the human situation might be greater. This is because the form of TSE infectivity in the blood of an infected animal is more likely to resemble that found in human blood, as compared to brain or spleen derived spiking material. Because of the very small amounts of agent likely to be present in endogenously TSE-infected blood, any failure of complete TSE clearance would be highly significant.

On September 18, 2006, the Committee was asked to comment whether a minimum TSE reduction factor, demonstrated using an exogenous model in scaled-down manufacturing experiments, might serve as an appropriate standard for demonstrating vCJD safety of pdFVIII products. The TSEAC was also asked to comment on what actions FDA might consider if such a minimum TSE reduction factor were not achieved. The discussion was deferred to the current meeting so that the Committee could respond in the context of recently completed FDA risk assessments.

Discussion

To determine a likely appropriate threshold level of TSE clearance for pdFVIII, two separate lines of evidence should be considered: the amount of clearance needed to assure that infectivity is removed (based on amount of starting infectivity in plasma), and the impact of clearance results on the pdFVIII risk assessment.

In the somewhat similar case of viral clearance validation studies, typical results accepted by FDA in support of label claims usually demonstrated at least 4 $\log_{10}$ of clearance by each of two mechanistically dissimilar (orthogonal) steps.[2] In the case of TSEs, the amount of infectivity in blood or plasma of experimentally infected animals has been estimated as 2-30 intracerebral infectious units (i.c. IU)/mL (2-4). An IU is the quantity of infectivity associated with a 100% probability of infection in recipients. An ID_{50} is the amount of infectivity associated with a 50% probability of infection in recipients. Therefore 1 IU = 2 ID_{50} . The amount of infectivity in the blood of BSE-infected and scrapie-infected ruminants and in the blood of vCJD-infected persons is unknown. If vCJD infectivity levels in human blood are similar to those found in rodent blood or plasma, then effective clearance might necessitate a reduction of infectivity by at least 4 $\log_{10}$, plus an additional margin of safety. Calculations of pathogen reduction are based upon removal of the absolute amount of infectivity, rather than upon infectivity concentration. For example, a plasma unit of 800 ml that contained infectivity of 2-30 IU/ml would contain 1,600 - 24,000 IU (3.2 - 4.4 $\log_{10}$).[3] A precise margin of safety for TSE clearance studies is difficult to specify, given current limitations in test methodology and uncertainties about the maximum infectivity titers in blood of asymptomatic vCJD-infected donors. In viral studies, an additional margin of safety that assures clearance of at least 2-3 $\log_{10}$ more than the highest anticipated titers of the viral pathogen has often been considered prudent.

The pdFVIII risk assessment provides additional information about TSE clearance and risk of vCJD exposure. The risk assessment was performed using $\log_{10}$ clearances of 2-3, 4-6, and 7-9. The level of risk is highly impacted by the amount of clearance achieved in product manufacturing. For example, assuming a higher prevalence of vCJD based on the UK tissue survey, a patient with severe hemophilia A who has no inhibitors and is on episodic treatment with pdFVIII is estimated to have a potential mean annual risk of vCJD of 1 in 159 if exposed to

[2] Estimated maximum levels of viremia range from 10^4 to 10^7 for enveloped viruses e.g. HIV-1, HCV, and HBV (5), and from 10^5 to 10^{10} for non-enveloped viruses, HAV(6) and B19 (7) virus.

