Table 4
Scrapie infectivity in samples generated during the P-15N/AT study

	Before filtration			Filtrate		
	Animal number			Animal number		
	1	2	3	1	2	3
Appearance of clinical signs (day euthanized)	87	87	87	94	143	105
PrP^{Sc} in brain by WB3	Detected	Detected	Detected	Detected	Detected	Detected
Lesions by histopathology	+ve	+ve	+ve	+ve	+ve	+ve
Medulla (oblongata)	D,V,P	D,V,P	D,V,P	D,V,P	D,V,P	D,V,P
Cerebellum (cortex)	D	D,V,P	D,V,P	D,V,P	D,V,P	D,V,P
Midbrain	D,P	D,V,P	V,P	D,P	D,P	D,V,P
Hypothalamus	D,P	D,V,P	D,P	D,V,P	D,P	D,P
Thalamus	D,P	D,V,P	D,P	D,P	D,P	D,P
Hippocampus	NR	D,V	D	D	D,V,P	D,V
Paraterminal body	D,P	D,P	D,P	NR	D,V,P	P
Cerebral cortex (posterior midline)	D,P	D,P	D,P	D,P	D,V,P	D,V,P
Cerebral cortex (anterior midline)	D,P	D,V,P	D,V,P	D,V,P	D,V,P	D,V,P

Abbreviations used: +ve, scrapie positive; NR, no remarkable change; D, degeneration of nerve cell; V, vacuolation; P, proliferation of glial cell.

corresponding hamster brain material on histopathological observation (Table 4). Typical nerve lesions are shown in Fig. 3. Thus, P-15N filtration did not result in the complete removal of infectivity, for this process step.

4. Discussion

In this study, we have investigated the capacity of P-35N, P-20N and P-15N filters to remove the 263K scrapie prion protein, PrP^{Sc}, under the conditions used for the manufacture of four different plasma-derived products, using spike preparations designed to present a serious challenge to the filters.

Validation studies to evaluate the capacity of manufacturing processes to remove potential contaminants, including prions, are required for biological or biopharmaceutical products intended for human use. When designing these studies, a worst-case challenge should be used wherever possible, to minimize the risk of over-estimating the capacity of the process to remove such contaminants. Virus removal filters (or nanofilters) are designed to remove contaminants predominantly on the basis of size. The worst-case challenge for such steps should therefore be a preparation containing the smallest possible form of the infectious agent.

TSE clearance studies provide a particular challenge in that the nature of the infectious agent is still uncertain, and the forms of infectious agent present in plasma, and/or during the different stages of a manufacturing process, are not clearly understood. The causative agent of TSE diseases is believed to be strongly associated with, if not solely composed of, the disease-associated prion protein, PrP^{Sc}. Normal cellular PrP is a membrane-bound glycoprotein, which associates with membranes through a glycosylphosphatidylinositol (GPI) anchor. Prion infectivity is associated with heterogeneous particles, including membranes, liposomes and protein aggregates, so called prion rods. Therefore, methods which result in solubilization of membrane proteins, or dispersal of membrane fragments, vesicles and/or protein aggregates, may be expected to reduce the size of particles associated with prion infectivity.

Treatment of MF preparations derived from brains of uninfected (normal) hamsters with either detergent (0.1% lysolecithin or 0.1% sarkosyl) or extensive sonication ("supersonication") resulted in a rapid reduction in the average particle size, to approximately 100 nm. SD treatment (1% Tween 80 and 0.3% TNBP for 6 h) also resulted in a reduction in particle size, although this was slower and less effective, reducing the average particle size to the order of 200 nm.

