､どの製番の製品がどのような構造をしていたのか分かるようになっていなければならない。生産管理・品質管理においては、設計の指示通り生産が行われ製品の安全が確保されるための管理が要求される。部品手配・購入、生産現場での検査結果など各種データは保管され、指示通りの生産が行われたことの裏づけ資料となる。

6.2 低電圧指令

技術文書に関しては、指令の付属書４の 3 項に記載がある。これら書類は、同項にあるように生産中止後 10 年の保管が要求される。

内容は；

- 製品の一般的な説明、
- 基本設計、生産関連図面、回路図/構成品/部品などの構成、
- これら図面や製品の操作を理解するための説明、
- 適合証明に用いた規格のリストや、規格を用いなかった場合はその方法、
- 計算結果、試験結果、など
- 試験レポート

である。

技術文書とは、当該製品が定電圧指令の要求事項に合致していることを裏付ける資料である。したがって、当然ながら適合宣言の作成に先立ち準備されているはずである。作成に当たっては、EU 圏内のいずれかの国の公用語とする。特に 1 冊のファイルにまとめる必要はなく、万が一当局により要求された場合に、速やかに提出することができればよい。すなわち、各関連部門で書類を管理しておき、必要に応じまとめて提出できれば良い。当局からの要求とは、最悪の場合、事故である。そのときにこれら技術文書は、「物言わぬ弁護人」の役割を担うわけであるから、指令などの記述だけにこだわらず、関係者以外でも分かるような記述方法が要求される。

製品概要は、一般的に取扱説明書に記載されていると考えられるが、それを保管することで目的は達成される。特殊製品で、取扱説明書で説明しきれない場合には、別途説明資料の作成した方がよい。

設計の基本理念や図面などの構成については、企画書や仕様書、またサービスマニュアルが該当する。もし該当する書類がない場合は、新たに作成を要するが、その他書類の関連を示すことで、第三者が技術文書を理解しやすいかどうかが決まってしまうといった重要な書類であるので慎重に用意したい。

説明資料については、取扱説明書が該当する。もし、図面類に特有の記号や表現を用いる場合は、その記述も要する。IEC 規格や ISO 規格に規定されていない記号や表現を使う場合は特に注意が必要である。

規格のリストは、適合宣言書への記載も必要である。部分的に参照した規格もリストする必要がある。特定の規格に拘らず、製品評価に用いたすべての規格を記載する。規格には、その発行年により失効している規格がある。失効している規格で評価をした場合、再度有効な規格を用いて評価をやり直す必要がある。原則的には整合規格を用いて評価しなければならない。もし、整合規格が無く、独自に評価した場合は、その方法の詳細を記載しなければならない。独自の評価といっても、できうる限りの国際規格や各国規格を採用する必要があり、科学的・技術的・理論的裏づけの無い評価方法は認められない。

上記、規格での評価試験の結果を示した書類が、計算結果であり試験結果である。試験の再現性の必要性から、いつ・どこで・だれが…といった記載が必要である。試験日、場所、試験員、使用測定器、測定器の有効性、温度・湿度・気圧などの試験環境などは、最低限試験結果に記載する必要がある。当然ながら、試験した者の署名や、試験結果を検証した者の署名なども必要である。指令には具体的記述は無いが、リスクアセスメントなどもここに該当する。今後、リスクベースの安全対策が要求されるので、リスクアセスメントレポートは重要な書類のひとつになる。

これら試験データなどをもとに、該当する規格により評価した結果を述べた書類が試験レポートである。該当する全項目の適合·不適合の判定を記載するとともに、該当しない項目の記載も重要である。レポート作成者の署名とともに、検証者の署名も記する。

以上が、指令での記載事項である。原則的には、すでに存在する書類を用いることが可能である。また、設計変更などにより常に変更される性格の書類が多いので、特別に作成することは勧めない。（ただ、言語の問題があるので、運用の現実には難しい点がある。）

上記以外にも、写真などは適合を証明する有効は手段であるので、適合評価に用いた製品の写真などの保管を勧める。また、設計管理・生産管理・品質管理なども常用事項である。すべての関連手順書を翻訳する必要は無いであろうが、重要な文書の翻訳は必要である。

基本は、「安全な製品を供給する」ことにある。技術文書は安全な製品を設計生産する段階で必要に応じて作成されるものであるから、規格に記載の項目や、指令の要求項目は最低限の要求と考え、必要な書類を整えることが肝要である。

6.3 EMC 指令

技術文書は指令の要求への適合の根拠を示す文書であり、通常、以下の情報を含む。

- 機器に関する説明
- その機器の仕様書、取扱説明書など
- 整合規格を適用した場合、その規格の一覧、及びそれらの整合規格への適合の証拠 (試験報告書)
- 整合規格を適用しなかった、あるいは部分的にのみ適用した場合には、EMC に関する評価の記述、設計上の計算の結果、実施した試験、試験報告書を含む保護要求への適合のために用いた手順の説明
- 電気回路図、部品配置図、その他の図面類 (これはEMC指令では明示的には要求されてはいないが、評価の資料として必要となると考えられる)
- 上記の図面類を理解するために必要な情報 (同上)
- 89/336/EECのTCF ルートを適用した場合には、コンピテント・ボディからの技術報告書又は証明書
- 2004/108/EC で、ノーティファイドボディの関与を選択した場合には、ノーティファイドボディからのステートメント(意見書)
- 適合宣言書 (これを技術文書に含めることは EMC 指令では要求されてはいない)

89/336/EEC の TCF ルートを適用した場合、この文書は TCF と呼ばれる。また、89/336/EEC は、規格ルートを適用した場合に関しては技術文書の作成を要求していないが、その場合でもこのような文書化は行なっておくことが望ましい。技術文書は、EU の公用語のいずれか (例えば英語) で書く必要がある。技術文書に含めようとする資料 (特に、図面などの)に日本語が含まれている場合も少なくないと考えられるが、日本語を読めない専門家でもその資料を理解できるように、最低限、重要な部分についてだけでも英語で記述する(あるいは英語を併記する)ようにすべきである。複数の指令(例えば EMC 指令と低電圧指令) への適合宣言を行なう際には、単一の技術文書でそれら全ての指令のための文書を兼ねさせることが可能だが、その場合にはそれらの指令が技術文書に対して何を要求しているのかを確認し、それらの要求を同時に満足させることが必要となる。

6.4 医療機器指令

製造業者は、自己責任において当該医療機器が本指令の基本要件に対してどのように適合しているかを適切かつ十分に証明するため、技術文書を作成することが求められている。技術文書は、いわば製品に関しての身分証明書の役割を果たし、規制当局を含め第三者に対して適性な身元（基本要件を満たした製品であること）であることを証明する唯一のものであり、要請に応じて常に提出可能でなければならない。つまり技術文書は、製造業者内部に対してではなく外部の第三者に対して十分に説得力があるように記述されることが求められる。また、その目的によりEU域内の公用語で編さんされる必要がある。

技術文書作成の実践的手順は、以下の通りである。

技術文書の基本的な構成としてー１

分類	項目	内容
製品情報	製品の一般情報	・対象製品：欧州域内に出荷しようとする全ての機種を明確にする ・包装仕様 ・製品の仕様が理解できるような概要説明
	製品の意図した目的	・製品の意図した目的：使用方法、対象患者群、使用環境 及び ユーザーの特定 等 この部分の内容は非常に重要になるので明確に記述します。 意図した目的は、クラス分類にも影響を与えます。
	製造方法の概要	・製造方法の要約：一般的表現を用いて製造技術の概要が分かるように記述します。（包装、滅菌方法を含む） ・製品の特性の確認方法
	当該機器と組み合わせる機器	・機器と組み合わせて使用する機器の記述：付属品，オプション，システム構成 等
	クラス分類	・適用した付属書９でのクラス分類ルール及び適用した理由
技術情報	技術的要求事項の明確化	・適用指令 ・明らかでない場合適用指令選択の理由
	基本要件チェックリスト	・基本要件への適合状況（適用整合規格 等） ・適合性を示す証拠（試験・評価レポート 等） ・基本要件に該当しない場合の理由付け

基本的な構成としてー２

分類	項目	内容
設計	リスク分析	・製品に潜在する全てのリスク（重篤度＋発生率）の特定及びリスクの軽減対策 ・リスク分析の結論：製品の効能に対してリスクが許容できるか否か
	製造・特殊工程	・原材料の仕様（生体適合性等） ・特殊工程（クリーンルーム、洗浄、滅菌）及びそれぞれの適用規格 ・適用品質システム規格
	製品試験	・製品の要求性能・安全性を確認する基準・手段（標準・手順等）
	表示類	・取扱説明書，添付文書 等
	製品の有効期間	・製品の有効期間：材料の劣化、包装、滅菌の有効性、能動機器の場合は、スペアパーツの入手可能性等を考慮しての設定
	臨床評価	・製品の臨床的有効性の評価：臨床試験 或いは 文献情報調査
	変更情報	・製品仕様の変更履歴：製品の変更後の仕様、変更の検証 或いは 妥当性確認のデータ等

6.5　R&TTE 指令

Annex　II　4 項の記載通り、設計、生産、製品オペレーションの記載、特に、以下が問われている。

- 製品の一般的な説明
- 基本設計、生産関連図面、回路図/構成品/部品などの構成
- 適合証明に用いた規格のリストや、規格を用いなかった場合はその方法
- 図面や製品の操作を理解するための説明
- 計算結果、試験結果
- 試験レポート

製造者、または代表者は、適合宣言書を技術文書の中に組み込む必要がある。

6.6　玩具指令

玩具指令には、技術文書といった呼称は出てこないが、Article 8 に製造者もしくは、EU 域内の代理人が保持すべき情報に関する記載がある。それらは、適合の確認に用いた規格の番号、それらの試験レポートや関連技術文書、必要に応じ、ノーティファイドボディ発行の EC 型式認定書、ノーティファイドボディへ提出した書類の写しなどが必要になる。もちろん、製造業者名や製品の保管場所なども明確にする必要がある。その他、設計、生産関連書類の保管も義務付けられている。

これら書類は、当該玩具が玩具指令の要求事項に合致していることを裏付ける資料である。特に 1 冊のファイルにまとめる必要はなく、万が一当局により要求された場合に、速やかに提出することができればよい。すなわち、各関連部門で書類を管理しておき、必要に応じまとめて提出できれば良い。当局からの要求とは、最悪の場合、事故である。そのときにこれらの書類は、「物言わぬ弁護人」の役割を担うわけであるから、指令などの記述だけにこだわらず、関係者以外でも分かるような記述方法が要求される。作成に当たっては、PL 事故などの場合に、EU 圏内で使用されるわけであるから、EU 加盟国のいずれかの国の公用語とする。

適合の確認に用いた規格の番号、それらの試験レポートや関連技術文書に関する記述は、他の指令で言うところの適合宣言書に該当する。特に様式の指定はないので、英文などで書類を作成する際に、レポートや書類の名称、特定番号、発行者、総ページ数、改版番号など必要事項を記載した書類を作成した書類と一緒に保管しておけばよい。試験レポートや、関連技術文書に関しては、場合によっては、関連書類の目次に相当させることもできる。

生産者や保管場所の名称、住所、連絡先などは、表紙として作成しても良いし、上記書類の一部として記載しておくこともできる。もちろん別途作成も可能である。

EC 型式認定書や提出書類などはそれらをそのままファイルすればよい。

前述の提出技術書類には、設計関連の書類などが含まれる。機構図、電気回路図、写真などが考えられるが、玩具の場合は、強度計算や毒性の確認が重要であるので、それらの証明書などを忘れてはならない。

機構図には、必要に応じ、組み図も含める必要がある。これは、各々の部品の組み合わせ状態での勘合や隙間を確認するためのものである。周知のように、規格ではこれら隙間での手指の挟みこみや切断を考慮しており、部品許容誤差の最大値、最小値での挟み込みの確認が必要である。

機構図には、警告文やラベルの図面も含まなくてはならない。その記述内容がはっきりとわかる必要がある。図面の保管だけでなく、ラベルや印刷物そのものの保管をお勧めする。ラベルの材質や使用しているインクのメーカや型番の記述も必要であろう。もちろん機構部品の材料の特定に必要な情報、塗料の特定のための情報なども必要となる。そして、おそらくそれらの材料、インク、塗料に対して毒性の有無や含有量を確認して関連書類を入手、保管しておく必要がある。含有量の測定結果を入手した場合は、その結果が指令は関連規格の規定値を満足していることを確認しておくことを忘れてはならない。

機構、表示、毒性などは当該整合規格に従って適合を確認される必要がある。それらは、試験レポートとしてまとめられ、保管されなければならない。この適合確認は、関連する規格のすべての項目に関して行われる必要がある。製品によっては、多くの項目が非該当になる場合もあるが、非該当であることの確認も重要なことである。

電気回路図には、回路の詳細を記載し、各部品の特定ができる必要がある。使用電圧は、２４V 以下であろうが、各部の電圧などの記入も有効である。ちなみに、電気を使う玩具の場合は、たとえ２４V 以下であっても、今後は定電圧指令を考慮する必要性が出てくるので注意を要する。設計、生産関連書類には、上記書類だけではなく、設計管理や生産管理の書類を含む。近年、ISO9001 認証取得が進んでいるので、おそらく揃っていることと考えられる。適合を確認して設計･生産が行われていること、最低限、指令や規格の適合を確認しない設計変更がなされないような仕組みがつくられていることの確認が必要である。

7. マニュアル作成時の留意点

7.1 機械指令

機械の取り扱いを説明する取扱マニュアル、点検整備などを記述する整備マニュアル、修理に関する記述のサービスマニュアルを念頭において記述する。

機械は手入れをされながら長い間使用されるものである､従って日々使用されるマニュアルも機械の寿命に応じた丈夫さが要求される。当然ながら、読み易いものでなくてはならない。「どんな人が使うのか？」を考慮し、それに見合った表現が求められる。また、読む人にとって｢外国語｣で書かれたマニュアルは不適切であり､使用する言語は､機械が使用される国の公用語であることが要求される。作業の流れに沿って、「何故？」「どうして？」といった質問を前提にした記述を行い、わかりやすく作成することが要求される。操作説明はできるだけ単純明快に記述し、使用する単位、あるいは各部の呼び名などはすべての文書で統一させる。安全に関する説明、警告などは文字の色や大きさ形を変えることにより強調する。説明文だけでなく、絵や図表による理解の促進も考量されるべきである。機械が複雑で多くの書類からなる場合は、それらの相互関連が分かるようにする必要がある。各マニュアルや書類には識別番号をつけるなどして誤解が生じないようにする必要がある。また、特定の技術を有する人や訓練を受けた人が行う点検、整備、修理に関する記述は、一般作業員が行う作業に関する記述と明確に分ける必要がある。

マニュアルの第一目的は､設計により排除できなかった危険に関する記述を行うことにより機械を安全に使用するところにある。すなわち、｢残留危険の伝達｣である。また、万が一故障あるいは事故などが生じた場合の責任所在を示すものでもある。責任所在の表示として、輸入業者や修理拠点の住所など連絡を取ることができるだけの情報の記述が要求される。さらに、製品を特定するための製品番号などが必要となる。

機械を安全に使うためには､「意図した使用｣が何であるかを記述することは当然のことである。意図した使用に関しては、大きく分けて、組み立て分解に関すること、使用開始前に関すること、使用に関すること、手入れや定期点検に関すること、そして廃棄に関することなど　製品のライフサイクルを考慮し漏れなく記載する必要がある。また、｢予測可能な誤使用｣による危険回避の必要性から、正しい使用方法を記述しリスクを免れると同時に｢誤使用｣や｢禁止行為｣なども必要に応じ記述する。

以下、機械指令に特有と思われる記述項目を示す。

1) **安全に使用するための設置方法、保管方法、運搬方法の記述。機械や、その部品の寸法,重量,重心また、クレーンのフックの場所やフォークを入れる場所などの記述も必要。**
2) **機械に取り付ける工具に関する記述や、工具選択のために必要な情報の記述。**
3) **騒音に関する記述および騒音の減少に必要な設備の記述。**70dBA **未満であれば､その旨の記述。**

ピークが 63Pa を越えるならばその旨の記述。85dBA を超えるならばその記述。および測定時の運転条件の記述。

4) 爆発環境での使用が予測される場合は、そのための必要な情報の記述。

5) 機械の使用に関する記述として：

5.1) 使用や保守点検に要する作業スペースを示す。

5.2) 温度,湿度,振動,電磁放射,使用高度制限など使用環境に関する記述。その他、電気やエアーなどのエネルギー源に要求される条件(定格)の表示。

5.3) 動力への接続方法の記述。特に電気の過負荷に関して、短絡電流定格の表示や、過負荷保護装置の定格、取り付け方などに関する情報。

5.4) ごみの除去廃棄に関する記述。必要があれば、その処理方法なども記述する。

5.5) 作業者が取るべき保護装置、安全距離、安全表示などの保護手段に関する記述。

6) 機械そのものに関する記述として：

6.1) 設置方法,保護柵などの条件。アンカーやピット、ダクトの設置方法。保護柵の寸法、形状・材料など。

6.2) 必要な安全装置の記述。安全マット、ライトカーテン、インターロックスイッチなどのつなぎ込み、上流･下流の機械との連携のさせ方など。

6.3) 安全回路の記述。他の回路図とは別に、安全回路をしめす。当該機械の安全回路のみならず、連携する機械とのインターフェースも明記する。

6.4) 騒音振動のデータ。振動に関してもその値と、測定条件を明記する。

6.5) 使用するガス･蒸気などの情報。装置内に発生するガス、蒸気、埃なども記載する。

7) 機械の使用に関する記述として：

7.1) マニュアル制御に関する記述。作業者が行う制御に関して記述する。通常作業のみならず、調整や、点検なども含む。

7.2) 調整方法。必要な調整の説明。調整方法、調整値、調整箇所、調整頻度なども記述のこと。

7.3) 非常停止に関する記述。非常事態への対処方法。非常時における人力(あるいは動力)による機械の動かし方。被害者の救出方法など。

7.4) 修理に関する記述。分解、修理、組み立て、調整の方法。補修部品の型番や選択の基準、入手方法など。

7.5) 修理後の再起動。各部動作確認、機能確認、初期化の方法、順序などの記述。

7.6) 安全用具の指示と使用方法。必要な人体保護具(ヘルメット、ゴーグル、手袋など)の選択に必要な情報（要求される特性など）およびそれらを使用する旨の指示とその使用方法の記述。

7.7) 点検整備に関する記述。頻度、補修部品・材料、作業内容などを記述。

7.8) 点検整備に必要な図面などを用意すること。

7.9) 分解廃棄に関する記述。分解方法、分解時の危険性。特に、残留ガス、化学物質などの危険性とその処理方法の記述。

7.10) 非常事態に当たって使用する消火器の種別。発生が予測される有害物質に関する警告と対処方法。作業により発生が予測されるガス、化学物質、埃などの危険性の記述、被害の防止策、被害への対処方法などを記述。

7.2　低電圧指令

技術文書に関する項で述べたように、マニュアル(取扱説明書)の第一目的は、設計により排除できなかった危険に関する記述を行うことにより製品を安全に使用するところにある。すなわち、「残留危険の伝達」である。当然ながら、読み易いものでなくてはならない。「どんな人が使うのか？」を考慮し、それに見合った表現が求められる。また、読む人にとって「外国語」で書かれたマニュアルは不適切であり、使用する言語は、製品が使用される国の公用語が要求される。

製品を安全に使うためには、「意図した使用」が何であるかを記述することは当然のことである。意図した使用に関しては、大きく分けて、組み立て分解に関すること、使用開始前に関すること、使用に関すること、手入れや定期点検に関すること、そして廃棄に関することなど　製品のライフサイクルを考慮し漏れなく記載する必要がある。また、「予測可能な誤使用」による危険回避の必要性から、正しい使用方法を記述しリスクを免れると同時に「誤使用」や「禁止行為」なども必要に応じ記述する。

意図した使用の説明としては、安全に使用するための注意事項、製品の操作方法、点検･手入れの方法、故障発見方法などがある。これらの記述は、操作の流れに沿って、「何故？」「どうして？」といった質問を前提にした記述を行い、わかりやすく作成する。操作説明はできるだけ単純明快に記述し、使用する単位、あるいは各部の呼び名などは統一させる。安全に関する説明、警告などは文字の色や大きさ形を変えることにより強調する。説明文だけでなく、絵や図表による理解の促進も考量されるべきである。もし製品が複雑で、多くの書類からなる場合は、それらの相互関連が分かるようにする必要がある。各マニュアルや書類には識別番号をつけるなどして誤解が生じないようにする必要がある。

製品本体への表示が要求される製造者関連情報、製品の型番、製品の電気定格など製品にかかわる情報は、マニュアルでも重複させることをお勧めする。

さらに、壁・天井･床などへの取り付けを必要とする製品に関しては、その設置方法の詳しい説明が必要となる。設置時に電源コードをつなぎこむ製品の場合は、必要に応じ過電流保護の設置方法や電源コードの選択に関しても説明を要する。その他、製品の手入れ･点検などに必要なスペースの記述や、放熱などに必要な換気のための空間や開口の大きさなどの記述も必要となる。

国際規格では電源コードの交換に関する記述も要求している。これは、電源コードの交換を前提として考えているためで、電源コードの接続方法の種類により、それに見合った交換方法の説明をマニュアルへ記述しなければならない。もちろん、ユーザーによる交換を勧めるのではなく、サービスステーションへ連絡するとか、交換不能なので製品を廃棄するとかいった内容になる。
特殊な環境で使用される電気製品の場合は、その使用環境も重要な記述事項になるので、使用と保管のための温度・湿度・高度など必要な条件を記述する。また、液体や気体を使用する装置ではそれらの取り扱い方などの説明が必要になる。最近では、レーザーや紫外線を使った製品の多くあるので、

それらからの保護に関する記述も必要になる。

上記のことを考えると、リスクアセスメントがいかに重要であるかが認識できる。使用･保管条件が悪いために起こるかもしれない危険の防止､試用する気体や液体により起こるかもしれない危険の防止、レーザーや紫外線による危険の防止など、冒頭で述べたように､｢残留危険の伝達｣を行うことが要求されるわけである。このため、リスクアセスメントをしっかりと行い、製品に(顕在・潜在両方を含み)存在する危険(源)を発見し､その危険性をユーザーへ連絡することにより､事故を防ぐ必要があることを認識いただきたい｡

7.3 EMC 指令

2004/108/EC は､機器とともに以下の情報を提供することを求めている｡これらの多くは 89/336/EEC では明示的には要求されていなかったが､89/336/EEC を適用する場合も、同様の情報を提供することを推奨する。

- その機器を特定するための情報 (型式、ロット番号、製造番号など)
- 製造業者の名前、及び住所
- 製造業者が EU 内にない場合には、EU 内の任命された代理人の名前、及び住所
- 保護要求への適合を確かとするために必要な、その機器の組み立て、設置、保守、あるいは使用に際しての注意事項。例えば、専門家による設置や機器への組み込みを意図した機器における、使用を意図している電磁環境 (特に、シールド・ルームなどの特殊な電磁環境での使用を意図している場合)、供給電源の品質 (歪み、電圧変動、サージなど)、機器の接地の方法、使用すべきケーブルの種類やシールドの処理方法、ケーブルの引き回しの方法、外部に取り付けるべきフィルタの指定など。
- その機器が住宅地での使用に適さない場合には、明確な使用上の制限
- その機器を意図されたように使うために必要な情報

これらの情報を記載する場所を下記に示す。

	機器本体	梱包	取扱説明書	適合宣言書	補足
CE マーキング	✔	✓			
機器の型式	✔	✔	✔	✔	
機器の製造番号	✔				対象を限定する場合は適合宣言書にも明記
製造業者の名前	✔		✔	✔	
製造業者の住所	✓		✔	✔	
代理人の名前と住所	✓		✔	✔	
使用上の注意事項			✔		

✔— 必須、あるいは記載を強く推奨、✓— 記載を推奨

規格によってはこの他の情報の提示が要求されていることもあり､そのような規格を適用する場合にはその要求にも従う必要がある。例えば、製品群規格の１つである EN 55011は、機器にそのグループとクラスを表示することを要求している。

また、予期される干渉を受けた際の性能の低下を許容するのであれば、その性能の低下に関しても明記すべきである。干渉を受けた際の性能がユーザーが期待するであろう性能を下回り、かつそのような性能の低下に関する情報が示されていないならば、その機器は指令の保護要求に適合していないと考えられる。

7.4　　医療機器指令

付属書1 (Annex I) に示される基本要件の 13 項に、製造業者が供給する情報に関する詳細の要求事項がある。製造業者は、各項目の要求に従って機器の表示や取扱説明書などを作成する必要がある。

7.5　　R＆TTE 指令

R&TTE指令の中には、マニュアルや取扱説明書作成について、特別の記載はない。

7.6　　玩具指令

指令には格別取扱説明書に関する記載はない。これは、子供が使うといった玩具の性格によるものである。むしろ、パッケージへの表示や、本体への警告文表示など親にたいする「残留危険の伝達」表示が多く規定されている。とはいえ、要求が皆無なわけではない。たとえば、組み立てにより危険が生じる可能性が考えられる場合などは、組み立て方法の説明が要求される。ブランコなどのように紐などを使う場合は、その固定方法や点検方法は重要事項であり、説明書への記載が必要である。

マニュアルは、当然ながら読み易く分かり易いものでなくてはならない。おそらく親が読むことが予測されるので、それに見合った表現が求められる。イラストの使用や、大きな活字や色の使用により重要事項を目立たせるような工夫も必要であろう。また、読む人にとって「外国語」で書かれたマニュアルは不適切である。使用する言語は、玩具が使用される国の公用語が要求される。

「予測可能な誤使用」による危険回避の必要性から、正しい使用方法を記述しリスクを免れると同時に「誤使用」や「禁止行為」なども必要に応じ記述する。たとえば、浮き輪などへの「救命具としての使用の禁止」がそれに相当する。これらは、多くの場合、本体表示として要求されるが、マニュアルが存在する場合には、その表示も必要となる。

本体や、パッケージ(個装箱、展示箱など)に対しても表示が要求される。CE マーキングの表示はもちろん、製造者や輸入業者の特定のためにその名称、ロゴ、ブランドマーク、住所、連絡先などが必要となる。もちろん、前述の警告文なども必須である。

玩具特有の表示としては、対象年齢がある。パッケージや本体への表示も重要であるが、試験機関にとっても、対象年齢により規定された判断基準が異なるので、非常に重要な項目である。設計時に対象年齢を決定したらその後の変更に対しては要注意である。

8.　適合宣言書の作成方法

- 製造業者またはEU内に設立された正式代表者は、ニューアプローチ指令が定める適合性評価手続きの一部として、適合宣言書を作成しなければならない。
- 適合宣言書には、製造業者、正式代表者、ノーティファイドボディ（該当する場合）、製品、整合規格その他の規範文書への参照（必要に応じて）に加え、宣言発行の基となる指令を識別するための関連情報がすべて記載されなければならない。

適合宣言書(Declaration of Conformity: DoC)は、その機器が指令の要求に適合する旨を、製造業者(あるいはその任命された代理人) が宣言する文書であり、以下の情報を含めなければならない。

- 適合を宣言する指令のリスト
- 宣言の対象となる機器を同定する、名称、型式、製造番号などの情報
- 整合規格を適用した場合には、そのリスト(規格の参照には、年及びAmendmentを含める)
- 製造業者の名前と住所
- 該当する場合、EU内の代理人の名前と住所
- ノーティファイドボディの名前と住所
- 宣言の日付
- 適合宣言書に署名する人に関する情報(所属、肩書など)
- 適切な権限を持つ個人による署名

適合宣言書はEUの公用語のいずれかで作成されなければならない。適合宣言書添付が必須の製品については、宣言書は使用される加盟国の公用語で記載されなければならない、あるいは製造業者、正式代表者、または流通業者が翻訳を提供しなければならない。適合宣言書の具体的な書式は規定はないが、参考までにサンプルを紹介する。

適合宣言書も技術文書と同様、機器の出荷から一定期間保管しなければならない。各指令によって、保管期間や保管場所については、それぞれの指令によって扱いが異なるため
(1) 指令ごとの扱いを記載した一覧表を用意する
(2) EU域内で10年間保管することを推奨し、それぞれの指令を参照する。

機械指令の場合、製品が使用される加盟国の言語に訳したものを製品に添付する必要がある。その他のEU指令の場合、適合宣言書を顧客に提供することは義務付けられていないが、多くの製造業者は、適合宣言書のコピーを機器に添付している。(例えば、取扱説明書に含める)

EC Declaration of Conformity

Black Monolith Inc.