[3] Plasma collection volumes recommended by FDA are 625-800 mL, depending upon the donor's weight (http://www.fda.gov/ber/bldmem/110492.pdf).

a product with 2-3 $\log_{10}$ vCJD reduction, 1 in 105,000 if exposed to a product with 4-6 $\log_{10}$ vCJD reduction, and 1 in 100 million if exposed to a product with 7-9 $\log_{10}$ vCJD reduction (Attachment 1). Assuming a lower prevalence of vCJD based on the number of cases that have occurred and are projected to occur in the UK, a patient with severe hemophilia A who has no inhibitors and is on episodic treatment with pdFVIII is estimated to have a potential mean annual risk of vCJD of 1 in 21,500 if exposed to a product with 2-3 $\log_{10}$ vCJD reduction, 1 in 9.4 million if exposed to a product with 4-6 $\log_{10}$ vCJD reduction, and 1 in 3.2 billion if exposed to a product with 7-9 $\log_{10}$ vCJD reduction (Attachment 1)

In spite of the limitations of clearance studies and the uncertainties of risk assessment, a scientifically-based opinion about meaningful clearance of infectivity would provide a useful interim target to assess pdFVIII safety. FDA is considering what level of clearance, demonstrated in a well-designed scaled-down study using an exogenous spiking model, might provide a sufficient assurance of safety with respect to TSEs.

Questions for the Committee:

1. Based on available scientific knowledge, please discuss whether a minimum TSE agent reduction factor, demonstrated using an exogenous (spiking) model in scaled-down manufacturing experiments, would enhance vCJD safety of the products.

2. If the Committee identifies a minimum TSE reduction factor that would enhance vCJD safety what actions should FDA consider in cases when a licensed pdFVIII has a lower reduction factor:
   a. Labeling that would differentiate the lower TSE clearance products from the higher TSE clearance products;
   b. Recommending addition of TSE clearance steps to the manufacturing method;
   c. Performance of TSE clearance experiments using endogenous infectivity models;
   d. Any other actions?

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Attachment 1 - Range of *Predicted Annual Mean Potential vCJD risk for pdFVIII per HA Patient* – at *three levels of clearance: 7-9 log_{10}, 4-6 log_{10}, and 2-3 log_{10} and at Higher Prevalence and Lower Prevalence estimates and at different treatment doses. (excerpted from table 5.3.A in the FDA' s Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US*

				7 - 9 Log_{10} Reduction		4 - 6 Log_{10} Reduction		2 - 3 Log_{10} Reduction	
				Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani (2005)	Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004)	Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on Clark and Ghani (2005)	Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004)	Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on Clark and Ghani (2005)	Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004)
Treatment Regimen	Inhibitor Status	Est. Total Number patients in US	Mean quantity of product used per person per year (5^{th} - 95^{th})[b]	Mean potential vCJD risk per person per year[a] (5^{th} - 95^{th} perc)[b]	Mean potential vCJD risk per person per year[a] (5^{th} - 95^{th} perc)[b]	Mean potential vCJD risk per person per year[a] (5^{th} - 95^{th} perc)[b]	Mean potential vCJD risk per person per year[a] (5^{th} - 95^{th} perc)[b]	Mean potential vCJD risk per person per year[a] (5^{th} - 95^{th} perc)[b]	Mean potential vCJD risk per person per year[a] (5^{th} - 95^{th} perc)[b]
Prophylaxis	No Inhibitor	578	157949 IU (21242 , 382316)	1 in 4.1 billion (0-0)[c]	1 in 50 million (0 - 1 in 11 million)	1 in 4 million (0-0)[c]	1 in 54,000 (0- 1 in 12,000)	1 in 15,000 (0-0)[c]	1 in 82 (0 - 1 in 17)
	With Inhibitor – No Immune Tolerance	63	190523 IU (26956 , 447639)	1 in 3.5 billion (0-0)[c]	1 in 40 million (0 - 1 in 8.8 million)	1 in 4.8 million (0-0)[c]	1 in 41,000 (0- 1 in9,000)	1 in 12,000 (0-0)[c]	1 in 65 (0 - 1 in 13)
	With Inhibitor – With Immune Tolerance	62	558700 IU (33235, 1592943)	1 in 551 million (0-0)[c]	1 in 15 million (0 - 1 in 3.4 million)	1 in 1.3 million (0-0)[c]	1 in 15,000 (0- 1 in3,700)	1 in 2,700 (0-0)[c]	1 in 24 (0 - 1 in 3)
Episodic	No Inhibitor	946	85270 IU (4633, 244656)	1 in 3.2 billion (0-0)[c]	1 in 100 million (0 - 1 in 24 million)	1 in 9.4 million (0-0)[c]	1 in 105,000 (0- 1 in 24,000)	1 in 21,500 (0-0)[c]	1 in 159 (0 - 1 in 34)
	With Inhibitor	151	160458 IU (5314 , 488906)	1 in 4 billion (0-0)[c]	1 in 50 million (0 - 1 in 11 million)	1 in 8 million (0-0)[c]	1 in 23,000 (0- 1 in 12,000)	1 in 23,000 (0-0)[c]	1 in 73 (0 - 1 in 16)

