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)
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

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

ヘモビジランス①（2003 年 10 月～2005 年 12 月）

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。

ヘモビジランス②（2005 年 5 月～2007 年 1 月）

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。

ヘモビジランス③ フランス（Ile de La Reunion）

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

ヘモビジランス④ フランス（Alsace）（2006年 9 月～2007 年 8 月）

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。

ヘモビジランス⑤

輸血数 4000PC 2008 年 6 月 International societ of blood transfusion で発表予定

ヘモビジランス⑥ ドイツ

輸血数 5000PC 2008 年より開始

2）血漿

ヘモビジランス①

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。

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 aftermarket 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.

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.

From Cerus Corporation, Concord, California.

Address reprint requests to: Tim McCullough, Cerus Corporation, 2411 Stanwell Drive, Concord, CA 94520; e-mail: tim_mccullough@cerusCorp.com.

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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

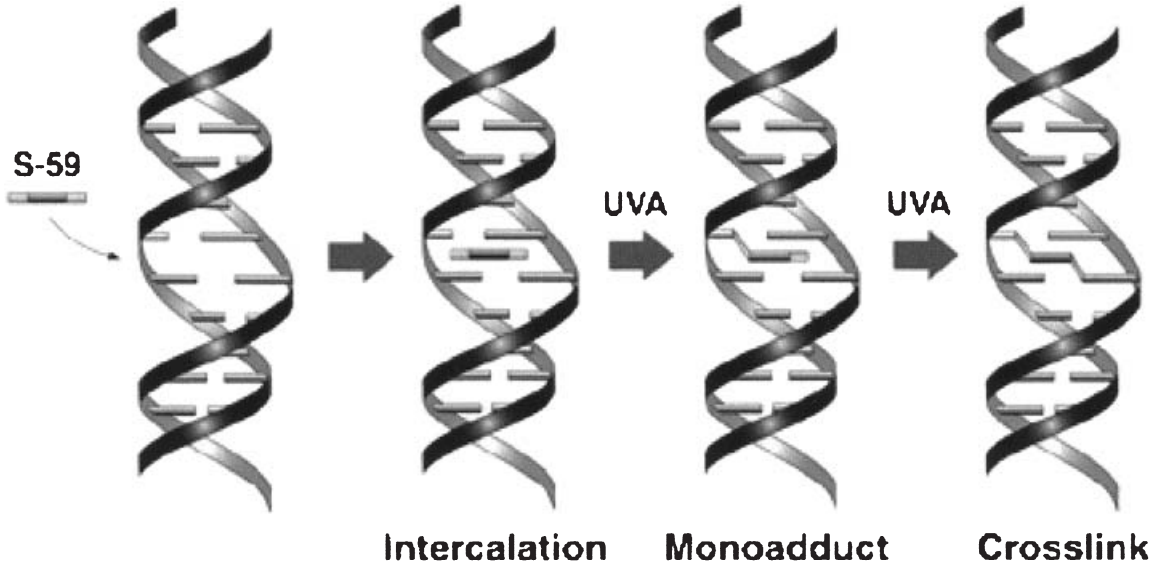

Fig. 1. Mechanism of action of PI. Amotosalen (S-59) is a synthetic psoralen that inhibits nucleic acid replication through UVA light-mediated covalent addition to nucleic acids. 1) The molecule is designed to penetrate cells, viruses, bacteria, or other pathogens and seek out DNA or RNA. 2) Amotosalen 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.

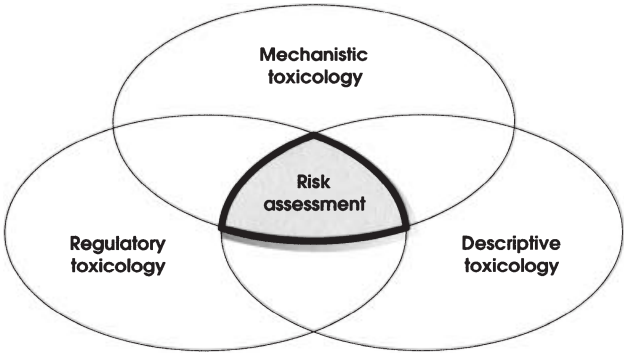

Fig. 2. The interconnections between different areas of toxicology.

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. Byproducts 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).