2001 Monolith Road, Sea of Tranquility, The Moon

We declare, under our solo responsibility, that the product:

Product	Super computer
Model	HAL 9000

to which this declaration relates complies with the provisions of following European Directives:

73/23/EEC as amended by 93/68/EEC — Directive on the harmonization of the laws of Member States relating to electrical equipment designed for use within certain voltage limits;

89/336/EEC as amended by 92/31/EEC and 93/68/EEC — Directive on the approximation of the laws of the Member States relating to electromagnetic compatibility.

Applied Harmonized Standards

EN 60950-1:2001
Information technology equipment – Safety – Part 1: General requirements

EN 55022:1998 + A1:2000 + A2:2003
Limits and methods of measurement of radio disturbance characteristics of information technology equipment

EN 61000-3-2:2000
Limits for harmonic current emissions (equipment input current up to and including 16 A per phase)

EN 61000-3-3:1995 + A1:2001
Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤ 16 A per phase and not subject to conditional connection

Authorized Representative

Black Monolith Europe GmbH
2010 Discovery Avenue, Neverland, Germany

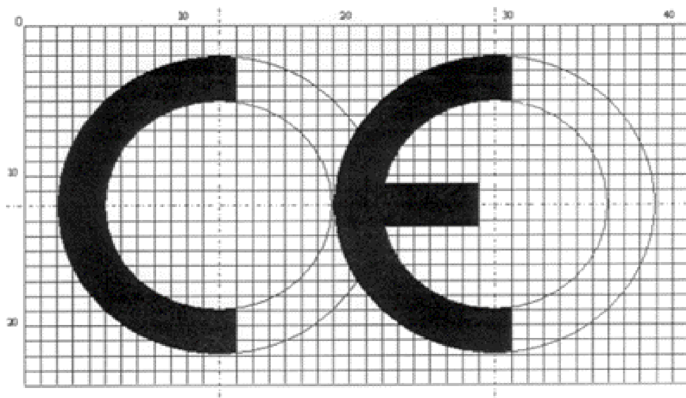
Signature: *Bowman*

David Bowman
Technical Director, Black Monolith Inc.

Date of Issue: *1 April, 2005*

適合宣言書のサンプル

9　CE マーキングの貼付



機器が指令の要求に適合していることを示すための方法として、CEマーキング貼付を規定している。そして、指令の要求への適合性を達成したならば、最終的に、機器にCEマーキングを貼付することによって、その機器をEU内で自由に流通できる。CEマーキングは、認証マークのように外部の機関から取得するものでなく、指令の要求に適合しているという宣言の証として、製造業者が自らの責任の元に機器に貼付する。

CEマーキングの貼付

CEマーキングは、原則としてその機器自身に貼付する。但し、機器に付けることが不可能な場合(例えば、それが小さ過ぎるために) には、その包装や添付文書に付けることも認められる。

CEマーキングは、高さが5mm以上であり、かつその形状の比率が保たれている限りは、任意に拡大/縮小することができる。このマーキングは、容易に見ることができ、かつ容易に剥がれたり消えたりしないような方法で行なう必要がある。

CEマーキングは、その機器に適用される、CEマーキングの貼付を規定している全ての指令への適合を示すものとなる。例えば、その機器が低電圧指令や機械指令の対象にもなる場合、その機器にEMC指令への適合のみに基づいてCEマーキングを付けることは認められない。

参考情報：欧州委員会サイト

ニューアプローチ指令

http://www.newapproach.org/

http://www.newapproach.org/ProductFamilies/Default.asp

●整合規格最新情報掲載サイト

The New Approach to technical harmonisation and standardisation - Harmonised Standards

http://europa.eu.int/comm/enterprise/newapproach/standardization/harmstds/index_en.html

●能動型埋め込み医療機器指令-整合規格リスト

Active Implantable Medical Devices Directive 90/385/EEC

http://europa.eu.int/comm/enterprise/newapproach/standardization/harmstds/reflist/implmedd.html

●EMC **指令-整合規格リスト**　EMC Directive 89/336/EEC

http://europa.eu.int/comm/enterprise/electr_equipment/emc/index.htm

http://europa.eu.int/comm/enterprise/newapproach/standardization/harmstds/reflist/emc.html

●IVD **指令**

In vitro diagnostic medical devices Directive 98/79/EC

http://europa.eu.int/comm/enterprise/newapproach/standardization/harmstds/reflist/invimedd.html

●低電圧指令-整合規格リスト　Low Voltage Directive

http://europa.eu.int/comm/enterprise/electr_equipment/lv/index.htm

http://europa.eu.int/comm/enterprise/newapproach/standardization/

harmstds/reflist/lvd.html

●機械指令-整合規格リスト　Machinery Directive 98/37/EC

http://europa.eu.int/comm/enterprise/mechan_equipment/machinery/index.htm

http://europa.eu.int/comm/enterprise/newapproach/standardization/harmstds/reflist/machines.html

●R&TTE **指令**

Radio and Telecommunications Terminal Equipment R&TTE Directive 1999/5/EC

http://europa.eu.int/comm/enterprise/newapproach/standardization/harmstds/reflist/radiotte.html

●医療機器指令-整合規格リスト　Medical Devise Directive 93/42/EEC

http://europa.eu.int/comm/enterprise/medical_devices/index.htm

http://europa.eu.int/comm/enterprise/newapproach/standardization/harmstds/reflist/meddevic.html

●玩具指令

http://europa.eu.int/comm/enterprise/toys/index_en.htm

題名：　自己宣言のための CE マーキング適合対策実務ガイドブック

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文献26

ドイツへの医療機器輸出に関する諸手続

2007 年 3 月

日本貿易振興機構（ジェトロ）

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■ 質問１：　本報告書は、ドイツ市場において医療機器を流通させる為に必要な諸手続きを照会し、日本企業の皆様が海外に輸出する際の参考資料になることを目的に作成いたしましたが、どの程度満足されましたか？（○をひとつ）

４：満足　　３：まあ満足　　２：やや不満　　１：不満

■ 質問２：　上記のように判断された理由、またその他本報告書に関するご感想をご記入下さい。

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ウェブ：　http://www.jetro.go.jp/jetro/activities/export/

はじめに

ドイツの連邦医療技術連合会によれば、ドイツ医療機器市場の市場規模は 2004 年の場合 200 億ユーロと、欧州医療市場中では最大であり、米国、日本に次いで世界第 3 位である。医療部門の世界市場規模は 1840 億ユーロで、2010 年までの同年間成長率は 4.5%と予測されており、その市場性は高いといえる。

ドイツの医療機器市場への参入に際しては、日本との認証手続の相違や、安全規格や治験制度および諸手続に対しての情報も不可欠であり、ジェトロでは、ドイツ市場における医療機器の認可と流通の法的基盤や、医療機器の流通開始に必要な手続きの概要などについて取りまとめた。

本報告書が、今後ドイツへの進出を検討する医療機器関係企業への参考となれば幸いである。

2007 年 3 月
日本貿易振興機構（ジェトロ）

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1．医療機器の認可と流通の法的基盤

1.1.　医療機器法

医療機器の認可と流通に関する規則は、『医療機器に関する法律（以下医療機器法）』(MPG: Gesetz über Medizinprodukte)　に定められている。医療機器法は 1994 年 8 月に施行された。最近の改訂は 2002 年 8 月である。

同法は、能動型埋め込み式機器に関する EU 指令(Active Implantable Medical Devices, AIMD) 90/385/EEC、医療機器に関する EU 指令(Medical Devices Directive, MDD) 93/42/EEC、およびインヴィトロ（体外）診断機器に関する EU 指令(In-Vitro Diagnostic Medical Devices, IVDD) 98/79/EC をドイツの国内法として整備したものである。内容は、以下の 2 つの部分で構成される。

- 製品を流通システムに載せることから使用開始に至るまで、製造者が必要とする全ての過程を規定する欧州法の部分
- 欧州枠組み条件に準じる市場の監視を規定し、国内の特別事情を実現に移す国内法の部分

医療機器法は、欧州経済地域 (EEA)内における医療機器製品の自由な流通に関する規則を定め、それにより医療機器の安全性、適性、性能の保証を図り、かつ患者、使用者及びその他の第三者に必要な防護を図り、その健康に配慮する (同法第 1 条 1 号)法規である。ドイツで取得された認可は欧州経済地域全域において有効であり、また医療機器の認証手続は EU 各国で概ね一律である。

医療機器法（ドイツ語）ページ： http://bundesrecht.juris.de/mpg/

1.2.　医療機器の定義

医療機器とは、その機能を介して、①疾病の認識・予防・監視・治療・軽減、②負傷もしくは障害の認識・監視・治療・軽減、③身体構造の、または生理機能の検査､代替もしくは変更、④または妊娠調節の目的で人間に対して使われ､薬理的もしくは免疫学的に作用する手段を用いずに人体の内外でその主要効果を発揮する、単独または相互に接続する器具、装置、素材、その他の物体｣である。医療機器が機能するために必要なソフトウェアもこれに含まれる。（医療機器法第 3 条 1 項）。

この定義により、医療機器は医薬品のような他の製品グループから明確に区別される。もっとも、医療機器が、医薬品とみなされる素材を含むこともありうる。だがその場合、その医薬品は、例えば薬剤をコーティングした薬剤溶出性ステントのように、当該医療機器の主目的とする機能を単に補佐するだけのものでなければならない。

2. 医療機器の流通開始に必要な手続の概要

医療機器をドイツ（および欧州連合経済地域）の市場で流通させるにあたっては、以下の二つの手続が必要である。

1. 医療機器の認証 (CE マーク取得)
2. 所轄州官庁(州)への医療機器流通開始届出

医療機器の認証は、医療機器がドイツ国内および欧州連合経済地域で流通するための安全性、適性、性能に関する基準を満たしていることを保証するものであり、同地域内での自由な流通の前提条件である。

さらに、州の所轄官庁への流通開始届出により、医療機器の流通開始後は、その監督下に置かれることになる。

本章では、上記の両手続の概要を示し、次章以降で各手続の詳細をまとめる。

2.1. 医療機器の認証 ― CEマークの取得

医療機器の認証は CE マークの取得をもって行われる。CE マークの取得、ドイツおよび他の欧州連合経済地域で医療機器を流通させるための前提条件である。

1998 年 6 月 14 日以降、ドイツおよび欧州連合（EU）内では「CE マーク」を付した製品のみが、医療機器としての流通と利用を認められている。CE とは「Conformité Europeen」の略であり、CE マークは、当該医療機器が、同種の製品に関する欧州整合規格(harmonisierte europäische Normen)に適合しており、医療機器法 (MPG)および 1.1.項で挙げた医療機器に関する EU 指令 93/42/EEC、90/385/EEC、98/79/EC に準じて安全性、適性、性能に対する基本的要項を満たしていることを示す標識である。自社の医療機器に CE マークを貼付することで、製造者は当該製品が必要な基準を全て満たしていることを表明でき、当該製品を欧州経済地域全域で流通させることが可能になる。

CE マーク取得は大半の医療機器の場合、医薬品医療機器連邦研究所（BfArM: Bundesinstitut für Arzneimittel und Medizinprodukte）の指定を受けた指定機関(Benannte Stelle)において EC 適合性評価手続(Konformitätsbewertungsverfahren)を済ませることにより行われる。指定機関により認証された医療機器には、CE マークと共に当該指定機関の 4 桁の認識番号が付される。(指定機関の詳細は 3.1.へ　EC 適合性評価手続の詳細は 3.5.へ)

CEマークの詳細はEU医療機器指令(MDD) 93/42/EEC付則Ⅰ～Ⅹ（英文）を参照：
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31993L0042:EN:HTML

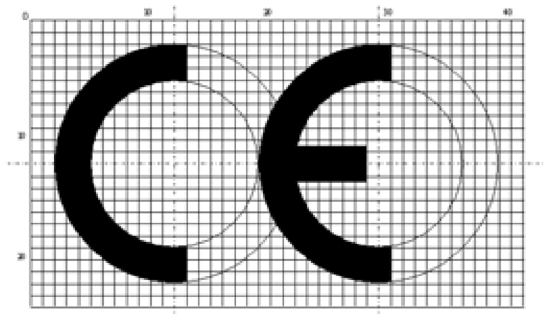

図 1：CE マークの寸法比率

出典：欧州委員会ホームページ http://ec.europa.eu/enterprise/faq/ce-mark.htm

CE マークは可能な限り、製品に直接にもしくは滅菌済みの包装材に貼付し、また取扱説明書、梱包ケースに表示しなければならない。マークの縦長は 5mm 以上、寸法比率は図 1 の通り決められている。

適合性評価手続の際に、EC 適合宣言書(Konformitätserklärung)を提出しなければならない。EC 適合宣言書は、当該医療機器が EU 指令の定める要求事項を満たしている旨を記した文書である。製品説明の記載とともに、技術仕様書、製造業者名、適用規格の一覧表の添付が求められる。（技術文書は 3.2.へ　EC 適合宣言は 3.4.へ）

医療機器は、一般医療機器に関する EU 指令 93/42/EEC の付則Ⅸに準じて等級分類されており(Ⅰ、Ⅱa、Ⅱb、Ⅲ)、そのうち、Ⅱa、Ⅱb、Ⅲに属する製品と、Ⅰのうちで滅菌の必要な製品のみが、指定機関において EC 適合性評価手続を通して認証される。

滅菌を必要としない等級Ⅰの製品に関しては、製造者自らが、EU 指令 93/42/EEC の付則Ⅶに準じて適合性評価を行い、指定機関の認識番号の伴わない CE マークのみを製品に貼付する。(等級分類の詳細は 3.3.へ)

2.2.　流通責任者による所轄官庁（州）への届出

CE マークを取得した医療機器をドイツ国内および欧州連合経済地域で流通させるには、流通責任者が自らの事業拠点のある州の所轄州官庁に届け出ねばならない(医療機器法第 25 条)。（詳細は 4.へ）この届出により、医療機器は所轄州官庁の監督下に置かれる。

流通責任者とは、医療機器が初めて流通する際に、医療機器の設計、製造、包装、商標について、自らの名においてその責任を負う者であり、製造者、製造者が指定した代理人、または輸入業者である(医療機器法第 5 条)。流通責任者は、届出後も官庁から当該医療機器について照会がある場合に対応しなければならない。（流通責任者については 2.3.へ）

届出はドイツ医療資料情報機構 (DIMDI: Deutsches Institut für medizinische Dokumentation und Information)のホームページ（www.dimdi.de）に掲載の指定書式を用いてオンラインで行

う（医療機器法第 33 条）。届出に際して手数料は発生しない。このオンラインシステムは医療機器分野の文書管理中央システムであり、医療機器情報システム(DIMDI, Deutsches Informationssystem Medizinprodukte)と呼ばれる。届出受理後２～３週間以内に、DIMDIデータバンクから申請者宛てに所轄州官庁の認可通達が届く。認可を得た医療機器は、欧州市場で流通可能となる。（届出の詳細は 4.へ）

また、届出時に、医療機器の流通責任者は、医療機器の保守受託人(Sicherheitsbeauftragte)を指名しなければならない。医療機器保守受託人は、自然科学・技術・医療専門分野の職業経験を有し、当該医療機器に関して生じた問題事例報告の収集・評価と官庁への届出、ならびに必要な対策の推進にあたる（医療機器保守受託人については 7.2.へ）。

2.3. 製造者と流通責任者、およびEU経済地域以外に事業拠点を持つ製造者

ドイツ（および欧州連合経済地域）において流通する医療機器には、包装上の表示、取扱説明書、その他の製品に関する書類に、製品の流通に関する責任者の氏名と所在地の記載が必要である。

医療機器法第 3 条では、『製造者』を、「医療機器が初めて流通する際に、医療機器の設計、製造、包装、商標について、自らの名においてその責任を負う者」であると規定している。ここでは、製造者とは、欧州経済地域で営業する者で、製品に対する責任者であればよく、必ずしも製品自体の製造者とは限らない。医療機器を流通システムに投入する責任者であり、小売業者、輸入業者、もしくはオリジナル製品を自身のロゴマークとブランド名で販売するライセンス取得者であってもよい。

欧州経済地域外に事業拠点を持つ製造業者は、『委任代理人(Bevollmächtigte)』を流通責任者として指名することができる。委任代理人は欧州経済地域に拠点を持ち、製造業者の名の下に法的責任を肩代わりし、所轄州官庁からの照会に対応する。この場合は、本来の外国の製造業者は引き続き製造業者として表記される（医療機器法第 3 条）。

委任代理人が製品の流通開始届出を行う際には、当該医療機器に関する必要書類を適切な期間内、通常 5 週間以内に受け取れることを証明しなければならない。この証明は、当該内容を記した製造業者の書面か、もしくはその他契約書類をもって行われる。

ヨーロッパ外の製造業者が委任代理人の指名を望まない場合は、その製品を輸入者の自己の責任において市場で販売することができる。この場合、輸入者が医療機器法で定める製造者となり、製品にも製造者としてその名前が明記される。この場合は、輸入者が必要な全書類を現地において保管しなくてはならない。

3. EC 適合性手続の詳細(CE マークの取得)

医療機器の認証手続は、EC **適合性評価手続**と呼ばれる。製造者は、EC 適合性評価手続を通して、製品が医療機器法および欧州整合規格の要求事項を満たしていることを示さなければならない。その結果、製造者に CE マーク貼付の権利が認められ、ドイツにおける流通と利用が可能となる。EC 適合性評価手続は、製品の危険度に準じて様々なタイプに分類されており、それに応じて手続費用も異なる。

医師の処方に従い、名前を指定した特定の患者に合わせて個別に仕上げる(量産品でない)特別仕様の製品には、EC 適合性評価を行う必要はない。

欧州の他の国で行われている官庁による医療機器認可手続はドイツでは実施されておらず、認証は次項で述べる指定機関(Benannte Stelle)により行われる。

3.1. 指定機関

医療機器の認証は、大半の製品の場合、指定機関(Benannte Stelle)を通して行われる。指定機関とは、EU 指令付則（1.1 参照）に準拠し、公的な認可･監督手続を実施する認証機関である。指定機関は、ドイツ連邦保険・健康省の連邦上級官庁である医薬品医療機器連邦研究所（BfArM: Bundesinstitut für Arzneimittel und Medizinprodukte）の指定を受け、欧州委員会に登録されている(医療機器法第 37 条 1 項)。ドイツの指定機関は、ほとんどが民間機関である。欧州連合加盟国の指定機関による認証は、欧州経済地域(EEA)全域で有効である。

これらの指定機関は、様々な製品分野に特化されている。自社の特定製品の認証が可能か否かについては、各機関に照会することが望ましい。各指定機関とも、認識番号を有し、認証された製品には CE マークと共にその指定機関の 4 桁の認識番号が付される。

表 1：ドイツの指定機関一覧

認識番号	指定機関名／所在地、Tel.　Fax、E-mail、URL	取り扱い製品
0481	ECM 欧州医療機器認証有限会社 ECM Zertifizierungsgesellschaft für Medizinprodukte in Europa mbH Eifelstraße 1c 52068 Aachen Tel: +49-241-501034 Fax: +49-241-501038 E-Mail :ecm@medi-online.com www.medi-online.com/ECM/	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器 - 非能動型機器：付則Ⅱ,Ⅴ、Ⅵに基づく EC 適合性評価を行う製品（インプラント創傷処置用器具、医用消費財、歯科器具、特殊部品と素材等） - 付則Ⅲ、Ⅳに基づく EC 適合性評価を行う製品（埋め込み式カテーテル、経口カテーテル、脊髄カテーテル、包帯、創傷被覆材等） - EU 指令 2003/32/EC で規定する機器（動物組織利用医療製品） - 付則ⅡとⅤに基づく EC 適合性評価を行う製品で、素材が欧州医薬品品質規則の TSE 適性認証を得ているもの
0633	Berlin Cert ベルリン工科大学医療機器試験・認証機関有限会社	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器：

	Berlin Cert Prüf- und Zertifizierungsstelle für Medizinprodukte GmbH an der Technischen Universität Berlin Dovestraße 6 10587 Berlin Tel: +49-30-31425111 Fax: +49-30-31423719 E-Mail :info@berlincert.de www.berlincert.de	- 能動型医療機器：付則III、IVに基づく EC 適合性評価を行う全製品。ただし、結石破砕機器、超音波・レーザー利用外科機器、MRI、レーザー治療機器、イオン照射治療、イオン放射造影装置は除く
0535	EUROCAT 認証・試験機関有限会社 EUROCAT Institute for Certification and Testing GmbH Wittichstraße 2 64295 Darmstadt Tel: +49-6151-500350 Fax: +49-6151-5003550 E-Mail :info@eurocat.de www.eurocat.de	医療機器に関する EU 指令(MDD) 90/385/EEC に準拠する機器： - 能動型埋め込み式機器：付則 2、5 に基づく EC 適合性評価を行う全製品 医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 非能動型機器：付則II,V、VIに基づく EC 適合性評価を行う製品（インプラント、創傷処置用器具、歯科器具、特殊部品と素材）。等級IIIは除く。 - 能動型機器：付則II～VIに基づく EC 適合性評価を行う全製品。ただし、加圧療法機器を除く。 体外診断機器に関する EU 指令(IVDD) 98/79/EC に準拠する機器： - 付則II リスト B に基づく製品 - 付則IV3、5、付則VII3、4 に基づく EC 適合性評価を行う血糖値測定、比較、コントロール素材 - 付則IV3、5、付則VII3、4 に基づく EC 適合性評価を行う(病院などで)自家利用の製品
0432	MPA NRW ノルトライン=ヴェストファーレン材料試験局 MPA NRW Materialprüfungsamt Nordrhein-Westfalen Marsbruchstraße 186 44287 Dortmund Tel: +49-231-4502324 Fax: +49-231-4502501 E-Mail :info@mpanrw.de www.mpanrw.de/	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 能動型機器：付則II、V、VIに基づく EC 適合性評価を行う聴覚計、補聴器
0044	テュフ(技術監査協会)・ノルト・ツァート有限会社 TÜV NORD CERT GmbH Langemarckstraße 20 45141 Essen Tel: +49-201-8253455 Fax: +49-201-8253243 E-Mail :medical@tuev-nord.de www.rwtuev-at.de	医療機器に関する EU 指令(MDD) 90/385/EEC に準拠する機器： - 能動型埋め込み式機器：付則 2、5 に基づく EC 適合性評価を行う全製品 医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 非能動型機器：付則II～VIに基づく EC 適合性評価を行う全製品 - 能動型機器：付則II～VIに基づく EC 適合性評価を行う全製品 - EU 指令(MDD) 93/42/EEC 付則IIとVに基づく EC 適合性評価を行う、EU 指令 2003/32/EC（動

		物組織利用医療製品）で規定する機器
0297	DQS ドイツ認証・管理システム有限会社 DQS GmbH Deutsche Gesellschaft zur Zertifizierung von Managementsystemen August-Schanz-Straße 21 60433 Frankfurt am Main Tel: +49-69-954270 Fax: +49-69-95427111 E-Mail :medical.devices@dqs.de www.dqs.de/futuretense_cs/dqs/static/index.html	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 非能動型機器：付則Ⅱ、Ⅴに基づく EC 適合性評価を行う全製品 - 能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う全製品 - EU 指令(MDD) 93/42/EEC 付則ⅡとⅤに基づく EC 適合性評価を行う、EU 指令 2003/32/EC で規定する機器（動物組織利用医療製品）
0482	MEDCERT 医療認証・試験有限会社 MEDCERT Zertifizierungs- und Prüfungsgesellschaft für die Medizin GmbH Vorsetzen 35 20459 Hamburg Tel: +49-40-369517982 Fax: +49-40-369517983 E-Mail :info@medcert.de www.medcert.de	医療機器に関する EU 指令(MDD) 90/385/EEC に準拠する機器： - 能動型埋め込み式機器：付則 2、5 に基づく EC 適合性評価を行う全製品 医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 非能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う全製品 - 能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う全製品。ただしイオン照射療法機器、CT・血管造影用機器を除く - EU 指令(MDD) 93/42/EEC 付則ⅡとⅤに基づく EC 適合性評価を行う、EU 指令 2003/32/EC で規定する機器（動物組織利用医療製品）
0494	SLG 試験・認証有限会社 SLG Prüf- und Zertifizierungs GmbH Burgstädter Straße 20 09232 Hartmannsdorf Tel: +49-3722-73230 Fax: +49-3722-732399 E-Mail :slg@slg-pruef-zert.de www.slg-pruef-zert.de	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う全製品。ただし、加圧療法機器を除く。
0118	テューリンゲン計測・計量検定所 Landesamt für Mess- und Eichwesen Thüringen Unterpörlitzer Straße 2 98693 Ilmenau Tel: +49-3677-8500 Fax: +49-3677-850400 E-Mail :certif-thueringen@lmet.de www.lmet.de -	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 非能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う、有計測機能医療機器（医療用温度計、医療用注射器、トノメータ、非能動型血圧計、容量・流量計測器、バイタル機能特定用機器、秤） - 能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う機器（バイタルパラメータの特定と監視用機器、医療用秤、トノメータ、非イオン放射画像診断、非イオン照射の放射線療法機器と透熱療法機器（外科用レーザー治療機器を除く）、刺激療法機器）
0197	テュフ(技術監査協会)・ラインラント製品安全有限会社 TÜV Rheinland Product Safety GmbH Am Grauen Stein 51105 Köln Tel: +49-1803-112112 Fax: +49-1803-000169	医療機器に関する EU 指令(MDD) 90/385/EEC に準拠する機器： - 能動型埋め込み式機器：付則 2、5、6 に基づく EC 適合性評価を行う全製品 医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器：

	E-Mail :medical-products@de.tuv.com www.de.tuv.com/de/produkte_leistungen/produkte/index.html -	- 非能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う医療機器 - 能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う全製品。 - EU 指令(MDD) 93/42/EEC 付則ⅡとⅤに基づく EC 適合性評価を行う、EU 指令 2003/32/EC で規定する機器（動物組織利用医療製品） 体外診断機器に関する EU 指令(IVDD) 98/79/EC に準拠する機器： - 付則Ⅱリスト A に基づく製品 - 付則Ⅳ3、4、5、6、付則Ⅴ、Ⅶ3、4、5 に基づく EC 適合性評価を行う全製品 - 付則Ⅳ3、5、付則Ⅶ3、5、Ⅴ、Ⅵ、Ⅶ3、4 に基づく EC 適合性評価を行う全製品 - 付則Ⅲ6、Ⅳ3、5、付則Ⅶ3、5、Ⅶ3、4 に基づく EC 適合性評価を行う全製品
0123	テュフ(技術監査協会)・ズート製品サービス有限会社、医療機器認証 TÜV SÜD Product Service GmbH Zertifizierung Medizinprodukte Ridlerstraße 65 80339 München Tel: +49-89-50084477 Fax: +49-89-50084327 E-Mail :info@tuev-sued.de www.tuev-sued.de	医療機器に関する EU 指令(MDD) 90/385/EEC に準拠する機器： - 能動型埋め込み式機器：付則 2、5 に基づく EC 適合性評価を行う全製品 医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 非能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う医療機器 - 能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う全製品 - EU 指令(MDD) 93/42/EEC 付則ⅡとⅤに基づく EC 適合性評価を行う、EU 指令 2000/70/EC で規定する機器、および 2001/104/EC（EU 指令 MDD 93/42/EEC の変更）で規定する機器（ヒト血液またはプラスマを含む医療製品） - EU 指令(MDD) 93/42/EEC 付則ⅡとⅤに基づく EC 適合性評価を行う、EU 指 2003/32/EC で規定する機器（動物組織利用医療製品） 体外診断機器に関する EU 指令(IVDD) 98/79/EC に準拠する機器： - 付則Ⅱリスト A に則る製品：付則Ⅳ3、4、5、6、付則Ⅴ、Ⅶ3、4、5 に基づく EC 適合性評価を行う全製品 - 付則Ⅱリスト B に則る製品：付則Ⅳ3、5、付則Ⅶ3、5、Ⅴ、Ⅵ、Ⅶ3、4 に基づく EC 適合性評価を行う全製品 - (病院などで)自家使用のための製品：付則Ⅲ6、Ⅳ3、5、付則Ⅶ3、5、Ⅶ3、4 に基づく EC 適合性評価を行う全製品
1275	LGA InterCert 認証有限会社、環境鑑定機関 LGA InterCert Zertifizierungsgesellschaft mbH, Umweltgutachterorganisation Tillystraße 2	医療機器に関する EU 指令(MDD) 90/385/EEC に準拠する機器： - 能動型埋め込み式機器：付則 2、5 に基づく EC 適合性評価を行う全製品 医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器：