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The 5^{th}- 95^{th} perc (percentiles) are the minimum and maximum numbers that define the range constituting the 90% confidence interval. Accordingly, the mean risk estimates from the model should fall within this defined interval at least 90% of the time.

[c] For a 5^{th} and 95^{th} percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

Attachment 2 – TSE Clearance Study Results for pdFVIII, presented by the Plasma Protein Therapeutics Association at the TSEAC meeting of 9/18/06 at http://www.fda.gov/ohrms/dockets/ac/06/slides/2006-4240S1_7_files/frame.htm.

PPTA
Plasma Protein Therapeutics Association

Company A

Step	MAB column	Q-Sepharose chromatography
Spike	Scrapie strain 263K	Scrapie strain 263K
Preparation	10% brain homogenate	10% brain homogenate
Prion detection / quantification method	- Hamster bioassay - Western blot confirmation	- Hamster bioassay - Western blot confirmation
No. of independent runs per spike preparation	one	one
Log reduction(s), ID_{50}	**4.6**	**3.5**

TOTAL REDUCTION: 8.1 $\log_{10} ID_{50}$

→ Product is licensed in the USA

PPTA
Plasma Protein Therapeutics Association

Company B

Step	3.5 % PEG Precipitation	Heparin Affinity Chromatography*	Saline Precipitation and Final Filtrations	TOTAL
Spike	PrP^{Sc} 263K Scrapie	PrP^{Sc} 263K Scrapie	PrP^{Sc} 263K Scrapie	
Preparations	1) Microsomal fraction 2) Detergent treated preparation	1) Brain homogenate 2) Detergent treated preparation	1) Microsomal fraction 2) Detergent treated preparation	
Prion detection / quantification method	WB	WB	WB	
No. of independent runs per spike preparation	2	2	2	
Log reduction(s)	3.21 – 3.43	≥3.44 – ≥3.45	2.08 – 2.47	
Mean	**3.32**	**≥3.45**	**2.28**	**≥9.05**

* Preliminary results

→ Product is licensed in the USA

PPTA Plasma Protein Therapeutics Association

Company D

Steps	Subsequent Precipitation Steps	Precipitation Step Followed by Polishing Step and Sterile Filtration
Spike	263K Scrapie	263K Scrapie
Preparation	Microsomes // purified PrP^{Sc}	Microsomes // purified PrP^{Sc}
Prion detection/quantification method	CDI (conformation-dependent immunoassay)	CDI (conformation-dependent immunoassay)
No. of independent runs/spike preparation	2 per spike preparation	2 per spike preparation
Log reduction(s), Mean	**3.5 // 3.9**	**2.9 // 4.0**

→ Product is licensed in the USA

PPTA Plasma Protein Therapeutics Association

Company E

Steps	Adsorption, Precipitation, and Chromatography
Spike	263K Scrapie
Preparation	Clarified Scrapie Brain Homogenate (cSBH) and Microsomal Fraction
Prion detection/quantification method	PK treatment, 0.5 log titration, and one-step Western blot
No. of independent runs/spike preparation	1 per spike preparation
Log reduction(s)	3.8 for cSBH spike, 3.7 for microsomal spike
Mean	**3.7 to 3.8**
Comments: Consistent results were also obtained from partially combined experiments. An additional step is under evaluation.	

