were >99% identical in sequence to those detected in patients with prostate cancer. To exclude the possibility that we were detecting a murine leukemia virus (MLV) laboratory contaminant, we determined the phylogenetic relationship among endogenous (non-ecotropic) MLV sequences, XMRV sequences, and sequences from CFS patients 1104, 1106, and 1178 (fig. S2). XMRV sequences from the CFS patients clustered with the XMRV sequences from prostate cancer cases and formed a branch distinct from non-ecotropic MLVs common in inbred mouse strains. Thus, the virus detected in the CFS patients' blood samples is unlikely to be a contaminant.

To determine whether XMRV proteins were expressed in PBMCs from CFS patients, we developed intracellular flow cytometry (IFC) and Western blot assays, using antibodies (Abs) with novel viral specificities. These antibodies included, among others, (i) rat monoclonal antibody (mAb) to the spleen focus-forming virus (SFFV) envelope (Env), which reacts with all polytropic and xenotropic MLVs (*7*); (ii) goat antisera to whole mouse NZB xenotropic MLV; and (iii) a rat mAb to MLV p30 Gag (*8*). All of these Abs detected the human VP62 XMRV strain grown in human Raji, LNCaP, and Sup-T1 cells (fig. S3) (*5*). IFC of activated lymphocytes (*6*, *9*) revealed that 19 of 30 PBMC samples from CFS patients reacted with the mAb to MLV p30 Gag (Fig. 2A). The majority of the 19 positive samples also reacted with antisera to other purified MLV proteins (fig. S4A). In contrast, 16 healthy control PBMC cultures tested negative (Fig. 2A and fig. S4A). These results were confirmed by Western blots (Fig. 2, B and C) (*6*) using Abs to SFFV Env, mouse xenotropic MLV, and MLV p30 Gag. Samples from five healthy donors exhibited no expression of XMRV proteins (Fig. 2C). The frequencies of CFS cases versus healthy controls that were positive and negative for XMRV sequences were used to calculate a Pearson $\chi^2$ value of 154 (two-tailed $P$ value of $8.1 \times 10^{-35}$). These data yield an odds ratio of 54.1 (a 95% confidence interval of 23.8 to 122), suggesting a nonrandom association with XMRV and CFS patients.

To determine which types of lymphocytes in blood express XMRV, we isolated B and T cells from one patient's PBMCs (*6*). Using mAb to MLV p30 Gag and IFC, we found that both activated T and B cells were infected with XMRV (Fig. 2D and fig. S4A). Furthermore, using mAb to SFFV Env, we found that >95% of the cells in a B cell line developed from another patient were positive for XMRV Env (fig. S4B). XMRV protein expression in CFS patient–derived activated T and B cells grown for 42 days in culture was confirmed by Western blots (fig. S4C) using Abs to SFFV Env and xenotropic MLV.

We next investigated whether the viral proteins detected in PBMCs from CFS patients represent infectious XMRV. Activated lymphocytes (*6*) were cocultured with LNCaP, a prostate cancer cell line with defects in both the JAK-STAT and RNase L pathways (*10*, *11*) that was previously shown to be permissive for XMRV infection (*12*). After coculture with activated PBMCs from CFS patients, LNCaP cells expressed XMRV Env and multiple XMRV Gag proteins when analyzed by Western blot (Fig. 3A) and IFC (fig. S5A). Transmission electron microscopy (EM) of the infected LNCaP cells (Fig. 3B), as well as virus preparations from these cells (Fig. 3C), revealed 90- to 100-nm-diameter budding particles consistent with a gamma (type C) retrovirus (*13*).

We also found that XMRV could be transmitted from CFS patient plasma to LNCaP cells when we applied a virus centrifugation protocol to enhance infectivity (*6*, *14*, *15*). Both XMRV gp70 Env and p30 Gag were abundantly expressed in LNCaP cells incubated with plasma samples from 10 of 12 CFS patients, whereas no viral protein expression was detected in LNCaP cells incubated with plasma samples from 12 healthy donors (Fig. 4A). Likewise, LNCaP cells incubated with patient plasma tested positive for XMRV p30 Gag in IFC assays (fig. S5B). We also observed cell-free transmission of XMRV from the PBMCs of CFS patients to the T cell line SupT1 (Fig. 4B) and both primary and secondary transmission of cell-free virus from the activated T cells of CFS patients to normal T cell cultures (Fig. 4C). Together, these results suggest that both cell-associated and cell-free transmission of CFS-associated XMRV are possible.