	90431 Nürnberg Tel: +49-911-6554161 Fax: +49-911-6554170 E-Mail :lga@lga.de www.lga.de -	- 非能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う医療機器 - 能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う全製品 - EU 指令(MDD) 93/42/EEC 付則Ⅱと V に基づく EC 適合性評価を行う、EU 指令 2003/32/EC で規定する機器（動物組織利用医療製品） 体外診断機器に関する EU 指令(IVDD) 98/79/EC に準拠する機器： - 付則Ⅱリスト B に則る製品：付則Ⅳ3、5、Ⅴ、Ⅵ、Ⅶ3、4 に基づく EC 適合性評価を行う血糖特定、比較、コントロールのための製品 - 自家使用のための製品：付則Ⅲ6、Ⅳ3、5、付則Ⅶ3、5、Ⅶ3、4 に基づく EC 適合性評価を行う全製品
0366	VDE 電気電子情報技術連合会 VDE 検査認証研究所 VDE Verband der Elektrotechnik Elektronik Informationstechnik e.V. VDE Prüf- und Zertifizierungsinstitut Merianstraße 28 63069 Offenbach am Main Tel: +49-69-8306228 Fax: +49-69-8306855 E-Mail :vde-institut@vde.com www.vde.com/vde	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う全製品。ただし高温透熱機器、医療機器用ソフトウエアは除く。
0124	DEKRA 認証有限会社 DEKRA Certification GmbH Handwerkstraße 15 70565 Stuttgart Tel: +49-711-78612566 Fax: +49-711-78612615 E-Mail :info@dekra-certification.com www.dekra.de/its -	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 非能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性評価を行う医療機器 - 付則Ⅲ、Ⅳに基づく EC 適合性評価を行うコンドーム - 能動型機器：付則Ⅱ、Ⅴ、Ⅵに基づく EC 適合性評価を行う全製品 - 能動型機器：付則Ⅲ、Ⅳに基づく EC 適合性評価を行う全製品。ただし人工呼吸器、酸素療法機器、麻酔吸入器、イオン照射放射線療法機器、眼科用機器、滅菌用機器、リハビリ用機器、能動型義肢・義手をのぞく。 - EU 指令(MDD) 93/42/EEC 付則Ⅱと V に基づく EC 適合性評価を行う、EU 指令 2003/32/EC で規定する機器（動物組織利用医療製品） 体外診断機器に関する EU 指令(IVDD) 98/79/EC に準拠する機器： - 付則Ⅱリスト B に則る製品：付則Ⅳ3、5、Ⅴ、Ⅵ、Ⅶ3、4 に基づく EC 適合性評価を行う血糖特定、比較、コントロールのための製品 - (病院などで)自家使用のための製品：付則Ⅲ6、Ⅳ3、5、付則Ⅶ3、5、Ⅶ3、4 に基づく EC 適合性評価を行う全製品
0483	mdc 医療機器認証有限会社 mdc medical device certification GmbH	医療機器に関する EU 指令(MDD) 93/42/EEC に準拠する機器： - 非能動型機器：付則Ⅱ～Ⅵに基づく EC 適合性

	Kriegerstraße 6 70191 Stuttgart Tel: +49-711-2535970 Fax: +49-711-25359710 E-Mail :mdc@mdc-ce.de www.mdc-ce.de	評価を行う医療機器 - 付則III、IVに基づくEC適合性評価を行うコンドーム - 能動型機器：付則II（No4を除く）、V、VIに基づくEC適合性評価を行う製品（バイタルパラメータの監視および特定用機器、点滴機器、人工呼吸器、酸素療法機器、外科用機器、イオン照射および非イオン照射画像診断機器、イオン照射および非イオン照射放射線療法機器、眼科用機器、刺激療法機器、歯科用機器、患者移送用装置、リハビリ用機器、能動型義肢・義手。ただし加圧療法機器、結石破砕機器、体外型心臓ペースメーカー、除細動器を除く） 体外診断機器に関するEU指令(IVDD) 98/79/ECに準拠する機器： - 付則IIリストAに則る製品：付則IV3、4、5、6、付則VII3、4、5に基づくEC適合性評価を行う全製品 - 付則IIリストBに則る製品：付則IV3、5、VII3、4に基づくEC適合性評価を行う血糖特定、比較、コントロールのための製品 - 自家使用のための製品：付則III6、IV3、5、付則VII3、5、VII3、4に基づくEC適合性評価を行う全製品

出典：ドイツ医療機器情報システム（DIMDI: Deutcshes Infromationssystem Medizinprodukte）

http://www.dimdi.de/static/de/mpg/adress/benannte-stellen/bs-akt.htm

表1のうち、テュフ(技術監査協会)・ラインラント（0197）とテュフ・ズート（0123）が以下のとおり日本に進出し、医療機器認証に関する包括的サービスを提供している。

テュフ・ラインランド ジャパン株式会社
新横浜本社

〒222-0033
横浜市港北区新横浜3-19-5 新横浜第二センタービル
045-470-1850 (TEL)
045-473-5221 (FAX)
ホームページ：http://www.tuv.com/jp/index.html
医療機器認証に関するサイト：http://www.tuv.com/jp/medical_products.html

同社は、大阪にもラボとオフィス、　広島、九州、沖縄にオフィスを有する。

テュフ・ズードジャパン株式会社

〒160-0023
東京都新宿区西新宿4丁目15番3号　住友不動産西新宿公園3号館 7F
TEL: (03)-3372-4821（代）
FAX: (03)-3372-4163（代）

問い合わせは：
Tel：03-3372-4849 (部門直通)
Fax：03-3372-4122
E-mail：mhs@tuv-sud.jp
ホームページ：http://www.tuv-sud.jp/
医療機器に関する総合サービスのサイト：http://www.tuv-sud.jp/mhs/index.html

同社は大阪に関西本部、川崎市に東京試験所を有する。

3.2. 技術文書の作成

製造者は、EC 適合性評価手続に際して技術文書を作成しなければならない。技術文書は、EU 指令が医療機器に求める要求事項が遵守されていることを示す書類である。危険性の少ない製品に関しては技術記録の提出は不要であるが、製品の耐用期間中、最低でも最終製品製造から 5 年間は、監督官庁の監査に備えて技術文書を保管しておかなければならない。製造業者が欧州経済地域を拠点としない場合は、委任代理人もしくは欧州経済地域に拠点を置く他の製品責任者（例：輸入業者）に、監査に備えての技術文書保管義務がある。

技術文書に必須の項目は以下の通りである。

- 製品の一般説明
- 製品・部品・部品の組合せ・回路等の図面と製作図
- 図面・製品の機能に関する解説
- リスク分析の結果
- 適用規格の一覧表
- 試験、計算等の結果、試験報告
- 臨床の評価、場合によってはそのデータ
- EU 指令の要求事項を満たすために実施した解決策の説明
- 重要な参考文献、学術調査の結果
- 取扱説明書
- 必要に応じて、適用した品質管理システムに関する文書

技術文書に関する詳細は、EU勧告「技術文書」(Technical Documentation, NB-MED/2.5.1/Rec5)英語）に記されている。www.meddev.info/_documents/R2_5_1-5_rev4.pdf

3.3. 医療機器の等級分類

医療機器は、一般医療機器に関する EU 指令 93/42/EEC の付則IXに準じて、製品使用の際に発生し得る危険度に応じて、製品等級 I、IIa、IIb、III（最大の危険度）に分類される。この製品等級により適合性評価手続の種類が異なるため、製造業者は、適切な手続を選択する前にまず等級分類を行う。ただし、インヴィトロ(体外)診断用機器と能動型埋め込み式医療機器は、この等級分類に含まれない。

表 2：EU 指令 93/42/EEC 付則 IX による危険度等級の分類基準

侵襲性	- 非侵襲 - 人体開口部からの侵襲、例：口腔、聴覚器官 - 外科的侵襲 - 埋め込み型
人体との接触時間	- 一時的：60 分未満 - 短期間：30 日未満、もしくは断続的 - 長期間：30 日以上
人体での適用場所	- 生命機能に障らない - 中心循環系 - 中枢神経系
エネルギー源の利用	- 能動型製品（電気・エンジンを動力とする） - 非能動型製品

表 2 の危険度等級の分類基準に準じて以下の等級が定められている。例示した機器は、その種の機器が当該等級に分類される頻度が多いことを示したものであり、あくまでも目安に過ぎない。類似の機能を有する機器が別の等級に分類されることもある[1]。

等級I：滅菌指定、計測機能ともなし

潜在的危険が少なく、人体との接触がわずかで長時間に渡らない（使用時間 1 時間未満）。非侵襲型の製品

例：メガネのフレームや歩行用の杖など。

等級I：滅菌指定、または／及び計測機能つき

危険性は低いが滅菌指定のあるもの、もしくは計測機能を備えたもの。大半が非侵襲型。例：聴診器、外科用器具(複数回使用)、臍帯クリップ、体温計、血圧計、カニューレ（複数回使用）、など。

指定機関は、殺菌もしくは計測機能に関して検査をする必要がある。

等級IIａ

中程度の危険性。製品使用は短期間（30 日以下）、もしくは同じ製品を繰り返し断続的に使用。侵襲度の大きくない製品。外科的に設けられた開口部での短期間の使用。

例：カニューレ(使い捨て)、カテーテル、心電計（Ⅱb に分類されるものもある）、多くの診断用機器(大半の内視鏡など)、一部の点滴ポンプ(インシュリン用)、輸血用機器、外科用器具(1 回のみ使用)、外科用縫合素材、注射器、補聴器、消毒用具、手術用手袋等。

[1] 例えば、内視鏡は、人体のどの部分に挿入されるかにより、異なる等級に分類される。一般には等級Ⅱだが、中枢神経系に使用の場合は等級IIIである。

等級IIｂ

人体の全組織に影響を与える中程度の危険性、30 日以上の長期に渡って使用。

例：患者モニター、非侵襲型避妊器具、体外型除細動器、レントゲン装置、コンタクトレンズ、レーザー機器、人工呼吸器、保育器、人工透析器、一部の点滴ポンプ（経静脈栄養、経管栄養）、長期使用の呼吸装置、心電計（IIａに分類されるものもある）、カテーテル(30 日以上継続使用)、インプラント（中枢神経系や中枢循環器系以外に使用）[2]、結石破砕機器、等。

等級III

人体の生体機能を侵害しかねないため、危険性が特に大。心臓・循環系・神経系に直接使用の製品。

例：埋め込み式心臓ペースメーカー、非能動型埋め込み式医療機器（中枢神経系や中枢循環器系に使用するステント、人工血管、人工心臓弁など）、硬膜外カテーテル等中枢神経系や中枢循環器系に挿入のカテーテル、内視鏡（中枢神経系や中枢循環器系に使用）、外科用縫合素材（吸収性のもの、また吸収性でなくとも中枢神経系や中枢循環器系に使用のもの）。

EU 医療機器等級分類ガイドライン(MEDDEV 2.4/1 Rev.8 Guidelines for the classification of medical devices)中には、医療機器の等級分類に関する詳細が記されている。

第 1 部：http://www.meddev.info/_documents/2_2_4-1part1_07-2001.pdf

第 2 部： http://www.meddev.info/_documents/2_2_4-1part2_07-2001.pdf.

3.4.　EC 適合性評価手続に採用される検査と管理システムの種類

EU 指令ではその付則において、危険度等級に応じ EC 適合性評価のための様々なモジュールを定めている。潜在的な危険が高い製品に関しては、製造業者に品質管理システム（QM システム）と呼ばれる適切な品質保証システム（QS システム）の導入を求めている。指定機関は、EC 適合性評価手続の一環として品質管理システム(QM)の監査を行い、その品質管理システムが EU 指令の要求事項を満たすにふさわしいかどうかを評価する。また、製造業者には、その品質管理システムがどのような EU 指令要求事項に基づき、いかなる対策が盛り込まれているかについての詳細な説明が求められる。説明の基本的内容は、管理システムの戦略、行動指示書、手引き、プログラム、計画等が記載された品質管理システム文書からなっている。

以下の検査もしくは品質管理システムが、EC 適合性評価手続に適用される。EU 指令 93/42/EEC の付則IXにおける等級（3.3 参照）に応じて、組み合わせて用いられる（3.5 参照）。

[2] ただし、乳房インプラントは等級III。また、肩・腰・膝部インプラントは、等級IIIへの昇格を予定（EU指令 2005/50/ECに基づき）

(1)EC適合宣言（EU指令 93/42EEC付則VII）

EC 適合宣言書と技術文書のみが必要である。製造業者は自己の責任において、その製品が EU 指令の要求事項に適っていることを、EC 適合宣言書を作成して保証する。また技術文書を作成する。その後 CE マークを貼付することができる。この手続は危険度の最も低い等級の製品にのみ適用できる。

EC適合宣言の見本は、指定機関mdc医療機器認証有限会社(表 1) のホームページ(英語版)からダウンロードできる。 http://www.mdc-ce.de/doc_01.htm

(2)ECサンプル検査（EU指令 93/42EEC付則III）

指定機関が、製品の設計図の審査とそれに加えて一つもしくは複数の代表的な製品（すなわちサンプル）の検査を行う。サンプル検査は常に EU 指令の他の付則と組み合わせて行わなければならない。

(3)EC検査（EU指令 93/42EEC付則IV）

全製品もしくは全製品中から無作為抽出した製品が EU 指令の要求事項に適っているかどうかを、指定機関が検査する。危険度の高い等級では、合わせてサンプル検査の実施も必要となる。

(4)製造品質保証（EU指令 93/42EEC付則V）

製造業者は、原料調達から製品の最終検査までの全製造工程の品質保証システムを導入する。この品質保証システムにより、製品と技術文書の一致が保証されなければならない。危険度の高い等級の場合、本手続とサンプル検査を組み合わせて、製品とサンプル検査のサンプルが一致するという保証が必要である。この品質保証システムには ISO 9002、EN 46002、ISO 13488 等の規格が対応する。

(5)製品品質保証（EU指令 93/42EEC付則VI）

製造業者は製品の最終検査のみを対象範囲とする品質保証システムを導入する。この品質保証システムでは、製品と技術文書の一致が保証されなければならない。危険度の高い等級では本手続とサンプル検査を組み合わせて、製品とサンプル検査のサンプルが一致するという保証が必要である。この品質保証システムには ISO 9003 と EN 46003 の規格が対応する。

(6)完全品質保証システム（EU指令 93/42EEC付則II）

製造業者は、製品開発から最終検査までを監視する総合的な品質管理システムを導入する。指定機関は技術文書と設計データを検査する。個々の製品の検査は必要でない。規格としては、ISO 9001、EN 46001、ISO 13485 が適用できる。

3.5. EC 適合性評価手続

3.4.で上げたモジュールは、EU 指令 93/42/EEC の付則IXによる医療機器の等級に対応して、以下のよう EC 適合性評価手続が行われる。

本項で『付則』と記す場合は、すべて医療機器に関する EU 指令 93/42EEC の付則を示す。

等級I（滅菌指定も計測機能もなし）

認証には付則 VII に準じた EC 適合性評価手続（3.4.(1)）で十分である。この場合、手続はいわば自己認証に等しい。責任者は EC 適合宣言書と合わせて、必ず技術文書を作成し監査の際に開示できるようにしておく。

危険度等級がこれより高い場合は、以下に示すように指定機関の審査が必要となる。

等級I（滅菌指定、または／及び計測機能つき）

付則 VII に準じ EC 適合性評価手続（3.4.(1)）を実施する。さらに殺菌または計測機能に関する要求事項のみに限定して、以下 3 種類の手続のうち１つを行う必要がある。

- 付則 IV に則る EC 検査。指定機関は、全製品もしくは無作為抽出の製品が技術文書と一致しているかどうかを検査（3.4.(3)）
- 付則 V に則る製造品質保証。指定機関は品質保証システムを監督、監査する。品質保証システムにより製品と技術文書との一致が保証されるため、製品の検査はしない。（3.4.(4)）
- 付則 VI に則る製品品質保証。上記と同様、指定機関は品質保証システムを監督し、監査（3.4.(5)）

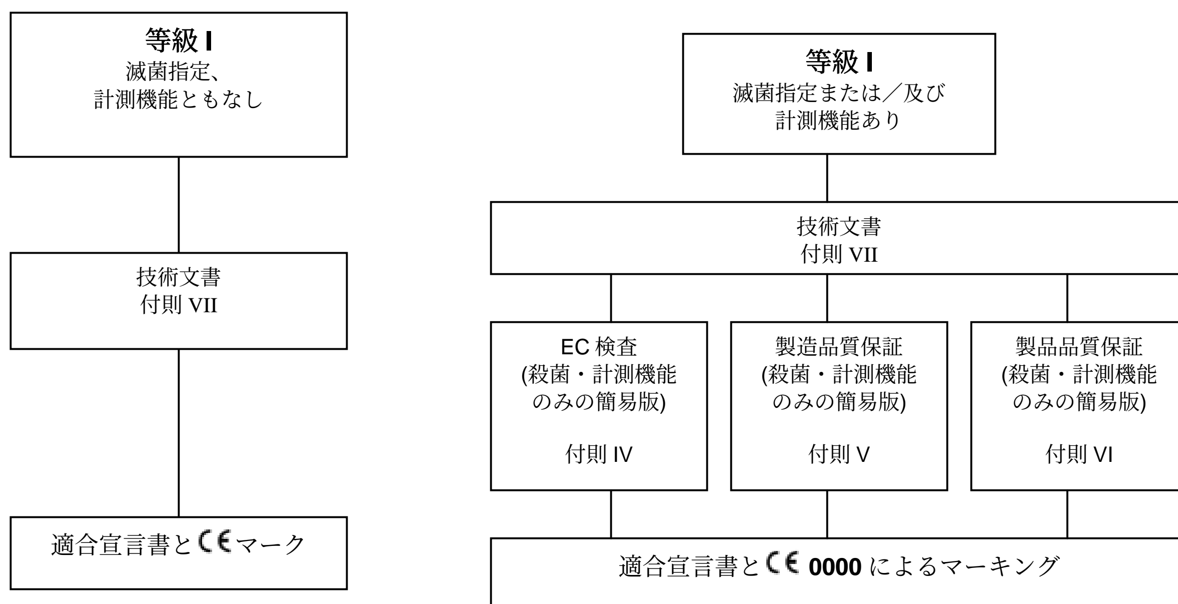

図 2：等級Ｉの医療機器に関する EC 適合性手続評価

等級IIａ

付則 VII に則る適合性評価手続（3.4.(1)）と合わせて、さらに以下の手続のうちいずれか一つを実施する。

- 付則 IV による EC 検査（3.4.(3)）

- 付則 V による製造品質保証（3.4.(4)）
- 付則 VI による製品品質保証（3.4.(5)）

指定機関は上記の追加手続において、製品もしくは品質保証システムの要求事項が技術文書と一致するかどうかを検査する。

付則II（3.4.(6)）に準じて完全品質保証システム（製品設計検査なしの簡易版）[3]を導入して、それを指定機関に監査、監視させるという選択肢もある。この場合は付則VIIに則る適合性評価手続（3.4.(1)）の追加は不要である。

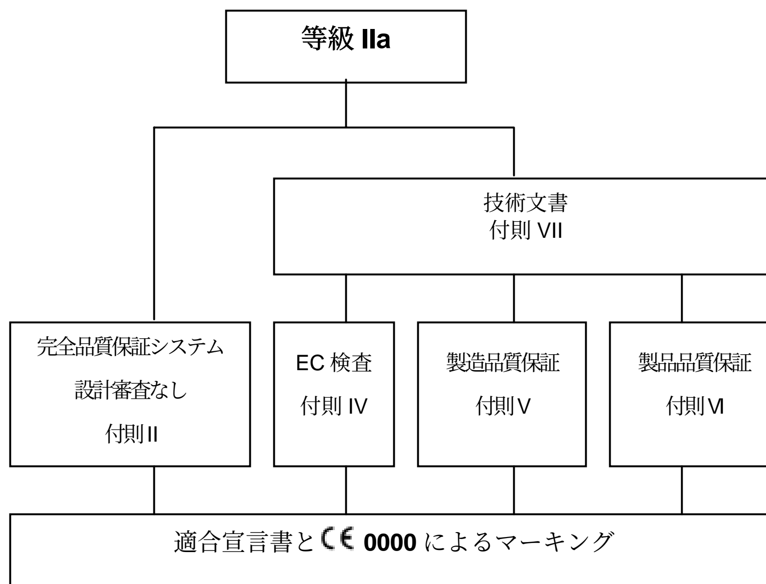

図 3：等級Ⅱa の医療機器に関する EC 適合性評価

等級IIｂ

EC サンプル検査（3.4.(2)）を実施する。指定機関は、代表的なサンプルと EU 指令要求事項の一致を確認する必要がある。サンプル検査と合わせて、等級 IIａの製品と同様に、以下の手続のうち一つを実施しなければならない。

- 付則 IV による EC 検査（3.4.(3)）
- 付則 V による製造品質保証（3.4.(4)）
- 付則 VI による製品品質保証（3.4.(5)）

[3] 人体の生体機能を侵害しかねないため、危険性が特に大きい等級IIIの医療機器は、製造過程で発生し得る瑕疵を可能な限り早期に察知する必要がある。したがって、この等級では指定機関が完全品質保証システムの枠内で設計審査を行い、危険の早期認識と防止を図る。それより危険度の低い機器の場合は、簡略化した品質保証システムでよい。

指定機関は上記 3 種の追加手続において、製品あるいは品質保証システムの要求事項がサンプル検査で使用されたサンプルと一致するかどうかを検査する。

製造業者はこの等級においても、付則 II（3.4.(6)）に則る完全品質保証システム（設計審査の伴わない簡易版）を導入し、指定機関に監査、監視させることができる。

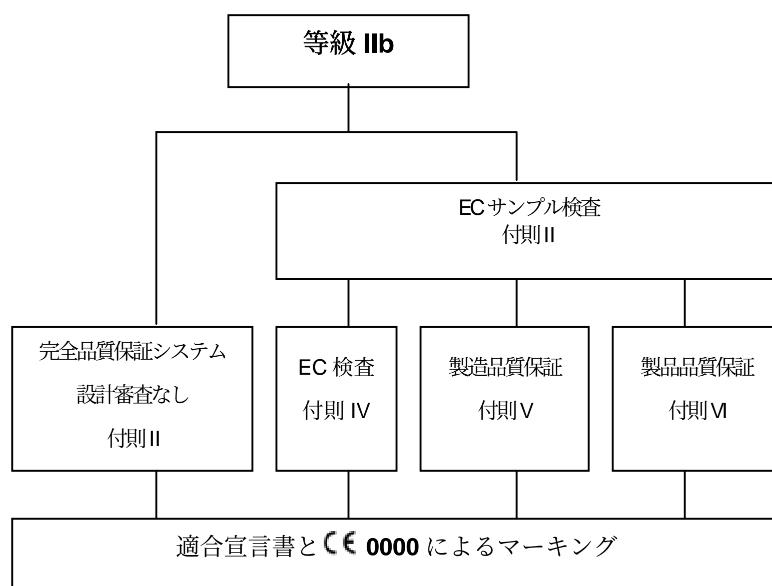

図 4：等級Ⅱb の医療機器に関する EC 適合性評価

等級III

付則 II に準じて完全品質保証（3.4.(6)）を実施する。これは指定機関が、製造開始に先立ってあらかじめ製品の設計文書を審査することを意味する。

EC サンプル検査（3.4.(2)）と合わせて、以下の両手続のうち一つを実施するという選択肢もある。

- 付則 IV に則る EC 検査（3.4.(3)）
- 付則 V に則る製造品質保証（3.4.(4)）

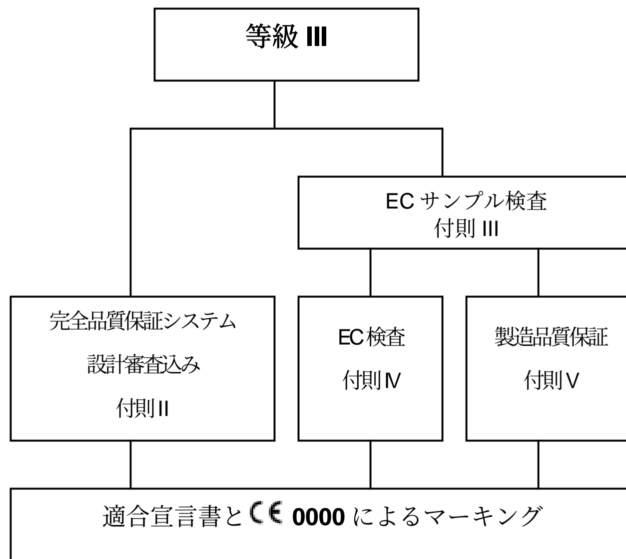

図 5：等級Ⅲの医療機器に関する EC 適合性評価

図 6 に、テュフ(技術監査協会)・ズート・アカデミー有限会社の作成した EC 適合性手続の概観を、参考までに示す。

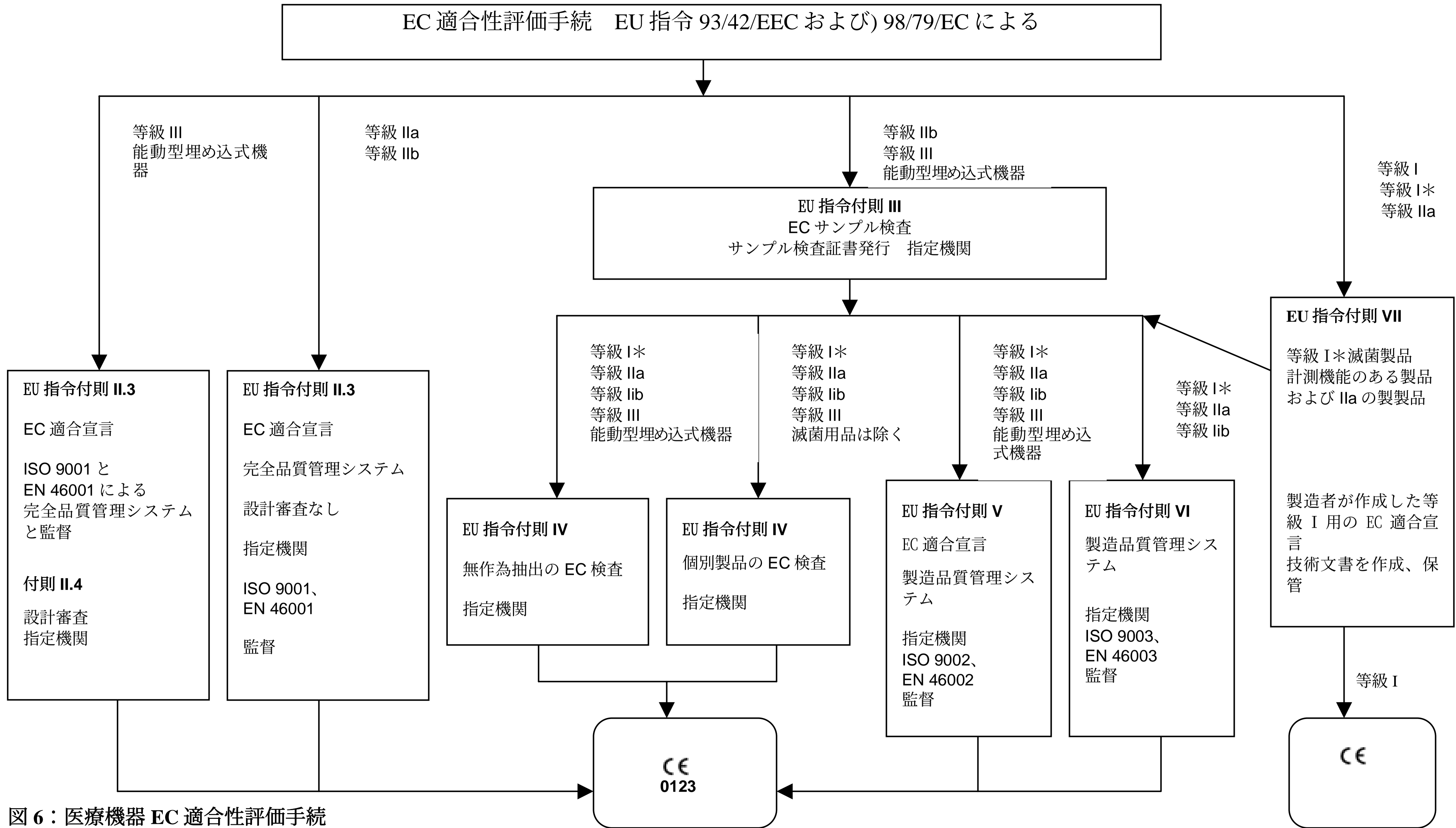

図 6：医療機器 EC 適合性評価手続

出典： テュフ(技術監査協会)・ズート・アカデミー有限会社(TÜV SÜD Akademie GmbH)　http://193.30.192.77/medtech/doku/Kon_akd_deutsch.pdf