→ Product is licensed in the USA

Attachment 3 – Summary of Topic I, TSE meeting 9/18/06 (at http://www.fda.gov/ohrms/dockets/ac/06/minutes/2006-4240M-updated.pdf

Abbreviated Summary
For the
TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES
ADVISORY COMMITTEE MEETING
September 18 & 19, 2006
Gaithersburg, MD
At: http://www.fda.gov/ohrms/dockets/ac/06/minutes/2006-4240M-updated.pdf

Topic I: Experimental Clearance of Transmissible Spongiform Encephalopathy Infectivity in Plasma-derived Factor VIII Products

FDA asked the Committee to discuss whether standardized methods and assessment criteria are feasible and appropriate for determining clearance of TSE agents by the manufacturing processes for plasma-derived FVIII (pdFVIII) products.

Dr. Dorothy Scott introduced the topic summarizing TSE safety concerns, the importance of TSE clearance, upstream pdFVIII manufacturing processes, and methodological and logistical challenges of TSE clearance studies using exogenous spiking materials or endogenously infected blood. She also discussed the question of whether a minimum TSE agent reduction factor might serve as an appropriate standard for demonstrating vCJD safety, similar to analogous precedents from viral validation studies. Then Dr. Thomas Kreil, PPTA, discussed specific TSE clearance study challenges with regard to scale-down and conditioning. Dr. Kreil also presented data from industry-sponsored TSE clearance studies for pdFVIII.

Questions for the Committee

1. a. Please comment on the feasibility and scientific value of adopting standardized exogenous (spiking) study methods to assess TSE clearance in manufacturing of pdFVIII including the following:

Optimal spiking material and its preparation from the standpoint of relevance to blood infectivity

The committee discussed several possibilities, including TSE-infected brain-derived spiking materials, such as hamster 263K brain homogenate which is frequently used, is partially characterized with regard to partitioning during fractionation, and provides sufficiently high titers of infectivity and PrP^{TSE} to allow demonstration of a broad range of clearance in studies. Spleen-derived spikes have lower titers, and there is no guarantee that they represent the physical form of TSE agent in blood better than do brain spikes. It was suggested that, since VLDL fractions of blood may preferentially contain TSE infectivity (based on data from Dr. Safar), such fractions might usefully represent endogenous infectivity. Committee members felt that current experiments might begin with brain homogenate preparations, and that more definitively blood-relevant spikes or endogenous infectivity needed further study. It was widely acknowledged that the physical form of TSE agents in endogenously infected blood must be better understood before the most relevant spiking materials can be selected.

II) Selection of a TSE strain and animal model

Several models were discussed (e.g., PrP-bovinized transgenic mice, sheep, and chimeric transgenic mice). Bovinized mice are very susceptible to infection with vCJD agent, and conventional RIII mice can be used to model vCJD as well. It was suggested that, in theory, TSE-infected sheep blood could be assayed with RIII mice, enabling titration of large amounts of plasma or product intermediates. Mice lacking the PrP GPI anchor were also suggested as a possible model, since their blood titers of infectivity have been very high (although it is not known whether the form of infectious TSE agent and its associations in such deficient mice would faithfully model more typical infections). Some members of the committee felt that the most relevant strains of TSE agent to be studied would be derived from humans with vCJD or cows with BSE.

III) TSE immunoassays for PrP^{TSE} and bioassays for infectivity

Members commented that conformation-dependent immunoassay (CDI) or protein misfolding cyclic amplification (PMCA) technique showed preliminary promising results. However, the committee discussed the need to compare and carefully validate CDI, PMCA, and other binding assays with bioassays, and some members felt that infectivity still should be demonstrated by bioassay.