We next investigated whether XMRV stimulates an immune response in CFS patients. For this purpose, we developed a flow cytometry assay that allowed us to detect Abs to XMRV Env by exploiting its close homology to SFFV Env (*16*). Plasma from 9 out of 18 CFS patients infected with XMRV reacted with a mouse B cell line expressing recombinant SFFV Env (BaF3ER-SFFV-Env) but not to SFFV Env negative control cells (BaF3ER), analogous to the binding of the SFFV Env mAb to these cells (Fig. 4D and S6A). In contrast, plasma from seven healthy donors did not react (Fig. 4D and fig. S6A). Furthermore, all nine positive plasma samples from CFS patients but none of the plasma samples from healthy donors blocked the binding of the SFFV Env mAb to SFFV Env on the cell surface (fig. S6B). These results are consistent with the hypothesis that CFS patients mount a specific immune response to XMRV.

Neurological maladies and immune dysfunction with inflammatory cytokine and chemokine up-regulation are some of the most commonly reported features associated with CFS. Several retroviruses, including the MLVs and the primate retroviruses HIV and HTLV-1, are associated with neurological diseases as well as cancer (*17*). Studies of retrovirus-induced neurodegeneration in rodent models have indicated that vascular and inflammatory changes mediated by cytokines and chemokines precede the neurological pathology (*18*, *19*). The presence of infectious XMRV in lymphocytes may account for some of these observations of altered immune responsiveness and neurological function in CFS patients.

We have discovered a highly significant association between the XMRV retrovirus and CFS. This observation raises several important questions. Is XMRV infection a causal factor in the pathogenesis of CFS or a passenger virus in the immunosuppressed CFS patient population? What is the relationship between XMRV infection status and the presence or absence of other viruses that are often associated with CFS (e.g., herpesviruses)? Conceivably these viruses could be cofactors in pathogenesis, as is the case for HIV-mediated disease, in which co-infecting pathogens play an important role (*20*). Patients with CFS have an elevated incidence of cancer (*21*). Does XMRV infection alter the risk of cancer development in CFS? As noted above, XMRV has been detected in prostate tumors from patients expressing a specific genetic variant of the *RNASEL* gene (*5*). In contrast, in our study of this CFS cohort, we found that XMRV infection status does not correlate with the *RNASEL* genotype (*6*) (table S2).

Finally, it is worth noting that 3.7% of the healthy donors in our study tested positive for XMRV sequences. This suggests that several million Americans may be infected with a retrovirus of as yet unknown pathogenic potential.


References and Notes

1. L. D. Devanur, J. R. Kerr, *J. Clin. Virol.* 37, 139 (2006).
2. T. L. Whiteside, D. Friberg, *Am. J. Med.* 105, 27S (1998).
3. R. J. Suhadolnik *et al.*, *J. Interferon Cytokine Res.* 17, 377 (1997).
4. G. Casey *et al.*, *Nat. Genet.* 32, 581 (2002).
5. A. Urisman *et al.*, *PLoS Pathog.* 2, e25 (2006).
6. Materials and methods are available as supporting material on *Science* Online.
7. R. Wolff, S. Koller, J. Ruscetti, *Virology* 43, 472 (1982).
8. B. Chesebro *et al.*, *Virology* 127, 134 (1983).
9. K. A. Smith, F. W. Ruscetti, *Adv. Immunol.* 31, 137 (1981).
10. G. Dunn, K. Sheehan, L. Old, R. Schreiber, *Cancer Res.* 65, 3447 (2005).
11. Y. Xiang *et al.*, *Cancer Res.* 63, 6795 (2003).
12. B. Dong *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 104, 1655 (2007).
13. B. J. Poiesz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 77, 7415 (1980).
14. G. R. Pietroboni, G. B. Harnett, M. R. Bucens, *J. Virol. Methods* 24, 85 (1989).
15. S. M. Yoo *et al.*, *J. Virol. Methods* 154, 160 (2008).
16. L. Wolff, E. Scolnick, S. Ruscetti, *Proc. Natl. Acad. Sci. U.S.A.* 80, 4718 (1983).
17. C. Power, *Trends Neurosci.* 24, 162 (2001).
18. X. Li, C. Hanson, J. Cmarik, S. Ruscetti, *J. Virol.* 83, 4912 (2009).
19. K. E. Peterson, B. Chesebro, *Curr. Top. Microbiol. Immunol.* 303, 67 (2006).
20. A. Lisco, C. Vanpouille, L. Margolis, *Curr. HIV/AIDS Rep.* 6, 5 (2009).
21. P. H. Levine *et al.*, *Cancer Res.* 52, 5516s (1992).