適合性評価手続の終了後、製品への CE マーク貼付が可能になる。指定機関による適合性評価手続を経た医療機器には、CE マークと合わせて 4 桁の指定機関認識番号が表示される。

滅菌指定も計測機能もない等級 I の製品に対しては、付則 VII に準じた EC 適合性評価手続（3.4.(1)）のみを実施するため、CE マークのみが添付され、指定機関認識番号はつかない。

指定機関の発行する認証書の有効期間は 5 年である。5 年経過後は、申請によりさらに 5 年の延長が可能である。延長の条件は、製品製造の際の条件（例：基礎を成す規格など）がどう変化したかに左右される。製品の危険度が高いほど、延長のための再検査は早い時期に行われる。ただし再検査は初回の認証に比べれば、通常は小規模で済む。

3.6. 指定機関で行われる認証手続とその手順

等級 I のうちで滅菌指定または／及び計測機能のある機器、等級 IIａ、等級 IIｂ、等級 III に属する医療機器、および能動型埋め込み式機器と大半のインヴィトロ(体外)診断機器に関する整合性評価手続は、指定機関が行う。

指定機関が手続を行わないのは次のものである。

- 等級 I で滅菌指定がなく計測機能もないもの
- 特別注文による製品
- 臨床試験用の製品
- システムおよび処置用ユニット
- インヴィトロ（体外）診断機器に関する EU 指令(IVDD) 98/79/EC 第 9 条第１項でグループ１に属し、付則Ⅲに該当するインヴィトロ(体外)診断機器

図７に、指定機関（表 1）のうち、mdc 医療機器認証有限会社とベルリン・ツァートの両指定機関の事例を参考にして、指定機関が関わる認証プロセスの概要を示す。

ただし、認証プロセスの厳密なフローは事例ごとに異なる。臨床検査等の検査実施前に検査の計画と方法について指定機関と打ち合わせし、その取り決めに従いデータ評価を行うことが望ましい。製造業者が認証手続以前に既に質の高い検査を実施した場合には、その検査内容を認証手続に取り入れることができる。

欧州委員会は認証手順に関する様々な文書を手引書として公開している。主要文書は www.meddev.info に掲載されている。

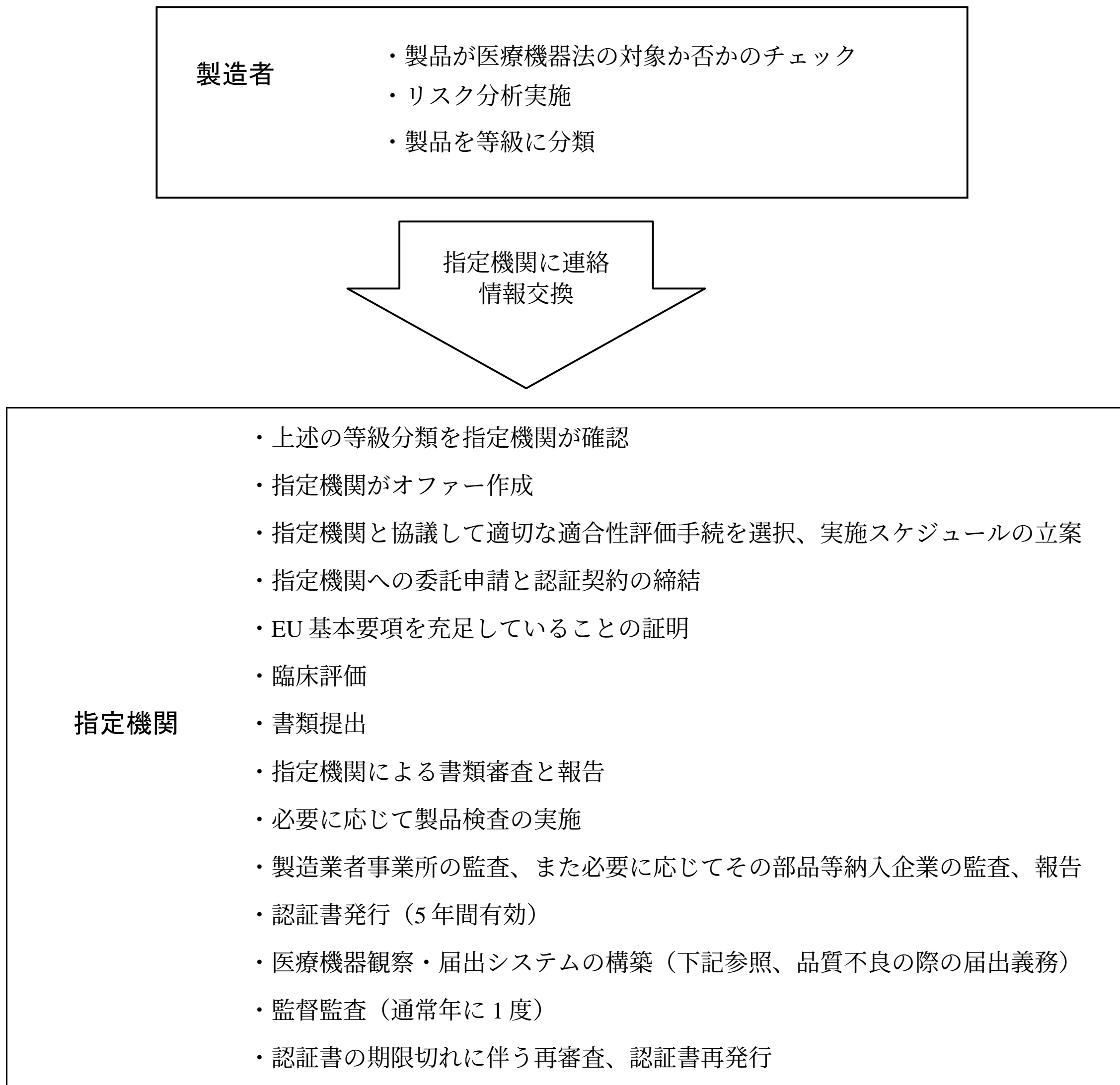

図 7：指定機関がかかわる医療機器認証のプロセス

3.7. 臨床評価と臨床試験

医療機器はすべて、認証手続の一環として臨床評価を受けなければならない。危険性の低い等級Ｉの製品の場合も、監督官庁の監査に備えて作成する技術文書の一部として、臨床評価データが必要である。臨床評価は医療機器法第 19 条および EU 指令 93/42/EEC 付則 X に則り行われる。医療機器の臨床面での性能、つまり意図する用途への適合性、望ましくない副作用の評価、便益・リスク分析がその際必要とされる。

臨床評価とは、以下の内容のものである。

- 主要な関連学術文献のまとめ、ならびにそれに対する批判的評価の報告書
- 評価対象となる製品もしくはそれと比肩しうる製品の市場における経験
- 製品の臨床試験結果、もしくは他の医療機器に関する公表済みの臨床研究で、その医療機器が評価対象の製品と比肩しうるものであることを製造者が証明できるもの

臨床評価の手法としては、臨床試験および学術文献分析がある。臨床評価に際して、臨床試験は常に必要なわけではない。学術文献についての分析、そして当該医療機器とその相応の使用法に関する最新の学術水準に関する分析のみで充分な場合もある。なお製造者は、臨床評価と臨床試験が EU の要求基準に基づくものであることを示さねばならない。

文献分析以外にさらに臨床試験が必要かどうかの判断は、専門文献中に記された製品に関するデータが十分なものであるかどうか、また製品の危険度がどの程度であるかによる。埋め込み型医療機器と等級IIIの機器では臨床試験は一般的であり、また侵襲型、等級IIの長期使用機器にも臨床試験が頻繁に行われる。

ドイツで臨床試験を行う場合は、被験者に発生し得る危険性に対して、国籍を問わず、一人当たり最低 500,000 ユーロの保険契約締結が義務付けられている。

ドイツで行われる臨床試験の場合、申請者ならびに審査機関は、試験開始の少なくとも 60 日前までに州の所轄官庁に届け出ねばならない。その際も、申請はドイツ医療機器情報システム(DIMDI)上で行われる。所轄州官庁は下記のリンクで検索可能である。参考までにバイエルン州の所轄州官庁を挙げておく（表 3）。同州やバーデン・ヴュルテンブルク州等幾つかの州では、能動型機器と非能動型機器の所轄州官庁が異なるが、ベルリン州、ブランデンブルク州等多くの州は、同一官庁が全種類の機器を扱っている。

所轄州官庁一覧：http://www.dimdi.de/static/de/mpg/adress/behoerden/klifo-liste.htm

企業は定期的に臨床評価を行い、製品の最新水準を維持するとともに、市場での経験を、利用者からのクレーム分析等を含めて評価する必要がある。

表 3：バイエルン州の臨床試験申請官庁

コード	所轄州官庁	能動型医療機器	非能動型医療機器	能動型体外診断用機器	非能動型体外診断用機器	所在地／連絡先
DE/CA126	バイエルン州保健・食品安全庁 Bayerisches Landesamt für Gesundheit und Lebensmittel-sicherheit	○		○		Pfarrstraße 3 80538 München Tel: +49-89-21840 Fax: +49-89-2184297 E-Mail :poststelle@lfas.bayern.de
DE/CA61	オーバーバイエルン行政府 Regierung von Oberbayern		○		○	Maximilianstraße 39 80534 München Tel: +49-89-21760 Fax: +49-89-21762914 E-Mail: medizinprodukteanzeigeverfahren@reg-ob.bayern.de
DE/CA62	ニーダーバイエルン行政府 Regierung von Niederbayern		○		○	Regierungsplatz 540 84028 Landshut Tel: +49-871-80801 Fax: +49-871-8081002 E-Mail :poststelle@reg-nb.bayern.de
DE/CA63	オーバーファルツ行政府 Regierung Oberpfalz		○		○	Emmeramsplatz 8 93047 Regensburg

						Tel: +49-941-56800 Fax: +49-941-5680699 E-Mail :poststelle@reg-opf.bayern.de
DE/CA64	オーバーフランケン行政府 Regierung von Oberfranken		○		○	Ludwigstraße 20 95444 Bayreuth Tel: +49-921-6062229 Fax: +49-921-6062280 E-Mail :poststelle@reg-ofr.bayern.de
DE/CA65	ミッテルバイエルン行政府 Regierung von Mittelfranken		○		○	Promenade 27 91522 Ansbach Tel: +49-981-530 Fax: +49-981-531206 E-Mail :poststelle@reg-mfr.bayern.de
DE/CA66	ウンターフランケン行政府 Regierung von Unterfranken		○		○	Peterplatz 9 97070 Würzburg Tel: +49-931-38000 Fax: +49-931-3802222 E-Mail :pharmazie@reg-ufr.bayern.de
DE/CA67	シュヴァーベン行政府 Regierung von Schwaben		○		○	Fronhof 10 86152 Augsburg Tel: +49-821-32701 Fax: +49-821-3272289 E-Mail :poststelle@reg-schw.bayern.de

出典：http://www.dimdi.de/static/de/mpg/adress/behoerden/klifo-liste.htm

さらに、連邦薬剤・医療製品研究所(BfArM)に登録されており、不偏不党の各分野の代表で構成される倫理委員会の同意が必要である。

外国で実施された臨床試験の結果は、それが ISO 規格を満たしており、試験結果の詳しい報告が付されているのであるならば、通常は問題なく認められる。製品検査が EU 域外で実施された場合には、製造者は、その際の医療機器の使われ方ならびに無作為抽出で選ばれた患者が、機器が EU 内で使用される際の使われ方および対象患者層と比肩しうるものであることを示さねばならない。

EU指令の付則 X には、臨床評価に関する基本的な指針がまとめて記されている。英語版は http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31993L0042:EN:HTML

また、EUガイドライン「臨床データの評価　―　製造者と公認機関用の手引き」(MEDDEV 2.7.1 Evaluation of Clinical Data – A Guide for Manufacturerers and Notified Bodies)は、医療機器の認証の際に、臨床評価と臨床試験報告のどの点にウエイトが置かれるかを詳細に記しており、臨床評価に関して大変参考になる。http://www.meddev.info/_documents/2_7.pdf.

表 4 に、臨床試験の代行機関・企業の例を挙げる。

表 4：臨床試験の代行機関の例

機関名、所在地	住所、電話・ファックス、E-mail、URL
アマンテック ノルトライン＝ヴェストファーレン州 ミュンスター市 (フランクフルトとベルリンにも事務所あり)	Amantec Technologiehof Mendelstr. 11 48149 Münster Tel.:+49 (0)251 9801490 Fax: +49(0) 251 9801491 E-Mail :gabriele.hartwig@amantec.de www.amantec.de
エムデーテー・メディカルデヴァイス・テスティング有限会社 バイエルン州 オクセンハウゼン市	mdt medical device testing GmbH Grenzenstr. 13 88416 Ochsenhausen Tel.: +49 (0) 7352 9114 0 E-Mail :info@mdt-gmbh.com www.mdt-gmbh.com
フライブルク大学臨床試験センター バーデン＝ヴュルテンベルク州 フライブルク市	Universitütsklinikum Freiburg ZKS-Zentrum Klinische Studien Elsässer Straße 2 79110 Freiburg Tel.: +49 (0) 761 270 7384 Fax. +49 (0) 761 270 7383 E-Mail :eva.hermann@uniklinik-freiburg.de www.uniklinik-freiburg.de/zks/live/leistungen/monitoring.html
ゲーヴェーテー・テーウーデー有限会社 ザクセン州 ケムニッツ市	GWT-TUD GmbH Chemnitzer Str. 48B 01187 Dresden Tel.: +49 (0) 351 87 34 17 20 E-Mail :contact@GWTonline.de www.gwtonline.de
ゲーツェーペー・サーヴィス ブレーメン市	GCP-Service Kurfürstenallee 35a 28211 Bremen Tel.: +49 (0) 421 43 48 658 Fax: +49 (0) 421 43 48 659 E-Mail :germany@gcp-service.com www.gcp-service.com

3.8.　認証手続における外国の規格とその証明

EU 指令の要項が守られていることを証明するには、EU 整合規格を使用することが望ましい。EU 整合規格のどれが該当するかは、製品グループにより異なり、DIN（ドイツ産業規格）、EN（欧州規格）、ISO 規格（国際標準化規格）と幅がある。

諸外国の国内規格は、基本的に医療機器法の要求事項の証明に適用できる。しかし製品に異常が認められた場合には、製造業者は EC 適合宣言書を根拠にその責任を問われることになる。その際に、EU 整合規格に基づいていることが有利に働くことはありうる。指定機関は認証手続時に、適用されている規格が EU 整合規格と比肩しうるものであるかに留意する。

EC 適合性評価手続では当該製品の過去における検査と分析も考慮される。日本からの製品検査報告書も、それが質の高いものであり、基本となっている規格が明示されている場合には､しばしば認められる。

ただし、等級Ⅲ（3.3 参照）に該当する製品の EC サンプル検査については、試験およびその報告は欧州基準に準じて行われる。

流通開始の届出に際しては、国外の製造業者は、欧州における委任代理人もしくは輸入業者を、所轄州官庁からの照会に対する担当者として指名しなければならない。ただし、認証手続に際して指定機関が直接連絡を取るのは、通常は製造者（2.3 参照、輸入業者が務めることも可能）であるため、技術文書と品質保証システムの文書等はドイツ語もしくは英語で用意することが望ましい。特に、重要書類をドイツ語で作成することは製造者にとって有利であろう。報告書が複雑な内容の場合は、監査の際に当局から英語文書のドイツ語訳を求められることがありうる。

認証手続を進めるに当たっては、指定機関が直接製造者に連絡をとることが多いため、企業内に認証手続を理解した従業員がいて、指定機関に直接対応できるようにするのが最善である。日本に進出しているドイツの指定機関テュフ（技術監査協会）2 社が、医療機器認証に関して包括的なサービスを提供しているので、アドバイスを得るのも一方法である（日本のテュフの連絡先は　→　P.13～14）。

3.9.　認証手続にかかる費用

認証手続にかかる費用は、簡単な適合性評価手続で数千ユーロから、手間のかかる品質管理システムの監査では数万ユーロと幅が広い。費用は製品の危険度等級と企業の規模、製造プロセスの複雑さ等の条件に左右される。等級Ⅰの製品であれば、費用は 1,000～10,000 ユーロでほぼカバーできる。等級Ⅲのステントになるとその 2 倍から 3 倍の 20,000～30,000 ユーロかかる。医療機器が医薬品を伴うものであれば、費用はさらにその 2 倍になる。認証の対象となる製品パターンが多ければ多いほど、認証費用も増加する。

以上の費用は純粋な認証手続費用である。コスト計算の際はその他に、5 年間の監督監査の実施が義務付けられていることを考慮しなければならない。品質保証システム等の監査は

製造の行われている場所で実施されるため、外国の製造業者には、加えて渡航費及びこれに類するコストが発生する。

指定機関は互いに競合関係にあるため、製造業者は監督・監査等全てのサービス業務を含む種々のオファーを吟味するべきである。

4. 医療機器の流通開始届出

医療機器を流通させるには、適合性評価手続実施とならんで、医療機器法第 25 条と 30 条に基づき、所轄州官庁への届出が必要となる。

届出はオンラインでのみ可能であり、ドイツ医療資料情報機構 (DIMDI: Deutsches Institut für medizinische Dokumentation und Information)のホームページ（www.dimdi.de）に掲載の指定書式を用いる（医療機器法第 33 条）。届出の手数料は発生しない。医療機器情報システム(Deutsches Informationssystem Medizinprodukte, DIMDI)は医療機器分野の中央管理システムであり、届出のうちのいくつかは、この文書管理システム内でのみ処理される。届出の受理から２～３週間以内に、DIMDIデータバンクから製造業者に州官庁の認可通達が届く。（流通届出所轄州官庁リストは 5.参照）

認可前に、所轄州官庁が製品責任者に連絡を取ることがしばしばある。これは製品の情報を得ると共に不明な点を明らかにする目的のものだが、規定にはなく、当該官庁担当者の判断によるものである。危険性の低い等級 1（滅菌指定、計測機能ともなし）の製品の場合は、当局側からの照会なしに申請が受理されることも少なくない。

州官庁による認可済み医療機器に対する無作為抽出の検査は定期的に行われ、その際には当該製品に関する書類が詳細に審査される。.

指定機関による認証が必要な製品の場合は、届出に際して当局が製品責任者に連絡を取ることは稀である。これは、製品の認証が指定機関により十分な審査に基づき実施されているとの判断によるものである。

医療機器情報システム(DIMDI)上での医療機器の流通開始届

医療機器法 25 条及び 30 条に則る医療製品の流通開始届は、DIMDI のオンライン文書管理システム上で行われる。届出ならびにその後の変更届けはドイツ語で行われる。届出の詳細は以下のホームページに記載されている（ドイツ語のみ）。

http://www.dimdi.de/static/de/mpg/ismp/mp_erfassung/de/mp_ivd/anzeigende/organigramm.pdf

届出は、次の手順を踏んで行われる。

- 医療機器情報システム(DIMDI)のユーザーコード申請
- 医療機器流通責任者の初回住所登録
- 医療機器の初回登録

ユーザーコードの取得後、利用者には同システムへのログインが常時可能となる。申請手続の処理過程で、利用者(流通責任者)の提出した届出に書類番号とコードが付される。当該届出に関する事後の変更は、この番号を用いて行われる。

ユーザーコードにより、医療機器情報システム(DIMDI)のデータバンク利用が可能になる。70 を超えるデータバンクには多数のドイツ語専門文献が備えられている。

後日の製品に関する変更、医療機器保守受託人の変更、住所の変更、製品登録の取り消しなどの変更も、このシステム経由で行われる。

5. 所轄官庁

医療機器の流通開始届出と医療機器監督の所轄官庁

ドイツでは、医療機器の流通開始届出受付と医療機器法遵守の監督の所轄は、州官庁にある。官庁の管轄領域は連邦州ごとの医療機器管轄規定（Medprod-ZustVO: Medizinproduktezuständigkeitsverordnung）に定められている。所轄州官庁の名称や組織は州により異なるが、医療機器の届出手続には連邦州による差はほとんどない。

監督官庁は医療機器に異常が認められた場合の検査のみならず、製造者の元で抜き打ち的に技術文書と製造条件の検査も実施する。欧州外の製造業者が欧州経済地域内で委任代理人を指名しなければならないのはそのためで、代理人は最新の技術文書を管理し、官庁に対する窓口の役割を引き受ける。

製品の流通開始の申請先官庁は、以下に示すリンクで検索可能である。所轄州官庁は、連邦州別に、官庁コードを付して列挙されている。医療機器情報システム(DIMDI)上で申請する際に、このコードを入力する。

面積の広い州では所轄州官庁の数が多いが、一州に１官庁のみの州もある。また、能動型医療機器と非能動型医療機器の申請を別の官庁が担当する州もあれば、全機器を 1 官庁で扱う州もある。官庁の所轄部門も、営業監督庁であったり、環境庁であったりと、さまざまである。バイエルン州は州内の７行政府に、能動型医療機器と非能動型医療機器の申請部署を分けて設けており、官庁の数は 14 に上る。下にその一部を例示した。行政府営業監督庁が能動型機器を、行政府が非能動型機器を扱っていることが、オーバーバイエルン行政区の例から明らかである。他方、ザールラント州は１官庁を置いているのみで、同部署で全機器を扱う。

流通開始届所轄州官庁リスト(ドイツ語)： www.dimdi.de/static/de/mpg/adress/behoerden/beh-liste.htm

表 5：流通開始届の所轄州官庁の例

バイエルン州の例（一部官庁のみ掲載）

コード	官庁	能動型医療機器	非能動型医療機器	測定機能付機器	能動型体外診断	非能動型体外診断	
DE/CA52	シュヴァーベン行政府営業監督庁 Regierung von Schwaben Gewerbeaufsichtamt	○		○	○		Morellstraße 30d 86159 Augsburg Tel: +49 821 32701 Fax: +49 821 3272700 E-Mail :gaa@reg-schw.bayern.de
DE/CA54	オーバーフランケン行政府営業監督庁 Regierung von Oberfranken Gewerbeaufsichtamt	○		○	○		Oberer Bürglaß 34-36 96450 Coburg Tel: +49 9561 74190 Fax: +49 9561 7419100 E-Mail :medizinprodukte-gaa@reg-ofr.bayern.de
DE/CA55	ニーダーバイエルン行政府営業監督庁 Regierung von Nieder-bayern Gewerbeaufsichtamt	○		○	○		Neustadt 480 84028 Landshut Tel: +49 871 8040 Fax: +49 871 804219 E-Mail :gaa-mpg.vorkommnisse@reg-nb.bayern.de
DE/CA57	オーバーバイエルン行政府営業監督庁 Regierung von Oberbayern Gewerbeaufsichtamt	○		○	○		Maximilianstr. 39 80534 München Tel: +49 89 21761 Fax: +49 89 21763121 E-Mail :dimdiml@reg-ob.bayern.de
DE/CA61	オーバーバイエルン行政府 Regierung von Oberbayern		○			○	Heßstraße 39 80797 München Tel: +49 89 21760 Fax: +49 89 21762914 E-Mail : medizinprodukteanzeigeverfahren@reg-ob.bayern.de
DE/CA62	ニーダーバイエルン行政府 Regierung von Nieder-bayern		○			○	Regierungsplatz 540 84028 Landshut Tel: +49 871 80801 Fax: +49 871 80801002 E-Mail :poststelle@reg-nb.bayern.de

ザールラント州の例

コード	官庁	能動型医療機器	非能動型医療機器	測定機能付機器	能動型体外診断	非能動型体外診断	
DE/CA70	州環境・労働保護庁 Landesamt für Umwelt- und Arbeitsschutz	○	○	○	○	○	Don-Bosco-Straße 166119 Saarbrücken Tel:+49 681 85000 Fax:+49341 6973110

出典：www.dimdi.de/static/de/mpg/adress/behoerden/beh-liste.htm

医薬品医療機器連邦研究所（BfArM）

医薬品医療機器連邦研究所（BfArM: Bundesinstitut für Arzneimittel und Medizinprodukte）はドイツ連邦保険・健康省内に置かれた連邦上級官庁である。

医薬品医療機器連邦研究所ホームページ：www.bfarm.de

医薬品医療機器連邦研究所（BfArM）の所轄業務は、医療機器の使用の際に発生し得る危険を、中央において把握し評価することである。医療機器使用の際に甚大な危険が発生したり、製品に瑕疵が生じたりした場合、製造者、運用者、使用者はドイツ医療機器情報システム(DIMDI)を通じて医薬品医療機器連邦研究所に報告する義務がある。医薬品医療機器連邦研究所は、個々の医療機器に関する危険性評価を実施し、製品欠陥の改良措置を提案し、対応処置の調整を行う。

6. CE マーク以外の標識

CE マーク以外の安全性と品質を示すマークは、基本的に必要ではなく、その取得には経費が嵩むだけである。医療機器法第 9 条によると、その標識が CE マークと異なった機能を果たす場合のみ、追加的に他のマークを貼付しても良い。追加認証として価値があるのは、例えば製品の環境へのやさしさをアピールする標識である。

したがって、輸入業者でも、小売業者でも、医療機器を扱うドイツの販売業者は、CE マークのみを、また場合によっては法定健康保険の補助器具目録（7 .3.参照）への登録を、外国製品販売のための前提条件としている。

指定機関によっては、ＣＥマークに加えて、ＧＭ（検査済み医療機器 Geprüftes Medizinprodukt）マークを発行しているところもある。しかし、これはあくまでも任意で付加する標識である。

環境負荷の特に少ない製品・サービスに交付される標識であるブラウアーエンゲルのホームページを以下に記す。www.blauer-engel.de.