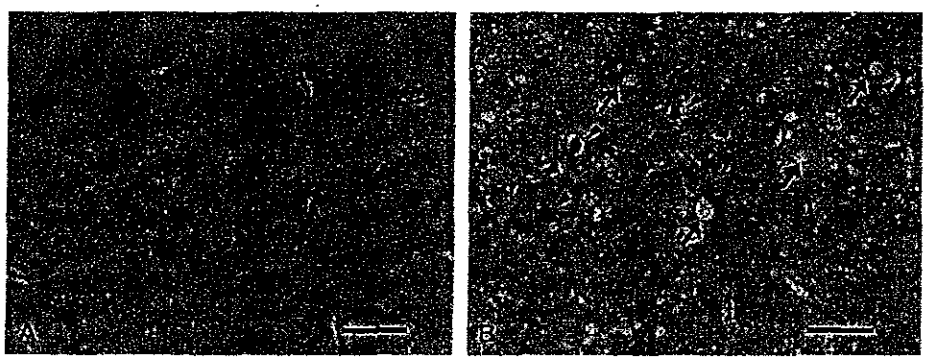

Fig. 3. Typical nerve lesions in the hippocampus of a hamster brain, taken from an animal inoculated with a P-15N-filtered sample (B), in comparison with the corresponding region from an uninfected animal (A). Arrows, vacuolation; Arrowheads, degeneration of nerve cells; scale bar = 50 μm; HE staining used.

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"Super-sonication" has the advantage that it is a physical disruption process, and does not alter the chemical composition of the spike material, thus minimizing changes to the start material used for nanofiltration. SD treatment is included in many manufacturing processes for plasma-derived products, and therefore, although not as effective as "super-sonication", use of this treatment might be expected to result in a spike material more closely mimicking the form of infectious prion present in the relevant start material during the manufacturing process. Use of these treatments alone or in combination may therefore be useful in reducing the size of infectious particles present in TSE spike materials for prion clearance studies.

The effect of the above treatments was studied using normal MF, as the facility was unable to handle infectious TSE materials. Although some care should be taken in extrapolating these results to TSE-infected brain material, "super-sonication" of 263K MF preparations appeared to reduce the removal of PrP^{Sc} following filtration, while detergent-treated spike preparations have previously been shown to present a more significant challenge to nanofiltration steps than untreated preparations ([9,10] and own unpublished observations). Furthermore, "super-sonication", with or without SD treatment, does not appear to reduce the level of infectivity present within the 263K MF, supporting the use of such preparations for prion clearance studies.

Using 263K MF treated with 0.1% sarkosyl, "super-sonication" or SD plus "super-sonication", we investigated the prion removal capacity of P-15N, P-20N and P-35N filters in the manufacturing processes used for four different plasma products. The results obtained suggest that both the composition of the materials to be filtered and the prion load influences the removal of prions. PrP^{Sc} was recovered in the filtrate fraction from three out of the four processing steps performed for P-20N and P-35N. In contrast, under all conditions tested, P-15N filtration resulted in removal of PrP^{Sc} to below the limit of detection of the Western blot assays used. Thus, P-15N would appear to be a more robust method for the removal of prions, reproducibly giving LRF in the order of 3 logs, under the conditions tested. In practice, however, it is not feasible to incorporate P-15N filtration into the manufacturing process for all plasma derivatives. From the results shown in Table 2, it may also be possible to optimize processing conditions to allow effective removal of PrP^{Sc} using P-20N or P-35N filters.

WB assays were used to monitor the partitioning of PrP^{Sc} during the nanofiltration processes. WB assays are semi-quantitative and serve to provide an indication of the relative levels of PrP^{Sc} present in different samples. However, there are limitations to the sensitivity of available WB assays, and these assays provide only an indirect measure of infectivity. Therefore, to confirm that removal of PrP^{Sc} does reflect removal of infectivity, bioassays need to be performed.

Although PrP^{Sc} was not detected in any of the P-15N filtered samples by WB assay, infectivity was recovered in a filtrate fraction tested by bioassay for one process run. Foster also noted that infectivity was detected in a filtrate fraction after P-15N filtration ([8] reported as personal communication; data not shown). Thus, even with P-15N, depending on the processing conditions, there may be incomplete removal of prion contaminants.

Although infectivity was detected in the filtrate fraction from the one process step studied, longer and more variable incubation periods were observed in the animals inoculated with the filtrate sample (Table 4), suggesting a lower prion titer following filtration. However, it was not possible to estimate the relative levels of prion infectivity present in the input and filtrate samples, as no data was available to correlate incubation periods and prion titers for this study. Based on the titers typically observed for 263K MF stocks, the bioassay used could theoretically detect reductions in prion infectivity in the order of 4 logs for this process step. Detection of infectivity in the filtrate fraction by bioassay is therefore not necessarily incompatible with the WB results obtained (LRF $\geq$2.8 logs), and may simply reflect a difference in sensitivity between the two assays used.