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*Note added in proof:* V.C.L. is operations manager of Viral Immune Pathologies Laboratory, which is in negotiations with the Whittemore Peterson Institute to offer a diagnostic test for XMRV.

**Supporting Online Material**
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Materials and Methods
Figs. S1 to S6
Tables S1 and S2
References

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# Complete Reconstitution of a Highly Reducing Iterative Polyketide Synthase

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Highly reducing iterative polyketide synthases are large, multifunctional enzymes that make important metabolites in fungi, such as lovastatin, a cholesterol-lowering drug from *Aspergillus terreus*. We report efficient expression of the lovastatin nonaketide synthase (LovB) from an engineered strain of *Saccharomyces cerevisiae*, as well as complete reconstitution of its catalytic function in the presence and absence of cofactors (the reduced form of nicotinamide adenine dinucleotide phosphate and *S*-adenosylmethionine) and its partner enzyme, the enoyl reductase LovC. Our results demonstrate that LovB retains correct intermediates until completion of synthesis of dihydromonacolin L, but off-loads incorrectly processed compounds as pyrones or hydrolytic products. Experiments replacing LovC with analogous MlcG from compactin biosynthesis demonstrate a gate-keeping function for this partner enzyme. This study represents a key step in the understanding of the functions and structures of this family of enzymes.

Nature uses an amazing array of enzymes to make natural products (*1*). Among these metabolites, polyketides represent a class of over 7000 known structures of which more than 20 are commercial drugs (*2*). Among the most interesting but least understood enzymes making these compounds are the highly reducing iterative polyketide synthases (HR-IPKSs) found in filamentous fungi (*3*). In contrast to the well-studied bacterial type I PKSs that operate in an assembly line fashion (*4*), HR-IPKSs are megasynthases that function iteratively by using a set of catalytic domains repeatedly in different combinations to produce structurally diverse fungal metabolites (*5*). One such metabolite is lovastatin, a cholesterol-lowering drug from *Aspergillus terreus* (*6*). This compound is a precursor to simvastatin (Zocor, Merck, Whitehouse Station, NJ), a semi-synthetic drug that had annual sales of more than $4 billion before loss of patent protection in 2006 (*7*).

Biosynthesis of lovastatin proceeds via dihydromonacolin L (acid form 1, lactone form 2), a product made by the HR-IPKS lovastatin nonaketide synthase (LovB), with the assistance of a separate enoyl reductase, LovC (*8*) (Fig. 1). LovB is a 335-kD protein that contains single copies of ketosynthase (KS), malonyl–coenzyme A (CoA) acyltransferase (MAT), dehydratase (DH), methyltransferase (MT), ketoreductase (KR), and acyl-carrier protein (ACP) domains, as well as a section that is homologous to the condensation (CON) domain found in nonribosomal peptide synthetases (NRPSs) (*9*). It also contains a domain that resembles an enoyl reductase (ER) but lacks that activity. LovB must catalyze ~35 reactions and use different permutations of tailoring domains after each of the eight chain-extension steps to yield the nonaketide, dihydromonacolin L (2). This enzyme also catalyzes a biological Diels-Alder reaction during the assembly process to form the decalin ring system (*10*). In vitro studies of LovB (*11*) have been hampered by an inability to obtain sufficient amounts of the functional purified megasynthase from either *A. terreus* or heterologous *Aspergillus* hosts. As a result, the programming that governs metabolite assembly by LovB or other HR-IPKSs is not understood. Key aspects that remain to be elucidated include (i) the catalytic and structural roles of each domain in the megasynthase, (ii) substrate specificities of the catalytic domains and their tolerance to perturbation in megasynthase functions, and (iii) factors governing the choice of different combinations of domains during each iteration of catalysis. To initiate such studies, we engineered an expression system in yeast to produce large amounts of LovB and examined the influence of cofactors and the ER partner (e.g., LovC) on product formation.