7. その他の規則

7.1. 製造物責任

医療機器法（MPG:Medizinproduktegesetz）と製造物責任法（PHG: Produkthaftungsgesetz）は、医療機器使用の際に患者に発生した損害の責任について定めている。製造業者または販売業者は使用者（医師）に対して責任を負い、同様に使用者は最終消費者（患者）に対する責任を負う。医療機器の瑕疵により、もしくは不適正な使用から患者に損害が生じた場合、損害補償額を調整するために、因果関係が立証されなければならない。製造物責任は、製品に自らの名称、商標を表示し、流通責任者（流通責任者については → 2.3.）として事業を行う輸入業者、小売業者等にも課せられる。

製造物責任法（ドイツ語）：http://www.ucl.ac.uk/laws/global_law/german-statutes/print_statute.shtml?product_1989

7.2.　製品の瑕疵の届出義務

医療機器安全性計画規定（MPSV:Medizinprodukte-Sicherheitsplanverordnung）は、医療機器の安全性にとって重要な観察事項やデータの届出義務を定めている。医療機器法第 5 条で定める製造者、ならびに販売業者や医療機器を扱う全ての職業集団は、医療機器の特徴や性能に変化が認められた場合、医薬品医療機器連邦研究所(BfArM)に届け出る義務を有する。これには、製造者、販売業者に加えて、医師、看護業務スタッフ、医療技術職、検査・監督・認証機関の責任者、医療機関の保守技術員が該当する。届出義務の対象となるのは、特に危険な副作用、安全上の欠陥、機能不良、品質上の瑕疵である。また、製品のマーキング上の瑕疵、取扱説明書の甚大な瑕疵も同様である。

医療機器法第 30 条により、医療機器の流通開始責任者は、流通責任者としての活動開始後直ちに、医療機器の保守受託人(Sicherheitsbeauftragte)を指名し、州所轄州官庁に届け出なければならない。医療機器保守受託人には以下の専門分野での経歴が必要である。

- 自然科学・医療・技術系の大学を修了
- もしくは、それに代わる職業教育修了
- 最低 2 年の職業経験

医療機器保守受託人は、危険性が判明した報告事例を集め、評価し、官庁に届け出て、必要な措置が講じられるよう調整を図る義務がある。

7.3.　法定健康保険の補助器具目録

法定健康保険（GKV: Gesetzliche Krankenkassenversicherungen）では、給付対象の補助器具を登録した目録を作成している。補助器具とは、治療用・介護用医療機器であり、患者処置後の患者の安全を図ること、または障害機能を補うことを目的としている。補助器具に属するものは以下のとおり。

- 身体補填器具
- 整形外科補助器具
- 視覚、聴覚補助器具
- 失禁・ストーマ用品
- 薬剤投与補助器具・吸入器具等の技術製品

法定健康保険は、わずかの例外を除けば、補助器具目録に登録された補助器具のみを費用負担する。補助器具とみなされる医療機器は、補助器具・介護補助器具目録への登録を行うことが薦められる。

製品の補助器具目録への登録は、健康保険同業組合連合会 (IKK: Bundesverband der Innungskrankenkassen)に申請する。

健康保険同業組合のホームページ（ドイツ語）：www.ikk.de

補助器具製造者向け解説ページ(ドイツ語)：http://www.ikk.de/ikk/generator/ikk/fuer-medizinberufe/hilfs--und-pflegehilfsmittel/3366,i=l.html

8. 医療機器輸入時の諸手続

8.1. 関税と税関手続

EU 以外の国で製造された医療機器は、連邦税関の通関手続を通してドイツおよび欧州共同体域内の自由流通市場へ導入される。

ＣＥマークが取得されていない場合は、税関事務局により、当該製品の欧州経済地域およびドイツ市場への導入は認められないことになっている。

通常の医療機器には基本的に関税はかからず、また貿易制限も存在しない。能動型ハイテク・侵襲型・埋め込み型機器（例：人工関節、心臓ペースメーカー、CT スキャナー、放射線療法機器）も、基本的に関税・貿易制限なしに輸入が可能である。

具体的な製品に関しては、連邦税関事務局の税関情報センターで輸入条件の正確な情報を照会することが望ましい。

税関情報センター (Zollinfocenter)

Hansaallee 141
60320 Frankfurt am Main
Tel.: 069-469976-00
Fax: 069-469976-99
E-Mail : info@zoll-infocenter.de

税関手続は以下のステップを経て行われる。

- EU 統一関税(TARIC, Tarif Intégré des Communatués Européennes)の商品コード番号(Codenummer für die Warenkategorie)を探し、 商品の輸入条件に関する情報と所轄税関を調べる。
- 輸入者に付される税関登録番号(Zollnummer)を申請する。
- 商品を税関に届け出る。

製品の輸入条件と商品コード番号は、申請前にオンラインの TARIC システム(Integrierter Tarif der Europäischen Gemeinschaft)で照会可能である。コード番号は、税関手続の際に必要である。

TARIC (英語)に関する情報は以下に記されている：

http://ec.europa.eu/taxation_customs/dds/en/tarhome.htm

TARIC に輸入売上税や付加価値税などのドイツ国内データを加えた電子関税 EZT (Elektronischer Zolltarif)システムがあり、その情報は以下に詳しい（ドイツ語）：

http://auskunft.ezt-online.de/ezto/Welcome.do

商品コード番号(Codenummer für die Warenkategorie)：

商品コード番号については、以下のサイトで詳細情報 (EZT または TARIC) が得られる（ドイツ語）：

http://www.zoll.de/b0_zoll_und_steuern/a0_zoelle/d1_zolltarif/b0_aufbau_ezt/index.html

大半の医療機器は、TARIC の 90 番台、XVIII に含まれる。この章には、光学機器、写真・映画用機器、計測機器、検査機器、精密機器、医用・外科用機器やその付属品がまとめられている。84 番台と 85 番台の XVI は機械装置や電気技術利用の機器と付属品を集めており、医療機器の一部はこの段にも含まれる。

当該医療機器に関して、TARIC の商品コードに該当するものがない場合は、製造者は所轄税関に輸入条件を照会することになる。

所轄窓口：

税関手続が行われる所轄税関窓口の所在地は、以下のサイトに掲載されている。

(ドイツ語)：http://zoll.de/service/dienststverz/index.html

ミュンヘン市所在の税関とフランクフルト・アム・マイン空港の税関を事例として示す。

医療機器の税関手続は、基本的にどの税関でも可能である。したがって、商品の受け取り手（輸入業者等）は、自らの事業所に近い税関を指定し、そこで手続を終了できる。フランクフルト空港の場合、空輸貨物を使う貨物税関は、貨物センター別に設けられている（すなわち利用する航空会社や運輸会社により窓口が異なる）。

表 6：税関窓口の事例、ミュンヘン、フランクフルト

ニュルンベルク上級財務局(Oberfinanzdirektion Nürnberg, OFD Nürnberg)管轄
所轄税関／所在地／キー番号
ミュンヘン中央税関 **Hauptzollamt München** Sophienstr. 6 80333 München Postfach 20 09 45 80009 München Tel. (0 89) 59 95-00 Fax (0 89) 59 95-24 88 E-Mail poststelle@hzam1.bfinv.de **7600**
ミュンヘン中央税関 －ランズベルガー街事務所 **Hauptzollamt München** **- Dienstsitz Landsberger Straße -** Landsberger Str. 124 80339 München Postfach 20 09 45 80009 München Tel. (0 89) 51 09-00 Fax (0 89) 51 09-20 15 E-Mail poststelle@hzam.bfinv.de

E-Mail mkg.muenchen@hzam.bfinv.de E-Mail fks_m@hzam.bfinv.de **7622**
ミュンヘン中央税関 －空港税関 **Hauptzollamt München** **- ZA Flughafen -** Frachtgebäude Modul B, 5. OG 85356 München Postfach 23 20 53 85329 München Tel. (0 89) 9 75-9 07 00 Fax (0 89) 9 75-9 07 06 E-Mail poststelle@hzam-fhf.bfinv.de **7650**
ミュンヘン中央税関 －ガルヒング・ホッホブリュック税関 **Hauptzollamt München** **- ZA Garching-Hochbrück -** Dieselstraße 9 85748 Garching-Hochbrück Postfach 15 58 85743 Garching Tel. (0 89) 48 09 07-0 Fax (0 89) 48 09 07-2 50 E-Mail poststelle@zam.bfinv.de **7602**
ミュンヘン中央税関 ガルヒング・ホッホブリュック税関 －ミュンヘン見本市通関手続事務所 **Hauptzollamt München** **ZA Garching-Hochbrück** **- AbfSt Messe München -** Paul-Henri-Spaak-Str. 8 81829 München Tel. (0 89) 9 49-2 45 91, -2 45 97 Fax (0 89) 9 49-2 45 99 **7632**

コブレンツ上級財務局（**Oberfinanzdirektion Koblenz, OFD Koblenz**）管轄
所轄税関／所在地／キー番号
フランクフルト・アム・マイン空港中央税関 **Hauptzollamt Frankfurt a.M.-Flughafen** Flughafen, Gebäude 201, Tor 13 60549 Frankfurt am Main Postfach 75 04 63 60534 Frankfurt am Main Tel. (0 69) 6 90-2 17 31, -7 19 19 Fax (0 69) 6 90-5 01 51 E-Mail poststelle@hzaf-fhf.bfinv.de **3300**
フランクフルト・アム・マイン空港中央税関 －空港監督税関 **Hauptzollamt Frankfurt a.M.-Flughafen** **- ZA Flughafenüberwachung -** 60549 Frankfurt am Main Flughafen, Gebäude 123 Postfach 75 04 63 60534 Frankfurt am Main Tel. (0 69) 6 90-5 12 81 Fax (0 69) 6 90-5 94 77

E-Mail leitstelle.vorfeld@hzaf-fhf.bfinv.de
3301

フランクフルト・アム・マイン空港中央税関
－貨物税関
Hauptzollamt Frankfurt a.M.-Flughafen
- ZA Fracht -
Flughafen, Gebäude 453
60549 Frankfurt am Main
Postfach 75 04 63
60534 Frankfurt am Main
Tel. (0 69) 6 90-7 19 19, -7 83 71
Fax (0 69) 6 90-2 58 01
E-Mail poststelle@hzaf-fhf.bfinv.de
3302

貨物税関
－貨物センター、カーゴ・シティー・ノルト所轄
ZA Fracht
- Arbeitsgebiet CCN (CargoCity Nord)
Flughafen, Gebäude 453
60549 Frankfurt am Main
Postfach 75 04 63
60534 Frankfurt am Main
Tel. (0 69) 6 90-7 83 70, -7 83 71
Fax (0 69)6 90-2 58 01

貨物税関
－ルフトハンザ貨物センター 所轄
ZA Fracht
-Arbeitsgebiet LCC (Lufthansa Cargo Center)-
Flughafen, Gebäude 451
60549 Frankfurt am Main
Postfach 75 04 63
60534 Frankfurt am Main
Tel. (0 69) 6 90-2 98 31, -5 32 51
Fax (0 69)6 90-5 62 61

貨物税関
貨物センター、カーゴ・シティー・ズート所轄
ZA Fracht
-Arbeitsgebiet CCS (Cargo City Süd)-
Flughafen, Gebäude 537
60549 Frankfurt am Main
Postfach 75 04 63
60534 Frankfurt am Main
Tel. (0 69) 6 90-7 27 01, -7 25 50
Fax (0 69)6 90-5 98 14

貨物税関
－国際郵便センター所轄
ZA Fracht
-Arbeitsgebiet IPZ (Internationales Postzentrum) -
Flughafen, Gebäude 190
60549 Frankfurt am Main
Postfach 75 04 63
60534 Frankfurt am Main
Tel. (0 69) 6 90-7 10 52, -7 15 19

フランクフルト・アム・マイン空港中央税関
－税関　旅行

Hauptzollamt Frankfurt a.M.-Flughafen
- ZA Reise -
60549 Frankfurt am Main
Flughafen, Terminal
Postfach 75 04 63
60534 Frankfurt am Main
Tel. (0 69) 6 90-7 25 93, -2 21 24
Fax (0 69) 6 90-5 90 83, -5 90 68
E-Mail poststelle@hzaf-fhf.bfinv.de
3303

出典：http://zoll.de/service/dienststverz/index.html

税関登録番号（Zollnummer）：

輸出入業者はドイツで税関手続を行うにあたって、税関登録番号を必要とする。税関登録番号は 7 桁の番号で、連邦関税局から無償交付される。税関登録番号は、税関手続申請に際して必要である。国外在住者が申請を行う際にも、税関登録番号を要する。申請書は下記の対応窓口ATLASに送る。申請用紙は以下のリンクからダウンロードできる。
www.zoll.de/e0_downloads/b0_vordrucke/a0_vordruckgesamtliste/0870en_participant_master_data.pdf

対応窓口
Koordinierende Stelle ATLAS
Hertzstraße 10
76187 Karlsruhe
Postfach 10 02 65
Tel.: 0721/7909-0
Fax: 0721/7909-319

税関登録番号の照会：
Tel.: 0721/7909-200
Fax: 0721/7909-110
E-Mail: poststelle@kostatlas.bfinv.de

税関登録番号交付情報： www.zoll.de/english_version/c0_registration_numbers/index.html

税関手続：

商品の税関手続は、所轄税関に税関手続申請を提出して行う。この申請には、必要な情報と書類を全て添付する。

申請者は、通常、欧州域内在住でなければならす、商品の購入者もしくは輸入業者であることが普通である。

必要なデータは以下のものである。

- 商品の特定（商品名称、商品コード番号、数量）
- 商品の原産地
- 商品の価格
- 場合によっては取引契約

必要書類としては、商業送り状、運送書類などが上げられる。

必要書類に関する詳細情報（ドイツ語）：
http://www.zoll.de/b0_zoll_und_steuern/a0_zoelle/c0_zollanmeldung/c0_schriftliche_anmeldung/c0_unterlagen/index.html

申請は、書面もしくは ATLAS システムのオンライン税関申請により行う。

税関当局は、通常の場合、欧州共同体内に在住し、当該商品の輸入に関する現地での応対者となり、公課債務の生じたときに対処できる者を置くことを求めてくる。申請者が時間的もしくは地理的な理由で税関手続を自ら進められないときは、税関手続申請提出の代理人を立てることが出来る。代理人は、たいていの場合は運送会社である。.

申請者に関する情報（ドイツ語）：

www.zoll.de/b0_zoll_und_steuern/a0_zoelle/c0_zollanmeldung/a0_anmelder/index.html

書面による申請：

所定の統一書式を用いて行う書面による税関手続申請は、ドイツではもっともよく用いられる申請方式である。申請用紙は、EU 共通のコードに準じて記入するよう作成された EU 統一書式である。所定の用紙は専門店で求めるか、商工会議所(Industrie und Handelskammer)に請求する。EU 経済地域内での流通が自由な商品の輸入については、所定書式の中の６、７、８項の記入で足りる。

商工会議所(Industrie und Handelskammer)のリスト：http://www.dihk.de/inhalt/ihk/adressen.pdf

税関手続書式への記入に関する指針(ドイツ語)（連邦財務省）：

http://zoll.de/e0_downloads/b0_vordrucke/a0_vordruckgesamtliste/0781_merkblatt_2007.pdf

税関手続書面申請に関する情報全般（ドイツ語）：

http://www.zoll.de/b0_zoll_und_steuern/a0_zoelle/c0_zollanmeldung/c0_schriftliche_anmeldung/index.html

税関手続所定書式への一部項目のみ記入の場合の解説（ドイツ語）：

http://www.zoll.de/b0_zoll_und_steuern/a0_zoelle/c0_zollanmeldung/c0_schriftliche_anmeldung/a1_teilsaetze/index.html

ATLASシステムによるオンライン申請：

オンライン申請は統一書式申請に代わる手法である。下記のリンクより書式画面にログインできる（ドイツ語）：

https://www.einfuhr.internetzollanmeldung.de/portal/page/portal/myIZA/IZA

この書式も統一書式であり、必要データ記入後は、所轄の税関に送られる。ただし、申請者は送信した書式をプリントアウトし、申請に必要な書類の提出に税関に出向く必要がある。

オンライン申請の詳細解説(ドイツ語)：:

http://www.zoll.de/b0_zoll_und_steuern/a0_zoelle/c0_zollanmeldung/d10_atlas/e01_iza/index.html

完全な電子税関手続を望む場合は、専用ソフトウエア使用による ATLAS の IT 手続を申請しなければならない。IT による手続は、税関申請を頻繁に行う輸入業者等が利用する。

IT税関手続に必要なソフトウエア及び情報（ドイツ語）：

http://www.zoll.de/b0_zoll_und_steuern/a0_zoelle/c0_zollanmeldung/d10_atlas/d0_teilnvoraus/index.html

http://www.zoll.de/b0_zoll_und_steuern/a0_zoelle/c0_zollanmeldung/d10_atlas/index.html

製品の安全性検査：

税関当局は、製品が安全性の点で問題がないかどうかについて、無作為抽出の検査を行う。製品の利用の際に危険性がないか、これは安全性が要求される製品に CE マークなど現行の標識が添付されているかどうかの検査である。現行の安全基準を満たさない疑いがある場合は、税関は税関手続を中止し、所轄の監督官庁に通告する。

製品安全性規定と税関の任務に関する情報：

http://www.zoll.de/b0_zoll_und_steuern/d0_verbote_und_beschraenkungen/c0_schutz_menschl_gesundh/h0_produktsicherheit/index.html

8.2. 輸入取引高税（輸入付加価値税）

ドイツでは医療機器の売買に売上高税（付加価値税とも呼ばれる）が、また輸入時には付加価値税の一種である輸入取引高税が課される。

ドイツの付加価値税率は 2007 年 1 月以降 19%であるが、食品、書籍、雑誌等の特定の商品には軽減税率 7%が適用されている。医療機器部門では以下の製品に 7%の軽減税率が適用される。

- エンジン付き・なしを問わず、障害者の移動手段である車椅子とその他の乗り物や装置
- 身体補填器具と整形外科用装置
  - 人工関節（部品と付属品は除く）
  - 整形外科装置と松葉杖、医療外科用ベルト、サポーターを含むその他の整形外科器具（部品と付属品は除く）
  - 補装具（部品と付属品は除く）
  - 難聴用補聴器、心臓ペースメーカー、機能障害・欠陥の補助装置、身体取付けもしくは生体埋め込み型装置（部品と付属品は除く）

上記の製品グループの部品と付属品で軽減税率が適用されないものについては、通常の税率 19%が適用される。売上高税(付加価値税)の徴収は所轄税務署が行う。

ヨーロッパ外の国からドイツに商品を輸入する場合にも、輸入取引高税の形で付加価値税が徴収される。輸入品にも売上高税(付加価値税)と同様の 19%と 7%が適用される。

輸入取引高税は、所轄税務署ではなく連邦税関事務局が徴収する。商品輸入が営業目的であれば、輸入企業は商品売却の際に、一度支払った輸入取引高税（付加価値税）をいわゆる前段階税として税から控除できる。

欧州域内市場内の商品流通については、輸入取引高税（付加価値税）は徴収されない。

輸入取引高税（付加価値税）に関するサイト(ドイツ語)：

http://www.zoll.de/b0_zoll_und_steuern/a3_einfuhrumsatzsteuer/index.html

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8. 税関に関するデータはwww.zoll.de　および税関情報センターへの聞き取りに基づく。

文献 a

Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma

Ulrike F. Koenigbauer, Ted Eastlund, and John W. Day

BACKGROUND: Lipid-enveloped viruses such as HIV, HBV, and HCV can be inactivated by treatment with solvents and detergents. HAV and human parvovirus B19 lack lipid envelopes and are not inactivated. Solvent/detergent-treated pooled plasma (S/D plasma) contains neutralizing antibodies, but it is not known whether the parvovirus B19 antibody content is sufficient to prevent transmission of the disease. A patient is described who developed a clinical illness due to parvovirus B19 infection after the infusion of S/D plasma.

CASE REPORT: A 36-year-old woman with myasthenia gravis underwent five plasma exchange procedures from January 15 to January 25, 1999, using albumin, except for 5 units of SD plasma given because of a low fibrinogen level. Four of the 5 units were implicated in a recall after high levels of parvovirus B19 DNA were found in several lots. Two weeks after the infusion, the patient developed fatigue, a rash, and severe polyarthralgias. Parvovirus B19 IgG and IgM antibody titers were consistent with an acute infection.

CONCLUSION: Clinically apparent parvovirus B19 infection can follow the use of S/D plasma that contains high levels of parvovirus B19 DNA.

ABBREVIATIONS: IMIG = IM immune globulin; INR = international normalized ratio; IVIG = IV immune globulin; S:CO = signal-to-cutoff; S/D plasma = solvent/detergent-treated pooled plasma.

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Human parvovirus B19, a small nonlipid-enveloped DNA virus, is the causative agent of fifth disease (erythema infectiosum), a mild and usually self-limited childhood disease in which most infections occur between ages 5 and 15.[1] Approximately 40 to 60 percent of adults over 20 years old have protective antibodies.[2] Acute infections in adults usually have a benign course. If symptoms occur, symmetric polyarthralgias are prominent, especially in women, but rash, fever, malaise, and gastrointestinal symptoms can also occur.[3,4] Parvovirus B19 infects erythroid progenitor cells, and, in patients with compensated chronic hemolytic anemias, such as sickle cell disease, thalassemia, or spherocytosis, that can lead to a suppression of erythropoiesis with a sudden severe worsening of anemia.[5] A pregnant woman can transmit the infection to her fetus, causing anemia and heart failure, which can lead to hydrops fetalis and intrauterine death.[6,7] Chronic infection with persistent anemia can occur in patients with immune defects and was reported in patients with HIV infection, congenital immunodeficiency, and malignancies and in organ transplant recipients.[5] However, chronic infection has been observed in a presumably healthy person,[8] and reinfections seem to be possible.[9] Rare manifestations ascribed or linked to B19 infection include meningitis, encephalitis, hepatitis, myocarditis,[5] neuropathies,[10] fibromyalgia,[11] polyarteritis nodosa,[12] and vascular and Schönlein-Henoch purpura.[5]

The main route of parvovirus B19 transmission is thought to be through respiratory droplets. After intranasal inoculation of parvovirus B19 into healthy subjects, viremia was first detected by 6 days, persisted for up to 7 days, and was accompanied by mild symptoms and development of specific antibodies. Rash and arthralgia started to develop by Day 17 or 18 after inoculation.[9]

Parvovirus B19 can be transmitted from asymptomatic blood donors to the recipients of their blood components.[5,13] High rates of seroconversion,[14-19] as well as a few cases of symptomatic illness[20,21] and hypoplastic anemia,[22,23] have been described in patients receiving clotting factor concentrates, which were derived from large plasma pools.[5] The virus is relatively heat-stable, and it resists treatment with solvents and detergents.[13,15,18] B19 DNA was

found by PCR in plasma-derived clotting factor concentrates from various manufacturers and treated with different virus-inactivation methods by many investigators.[24-29] Clinically evident transfusion-transmitted B19 infection, however, is infrequent, even in susceptible hosts such as HIV-infected hemophilia patients receiving clotting factor concentrates.[5] There have been at least two reports of transfusion-transmitted parvovirus infection from single-donor components, namely RBCs[30] and platelets.[31]

We describe a case of symptomatic parvovirus B19 infection after the infusion of solvent/detergent-treated pooled plasma (S/D plasma), which was subsequently implicated in a voluntary recall after high levels of infectious parvovirus B19 DNA were detected in the corresponding plasma lots.

CASE REPORT

The patient is a 36-year-old woman with a 10-month history of myasthenia gravis that was poorly responsive to mestinon, prednisone, and azathioprine, as well as to IV immune globulin (IVIG), which had been administered on December 10 and 11, 1998. She also had a history of Hashimoto's thyroiditis and systemic lupus erythematosus with only mild arthralgias in the past that were readily treated with ibuprofen. She did not have any dermatologic manifestations. As treatment for her myasthenia gravis, she underwent five plasma exchange procedures from January 15 to January 25 using 2300 to 2900 mL of 5-percent albumin (Buminate 5%, Baxter Healthcare Corp., Glendale, CA; Albumarc 5%, Baxter; and Albutein 5%, Alpha Therapeutic Corp., Los Angeles, CA) as exchange fluid, which resulted in mild improvement of her proximal muscle weakness. The medications the patient received during the period of the plasma exchange procedures were azathioprine, mestinon, thyroxine, and estrogen. Her fibrinogen level was 98 mg per dL before the fourth procedure, and, even though she had no bleeding, she was given a total of 5 units (1000 mL total volume) of S/D plasma (PLAS+SD, V.I. Technologies, Melville, NY) at the end of her fourth and fifth treatment. Preprocedure international normalized ratio (INR) and partial thromboplastin time were in the normal range, and platelet counts were 159 and 166 $\times 10^9$ per L. Fibrinogen was 145 mg per dL on January 25, 1999. Her Hb level ranged from 10.7 to 11.5 g per dL and her WBC count from 5.1 to 6.7 $\times 10^9$ per L. One unit of S/D plasma infused on January 22, 1999, and all 3 units infused on January 25, 1999, were implicated in a subsequent recall of S/D plasma lots conducted by the American Red Cross after high levels of infectious parvovirus B19 DNA had been detected by the manufacturer in several lots.

We subsequently interviewed the patient, who reported that, during the second week of February, approximately 2 to 3 weeks after receiving S/D plasma, she had developed an illness, that began with mild rhinorrhea and fatigue. This was followed by severe generalized symmetric polyarthralgias that were poorly relieved by ibuprofen and a lacy erythematous rash on her extremities, which lasted about 7 days. She had no fever, sore throat, or gastrointestinal symptoms. When her symptoms started to resolve, she resumed work and was tested for parvovirus B19 antibodies on February 19, 1999. Her test results were positive for IgM and IgG. The signal-to-cutoff (S:CO) ratio for IgM was 1.68 and that for IgG was 1.49. A S/CO of $\geq$1.20 is interpreted as positive. The antibody test used was an EIA (Microbiology Reference Laboratory, Cypress, CA) with confirmation testing of IgM antibodies by an indirect immunofluorescence assay (Microbiology Reference Laboratory). On a follow-up test on May 27, 1999, performed in the same laboratory with the same test system, the IgM was no longer positive (IgM, 0.89 S/CO) and IgG rose to 5.42 S/CO, which is consistent with a recent infection. She recalled no history of contact with others infected with or recently exposed to parvovirus B19 infection. Her illness resolved without further treatment. Two weeks after her illness, her blood counts were similar to those performed previously: platelet count 206 $\times 10^9$ per L, WBC count 5.3 $\times 10^9$ per L, and Hb 10.9 g per dL.

DISCUSSION

Treatment of plasma with the solvent tri(n-butyl)phosphate and the detergent Triton-X 100 inactivates lipid-enveloped viruses such as HIV, HBV, and HCV.[32] Viruses such as HAV or parvovirus B19 that lack the lipid envelope are not inactivated. Because S/D plasma is prepared from the blood of donors who are representative of the general community, about half of the units of donated plasma contain antibodies to parvovirus. Neutralizing antibodies are therefore contained in S/D plasma, which is derived from pooled plasma from up to 2500 volunteer donations. These antibodies could potentially prevent infection in the recipient. However, the amount of antibody needed to prevent transmission by a blood component containing parvovirus B19 has not been established.[33]

IVIG is recommended as therapy in chronic parvovirus B19 infection,[34,35] as it has effectively eliminated viremia and symptoms.[31] The amount of antibody required to protect against or cure parvovirus B19 infection with IVIG is not known, and one possible parvovirus transmission from IVIG has been reported.[36] Using PCR, B19 DNA has been detected in 3 of 4 lots of IM immune globulin (IMIG) and 3 of 15 lots of IVIG by one group,[28] whereas McOmish et al.[27] did not find B19 DNA in 10 lots of IVIG preparations. Our patient had received two infusions of IVIG to treat myasthenia gravis approximately 60 days before the onset of her symptoms. Because rash and arthralgias occur 2 to 3 weeks after infection by the virus,[9] IVIG is unlikely to be the cause

of her parvovirus infection. Passive acquisition of the antibody from either IVIG or S/D plasma would not explain her seroconversion pattern of a declining IgM parvovirus antibody and a rising IgG level.

Our patient received albumin during the course of her treatment, and this should be considered as a potential source of the infection. Saldanha et al.[28] found parvovirus B19 DNA in 1 to 3 of 12 lots of albumin, which contained the lowest levels of B19 DNA of various tested blood components ($<10^2$-10^3 genome equivalents/mL). Higher levels were found in IVIG, IMIG, and clotting factor concentrates that had levels of 10^4 to 10^6 genome equivalents per mL. In contrast, Lefrère et al.[37] had negative results in all 29 albumin batches from two manufacturers. It is interesting that B19 DNA has also been found in 5 of 30 lots of recombinant factor VIII concentrates that were not derived from human plasma.[38] This finding was attributed to the albumin used as stabilizer.

The presence of B19 DNA in plasma derivatives, especially if in low concentrations, does not mean that these products can transmit infection, as the viability of the virus may have been destroyed during processing.[24,26-29] This may explain the results of PCR testing, which did not correlate with infectivity assays in factor VIII concentrates spiked with canine parvovirus before terminal dry-heat treatment.[39] B19 DNA is infrequently found in albumin, and, when it is detected, the B19 DNA content is low. In addition, albumin is heat-treated for 10 hours at 60°C, which results in a reduction of at least 10^4 genome equivalents.[28] Conversely, the parvovirus B19 content of some of the S/D plasma lots used in our case was reported by the manufacturer to be greater than 10^7 genome equivalents per mL, which was the level that was found by the manufacturer to have caused B19 viremia and seroconversion in healthy subjects. Thus, we do not consider albumin to be the source of parvovirus infection in our patient.

Our patient had been diagnosed with systemic lupus erythematosus 2 years previously, on the basis of antinuclear antibodies and mild arthralgias that were readily treated with ibuprofen. Before her recent parvovirus infection, she had never had severe joint pain, lupus skin eruptions, or any other severe manifestation. The rash she developed during her acute illness after S/D plasma treatment was not suggestive of lupus, and her severe symmetric polyarthralgias were consistent with acute parvoviral infection.