IV) Identification of manufacturing processes that might alter TSE agent properties

The Committee members commented that the manufacturing process itself is not standardized and varies from product to product and manufacturer to manufacturer so that developing a standard method for validation will require further consideration. Overall, efforts at standardization were felt by some to be premature, since characteristics of endogenous infectivity are still not well understood, and therefore difficult to model; standardization might even impede research to address remaining challenges in TSE clearance studies. A second viewpoint was expressed, that some standardization now might be useful, because as better methods are discovered they are inevitably adopted.

1. b. Please comment on the feasibility and scientific value of adopting standardized endogenous study methods to assess TSE clearance in manufacturing of pdFVIII.

The Committee discussed the merits of various models including the use of transgenic mice (e.g., PrP-cervidized mice for CWD, PrP-bovinized mice for BSE, and PrP GPI-deficient mice) and sheep models of infectivity. Dr. Kreil warned that a potential limitation of endogenous infectivity studies is that animal plasma is known to have characteristics somewhat different from those of human plasma when fractionated, so that manufacturing processes might not be comparable and results with animal models not predictive of those with human plasma. While data were not presented to support or refute this contention, the committee agreed that it might pose an additional limitation of studies using endogenous TSE infectivity in animal plasma.

2. Based on available scientific knowledge, please discuss whether a minimum TSE agent reduction factor, demonstrated using an exogenous (spiking) model in scaled-

down manufacturing experiments, might serve as an appropriate standard for demonstrating vCJD safety of the products.
A detailed discussion of this question was postponed until the next meeting when risk assessment results will be discussed. One member reminded the Committee of the need for a clear definition for "log reduction" of infectivity, recognizing that the 50-percent infectious dose (ID50) is a continuous rather than a discrete variable and that estimated reductions to less than a single ID50 do not guarantee safety.

3. Considering the outcome of the discussion on Item 2, in cases where a lower reduction factor is demonstrated for a pdFVIII, should FDA consider the following:

a. Labeling that would differentiate the lower clearance products from other products with sufficient TSE clearance;

b. Recommending addition of TSE clearance steps to the manufacturing method;

c. Performance of TSE clearance experiments using endogenous infectivity models;

d. Any other actions?

This answer depends on the answer to the previous questions, thus definitive discussions were deferred until more information is available. In limited discussion, some members felt that labeling of a product as having less clearance might unfavorably dispose consumers or physicians against certain products even though no vCJD infection has ever been attributed to any plasma derivative. A member felt that the patient community might favor adding effective clearance steps to a manufacturing process but that labeling of products with low clearance values is not indicated now and would not be helpful.

別紙様式第 2-1
番号 8

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数		報告日	第一報入手日 2006 年 11 月 7 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人ハプトグロビン	研究報告の公表状況	Plos Pathogens 2006; 2(10):956-963	公表国 フランス	
販売名 （企業名）	ハプトグロビン注－ヨシトミ(ベネシス)				

研究報告の概要

現在まで、BSE と vCJD は単一のプリオン株と関係していた。この株は、感染した動物およびヒトの脳から分離された異常なプロテアーゼ抵抗性プリオン蛋白（PrP^{res}）の唯一の且つ極めて安定な生化学的側面によって特徴付けられている。しかし、従来とは異なるウシの PrP^{res} が、最近、大規模なスクリーニングによって発見された。これらもまた別のプリオン株を代表するか否かを調査するため、我々は、より大きな分子量の PrP^{res}（H 型と呼ばれる）の特徴を有するフランスのウシの株を、ウシまたは羊の PrP を発現するトランスジェニックマウスに接種した。全てのマウスは神経学的症状を呈し、この株に感染したが、このことは、これらの株が感染性プリオンの新規の株であることを示している。重要なことにこの病原体は、同じマウス－継代感染が維持されたーに接種された BSE 病原体の特徴とは明らかに異なる株の特有の特徴を示した。加えて、この病原体は、これまで羊の PrP 発現マウスにおいて継代感染させた羊スクレイピー株とも異なっていた。我々の知見は、様々なプリオン株がウシに存在するか、または BSE 病原体がある種の動物において分岐し進化した可能性を示している。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)略
1)略
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
ウシから従来の BSE 病原プリオンとは異なるプリオン株の分離した報告である。 これまで血漿分画製剤によってvCJD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。	vCJD に関連する情報については、今後も注視することとする。