The engineered *Saccharomyces cerevisiae* strain BJ5464-NpgA, which contains a chromosomal copy of the *Aspergillus nidulans* phosphopantetheinyl (ppant) transferase gene *npgA* (*12*), was the expression host. A C-terminal hexahistidine-tagged LovB was placed under the control of the *S. cerevisiae ADH2* promoter (*13*, *14*) on an episomal plasmid (YEpLovB-6His). Abundant amounts of the intact LovB could be purified from the soluble fraction to near homogeneity with a final yield of ~4.5 mg/L (fig. S1). We used mass analysis of tryptic digest fragments to verify the identity of the recombinant LovB. The ACP domain of LovB was determined to be nearly completely phosphopantetheinylated by using a ppant ejection assay with high-resolution quadrupole orthogonal acceleration–time-of-flight mass spectrometry (fig. S2). To ascertain activity of the resulting LovB and to examine the necessity for cofactors, malonyl-CoA alone was first added to the purified enzyme in buffer. Whole-cell feeding studies of doubly [$^{13}$C, $^{2}$H]-labeled acetate to cultures of *A. terreus* showed that all three acetate hydrogens were incorporated into the acetate-derived starter units for both the nonaketide and diketide moieties in lovastatin (*15*). The purified LovB can use malonyl-CoA for both chain priming and chain elongation, loading malonate with decarboxylation to make the acetyl starter unit. Although LovB is able to prime with and elongate the chain by two further condensations with malonyl-CoA, in the absence of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), no ketoreduction occurs. The dominant product is lactone 3 (Fig. 2A, trace i), which forms by enolization and cyclization with off-loading of the unreduced triketide. Addition of NADPH to this system enables function of the KR domain. In this and subsequent experiments, the malonyl-CoA could be conveniently synthesized in situ by malonyl-CoA synthase (MatB) from *Rhizobium trifolii* using free malonate and CoA (*16*). With KR enabled, LovB makes penta-, hexa-, and heptaketide pyrones 4 to 6, as well as ketones 7 and 8 (Fig. 2A, trace ii). The structures were confirmed by chemical synthesis of authentic standards, except for heptaketide 6, which proved very unstable. However, the mass increase of 26 atomic mass units for 6 and its red shift in the ultraviolet spectrum when compared to 5 are consistent with its proposed heptaketide pyrone structure (table S3). Compounds 7 and 8 result from thioester hydrolysis of penta- and hexaketides stalling on the ACP at the β-keto stage. The resulting β-keto acids spontaneously decarboxylate to afford 7 and 8. Formation of compounds 4 to 8 illustrates that derailment in the normal programmed steps, namely the lack of methylation due to the absence of *S*-adenosylmethionine

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研究報告の概要

（目的）日本で採血された血漿由来の静注用免疫グロブリン製剤(IVIG)に Classical Swine Influenza A(H1N1)virus 及び Influenza A(H1N1)pdm virus に反応する抗体が含まれているかを調べ、ドナーが免疫を獲得している可能性について検討した。
（材料と方法）Classical Swine Influenza A(H1N1)virus は「A/Swine/Hokkaido/2/1981」を使用した。Influenza A(H1N1)pdm virus は大阪で分離したウイルスを用いた。IVIG は日本で採血された原料により2008年に製造されたロットを用いた。赤血球凝集抑制能（HI価）は、8HAのウイルス液に等量の希釈サンプルを加え、モルモット赤血球に対する完全凝集制御を示す最大希釈倍数で求めた。中和抗体価（NT活性）は100FFUのウイルスに対して50%以上の感染阻害を示す最大希釈倍数で求めた。
（結果）IVIGにブタ及び新型ウイルスに対するHI及びNT活性が認められ、その値はそれぞれ8倍、64倍であった。
（考察）日本で採血された血漿由来の IVIG にも HI 及び NT 活性が認められることから、日本においてもある程度の率で Influenza A(H1N1)pdm virus に反応する抗体を保有しているドナーが存在すると推測された。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 日本で採血された血漿を原料として製造された静注用免疫グロブリン製剤(IVIG)に新型インフルエンザウイルスに反応する抗体が含まれていたことについての報告である。<br>インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程にて十分に不活化・除去されると考えている。 | 本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。 |