Plasma was infused to our patient at the end of her fourth plasma exchange, because of a fibrinogen level below 100 mg per dL found before the plasma exchange. The patient was not bleeding and the hypofibrinogenemia was due to the use of albumin as a replacement fluid. S/D plasma was also given at the end of her fifth exchange, despite the absence of bleeding and a fibrinogen level above 100 mg per dL. This was inappropriate, and we have lowered our threshold to 50 mg per dL in nonbleeding patients.

Viremia in asymptomatic blood donors is of concern, especially in connection with S/D plasma derived from large pools. Titers of 5×10^{10} genome equivalents per mL have been found in some donors.[27] The prevalence of viremia in healthy blood donors has been studied by several groups with variable results that depend on the sensitivity of the assay; the rates are generally higher during epidemics.[25] In a study of 20,000 donors, the prevalence of B19 DNA detected by PCR was 1 per 3,300,[27] and, during an epidemic in Japan, it was as high as 1 per 167.[40] A more recent study in which US voluntary blood donors were screened for B19 DNA indicated a prevalence of 0.1 percent (11/9,568).[41] Therefore, most plasma pools from which S/D plasma is manufactured will contain parvovirus B19 DNA. Subsequent to the recall and in conjunction with the FDA, the S/D plasma manufacturer has instituted steps whereby all lots are now screened by PCR for parvovirus B19 DNA. Lots containing viral loads that have been associated with seroconversion in healthy volunteers are no longer distributed. S/D plasma released for transfusion may contain parvovirus B19 DNA and specific antibodies, but the B19 DNA antibody titers, if present, are at low levels that did not lead to seroconversion in experimental subjects. The S/D plasma is therefore considered noninfectious.

We conclude that our patient's clinical illness, which was consistent with parvovirus infection and accompanied by seroconversion, was acquired via infusion of S/D plasma containing high levels of parvovirus B19.

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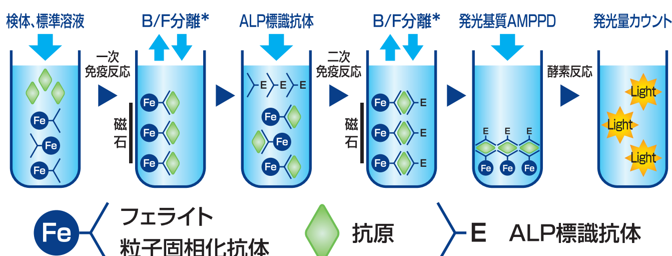
血液製剤の安全性の向上について

日本赤十字社では血液製剤の安全対策として、①献血受付時の問診、②血清学的検査(HBs抗原、HBs抗体、HBc抗体、HCV抗体、HIV-1,2抗体、HTLV-1抗体、梅毒トレポネーマ抗体、ヒトパルボウイルスB19)、③核酸増幅検査(NAT:HBV、HCV、HIV)、④新鮮凍結血漿及び血漿分画製剤の原料血漿の6ヵ月間の貯留保管等を実施しています。
2008年8月、血清学的検査を凝集法から化学発光酵素免疫法(CLEIA法)へ変更し、また、NATについても新NATシステムへ切り替え、当該システムによる検査を開始しました。

●血清学的検査(CLEIA法)

■測定原理

固相化抗体とALP標識抗体で検体中の抗原を挟み抗原抗体複合体を形成させる。ALP標識抗体と基質との反応で発光させ、発光量をカウントする。



フェライト粒子固相化抗体　抗原　ALP標識抗体

*B/F分離:抗原と抗体が結合しているものと結合していないものを分離する。

■検査機器システム

感染症検査の判定の効率化、均一化を目的として従来の凝集法に替わり化学発光酵素免疫法(CLEIA法)を2008年1月より九州血液センターから順次導入し、同年8月までに全国10ヵ所すべての検査実施施設への導入が完了しました。

●核酸増幅検査(NAT)

■検出感度

ウイルスの種類	平均検出感度
HBV	3.2 IU/mL
HCV	12.4 IU/mL
HIV-1 Group M	41.8 IU/mL
HIV-1 Group O	93.7 copies/mL
HIV-2	2.0 copies/mL

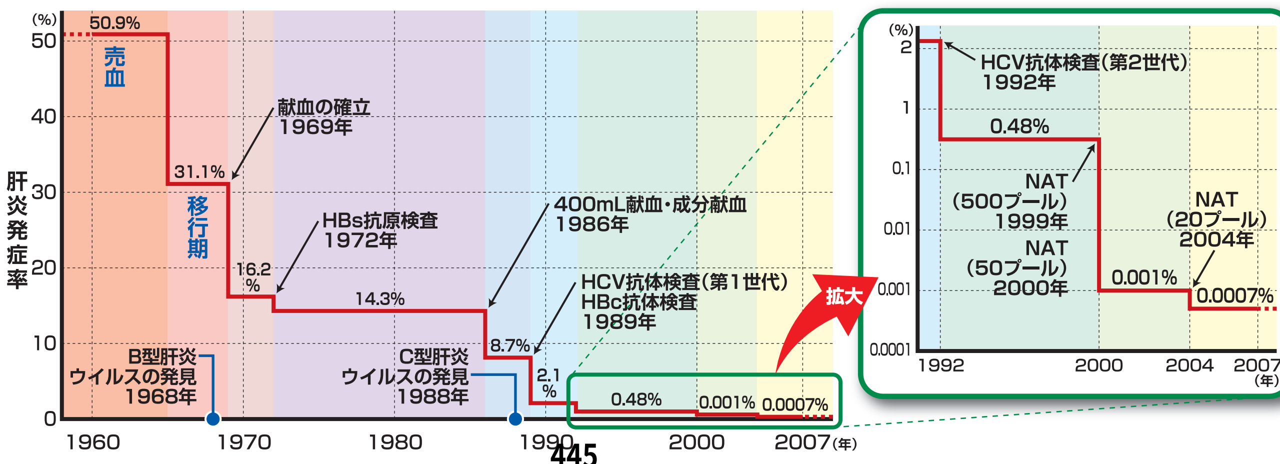
ロシュ社資料

■検査機器システム

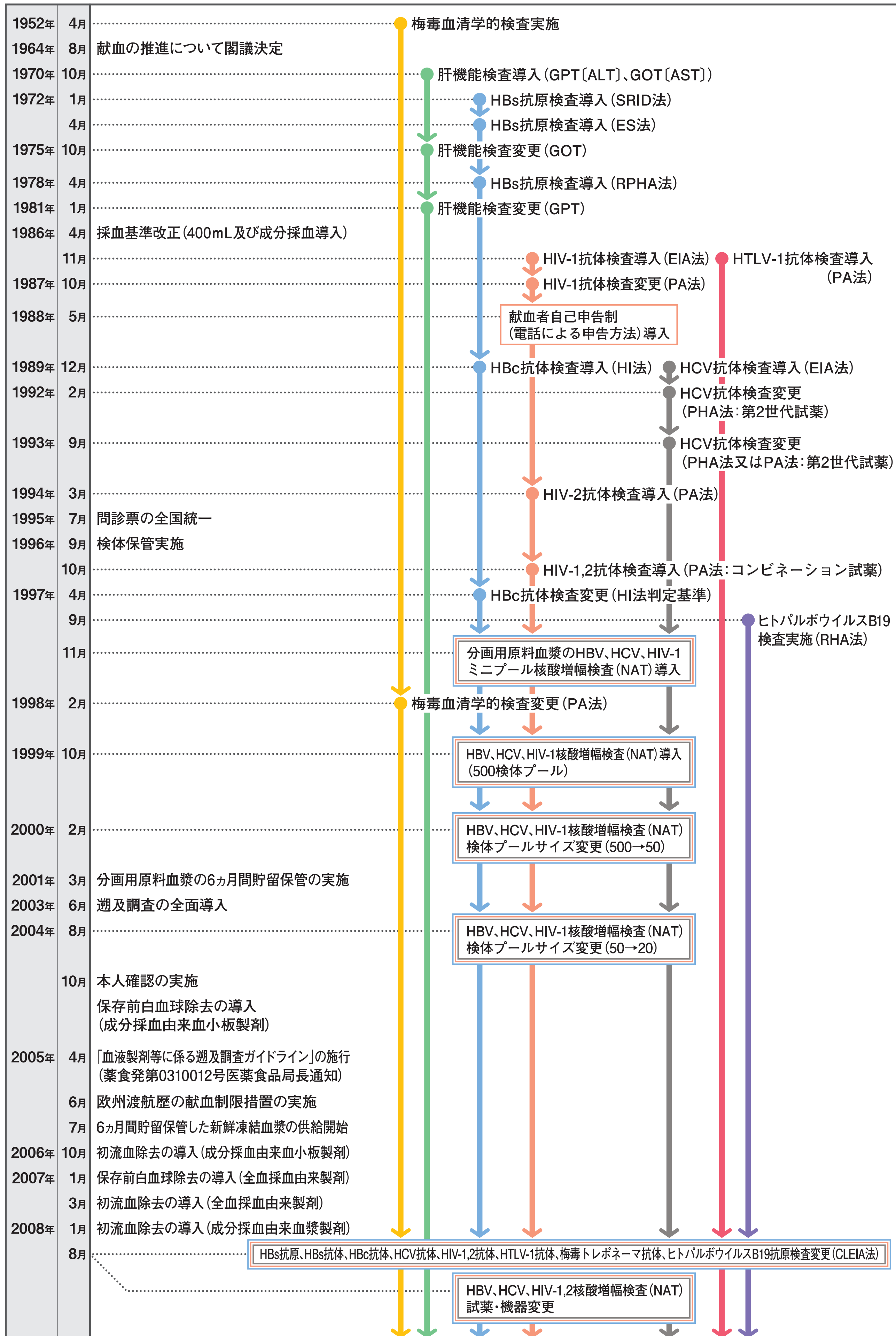
新NATシステムは、核酸抽出から増幅、検出まで1台で行う全自動タイプで、従来システムより感度が向上しました。また、HIV-1グループO及びHIV-2の検出も可能となりました。

➡これらの変更により 血液製剤の安全対策をこれまで以上に充実します。

●日本における輸血後肝炎の推移



●血液製剤の安全性確保対策の変遷



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0811

Mirasol Clinical Experience: Results from the MIRACLE Trial

Raymond P. Goodrich, Ph.D.
Chief Science Officer
Navigant Biotechnologies, LLC

Study Goals and Design

- Determine if the Mirasol PRT System for Platelets
  - Performs safely, and
  - Maintains adequate platelet performance in a clinical setting.

- Multicenter, Prospective, Randomized, Open Label, Blinded Endpoint
- Each investigational site:
  - Blood Establishment – Technical processing of platelet product, randomization of subjects
  - Clinical Site – Selection, transfusion and patient follow-up
- Subjects randomized to receive Mirasol vs. reference platelets
- Transfusion indications per attending MD
- On study for 28 days, or until no more platelet transfusion needed
- Followed additional 28 days for adverse events

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Endpoints

- 1- hour and 24-hour CCI for first 8 transfusions
- Days between platelet transfusions
- Number of platelet transfusions
- Number of transfusions per subject
- Number of platelets per day of support
- Length of time of transfusion support
- For subjects with > 8 transfusions: longitudinal regression analysis on 1-hour and 24-hour CCIs
- Number of subjects refractory to platelets
- Neoantigen analysis in refractory subjects
- Number of red cell transfusions
- Serious adverse events & bleeding (WHO scale)
- Infections

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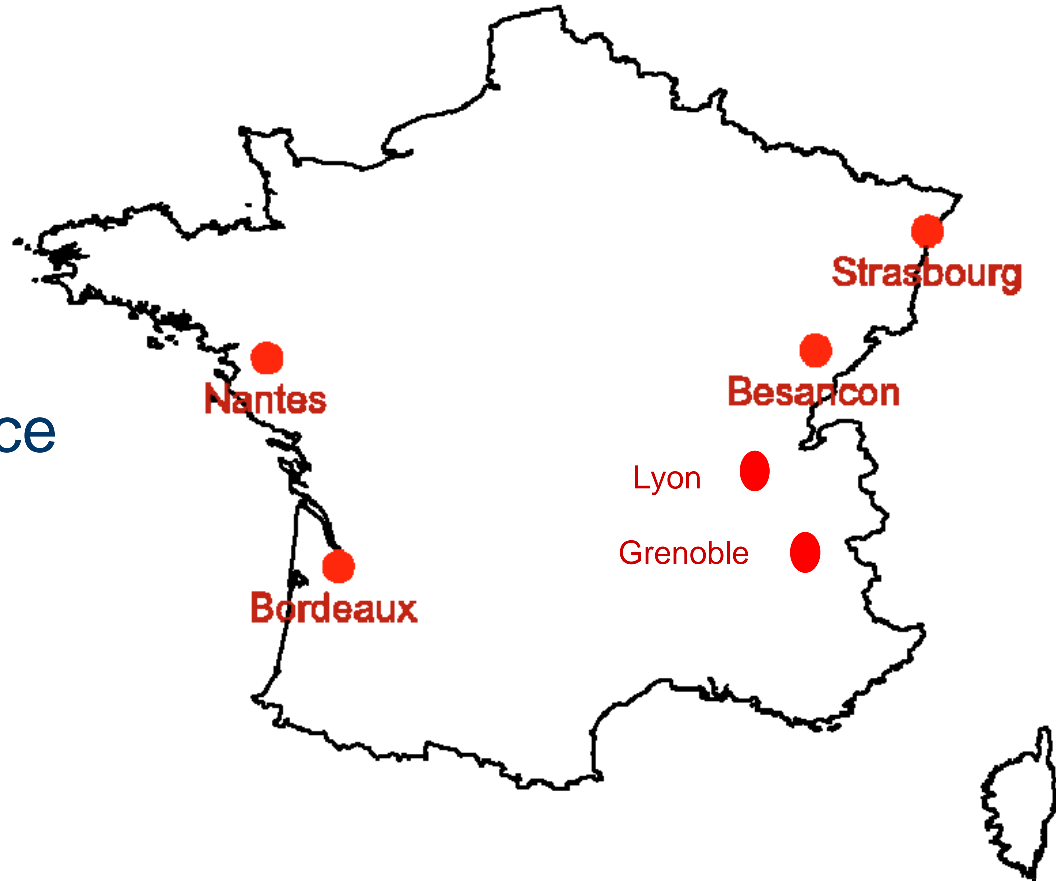
Platelet Products

- Mirasol treatment done at each site
  - Trima collections – 5 sites
  - Buffy coat platelets – 1 site
  - Gamma irradiated at 2 of the 6 sites
- Mirasol and Reference platelets stored in 100% plasma
  - No additive solutions
  - Initial volume = 170 – 360 mL
  - Initial Concentration = 1180 – 2100 X 10^3 / µL plasma
  - Final dose = 3.0 – 5.1 x 10^{11} platelets per product
- Stored for up to 5 days

Study Site Locations

6 sites in France



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Overview of Safety Approach

- Protocol required 3 levels of independent external review
  - Medical Monitor
  - Data Safety Monitoring Board
  - Data Monitoring Committee
- Reviews completed at defined intervals
- All reviews done independently to assure no introduction of bias between groups

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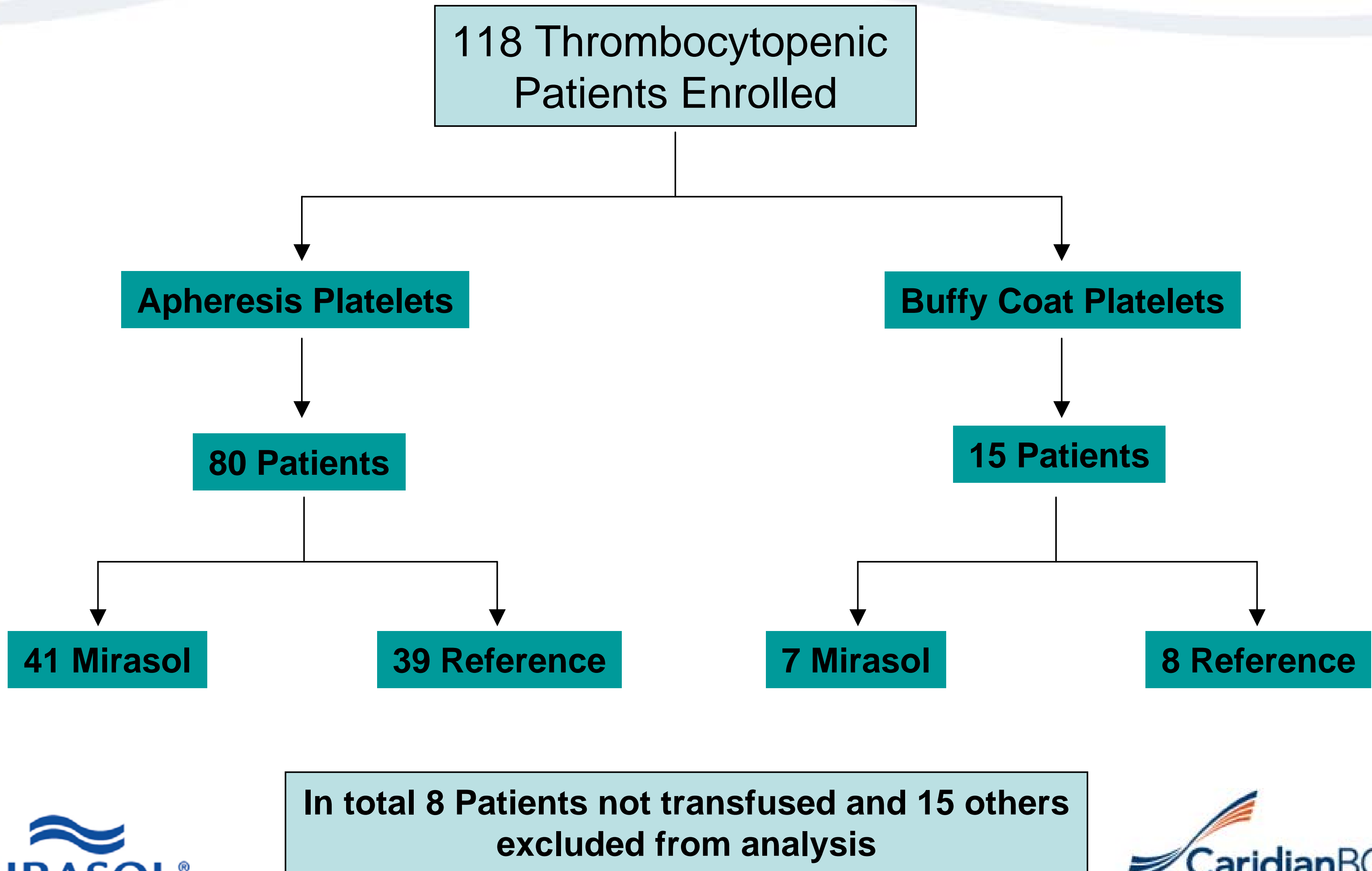
Subject Reconciliation
118 Thrombocytopenic Patients Enrolled
Apheresis Platelets
Buffy Coat Platelets
80 Patients
15 Patients
41 Mirasol
39 Reference
7 Mirasol
8 Reference
In total 8 Patients not transfused and 15 others excluded from analysis
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Platelet Product Characteristics

	Mirasol (N=47)	Reference (N=48)	P-value
Age of Platelets in Days (Range)	2.7 (1-5 Days)	2.7 (1-5 Days)	0.832 (NS)
Number of Gamma Irradiated products transfused	31 (11%)	182 **(74%)**	
Number of Non-Gamma Irradiated products transfused	262 **(89%)**	65 (26%)	

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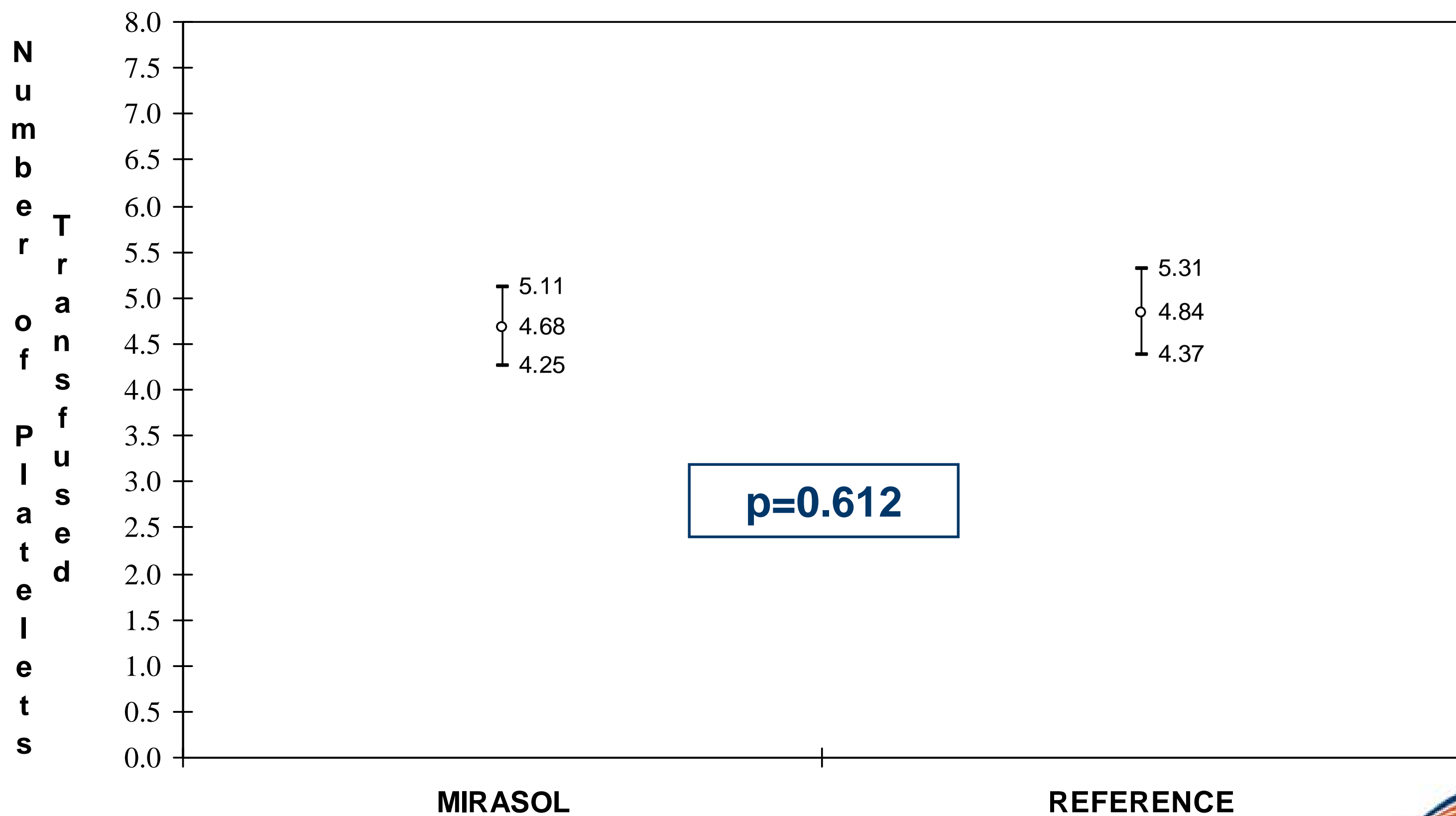
Platelet Dose in Products



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Platelet Usage: Platelet Dose

	Mirasol (N=47)	Reference (N=48)	P-value
Mean cumulative platelet dose transfused per patient in total (x 10^{11})	24.3	22.0	0.51 (NS)
Mean number of platelet units transfused divided by body surface area	2.6	2.7	0.50 (NS)
Mean number of platelet units transfused per day of platelet support	4.7	4.9	0.58 (NS)
Mean number of platelet units transfused over the 28 day treatment period	2.4	2.4	0.80 (NS)

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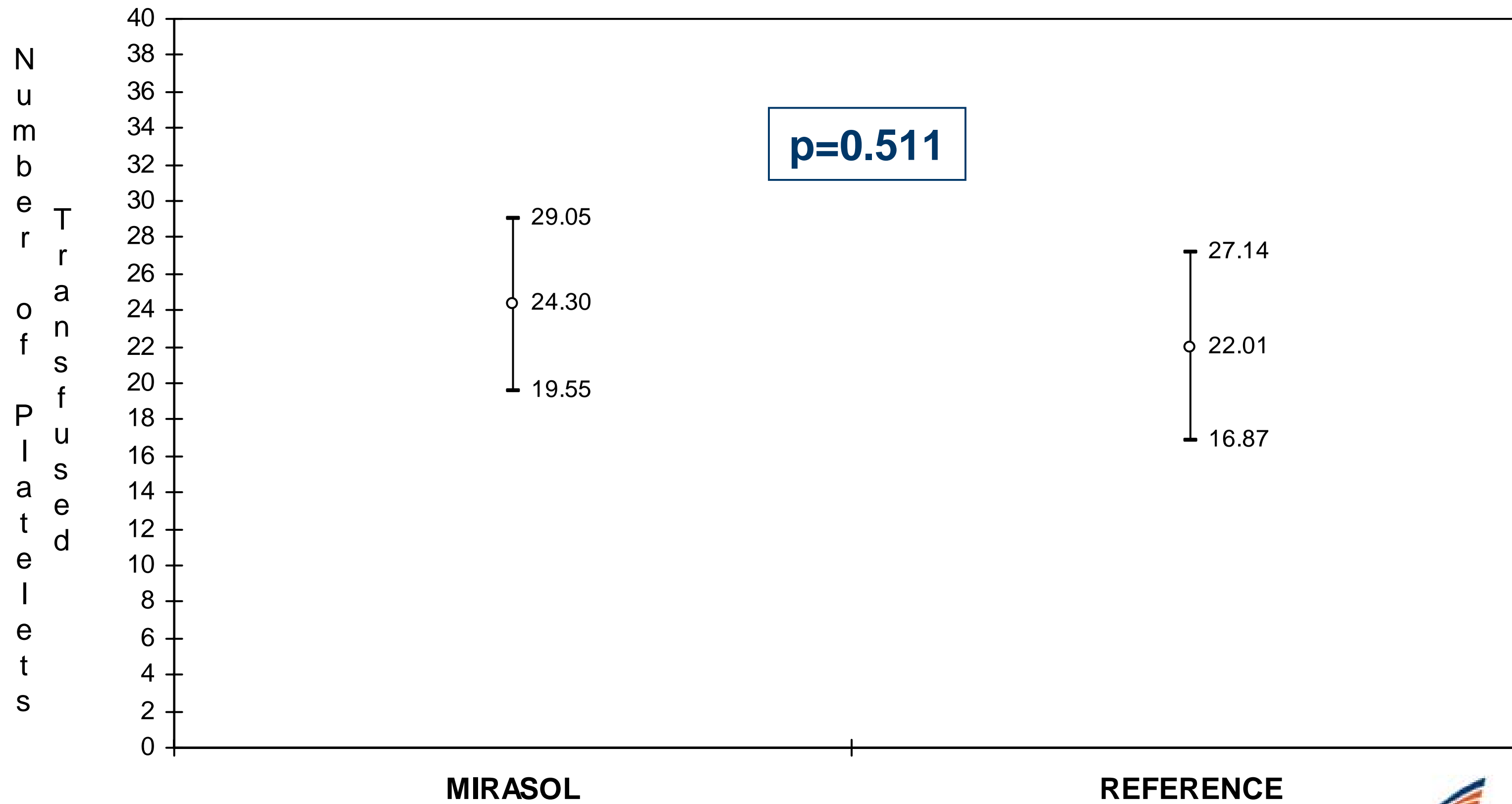
Cumulative Platelet Dose Transfused



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Platelet Usage – Transfusions

	Mirasol (N=47)	Reference (N=48)	P-value
Mean number of transfusions per patient	5.3	4.6	0.12 (NS)
Mean number of transfusions per day of platelet transfusion support	0.5	0.5	0.59 (NS)
Mean number days between transfusions:			
– Transfusions 1-8	2.4	3.3	<0.01
– Transfusions >8	2.2	1.2	0.10 (NS)
Cumulative number of days from transfusion 1 to 8	15.6	16.2	0.79 (NS)
Number of “off-protocol” platelet transfusions	60	72	<0.01