As discussed above, uncertainties about the nature of the infectious agent in plasma, and during the manufacturing process, raise concerns about the design and interpretation of prion clearance studies. No single spike preparation is likely to contain all potential forms of the infectious agent. Infectivity is associated with membranes and protein aggregates. In addition, it has recently been shown that the GPI anchor is not required for infectivity, suggesting that endogenous proteolytic release of PrP^{Sc} from cell surfaces may also contribute to the spread of the infectious agent *in vivo* [19,20]. Whether significant levels of infectivity in human plasma are associated with GPI-anchorless prion protein is not yet clear. These different forms of infectivity, with different biophysical properties, could show different partitioning properties through the same manufacturing process [7]. Furthermore, different forms of the agent may differ in their level of infectivity. For example, it was recently reported that particles in the order of 17–27 nm appeared to have the highest relative level of infectivity, in comparison to levels of PrP^{Sc} [21]. Therefore, a better understanding of the nature and forms of the infectious agent is essential to allow the design of more accurate models for prion clearance studies, and a more confident evaluation of the safety of manufacturing processes with respect to potential TSE contamination.

In summary, we used methods intended to reduce the size of particles present within MF preparations in an effort to present a worst-case (smallest) prion challenge during nanofiltration. Using such preparations, P-15N filtration consistently reduced the level of PrP^{Sc} to below the limits of detection of the Western blot assays used, suggesting that this process step is effective for the removal of prions. However, data from a single process step studied suggested that infectivity could be recovered following P-15N filtration, and thus even P-15N filtration may not result in complete removal of prions, at least when used under some conditions.

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A part of this study was presented at the Planova workshop 2003 and 2006 held by Asahi Kasei. Asahi Kasei Medical Co., Ltd. kindly gave us permission to publish the entire study on

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Planova filters. Some of the data presented in this study has been summarized in a recent review [22].