## 1P073

### 季節性インフルエンザウイルスに対するヒト免疫グロブリン製剤の抗体価

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【目的と意義】
インフルエンザワクチンは流行予測に基づいて接種株が決定され、国民に接種されている。このような背景をもつドナー数万人分のプール血漿から製造される静注用免疫グロブリン製剤（IVIG）は、当然ワクチン株に対して高い赤血球凝集抑制能（HI価）を有する。しかしながら、本質的にグロブリンに期待される機能は中和能（NT価）であるにも関わらず、IVIGとNT価に関する知見は少ない。また、IVIGの製造時を起点とし、その前後の時期に分離されたウイルス株への交差反応性に関する知見も少ない。そこで私たちはIVIGに含まれる抗インフルエンザウイルス抗体の特徴を明らかにするため、製造時期及び地域（米国と日本）の異なるIVIG及び分離時期の異なるウイルス株を用いて、HI価及びNT価の比較を行った。
【材料と方法】
製造時期（1999～2008年）または、原料の採漿場所（米国又は日本）が異なる複数のIVIGロットを用いて、各種インフルエンザウイルス株（ワクチン株及び野外分離株）に対するHI価およびNT価を確認した。HI価は、8HAのウイルス液に等量の希釈サンプルを加え、モルモット赤血球に対する完全凝集抑制を示す最大希釈倍数で求めた。NT価は、100FFUのウイルスに対して50%以上の感染阻害を示す最大希釈倍数で求めた。
【結果と考察】
製造時以前に分離され、且つその後ワクチン株として採用されたウイルス株に対して、IVIGは高い抗体価を示した。また、製造時以降新たに分離されたウイルス株に対しても交差反応性を示した。これらの結果からIVIGは製造時期や採漿場所に関わらず、様々な季節性インフルエンザウイルスに反応する抗体を含んでいると考えられた。今後はこの分離時期・地域をまたがって反応する抗体の本質を明らかにする必要がある。

## 1P074

### Classical Swine Influenza A(H1N1)virus及びInfluenza A(H1N1)pdm virusに対する静注用グロブリン製剤の抗体価

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【目的と意義】
2009年4月は顕在化した新型インフルエンザの世界的流行は想定されていたトリ由来のA/H5N1とは異なり、ブタ由来のA/H1N1であった。今回発生したInfluenza A (H1N1) pdmは抗ウイルス剤（タミフル・リレンザ）に感受性であるが、Seasonal Influenza A (H1N1) virusとは抗原性が異なり、従来の季節型ウイルスワクチンが効かないことが指摘されている。しかしながら、高齢者はこの新型インフルエンザに罹患しにくいという報告もあり、一部のドナーは免疫を獲得していることが考えられた。そこで、私たちは日本で採血された血漿を原料として製造された静注用グロブリン製剤（IVIG）にClassical Swine Influenza A (H1N1) virus及びInfluenza A (H1N1) pdm virusに反応する抗体が含まれているかを調べ、ドナーが免疫を獲得している可能性について検討を行った。
【材料と方法】
Classical Swine Influenza A (H1N1) virusは「A/ Swine/ Hokkaido/2/1981」を使用した。Influenza A (H1N1) pdm virusは大阪で分離されたウイルスを用いた。IVIGは日本で採血された原料により2008年に製造されたロットを用いた。HI価は、8HAのウィルス液に等量の希釈サンプルを加え、モルモット赤血球に対する完全凝集抑制を示す最大希釈倍数で求めた。中和抗体価（NT）は100FFUのウイルスに対して50%以上の感染阻害を示す最大希釈倍数で求めた。
【結果】
IVIGにブタ及び新型ウィルスに対するHI及びNT活性が認められ、その値はそれぞれ8倍、64倍であった。
【考察】
米国における保存血漿のNovel influenza A (H1N1) virusに対する抗体保有率に関する報告が2009年5月22日のMMWRに掲載された。日本で採血された血漿由来のIVIGにもHI及びNT活性が認められることから、日本においてもある程度の率でInfluenza A (H1N1) pdm virusに反応する抗体を保有しているドナーが存在すると推測された。