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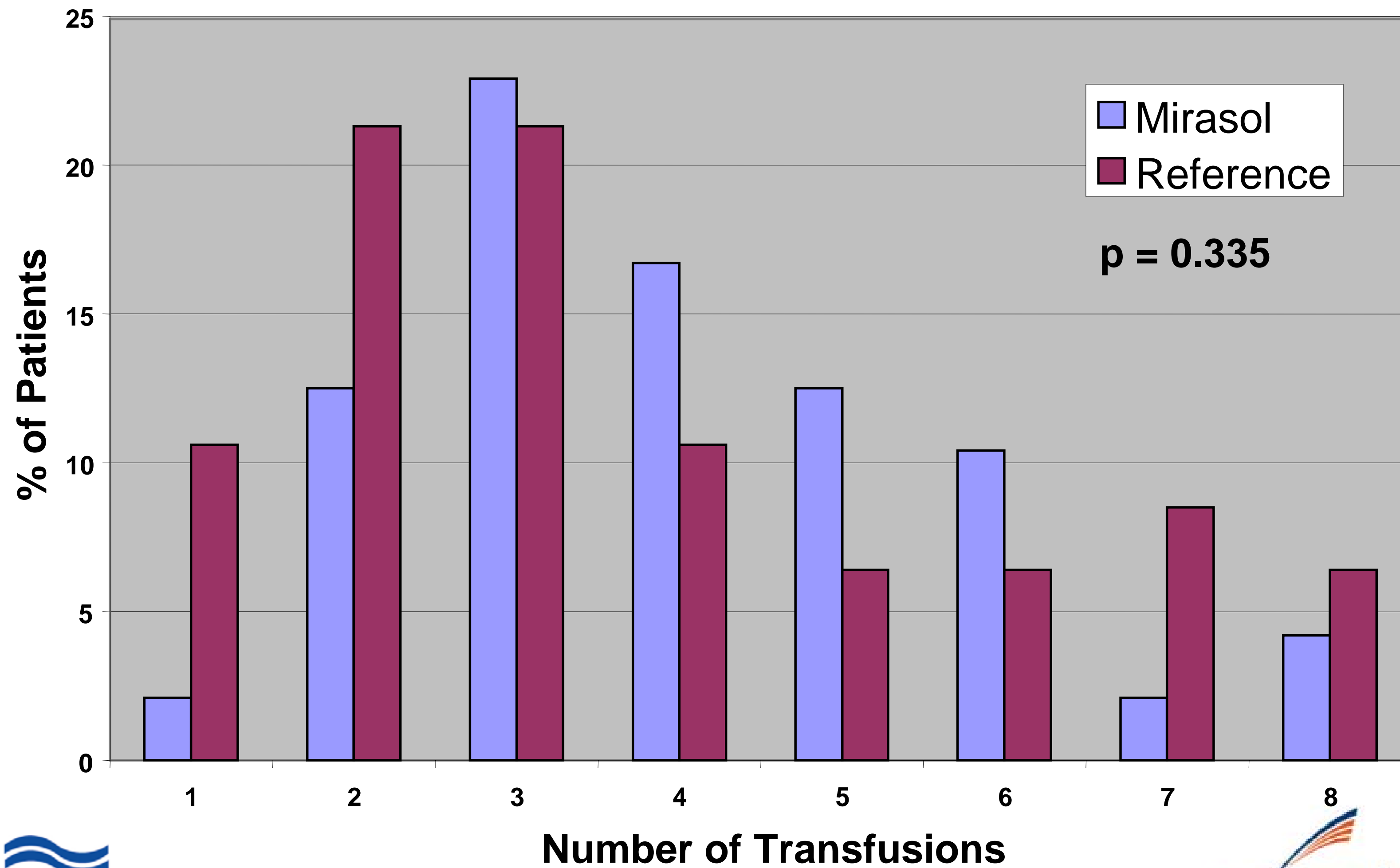
Frequency Distribution of Platelet Transfusions



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Refractoriness & HLA Alloimmunization

	Mirasol (N=47)	Reference (N=48)	P-value
% of subjects classified with refractory events (2 consecutive CCI-1 hr < 5,000)	10%	4%	0.48 (NS)
% of subjects with initial positive HLA test result	4%	8%	

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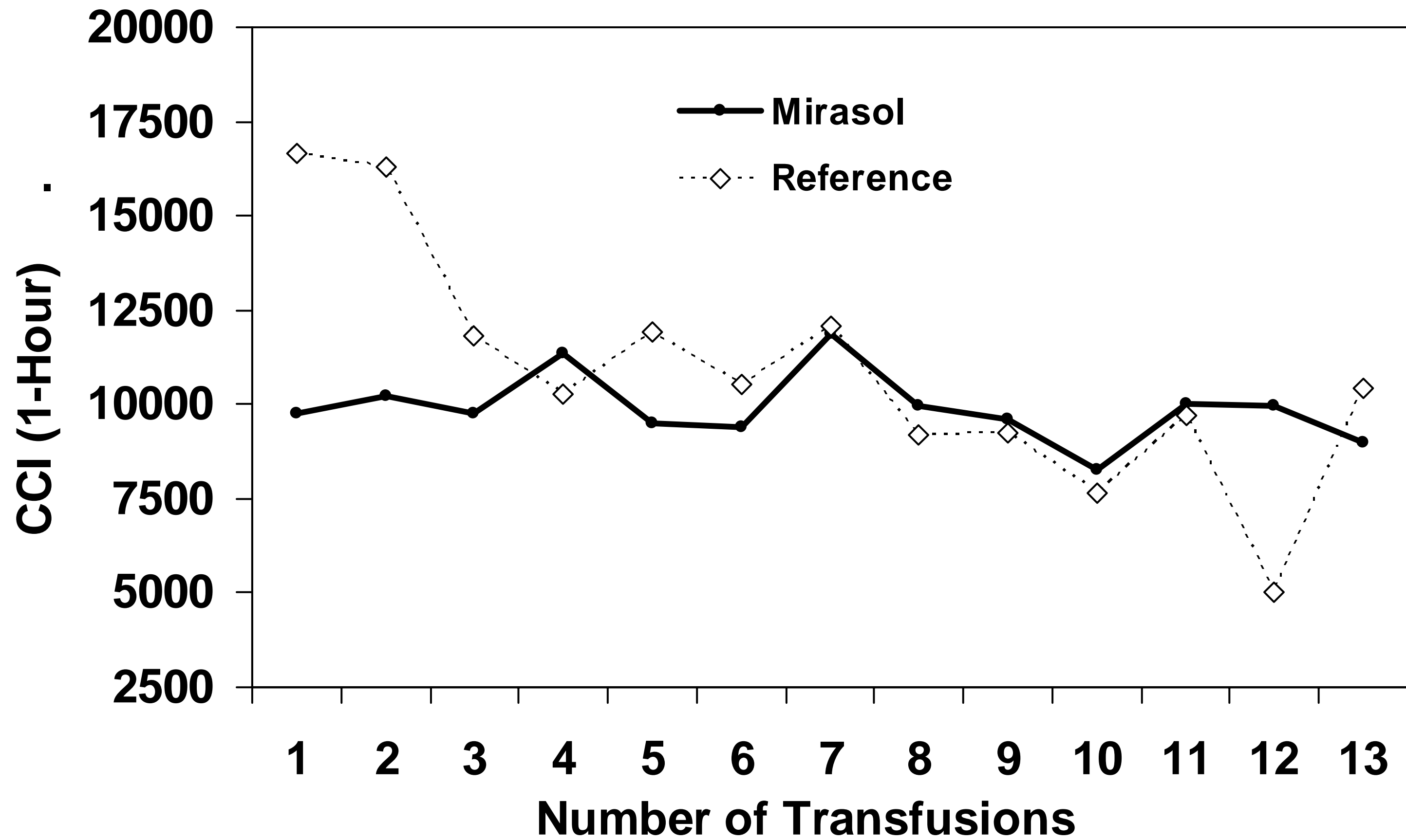
1-Hour CCI Values by Number of Transfusions



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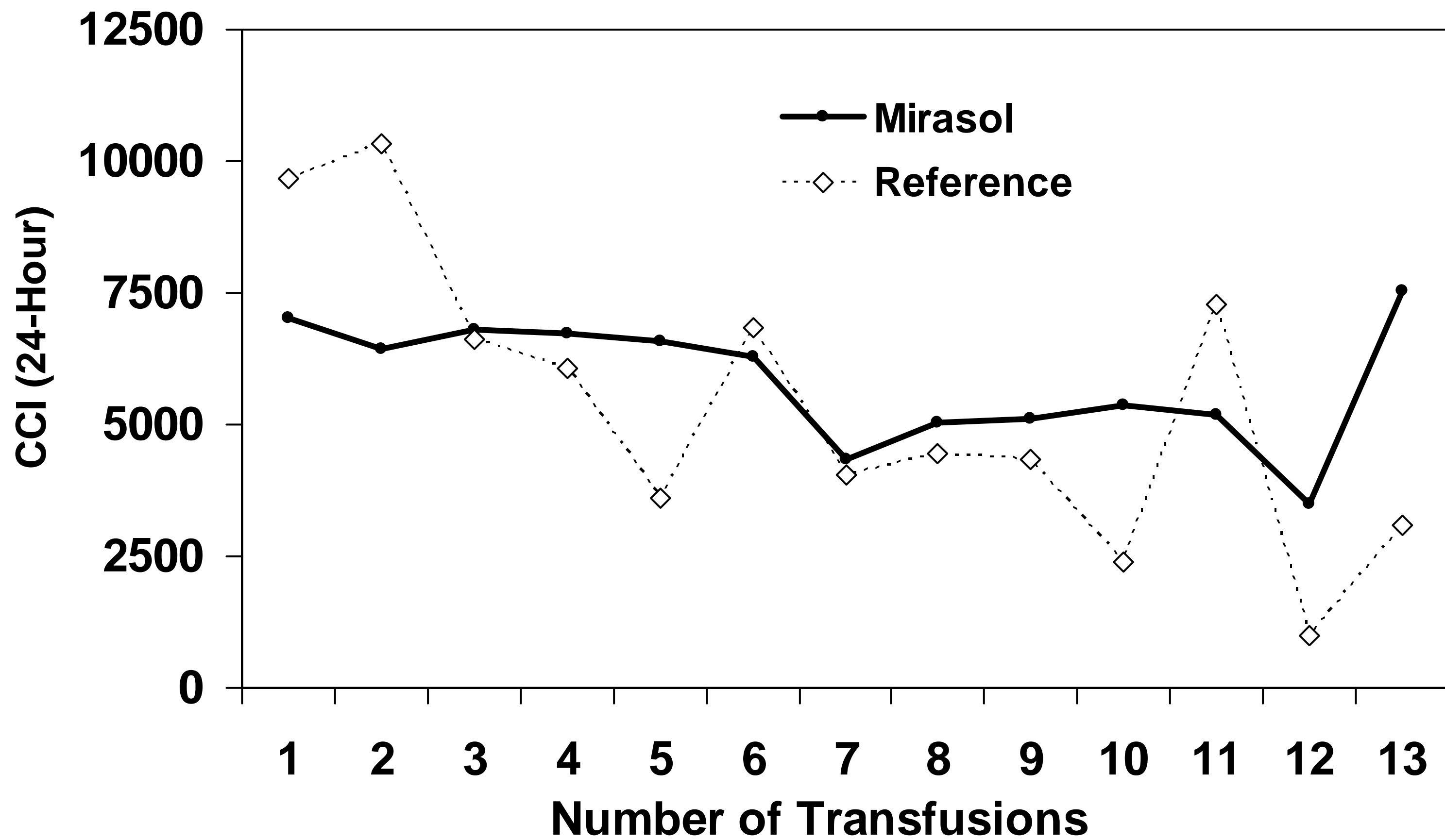
24-Hour CCI Values by Number of Transfusions



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Red Blood Cell Transfusion Needs

	Mirasol (N=47)	Reference (N=48)	P-value
Total number of RBC transfusions given	137	135	0.41 (NS)
% of subjects receiving RBC transfusions	96%	92%	
Mean number of RBC transfusions per patient	2.9 (1–9)	3.0 (1–14)	0.88 (NS)

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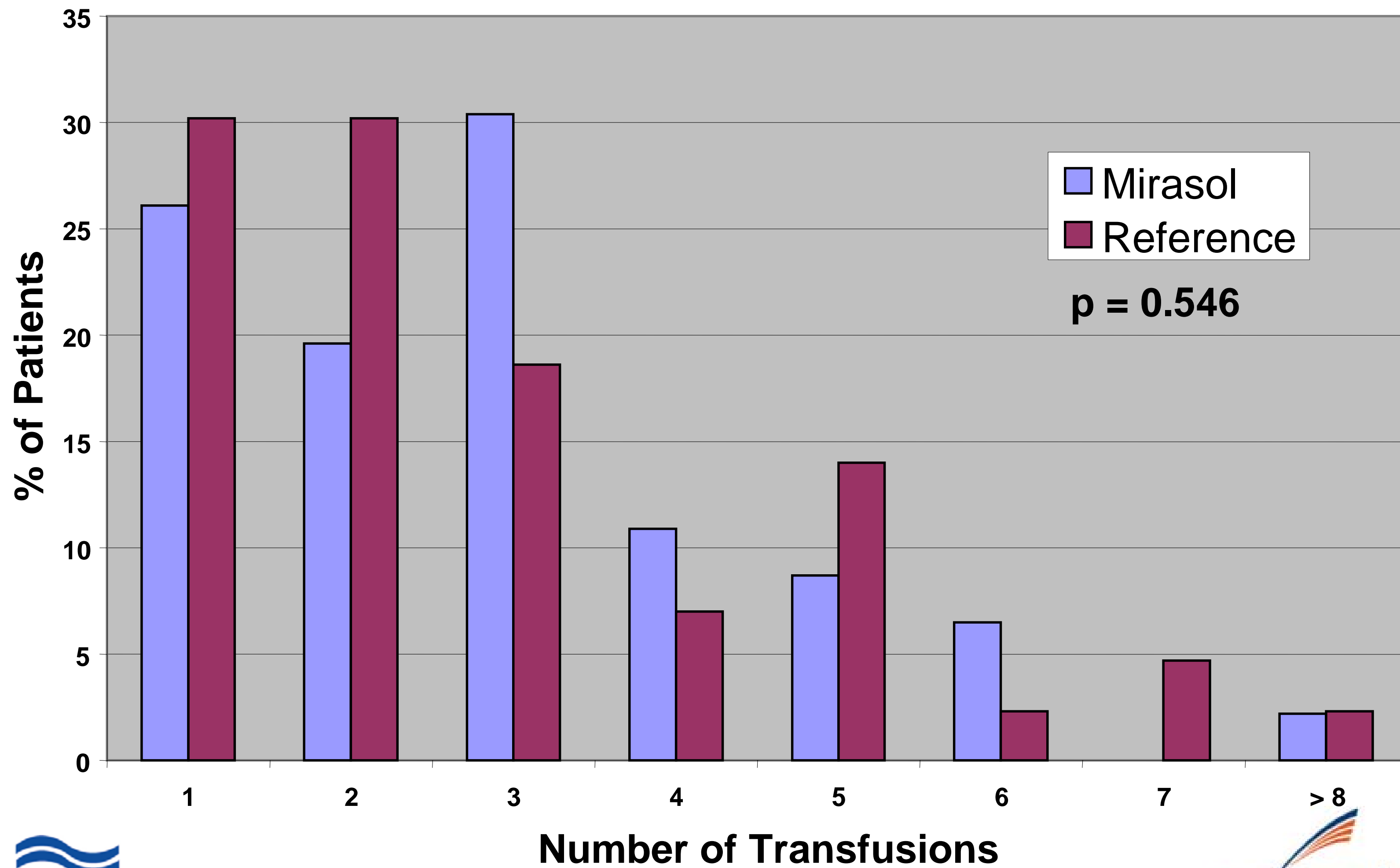
Frequency Distribution of Red Cell Transfusions



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Adverse Events and Serious Adverse Events

- Adverse events were observed in both patient groups
  - 1% of these events were rated as possibly, likely, or very likely related to the transfusion for Mirasol vs. 2% for Reference.
- There were 16 Serious Adverse Events (SAE's) in the Mirasol group, and 13 in the Reference group
  - No SAE's were adjudicated as due to transfusion of Mirasol
- No significant differences were observed in Grade II, III or IV bleeding between patients in the Mirasol or Reference groups

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Neoantigenicity and Photoproduct Testing

Neoantigen Testing

- Independent testing performed at Bonfils Blood Center (US) using clinical samples provided by study sites
- 44 patients exposed to Mirasol-treated platelets were evaluated — NONE demonstrated antibodies to neoantigens.

Photoproduct Analysis

- There was no evidence of accumulation of either riboflavin or photoproducts in patients, even after extensive repeat exposure.

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DSMB Conclusion

" **No adverse effect** appeared to be either related to Mirasol-treated platelets or due to the device used for the preparation of these platelets."

Final report from DSMB on MIRACLE STUDY

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Conclusions (1)

- No evidence of device related Adverse Events were observed.
- No evidence of neoantigen formation or photoproduct accumulation were observed.
- Elimination of gamma irradiation was possible.
- Interesting trends were observed in CCI values as a function of transfusion number.

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Conclusions (2)

- There was no evidence of increased use of platelets in the Mirasol group.
- There was no evidence of increased use of red cells in the Mirasol group.
- The platelet yield from collection to transfusion was virtually identical – minimal losses due to processing.

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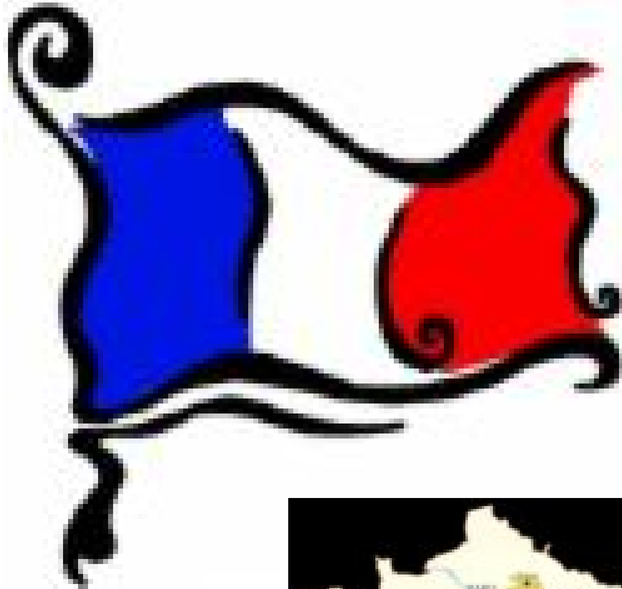
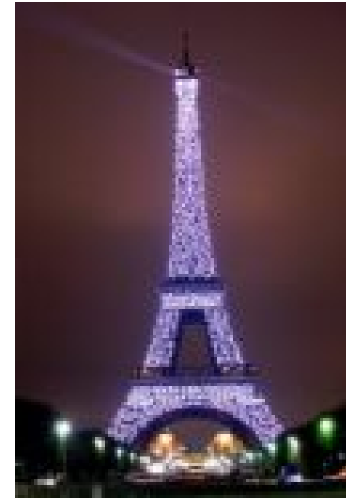
THANK YOU

Thank You to the EFS, Hospital Investigators and Patients
Who Took Part in This Trial!





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病原不活化による血液製剤の安全性と供給の確保 I

国際輸血学会アジア地区大会

2009年11月15日
名古屋国際会議場

シーラス社（米国）、日本バイオワン株式会社（日本）共催

CERUS　BioOne

IBS使用状況

- 35万回以上の不活化血小板輸血
- 5万人以上の患者の治療に使用
  - 13カ国53血液センターで臨床に使用中
- 5万回以上の不活化血漿の輸血
- 2万人以上の患者に使用
  - 4カ国12センターで使用中

CERUS

TRIAL INFO

The PREPAReS Study: Pathogen Reduction Evaluation & Predictive Analytical Rating Score.

- CANDIDATE NUMBER	6714
- **NTR NUMBER**	**NTR2106**
- ISRCTN	ISRCTN wordt niet meer aangevraagd.
- DATE ISRCTN CREATED	
- DATE ISRCTN REQUESTED	
- DATE REGISTERED NTR	13-nov-2009
- SECONDARY IDS	ABR30643
- PUBLIC TITLE	The PREPAReS Study: Pathogen Reduction Evaluation & Predictive Analytical Rating Score.
- SCIENTIFIC TITLE	Clinical effectiveness of standard versus pathogen-reduced buffy coat-derived platelet concentrates in plasma in acute myeloid leukemia patients.
- ACRONYM	The PREPAReS Study: Pathogen Reduction Evaluation & Predictive Analytical Rating Score
- HYPOTHESIS	Non-inferiority (defined as < 15% increase) of pooled buffy coat-derived PR platelet concentrates (PR-plasma-PCs) compared to plasma (plasma-PCs), stored for 1-5 days, in terms of clinical efficacy determined by CTCAE grade — 2 bleeding complications.
- HEALT CONDITION(S) OR PROBLEM(S) STUDIED	
- INCLUSION CRITERIA	1. Age — 18 years; 2. Expected — 2 platelet transfusion requirements; 3. Signed informed consent; 4. Having acute myeloid leukemia including those who undergo myelo ablative allogeneic stem cell transplant therapy.
- EXCLUSION CRITERIA	1. Micro-angiopathic thrombocytopenia (TTP, HUS) and ITP; 2. Bleeding > grade 2 at randomization (after treatment, the patient can be randomized in the study after 2 or more weeks after the last transfusion that was used to stop the bleeding); 3. Known immunological refractoriness to platelet transfusions; 4. HLA- and/or HPA-allo immunization and/or clinical relevant auto-antibodies; 5. Indications to use hyper-concentrated (plasma-reduced) platelet concentrates, i.e. patients with known severe allergic reactions and documented transfusion-associated circulatory overload (TACO); 6. Pregnancy (or lactating); 7. Prior treatment with pathogen-reduced blood products; 8. Known allergy to riboflavin or its photoactive products.
- MEC APPROVAL RECEIVED	no
- MULTICENTER TRIAL	yes
- RANDOMISED	yes
- MASKING/BLINDING	Single
- CONTROL	Active
- GROUP	Parallel
- TYPE	2 or more arms, randomized
- STUDYTYPE	intervention
- PLANNED STARTDATE	1-mrt-2010
- PLANNED CLOSINGDATE	1-sep-2012
- TARGET NUMBER OF PARTICIPANTS	375
- INTERVENTIONS	1. Pooled buffy coat-derived pathogen reduced platelet concentrates (PR-plasma-PCs), or; 2. Plasma (plasma-PCs), stored for 1-7 days.

	Time of intervention has a maximum of 6 weeks or a maximum of 8 transfusions whichever comes first.
- PRIMARY OUTCOME	CTCAE grade — 2 bleeding complications of PCs, stored for 1-5 days.
- SECONDARY OUTCOME	Using PCs, stored for 1-7 days: 1. The 1 and 24 hour CI; 2. The 1 and 24 hour CCI; 3. (1+24 hour CCI)/2; 4. Adverse transfusion reactions; 5. Total transfusion requirement of red cells and platelets; 6. Platelet transfusion interval; 7. Rate of HLA allo-immunization; 8. In vitro quality markers related with the 1-hour or 24-hour CCI; 9. Clinical factors interacting on primary endpoint, including in vivo variables of immunological responses and of hemostasis in the recipients after transfusion as compared prior to transfusion.
- TIMEPOINTS	Prior to, and 1 hr and 24 hr after PC-transfusion.
- TRIAL WEB SITE	N/A
- STATUS	planned
- CONTACT FOR PUBLIC QUERIES	Prof. Dr. A. Brand
- CONTACT FOR SCIENTIFIC QUERIES	Prof. Dr. A. Brand
- SPONSOR/INITIATOR	Sanquin Blood Bank (Stichting Sanquin Bloedvoorziening), CaridianBCT Biotechnologies LLC
- FUNDING (SOURCE(S) OF MONETARY OR MATERIAL SUPPORT)	CaridianBCT Biotechnologies LLC
- PUBLICATIONS	N/A
- BRIEF SUMMARY	The study is a prospective, randomized multicenter trial for the evaluation of platelet products in acute myeloid leukemia patients with thrombocytopenia or expected to become thrombocytopenic caused by myelosuppressive therapy or malignancy-related myelosuppression. In this trial patients will be randomized to receive one of two platelet products during a transfusion episode with a maximum of 6 weeks or a total of 8 platelet transfusions, whichever comes first. Because the Mirasol-treated platelet products show a color difference not allowing an appropriate placebo, the study will be single-blinded for investigators evaluating the bleeding score. Products will be stored up to 7 days. The primary endpoint is restricted to 5 days storage as this implies the most relevant information. Secondary endpoint evaluation requires that the patient continues treatment in the assigned study arm. Arm A: Plasma stored platelet concentrates (Plasma-PCs); Arm B: Pathogen reduced plasma-stored platelet concentrates (PR-plasma-PCs).
- MAIN CHANGES (AUDIT TRAIL)	
- RECORD	13-nov-2009 - 25-nov-2009

- Indien u gegevens wilt toevoegen of veranderen, kunt u een mail sturen naar **nederlands@trialregister.nl**

文献 f

Vox Sanguinis (2008) **94**, 315–323

ORIGINAL PAPER

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An active haemovigilance programme characterizing the safety profile of 7437 platelet transfusions prepared with amotosalen photochemical treatment

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[4]*EFS Auvergne Loire, St. Etienne, France*
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[6]*Centro de Hemoterapia y Hemodonacion de Castilla y Leon, Valladolid, Spain*
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[9]*Cerus Corporation, Concord, CA, USA*

Vox Sanguinis

Background An active haemovigilance programme was implemented to survey adverse events (AE) associated with transfusion of platelets photochemically treated with amotosalen and ultraviolet A (PCT-PLT). The results of 5106 transfusions have already been reported. Here we report the results of an additional 7437 PCT-PLT transfusions.

Methods The focus of this ongoing haemovigilance programme is to document all AEs associated with PCT-PLT transfusion. Data collected for AEs include: time of event after starting transfusion, clinical descriptions, vital signs, results from radiographs and bacterial cultures, event severity (Grade 0–4) and causal relationship to PCT-PLT transfusion.

Results One thousand four hundred patients (mean 60 years, range 1–96) received PCT-PLT transfusions. The majority of the patients (53·4%) had haematology–oncology diseases and required conventional chemotherapy (44·8%) or stem cell transplantation (8·6%). Sixty-eight PCT-PLT transfusions were associated with AE. Acute transfusion reactions (ATR), classified as an AE possibly related, probably related, or related to PCT-PLT transfusions were infrequent ($n = 55$, 55/7437 = 0·7%) and most were of Grade 1 severity. Thirty-nine patients (39/1400 = 2·8%) experienced one or more ATRs. The most frequently reported signs/symptoms were chills, fever, urticaria, dyspnoea, nausea and vomiting. Five AEs were considered severe (≥ Grade 2); however, no causal relationship to PCT-PLT transfusion was found. Repeated exposure to PCT-PLT did not increase the likelihood of an ATR. No cases of transfusion-related acute lung injury and no deaths due to PCT-PLT transfusions were reported.

Conclusions Routine transfusion of PCT-PLT is well-tolerated in a wide range of patients. ATRs related to PCT-PLT transfusion were infrequent and most were of mild severity.

Key words: PCT, platelets, haemovigilance, safety, INTERCEPT.

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E-mail: llin@cerus.com

Introduction

INTERCEPT Blood System™ uses a photochemical treatment methodology [PCT: amotosalen plus ultraviolet A (UVA) light] to inactivate viruses, bacteria, protozoa, and leucocytes in platelet (PLT) and plasma components. The PLT system received CE Mark registration in Europe in 2002. Several centres in Belgium, Spain, Norway and Italy began routine production of PCT-PLT in 2003. An active haemovigilance programme was immediately implemented to prospectively collect information on PCT-PLT transfusions administered to patients in routine clinical settings. Prior to CE Mark registration, the safety data of PCT-PLT were primarily obtained from controlled clinical trials with a limited number of patients and predetermined clinical and safety end-points [1–3]. The postmarketing haemovigilance programme provided a means to extend the characterization of the safety profile of PCT-PLT in routine use and in a broad patient population. The results of the first 5106 PCT-PLT transfusions have already been reported [4]. With additional centres in Belgium, Spain and France starting with the routine production of PCT-PLT, the database of this haemovigilance programme has been expanded [5].