References

[1] Health Protection Agency. Fourth case of variant CJD infection associated with blood transfusion. Press release, http://www.hpa.org.uk/hpa/news/articles/press_releases/2007/070118_vCJD.htm 18 January, 2007.
[2] Castilla J, Saá P, Soto C. Detection of prions in blood. Nature medicine 2005;11(9):982–5.
[3] European Medicines Agency/The Committee for Medicinal Products for Human Use (CHMP)/Biotechnology Working Party. CHMP position statement on Creutzfeldt–Jakob disease and plasma-derived and urine-derived medicinal products. EMEA/CPMP/BWP/2879/02/rev 1. London, http://www.emea.europa.eu/pdfs/human/press/pos/287902rev1.pdf 23 June, 2004.
[4] The European Agency for the Evaluation of Medicinal Products/The Committee for Medicinal Products for Human Use (CHMP)/Biotechnology Working Party. Guideline on the investigation of manufacturing processes for plasma-derived medicinal products with regard to vCJD risk. CPMP/BWP/5136/03 London, http://www.emea.europa.eu/pdfs/human/bwp/513603en.pdf 21 October, 2004.
[5] Strengthening of quality and safety assurance of drugs and medical devices manufactured using components of human origin as raw materials. PFSB notification no.0209003 dated February 9, 2005; Japan: MHLW (in Japanese).
[6] Stenland CJ, Lee DC, Brown P, Petteway Jr SR, Rubenstein R. Partitioning of human and sheep forms of the pathogenic prion protein during the purification of therapeutic proteins from human plasma. Transfusion 2002;42(11):1497–500.
[7] Vey M, Baron H, Weimer T, Gröner A. Purity of spiking agent affects partitioning of prions in plasma protein purification. Biologicals 2002; 30(3):187–96.
[8] Foster PR. Removal of TSE agents from blood products. Vox Sang 2004; 87(S2):7–10.
[9] Tateishi J, Kitamoto T, Ishikawa G, Manabe S. Removal of causative agent of Creutzfeldt-Jakob disease (CJD) through membrane filtration method. Membrane 1993;18(6):357–62.
[10] Tateishi J, Kitamoto T, Mohri S, Satoh S, Sato T, Shepherd A, et al. Scrapie removal using Planova® virus removal filters. Biologicals 2001;29(1): 17–25.
[11] Van Holten WR, Autenrieth S, Boose JA, Hsieh WT, Dolan S. Removal of prion challenge from an immune globulin preparation by use of a size-exclusion filter. Transfusion 2002;42(8):999–1004.
[12] Kimberlin RH, Walker CA. Characteristics of a short incubation model of scrapie in the golden hamster. J Gen Virol 1977;34(2):295–304.
[13] Kascsak RJ, Rubenstein R, Merz PA, Tonna-DeMasi M, Fersko R, Carp RI, et al. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J Virol 1987;61(12):3688–93.
[14] Lee DC, Stenland CJ, Hartwell RC, Ford EK, Cai K, Miller JLC, et al. Monitoring plasma processing steps with a sensitive Western blot assay for the detection of prion protein. J Virol Methods 2000; 84(1):77–89.
[15] Fraser H, Dickinson AG. The sequential development of the brain lesions of scrapie in three strains of mice. J Comp Pathol 1968;78(3):301–11.
[16] Kärber J. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak 1931;162:480–3.
[17] Somerville RA, Carp RI. Altered scrapie infectivity estimates by titration and incubation period in the presence of detergents. J Gen Virol 1983; 64(9):2045–50.
[18] Taylor DM, Fernie K. Exposure to autoclaving or sodium hydroxide extends the dose-response curve of the 263K strain of scrapie agent in hamsters. J Gen Virol 1996;77(4):811–3.
[19] Lewis PA, Properzi F, Prodromidou K, Clarke AR, Collinge J, Jackson GS. Removal of the glycosylphosphatidylinositol anchor from PrP^{Sc} by cathepsin D does not reduce prion infectivity. Biochem J 2006; 395:443–8.
[20] Trifilo MJ, Yajima T, Gu Y, Dalton N, Peterson KL, Race RE, et al. Prion-induced amyloid heart disease with high blood infectivity in transgenic mice. Science 2006;313(5783):94–7.
[21] Silveira RJ, Raymond JG, Hughson GA, Race ER, Sim LV, Hayes FS, et al. The most infectious prion protein particles. Nature 2005;437(7056): 257–61.
[22] Yunoki M, Urayama T, Ikuta K. Possible removal of prion agents from blood products during the manufacturing processes. Future Virol 2006; 1(5):659–74.

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研究報告の概要

英国血漿由来の第 XI 因子製剤が、1989-2000 年の間に米国内の様々なサイトにおいて新薬臨床(IND)試験に参加した 50 人以下と推定される少数の患者の治療に使用された。使用された英国製第 XI 因子製剤はいずれも、後で vCJD と診断されたドナーからの血漿を含む血漿プールからは製造されていなかった。しかしながら、英国の血漿提供者を含む英国人集団は、リスク評価は広範囲に変わるとはいえ、BSE への食物連鎖曝露により、米国人集団よりも vCJD リスクは非常に高い。
100 万人当り 4 人まで vCJD 感染者が存在するとする低い方の症例ベースの感染者推定値では、平均して約 1.6%のみの血漿プールが vCJD 病原体を含んでいる可能性があると予測できる。一方、一つの組織の調査に基づく 4,225 人に 1 人の英国人に vCJD 感染者がいるとする高い方の感染者推定値では、50%の血漿プールに vCJD に汚染されたヒト由来の血漿ドネーションが含まれていた可能性があると予測された。そして、げっ歯類動物モデルの実験結果をもとにした患者 1 人が第 XI 因子の治療当り受ける vCJD 感染リスクは、原料に用いた血漿ドナーに低い方の感染者推定値及び高い方の感染者推定値を適用し計算すると、各々①シナリオ 1(投与量が 3,000u の場合)：1/643、1/17、②シナリオ 2(投与量が 9,000u の場合)：1/214、1/5.6、③シナリオ 3(投与量が 15,000u の場合)：1/129、1/3.4 であった。
このモデルの結果は、vCJD 病原体の暴露があった可能性があり、潜在的感染リスクがあることを示唆しているものの、英国血漿から製造された第 XI 因子製剤を 2000 年まで使用した個々の患者に対して、本当に意味があり正確な vCJD リスクの推定値を提供することは不可能である。疫学的モデルを元にした英国 vCJD 感染率推定値が正しいと仮定すると、曝露の可能性及び感染リスクはかなり低い。また、リスクが低いにもかかわらず、リスク評価に使用できる情報の不足と不確かさから、実際のリスク評価は難しい。なお、このリスク評価の結果を考慮する際、血漿由来製剤の投与を受けた患者において、これまで世界中で一件も vCJD の症例が報告されたことがないことに注意を向けることが大切である。この中には、長い期間に亘って、英国の血漿ドネーションから製造された他の製剤を大量に投与された患者に加えて本製剤を投与された患者が存在する。
このリスク評価モデルは、製造工程の vCJD 病原体のクリアランス、ヒトの製剤使用量、脳内接種に対して静注ルートの効率及び英国ドナーの vCJD 感染陽性率がリスクに影響する最も重要な要因であると指摘している。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 略
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2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
英国血漿由来の第 XI 凝固因子製剤の投与を受けた米国レシピエントにおける vCJD 病原体に対する FDA のリスク評価案である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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FDA DRAFT Risk Assessment: Potential Exposure to the variant Creutzfeldt-Jakob Disease Agent in United States Recipients of Factor XI Coagulation Product Manufactured in the United Kingdom