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Isolation from Cattle of a Prion Strain Distinct from That Causing Bovine Spongiform Encephalopathy

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To date, bovine spongiform encephalopathy (BSE) and its human counterpart, variant Creutzfeldt-Jakob disease, have been associated with a single prion strain. This strain is characterised by a unique and remarkably stable biochemical profile of abnormal protease-resistant prion protein (PrP^{res}) isolated from brains of affected animals or humans. However, alternate PrP^{res} signatures in cattle have recently been discovered through large-scale screening. To test whether these also represent separate prion strains, we inoculated French cattle isolates characterised by a PrP^{res} of higher apparent molecular mass—called H-type—into transgenic mice expressing bovine or ovine PrP. All mice developed neurological symptoms and succumbed to these isolates, showing that these represent a novel strain of infectious prions. Importantly, this agent exhibited strain-specific features clearly distinct from that of BSE agent inoculated to the same mice, which were retained on further passage. Moreover, it also differed from all sheep scrapie isolates passaged so far in ovine PrP-expressing mice. Our findings therefore raise the possibility that either various prion strains may exist in cattle, or that the BSE agent has undergone divergent evolution in some animals.

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Introduction

While transmissible spongiform encephalopathies (TSEs) in small ruminants and humans are believed to involve distinct prion strains [1,2], a single prion strain has been associated so far with bovine spongiform encephalopathy (BSE) and its human counterpart, variant Creutzfeldt-Jakob disease (vCJD) [3–6]. In particular, the abnormal, protease-resistant form of prion protein (PrP^{res}) that accumulates in the brains of infected individuals [7] shows a consistently unique electrophoretic profile in immunoblots [8]. However, the biochemical testing of the brains of slaughtered and fallen cattle, which was intensified since 2000 in European countries as a means to protect the consumers, has led to the discovery of positive samples that showed distinct PrP^{res} profiles. These atypical profiles have been sorted into two groups so far, provisionally termed H-type when the size of the protease resistant fragments is higher than for BSE, and bovine amyloidotic spongiform encephalopathy, or L-type, when it is lower [9,10]. These observations raise the possibility that as yet unrecognised prion strains may exist in cattle as in other species [11], and have potential implications in terms of public health. Unlike bovine amyloidotic spongiform encephalopathy isolates, which derive from animals with defined histopathological abnormalities [10], precise information corroborating a prion disease is lacking for H-type cases. It was therefore crucial to determine through experimental transmission whether such cases reflect some alteration in PrP metabolism, possibly in aging animals, or involve a truly infectious agent.

In this study, we report the transmission of a TSE-like disease by inoculation of French cattle isolates identified as H-type variants to two lines of PrP transgenic mice. Furthermore, we provide compelling evidence that this agent has unique features compared to epizootic BSE and other related agents. We also establish that there is no link with ovine TSE isolates transmitted so far to these models.

Results

H-Type Isolates Are Transmissible to Mice

Two transgenic mouse lines were used as recipient for transmission experiments. The tg540 line is a newly established line that expresses bovine PrP (Protocol S1), resulting in an enhanced susceptibility to BSE agent compared to conventional mice [6,12]. The tg338 line, expressing the VRQ ($Val^{136}Arg^{154}Gln^{171}$) allele of ovine PrP, has allowed an efficient transmission of natural scrapie isolates from sheep and goat [13,14]. The rationale for including tg338 mice in this study was the possibility that characterisation of a prion

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Abbreviations: BSE, bovine spongiform encephalopathy; PrP^{res}, protease-resistant prion protein; TgBov, bovine tg540; TgOv, ovine tg338; TSE, transmissible spongiform encephalopathies; vCJD, variant Creutzfeldt-Jakob disease

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☯ These authors contributed equally to this work.