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**研究報告の概要**

米国FDAによる2009年11月付の業界向けガイダンス（案）「パンデミック（H1N1）2009インフルエンザウイルスへの対応における血液供給の保存，血液製剤の安全性，血液ドナーの適合性に関する推奨」が出された。
示された推奨の内容は以下のとおりである。
A. バックアップ要員の訓練
パンデミック（H1N1）2009インフルエンザウイルスにより引き起こされる疾患の範囲は未知であるので、要員不足を予期し、適正なバックアップ要員を持つことを推奨する。更に、最重要な機能については複数のバックアップ要員を訓練すべきである。バックアップ要員は継続する訓練プログラムで訓練すべきである。
B. 血液ドナー適合性、ドナーの延期そして製剤管理
一般的にドナーの医療歴は採血時に入手される。しかしながら、21 CFR 640.3(a)及び 640.63(a)の下では全血または原料血漿の供給源としてのドナーの適格性は採血日に確立されなければならない。これらの規則は、明確に採血日を定義してない。時々、採血前にドナーに示された質問に対する返答は血液事業者による再調査により不完全であることが発見される。採血から24時間以内に、ドナー歴問診票に対するドナーの返答を明確にするあるいは省略された質問に対する返答を入手する必要がある。
パンデミック（H1N1）2009ウイルスに感染したまたは感染した疑いのあるドナーは、解熱剤の投薬なしで熱が下がり、それ以外の症状もなくなってから、少なくとも24時間は採血を延期しなければならない。
献血後48時間以内にパンデミック（H1N1）2009インフルエンザあるいはインフルエンザ様疾患の可能性のあるドナーについて情報を入手した場合、メディカルディレクターは安全性を評価しなければならない。
C. 承認された申請に対する変更
認定血液事業者としての承認済みの申請について、以下の変更申請を提出してもよい。その試験所がFDAに登録され、ドナー試験を実施しているならば、その外部試験所を使用すること、等。

**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) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過膜処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| パンデミック(H1N1)2009インフルエンザウイルスへの対応における血液供給の保存、血液製剤の安全性、血液ドナーの適合性に関す業界向けガイダンス（案）である。<br>インフルエンザA(H1N1)はオルソミクソウイルス科に属し、ビリオンは球形で、直径80～120nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、インフルエンザA(H1N1)が原料血漿に混入したとしてもBVDをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程にて十分に不活化・除去されると考えている。 | 本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。 |

# Guidance for Industry

## Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus

### DRAFT GUIDANCE

**This guidance document is for comment purposes only.**

Submit comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. Submit electronic comments to http://www.regulations.gov. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD), (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or email ocod@fda.hhs.gov, or from the Internet at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm..

For questions on the content of this guidance contact OCOD at the phone numbers listed above.

**U.S. Department of Health and Human Services**
**Food and Drug Administration**
**Center for Biologics Evaluation and Research**
**November 2009**

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**Contains Nonbinding Recommendations**

*Draft – Not for Implementation*

# Guidance for Industry

# Recommendations for the Assessment of Blood Donor Suitability, Blood Product Safety, and Preservation of the Blood Supply in Response to Pandemic (H1N1) 2009 Virus

*This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.*

## I. INTRODUCTION

This guidance document provides recommendations for assessing blood donor suitability and blood product safety and maintaining blood and blood product availability in response to pandemic (H1N1) 2009 virus. It is intended for establishments that manufacture Whole Blood and blood components intended for use in transfusion and blood components intended for further manufacture, including recovered plasma, Source Plasma and Source Leukocytes. Within this guidance, "you" refers to blood establishments; "we" refers to FDA.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

## II. BACKGROUND

### A. Epidemiology and Pathogenesis

The 2009 H1N1 pandemic is caused by a novel influenza A virus of swine origin. On April 26, 2009, then Department of Health and Human Services (DHHS) Acting Secretary Charles E. Johnson, pursuant to section 319 of the Public Health Service Act, 42 U.S.C. § 247d, declared a public health emergency when a novel swine-origin 2009 influenza A (H1N1) virus was identified in California, Texas, Kansas, and New York. The pandemic influenza H1N1 virus has since spread quickly to all fifty states and globally. In June 2009, the World Health Organization (WHO) declared a Phase 6 Level of Pandemic Influenza Alert. This declaration was based upon a standard definition reflecting worldwide spread of the pandemic (H1N1) 2009 virus and the observed

efficiency of human to human transmission. Importantly, a declaration of a pandemic is independent of the severity of illness caused by the virus or the degree of infrastructure disruption. On July 24 2009, DHHS Secretary Kathleen Sebelius renewed DHHS' April 2009 determination that a public health emergency exists nationwide involving pandemic influenza H1N1 that has significant potential to affect national security.