In March 2007, the Canadian Blood Services and Héma-Québec organized a consensus conference to provide recommendations and guide decision-making about new pathogen inactivation technologies [6]. The panel, consists of nine healthcare professionals and members of the public, stressed the importance of postmarketing surveillance studies in the introduction of new technologies for blood safety. The panel recommended that specific studies should be mandated by the regulatory authorities and supported by the manufacturers and/or the blood suppliers. Postmarketing surveillance for adverse reactions to pathogen inactivation products should be linked to the national haemovigilance systems if possible. Depending on the new pathogen inactivation technologies implemented, specific additional surveillance outcomes may be identified. The panel also suggested that chronically transfused patients might serve as an ideal surveillance population to identify long-term toxicities of pathogen-inactivated products.

The active haemovigilance programme described in this study is in concordance with these recommendations. Although this programme is not directly linked to a specific country haemovigilance system nor designed to replace any existing haemovigilance system, the format of data collection is modelled after the data collection format of the French haemovigilance system for documentation of transfusion incidents [7]. The focus of the current programme is on all adverse events (AE), serious or non-serious, occurring after the start of PCT-PLT transfusion. Following the recent report of 5106 PCT-PLT transfusions [4], here we report the results of an additional 7437 transfusions of PCT-PLT.

Materials and methods

General study design

This was a prospective observational active haemovigilance study. The objective of this study was to document the transfusion safety profile for approximately 7500 PCT-PLT components prepared with the INTERCEPT Blood System™ for platelets (Cerus Europe BV, Leusden, the Netherlands). These components were prepared in three centres in Belgium (CTS UCL Mont Godinne, CTS Brabant-Hainaut and AZ Sint Jan AV), three centres in France (EFS-Alsace, EFS-Auvergne-Loire and EFS-Bretagne), and one centre in Spain (CHEMCYL Valladolid) and administered to thrombocytopenic patients under standard clinical practice in hospitals. There were no randomization requirements, no inclusion criteria and no exclusion criteria of patients other than the need to receive a platelet transfusion. Baseline demographical information was collected on all study participants. Patients were assigned a centre-specific study number to preserve anonymity.

Patients who received transfusions of PCT-PLT were monitored for any AEs after the start of each platelet transfusion, which is consistent with European Haemovigilance Network recommendations for surveillance of AE to transfusion of labile blood components, and with those of national transfusion services [7,8]. However, in this study, reporting was obligatory for all PCT-PLT transfusions in each participating clinical site. A transfusion report was required for each PLT transfusion regardless of whether or not an AE occurred. In case of occurrence of an AE, additional clinical and biological information was collected to allow diagnosis and assessment of causality and severity. The data in the final database were anonymous and were reported on a per-transfusion basis as well as on a per-patient basis. Transfusions associated with serious AEs were reported in greater detail.

Study report forms

The report form used for this haemovigilance programme was developed on the basis of haemovigilance report forms already in use. Information was collected in several broad categories: patient demographic/diagnosis data, platelet component characteristics, transfusion events and documentation of all AEs following transfusion. An acute transfusion reaction (ATR) was defined as an AE possibly related, probably related, or related to a PCT-PLT transfusion.

AEs were graded for clinical severity within the following categories: Grade 0, isolated dysfunction without clinical or biological manifestation; Grade 1, absence of immediate or long-term life-threatening effects; Grade 2, long-term life-threatening effects; Grade 3, immediate life–threatening effects; and Grade 4, death. For each transfusion, the following

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signs, symptoms and specific clinical syndromes were evaluated: fever, chills, cardiac arrhythmia, hypotension, itching, urticaria, skin rash, jaundice, pulmonary oedema, bronchospasm, dyspnoea, respiratory distress, nausea, vomiting, lower back pain, chest pain, abdominal pain, and shock. Any other findings could be entered as free text including refractoriness to platelet transfusion and transfusion-related acute lung injury. The following available clinical signs were recorded before and after each transfusion: temperature, blood pressure and heart rate. Abnormal clinical laboratory values, results of diagnostic procedures (chest X-ray) and bacterial cultures from patient and blood component sources were recorded when associated with an AE following a PCT-PLT transfusion.

Preparation of platelet components

Platelet components were collected by apheresis or from whole blood-derived buffy-coat procedures according to each centre's standard operating procedures. Volunteer donors were screened and tested for transfusion-transmitted pathogens according to each centre's standard operating procedures in compliance with respective national regulations. All components were leucocyte reduced, either by filtration (Sepacell PLS-5A, Asahi Biomedical, Tokyo, Japan) or process leucodepletion (Amicus Cell Separator, Fenwal, La Chatre, France; Haemonetics MCS+, Haemonetics, Braintree, MA, USA). Platelet components containing 2·5 to $6{\cdot}0 \times 10^{11}$ platelets were suspended in approximately 35% plasma and 65% InterSol™ (Fenwal) and prepared with amotosalen (nominal final concentration 150 μM) and a 3 J/cm^2 UVA light treatment (320–400 nm) according to the manufacturer's instructions for use (Cerus Europe BV). After treatment, PCT-PLTs were stored up to either 5 or 7 days under temperature-controlled conditions (22 ± 2 °C) before release for transfusion depending on country-specific regulations. PCT-PLTs were transfused before the expiration period of 5 days in France and Spain or 7 days in Belgium. PCT-PLTs were not cultured for bacterial contamination prior to release, and PCT was used in place of γ-irradiation for prevention of transfusion-associated graft-versus-host disease in all sites except EFS-Bretagne and EFS-Auvergne-Loire.

Platelet transfusion

PCT-PLT components for transfusion were ordered according to standard indications within each institution. The investigator was requested to report all AEs occurring after starting transfusion without time limitation. The severity of each AE (Grade 0 to 4) and the relationship of each AEs to the preceding platelet transfusion were assessed by the investigator. Serious adverse events were reported in greater detail with a narrative for each event.

Statistical analyses

All statistical analyses, summary tables and data listings were generated using SAS® version 8·2. The primary assessment of safety was the proportion of ATR for the transfusions reported. The safety profile of PCT-PLT transfusions included information on: the number of PCT-PLT transfusions by patient; the patient population profile; the characteristics of the PCT-PLT transfused, and the characteristics of the AE following platelet transfusion.

Data were analysed on a per-transfusion basis as well as on a per-patient basis. All PCT-PLT transfusions administered to a patient were included in the full analysis population, whether or not an AE was observed. Data were summarized for each parameter using descriptive statistics (mean, standard deviation, median, and range).

Statistical tests were performed for the exploration of risk factors only (multivariate logistic regression at 10% significant level). The variables included in the analysis are patient gender, age, previous transfusion history, type of platelet concentrate, γ-irradiation, antigen-matching and primary diagnosis. Variables with descriptive statistics were tested for P values and odds ratio. The number and proportion (%) of transfusions with one or more AEs were summarized overall, by seriousness and by relationship to platelet transfusion. Corresponding 95% confidence intervals (CIs) were calculated.

The non-survival analysis method is a univariate analysis of the number of transfusions received before the first occurrence of an AE. Only patients with at least one AE were considered in this analysis.

Results

Distribution of transfusions

A total of 7437 PCT-PLT transfusions were documented between May 2005 and January 2007 and constitute the full analysis population. The distribution of transfusion reports were: 3057 (41·1%) from CTS UCL Mont Godinne, 2048 (27·5%) from EFS-Alsace, 899 (12·1%) from CTS Brabant-Hainaut, 572 (7·7%) from EFS-Auvergne-Loire, 440 (5·9%) from AZ Sint Jan AV, 381 (5·1%) from CHEMCYL, and 40 (0·5%) from EFS-Bretagne.

Patient demographics

A total of 1400 patients underwent transfusion (Table 1). The majority of the patients were male (61·3%) and the mean age was 60 years (range < 1–96 years). Haematology–oncology diseases treated by chemotherapy (44·8%) and stem cell transplantation (8·6%) constituted 53·4% of the primary diagnoses and therapies among the transfused population. A significant number of patients receiving platelet transfusion (17·2%)

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Table 1 Patient and transfusion demographics

	Patient characteristics (*n* = 1400)	Transfusion characteristics (*n* = 7437)
Gender (*n*, %)		
Male	858 (61·3%)	4354 (58·5%)
Female	542 (38·7%)	3082 (41·4%)
Unknown		1 (< 0·1%)
Age (years)		
Mean ± SD	60·0 ± 17·8	
Median	63	
(minimum–maximum)	(<1–96)	
Location of transfusion		
Intensive care unit		1145 (15·4%)
Outpatient		382 (5·1%)
Regular ward		5908 (79·4%)
Unknown		2 (< 0·1%)
Haematology–oncology patients	748 (53·4%)	5463 (73·5%)
Conventional chemotherapy	627 (44·8%)	4481 (60·3%)
Stem cell transplant	121 (8·6%)	982 (13·2%)
Surgery patients	241 (17·2%)	480 (6·5%)
Cardiovascular surgery	209 (14·9%)	349 (4·7%)
Solid organ transplantation	32 (2·3%)	131 (1·8%)
Other diagnoses	397 (28·4%)	859 (11·6%)
Missing diagnosis	14 (1·0%)	635 (8·5%)
History of a previous transfusion		
Yes	837 (59·8%)	5029 (67·6%)
No	398 (28·4%)	1927 (25·9%)
Unknown	165 (11·8%)	481 (6·5%)
If 'Yes' – did they experience a transfusion-related adverse event?[a]		
Yes	53 (6·3%)	382 (7·6%)
No	779 (93·0%)	4639 (92·2%)
Unknown	5 (0·6%)	8 (0·2%)

[a]For per-patient basis, the denominator is 837; for per-transfusion basis, the denominator is 5029.

were undergoing cardiovascular surgery or solid organ transplantation. Other diagnoses included haematology–oncology diseases not treated by chemotherapy and/or stem cell transplantation and surgery other than cardiovascular surgery and solid organ transplantation.

Of all patients, 837 patients (59·8%) had already received another blood product before the first PCT-PLT transfusion (Table 1). Among these patients, 53 patients (6·3% of 837) had a history of a transfusion reaction of some type in the past.

Platelet component demographics

Most of the PCT-PLT units were manufactured from apheresis platforms (4822, 64·8% vs. 2615, 35·2% for buffy-coat products). The majority of the PCT-PLTs (7357, 98·9%) were not treated with γ-irradiation [9]. Among the 7437 PCT-PLTs transfused, only 2·5% (189 units) of platelet units were human leucocyte antigen-matched products.

A large proportion of the PCT-PLT components (5908, 79·4%) were transfused in non-intensive care hospital wards (Table 1). Intensive care units and day-hospital units were the location for 15·4 and 5·1% of the PCT-PLT transfusions (1145 and 382 units, respectively). While most of the PCT-PLT components (5463, 73·5%) were administered to haematology–oncology patients, only 480 PCT-PLT components (6·5%) were administered to surgery patients.

The majority of the PCT-PLT components (5029, 67·6%) were administered to patients who had already received another blood component before the first PCT-PLT transfusion (Table 1). Among these transfusions, 382 (7·6% of 5029) PCT-PLT components were transfused to patients reported to have experienced at least one transfusion reaction in the past.

Number of transfusions per patient

The range of PCT-PLT transfusions per patient was 1 to 129, with an average of 5·3 ± 10·8 (median: 2) transfusions per patient. Of the 1400 patients who received PCT-PLT transfusions, 529 patients (37·8%) received only one PCT-PLT transfusion during this study period, 418 patients (29·9%) received two to three transfusions, and 453 patients (32·4%) received more than four PCT-PLT transfusions during the study. The majority of patients who received multiple transfusions had a primary diagnosis of haematology–oncology diseases treated by chemotherapy and/or stem cell transplantation.

Two patients from CTS UCL Mont Godinne received more than 100 transfusions analysed in this haemovigilance plan. One 56-year-old man (J01-636) who was treated by conventional chemotherapy for haematology–oncology disease received 129 PCT-PLT components within an 8-month period (from April 2006 to November 2006). One 72-year-old woman (J01-071) who was also treated by conventional chemotherapy for haematology–oncology disease received 107 PCT-PLT components within a 10-month period (from August 2005 to November 2006).

Adverse events following PCT-PLT transfusion

On a per-transfusion basis, 68 (0·9% of 7437 transfusions, 95% CI: 0·7–1·2%) transfusions were associated with an AE (Table 2). Of which, 55 (0·7% of 7437 transfusions, 95% CI: 0·6–1·0%) were classified as ATR possibly related, probably related, or related to PCT-PLT transfusion. Only five events were classified as serious AEs (0·07%, 95% CI: 0·0–0·2%), and were judged as probably unrelated to the PCT-PLT transfusion based on the observation of alternative causes for symptoms and no evidence of causal relationship to the platelet transfusion. No cases of transfusion-related acute lung injury and no deaths due to PCT-PLT transfusions were reported.

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Table 2 Clinical characteristics of adverse events (AE)

	On a per-transfusion basis *n* (% = *n* × 100/7437)				On a per-patient basis *n* (% = *n* × 100/1400)			
	Any AEs	AE attributed to platelets (ATR)[b]	SAE[a]	SAE attributed to platelets[a,b]	Any AEs	AE attributed to platelets (ATR)[b]	SAEs[a]	SAE attributed to platelets[a,b]
Number with at least one event	68 (0·9%)	55 (0·7%)	5 (< 0·1%)	0 (0·0%)	45 (3·2%)	39 (2·8%)	4 (0·3%)	0 (0·0%)
Signs/Symptoms[c]								
Fever	8 (0·1%)	6 (< 0·1%)	0 (0%)	–	7 (0·5%)	5 (0·4%)	0 (0%)	–
Chills	45 (0·6%)	40 (0·5%)	2 (< 0·1%)	–	31 (2·2%)	28 (2·0%)	1 (< 0·1%)	–
Itching	2 (< 0·1%)	2 (< 0·1%)	0 (0%)	–	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–
Hypotension	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–
Urticaria	14 (0·2%)	14 (0·2%)	0 (0%)	–	13 (0·9%)	13 (0·9%)	0 (0%)	–
Skin rash	5 (< 0·1%)	5 (< 0·1%)	0 (0%)	–	4 (0·3%)	4 (0·3%)	0 (0%)	–
Dyspnoea	8 (0·1%)	6 (< 0·1%)	1 (< 0·1%)	–	8 (0·6%)	6 (0·4%)	1 (< 0·1%)	–
Respiratory distress	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–	1 (< 0·1%)	0 (0%)	1 (< 0·1%)	–
Nausea/vomiting	8 (0·1%)	5 (< 0·1%)	3 (< 0·1%)	–	5 (0·4%)	3 (0·2%)	2 (0·1%)	–
Lower back pain	6 (< 0·1%)	1 (< 0·1%)	0 (0%)	–	2 (0·1%)	1 (< 0·1%)	0 (0%)	–
Chest/abdominal pain	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–	1 (< 0·1%)	1 (< 0·1%)	0 (0%)	–
Shock	4 (< 0·1%)	0 (0%)	4 (< 0·1%)	–	3 (0·2%)	0 (0%)	3 (0·2%)	–
Tachycardia	4 (< 0·1%)	3 (< 0·1%)	1 (< 0·1%)	–	3 (0·2%)	2 (0·1%)	1 (< 0·1%)	–
Other	14 (0·2%)	11 (0·1%)	3 (< 0·1%)	–	12 (0·9%)	10 (0·7%)	3 (0·2%)	–

[a]Serious adverse event (SAE): long-term life threatening, immediate life threatening or death.
[b]Causal relationship that was possibly related, probably related, or related to PCT-PLT transfusion.
[c]Number of signs/symptoms can exceed number of AE due to multiple observed signs/symptoms per AE.

On a per-patient basis, 45 patients (3·2% of 1400 patients) who received at least one transfusion of PCT-PLT experienced the 68 AEs following PCT-PLT transfusions (Table 2). Only 39 patients (2·8% of 1400 patients) experienced the 55 ATRs attributed to the PCT-PLT transfusion. Four patients experienced serious AEs following transfusion; however, no causal relationship to PCT-PLT transfusion could be established.

All AEs regardless of the relationship with the PCT-PLT transfusion occurred within 4 h after the start of the platelet transfusion (mean time: 0·3 ± 0·51 h, 0–3·3 h). The majority of AEs (64, or 94·1% of 68 AEs) occurred in patients who were not premedicated. The other four AEs occurred in patients who were premedicated with antipyretic or antihistaminic drugs, or corticosteroids.

Characteristics of clinical signs and symptoms associated with adverse event

On a per-transfusion basis, the most frequently observed symptoms/signs (≥ 0·1% of the total 7437 transfusions) were fever, chills, urticaria, dyspnoea, nausea and/or vomiting (Table 2). The individual incidence of each of the following signs/symptoms was < 0·1%: itching, hypotension, skin rash, respiratory distress, lower back pain, chest or abdominal pain, shock and tachycardia. All additional symptoms included in the category of other, such as refractoriness to platelet transfusion, hypertension, cephalea, pain in the leg, flush, malaise, cyanosis, oxygen desaturation and volume overload were also reported but with an individual incidence of less than 0·1%. Most of ATRs were described principally as Grade 1 chills and urticaria (Table 2).

On a per-patient basis, the most frequently observed symptoms/signs (≥ 0·5% of the total 1400 patients) were fever, chills, urticaria and dyspnoea (Table 2). Approximately 0·1–0·4% of the population (from 2 to 5/1400) experienced the following signs/symptoms: skin rash, nausea/vomiting, shock, lower back pain and tachycardia. Clinical refractoriness to transfusion, hypertension, headache and flushing were additional symptoms reported in the category of 'other'. Less than 0·1% of the study population (only 1/1400) experienced the following signs/symptoms such as hypotension, itching, respiratory distress and chest/abdominal pain. Symptoms such as pulse increase, leg pain, cyanosis, oxygen desaturation, malaise and/or volume overload were also reported in the category of 'other'. Most of the ATRs consisted of various combinations of fever (0·4%), chills (2·0%), urticaria (0·9%), skin rash (0·3%), dyspnoea (0·4%), nausea/vomiting (0·2%), tachycardia (0·1%) and others symptoms (0·7%) (Table 2).

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Serious adverse events following platelet transfusion

During the course of this surveillance, five serious AEs were reported following transfusion of PCT-PLT (0·07%, 95% CI: 0·0–0·2). These serious AEs were assessed by the investigators as being 'unrelated or probably unrelated' to the PCT-PLT transfusions and were attributed to progression of underlying illness.

Patient B01-201 was admitted to hospital for a presumed pulmonary infection postchemotherapy. Additional co-morbidities at the time of admission were septic shock, acute renal insufficiency, neutropenia and thrombocytopenia. Intravenous (i.v.) antibiotic therapy was initiated and multiple transfusions of blood products (including PCT-PLT) were administered. One hour after administration of the second platelet unit, the patient complained of dyspnoea, respiratory distress was found to be hypotensive and tachycardic. Severe volume overload was determined to be the aetiology and treatment with oxygen, diuretics, and dialysis was initiated. The event was assessed by the investigator to be unrelated to the PCT-PLT transfusion.

Patient J01-382 experienced chills, nausea and sudden hypotension during transfusion with PCT-PLT. Prior to this, the patient had received at least four PCT-PLT transfusions with no AE. The transfusion was stopped and the patient was treated with i.v. fluids and recovered. Four days later, the patient experienced a second hypotensive episode after transfusion, which was spontaneously resolved. Subsequent to this, the patient received 19 additional PCT-PLT transfusions without any clinical sequelae. This patient did not receive any angiotensin-converting enzyme (ACE) inhibitors. Based on the patient's history and the lack of transfusion reaction with the subsequent transfusions, the investigator assessed both of these events as probably unrelated to PCT-PLT transfusion.

Patient J01-516 was admitted for ischaemic cardiomyopathy and underwent double vessel coronary artery bypass graft (CABG). The patient's postoperative recovery was complicated by a significant decrease in blood pressure, which occurred 10 min after start of transfusion of PCT-PLT. Despite vasopressor support and a 6-min period of circulatory arrest, the patient's condition continued to deteriorate and he died. Cause of death was attributed to an aortic dissection with major disseminated intravascular coagulopathy and mesenteric infarct and was assessed by the investigator as unrelated to the PCT-PLT transfusion.

Patient J01-780 experienced a hypotensive episode, cyanosis, oxygen desaturation and nausea approximately 30 min after receipt of PCT-PLT. The patient received oxygen therapy to treat the event and recovered. The patient had received two units of PCT-PLT before and one unit after this event with no adverse reactions. The patient had a history of hypotensive episodes, which occurred in the absence of transfusions. Based on the patient's history, the event was assessed by the investigator as probably unrelated to the PCT-PLT transfusion.

Risk factors associated with adverse event

The risk for AE was not correlated with the patient gender, age, or antigen-matching. The risk for AE for patients who already had been transfused before the first PCT-PLT transfusion appeared trending higher compared to patients who did not have any transfusion history; however, the difference did not reach statistical significance ($P = 0{\cdot}0675$; odds ratio: 1·875; 95% CI: 0·956–3·648). Buffy-coat-derived platelets were associated with a lower risk for AE compared to apheresis products ($P = 0{\cdot}0305$; odds ratio: 0·473; 95% CI: 0·240–0·932). Irradiated PCT-PLTs were of similar risk for AE compared to non-irradiated PCT-PLTs ($P = 0{\cdot}0848$; odds ratio: 6·344; 95% CI: 0·776–51·862). No trending can be concluded because, of the total 7437 platelet transfusions, only 80 PCT-PLT components were γ-irradiated in EFS-Bretagne and EFS-Auvergne-Loire. Haematology–oncology patients treated with conventional chemotherapy were at a higher risk for AE compared to the other patients ($P \leq 0{\cdot}0001$; odds ratio: 7·660; 95% CI: 3·014–19·467).

Number of transfusions prior to the first adverse event

Among the 45 patients who experienced at least one AE, repeated exposure to PCT-PLT did not appear to increase the likelihood of a transfusion reaction (Table 3). By using the non-survival analysis method (a subset analysis for patients with any AE only), the mean number of transfusions before first AE occurrence was 8·8 ± 10·1 (median = 4, minimum = 0 and maximum = 37).

Discussion

In accordance with the recommendations made by the panel of the Canadian Consensus Conference, an active haemovigilance programme has been implemented in Europe to document the occurrence of AE following transfusion of PCT-PLT [6]. To date, two reports have been prepared. The first report was on the transfusion of 5106 PCT-PLT components administered to patients in five European centres from October 2003 to December 2005 [4]. The second report as described here was on additional 7437 transfusions of PCT-PLT administered to patients in seven European centres between May 2005 and January 2007. This represents a total of 12 543 independent transfusions documented to date. There are no overlaps of PCT-PLT transfusions reported in this haemovigilance programme.

Overall, the incidence of ATR attributed to transfusion of PCT-PLT in both of the haemovigilance reporting periods was infrequent either on a per-transfusion basis (0·8% first period

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Table 3 Number of PCT-PLT transfusions per patient prior to the first adverse event (AE)

Number of PCT-PLT transfusions per patient until first occurrence of AE	Full analysis population (n = 1400)
1	11 (0·79%)
2	6 (0·43%)
3	3 (0·21%)
4	3 (0·21%)
5	1 (0·07%)
6–10	9 (0·64%)
11–19	6 (0·43%)
≥ 20	6 (0·43%)
N (non survival analysis method)	45
Mean ± SD	8·8 ± 10·1
Median	4
Minimum–maximum	0–37

vs. 0·7% second period) or on a per-patient basis (4·9% first period vs. 2·8% second period). The slightly higher occurrence of ATR per patient in the first reporting period was not surprising, because the mean number of transfusions per patient (7·8 ± 16·2) [4] was greater than those observed in the second period (5·3 ± 10·8). All ATRs were mild in severity and of Grade 1 or lower. No serious AE from both study periods were attributed specifically to transfusion of PCT-PLT.

On a per-transfusion basis, the prevalence of ATR has been reported in the literature to range from 18 to 31%; however, these studies were conducted some years ago with variable methods of platelet preparation [10–13]. More recently, the incidence of moderate and severe ATR has been reported from the trial to reduce alloimmunization to platelets (TRAP) study, which examined 8769 platelet transfusions in 598 patients during induction therapy for acute leukaemia [14]. In the TRAP study, platelet components were prepared by four methods: unfiltered pooled whole blood-derived platelets in plasma; filtered pooled whole blood-derived platelets in plasma; unfiltered pooled whole blood-derived platelets in plasma treated with ultraviolet B illumination to reduce human leucocyte antigen sensitization; and filtered apheresis platelets in plasma. None of these components were prepared with additive solutions. The overall incidence of ATR was 2·2% of transfusions, and 22% of patients experienced at least one ATR. In comparison to the TRAP trial, the current study in which all grades of reactions were reported, both the proportion of transfusions associated with a reaction was lower (0·7%) as well as the proportion of patients (2·8%) experiencing at least one ATR. The use of 65% InterSol, a platelet additive solution, in the preparation of PCT-PLT may partially contribute to the reduction in the observed incidence of ATR [15].

The incidence of ATR in this study can be compared to data from the haemovigilance network in France [7]. In France, data were reported for transfusion reactions, with an incidence of four events per 1000 platelet components (0·4%), during 2 years in which the reporting system was first implemented. However, this may be an underestimate since each whole blood platelet concentrate in a pool was tabulated as an individual component transfusion. More recently, Kerkhoffs *et al.* [16] compared the incidence of transfusion reactions for leucoreduced pooled platelet components in plasma and plasma with additive solution in a study of 168 patients and 765 transfusions. They observed an incidence of 5·5% of transfusions with reactions for platelets in plasma vs. 2·4% of transfusions for platelets in a mixture of plasma and additive solution. On a per-patient basis, 9·5% of patients transfused with platelets in plasma plus additive solutions had reactions compared to 15·5% of patients supported with platelets suspended in plasma. These results further support the role of the platelet additive solution, InterSol, in the reduction of ATR observed in this study.

During the conduct of this study, an interim analysis of 2497 PCT-PLT transfusions administered to 606 patients in the three regions of France (EFS-Alsace, EFS-Auvergne-Loire and EFS-Bretagne) was performed [5]. Of the 606 patients, the predominant recipients of PCT-PLT were haematology–oncology patients (46·2%); 39·9% treated with chemotherapy and 6·3% treated with stem cell transplantation. These proportions were only slightly lower than those in the overall study population of 1400 patients, yet only four of the 606 patients (0·7%) reported an AE, including one serious AE of volume overload classified as unrelated to PCT-PLT transfusion. This low rate of AE observed in the French regions could contribute to the overall low incidence of ATR per patient in this study.

Premedication in patients did not play a role in the overall low incidence of ATR reported in this study. Information on premedication was only requested in case of AE occurrence. Of the 68 transfusions with occurrence of at least one AE, only two antipyretic, two antihistaminic and one corticosteroid were prescribed to patients. For the majority (64/68, or 94·1%) of these transfusions, patients were not premedicated.

The active haemovigilance programme described here is a prospective observational study, which was designed to assess the safety profile of PCT-PLT in routine clinical practice. The data from this programme represent the largest prospective experience to date for recording potential AE associated with platelet transfusions compared to prior studies of retrospective design and limited in size [10,16–18]. The present study was designed to be consistent with European haemovigilance practices in which reporting of all grades of transfusion-associated reactions has been emphasized [7,8]. In contrast to other haemovigilance studies, obligatory reporting for all platelet transfusions was required irrespective of whether or not an AE was observed. The current study focused on AE that could be linked to PCT-PLT transfusions after starting transfusion, but there were no specific limitations

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on when adverse events could be reported following transfusion. Based on the patient population supported with platelet transfusion, the study was designed to capture repeated transfusions of PCT-PLT within patients to determine potential effects of repeated exposure to this new type of platelet component.

A limitation of the present study is the absence of a concurrent control group receiving conventional platelet components with which to determine a comparative baseline incidence of ATR. However, because reporting is obligatory, the expected outcomes of this active haemovigilance study are the increase in clinical experience with transfusion of PCT-PLT, the detection of unexpected AE following PCT-PLT transfusions in patient populations and for indications that were not studied previously in a formal clinical trial environment, and the establishment of a safety database for future reference.

In the current study, which was specifically designed to capture all grades of transfusion reactions, the prevalence of ATR per transfusion, was at the lower range of those reported in studies with conventional components. Prior exposure to PCT-PLT transfusions did not increase the likelihood of an ATR. The overall incidence of ATR was lower than that previously reported either on a per-transfusion or on a per-patient basis. Based on experience in a broad patient population, platelet components prepared with amotosalen photochemical treatment were well-tolerated in routine clinical practice.

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