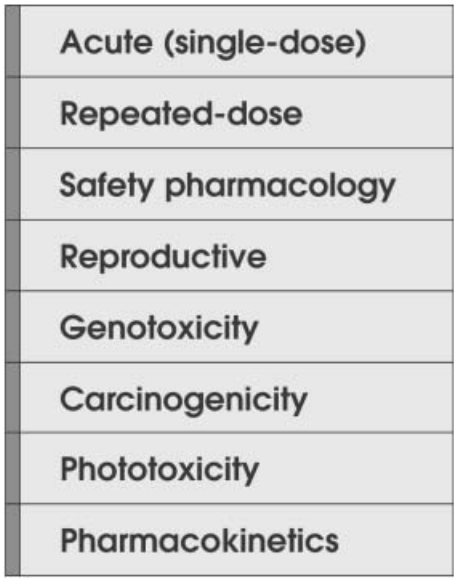

Fig. 3. Studies conducted in the preclinical safety evaluation of the INTERCEPT Blood System for PLTs.

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]

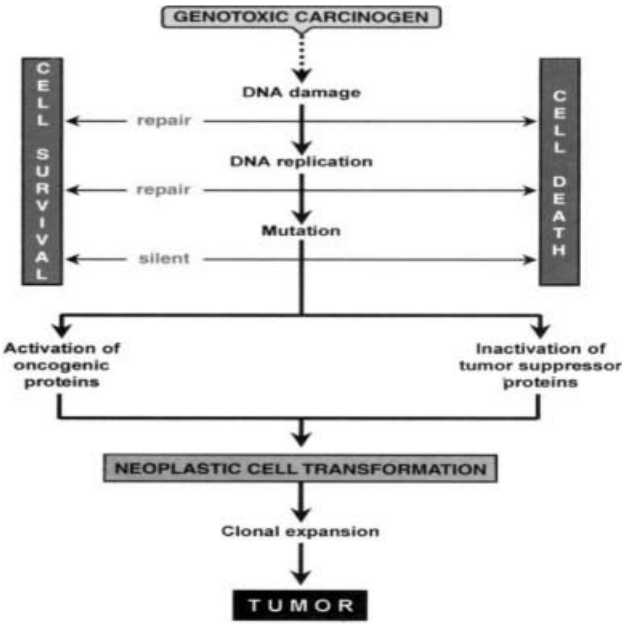

Fig. 4. The process of carcinogenesis initiated by genotoxic carcinogens.[27]

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-

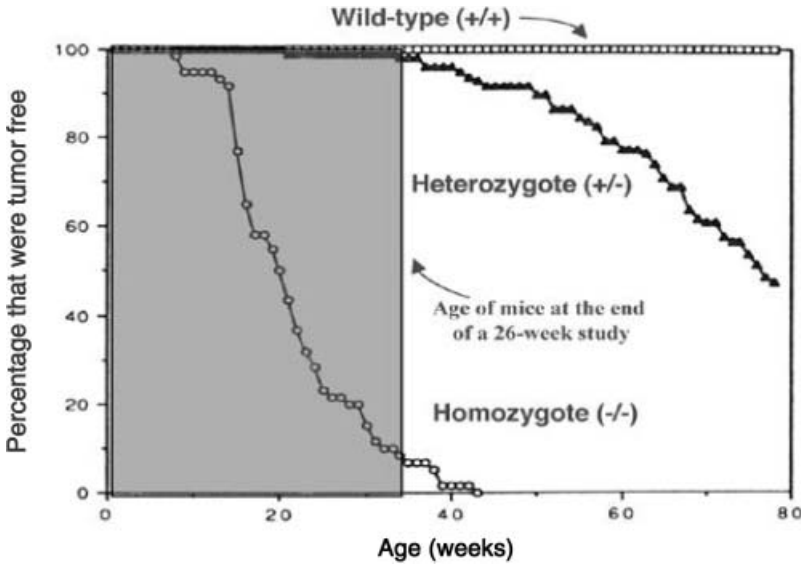

Fig. 5. Spontaneous tumor incidence in p53-deficient mice. (□) surviving wild type mice ($p53^{+/+}$); (▲) heterozygous mice ($p53^{+/-}$); and (○) homozygous mice ($p53^{-/-}$).[18]

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-

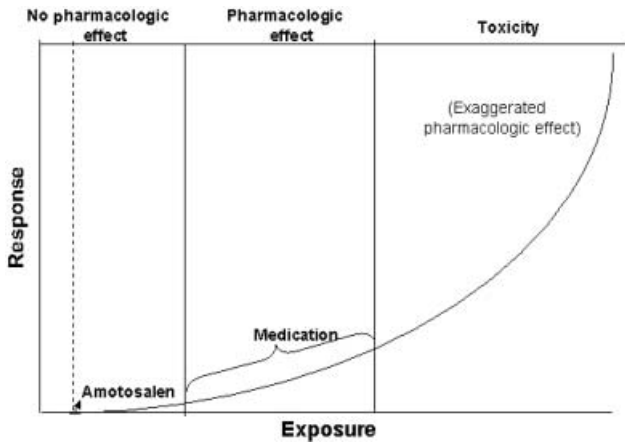

Fig. 6. Biologic response of a NCE ranges from no pharmacologic effect to desired pharmacologic action to toxicity. Amotosalen has no pharmacologic effects when administered at large multiples of clinical exposure.