November 7, 2006

Center for Biologics Evaluation and Research
US Food and Drug Administration

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EXECUTIVE SUMMARY

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease attributed to human infection with the agent of bovine spongiform encephalopathy (BSE) and is most often transmitted by the consumption of beef products from infected cattle. Cases of vCJD were first reported in humans in the UK in 1996, and as of August 2006, 195 cases have been reported worldwide, with 162 cases in the UK. Since December 2003, there have also been three reports in the United Kingdom (UK) of probable vCJD transmission by red blood cell transfusions. The donors were healthy at the time of donation, but later developed vCJD. Of the three red blood cell recipients who probably became infected with the vCJD agent after transfusion, two developed vCJD and died from the disease. The third died of an unrelated illness

The probable transmission of vCJD via red blood cell transfusions raised the possibility that plasma derivatives might also pose a risk of vCJD transmission, although there have as of yet been no reported cases of vCJD in any recipients of plasma derivatives in the UK, where the risk is considered greatest, or elsewhere. UK authorities have notified physicians in the UK and their patients who received plasma derivatives made from plasma from UK donors, about the potential for risk of vCJD from these products. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins. The derivatives of concern were manufactured from plasma collected from UK donors from 1980 through 1997. In 1998 UK manufacturers stopped using UK plasma. The last expiry date for any of the UK products was in 2001.

Problem: Some Factor XI (FXI) made from UK plasma was used between 1989 and 2000 to treat a small number of patients, estimated to be 50 or fewer, who participated in several Investigational New Drug (IND) studies at various sites in the US. No FXI product used in the US was manufactured from a pool containing plasma from a donor diagnosed with vCJD (that is, there were no known "implicated" lots). However, the UK population, including UK plasma donors, is at a considerably higher risk for vCJD than the US population due to food chain exposure to BSE, although the estimates of risk vary widely.

Question addressed by risk assessment: *Given the probable recent transmission of vCJD via transfusion of red blood cells, what is the estimated range of potential risk to US recipients who received a human plasma derived FXI product manufactured from UK plasma?*

FDA presented the "Draft Risk Assessment: Potential Exposure to the vCJD Agent in United States Recipients of Factor XI Coagulation Product Manufactured in the United Kingdom" at the February 8, 2005 meeting of the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) for review and comment. The risk assessment computer model predicted that recipients of UK manufactured FXI may have potentially been exposed to the vCJD agent, and although the risk of possible vCJD infection could potentially be significant, the actual risk is highly uncertain and could be low. On October 31, 2005 FDA sought advice and discussion on several risk assessment model inputs for plasma derivatives and potential vCJD risks. FDA has incorporated comments and advice provided by the TSEAC at the February 8, 2005 and October 31, 2005 meetings, and by FDA staff and Special Government Employee peer reviewers to develop this revised risk assessment (revisions summarized in Table 1 of the Risk Assessment) of potential vCJD risks for US recipients of FXI manufactured from UK donor plasma.