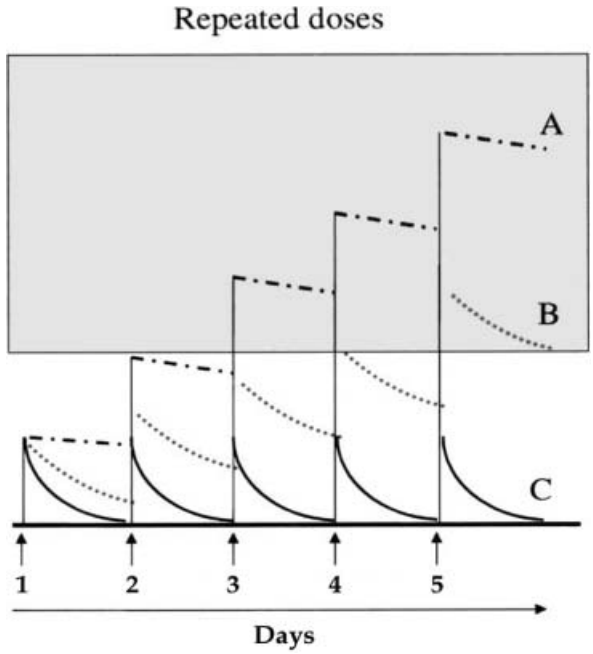

Fig. 7. Relationship between dose and concentration at the target site under different conditions of elimination rate. Lines A, B, and C represent chemicals with different t_{50} ranging from a long t_{50} to a short t_{50}, respectively. Shaded area is representative of the concentration of a chemical 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

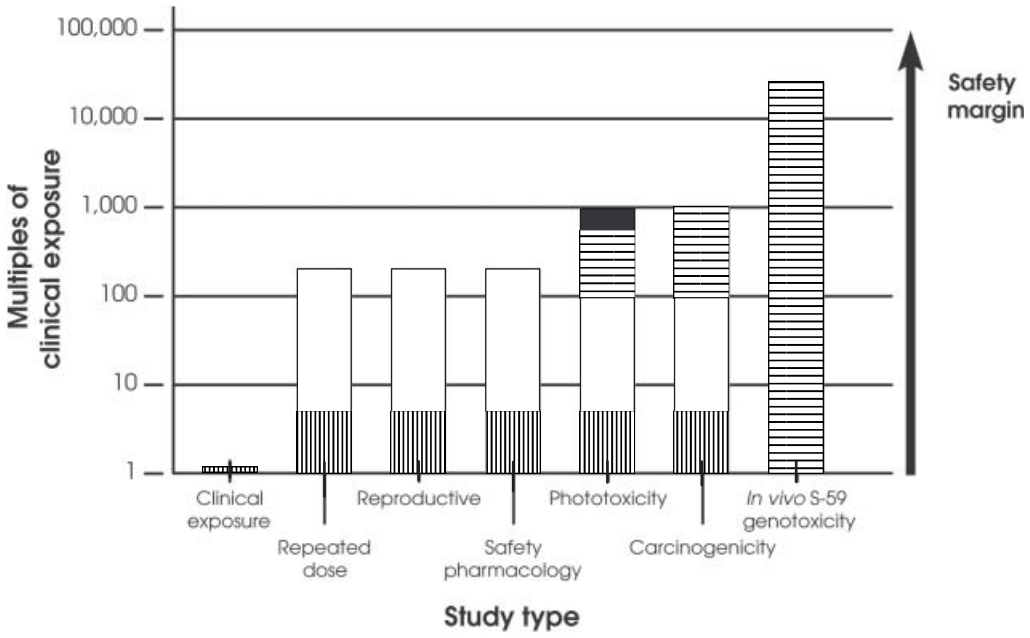

Fig. 8. Safety margins from toxicology studies compared to the clinical exposure. (■) Toxicity observed; (▤) no UVA; (□) no CAD; (▥) with CAD.

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

Patrice Rasonglès, Marie France Angelini-Tibert, Philip Simon, Caroline Currie, Herve Isola, Daniel Kientz, Marc Slaedts, Michele Jacquet, David Sundin, Lily Lin, Laurence Corash, and Jean Pierre Cazenave

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.

Address reprint requests to: Laurence Corash, MD, Cerus Corporation, 2411 Stanwell Drive, Concord, CA 94520; e-mail: lcorash@cerus.com.

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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.