From April 15, 2009 to July 24, 2009, states reported to the Centers for Disease Control and Prevention (CDC) a total of 43,771 confirmed and probable cases of novel influenza A (H1N1) infection. Of these cases reported, 5,011 people were hospitalized and 302 people died.[1,2] From August 30, 2009 to October 24, 2009, 25,985 hospitalizations and 2,916 deaths attributed to influenza and influenza-like illnesses have been reported in the United States (U.S.). CDC has developed a model to estimate the true number of cases in the U.S. The model took the number of cases reported by states and adjusted the figure to account for known sources of underestimation (e.g., not all people with pandemic influenza H1N1 seek medical care, and not all people who seek medical care have specimens collected by their health care providers). Using this approach, it is estimated that more than one million people became infected with novel influenza A (H1N1) between April and June 2009 in the U.S.[3]

The symptoms of human influenza disease caused by pandemic (H1N1) 2009 virus are similar to the symptoms of seasonal flu and include fever, cough, sore throat, runny or stuffy nose, body aches, headache, chills and fatigue. A significant number of people who have been infected with pandemic (H1N1) 2009 virus also have reported diarrhea and vomiting.[4]

The most severe outcomes have been reported among individuals with underlying health problems that are associated with high risk of influenza complications. Pandemic (H1N1) 2009 virus currently remains sensitive to oseltamivir (Tamiflu) and zanamivir (Relenza), though sporadic cases of resistance to oseltamivir have been reported. At this time, there is insufficient information to predict how severe the pandemic (H1N1) 2009 virus outbreak will be in terms of illness and death or infrastructure disruption, or how it will compare with seasonal influenza.

### B. Potential Impact of the H1N1 Pandemic on Blood Product Safety and Availability

There is limited information available on pandemic (H1N1) 2009 virus viremia, especially during the asymptomatic period. No case of transfusion transmitted seasonal

[1] http://www.cdc.gov/h1n1flu/update.htm, (Accessed Nov. 2, 2009).
[2] CDC discontinued reporting of confirmed and probable cases of novel H1N1 infection on July 24, 2009. The most recent total numbers of hospitalizations and deaths due to H1N1 are available on the CDC website. http://www.cdc.gov/h1n1flu/update.htm, (Accessed Nov. 2, 2009).
[3] http://www.cdc.gov/h1n1flu/surveillanceqa.htm, (Accessed Nov. 2, 2009).
[4] http://www.cdc.gov/h1n1flu/sick.htm, (Accessed Nov. 2, 2009).

influenza has ever been reported in the U.S. or elsewhere, and, to date, no cases of transfusion transmitted pandemic influenza H1N1 have been reported. At this time, the pandemic (H1N1) 2009 virus has not been isolated from blood or serum of asymptomatic, infected individuals; however, studies are ongoing. Furthermore, the potential for transmission of pandemic influenza H1N1 through blood transfusion remains unknown.

In some previous studies, other Influenza A viruses were isolated from blood, and throat secretions or nasopharyngeal mucosa of children with clinical manifestations of influenza (Refs. 1-2). The virus was isolated from blood and throat washings of 1/29 healthy asymptomatic contacts who became ill 12 hours after the specimens were obtained (Ref. 3). From another study, virus isolation was reported from lungs, adrenals and meninges (from autopsy) which indicated that viremia must have been present (Ref. 4). In humans experimentally infected by nasal inoculation, viremia was observed in 4/15 subjects using sensitive culture methods. Symptoms occurred 2 days after initial viremia and one patient remained asymptomatic throughout the study period (22 days) (Ref. 5). However, other investigators were unable to detect viremia in 27 subjects using a similar virus strain and assay methods (Ref. 6).

The pandemic influenza H1N1 virus is a large lipid-enveloped virus. Validation studies performed by product manufacturers have shown that viruses with similar characteristics to the pandemic influenza H1N1 virus are effectively inactivated and/or removed during manufacturing of plasma derivatives.

Due to its known potential for rapid spread, pandemic (H1N1) 2009 virus has the potential to cause disruptions in the blood supply. A significant number of blood donors, blood establishment staff, and vendors of blood-related supplies (e.g., manufacturers of reagents and blood bags) could be affected as individuals become ill or need to care for ill family members. At the same time, during a widespread outbreak of disease caused by the pandemic (H1N1) 2009 virus, it is anticipated that the demand for blood and blood components may be reduced due to postponement of elective surgery, were that to become necessary in some affected healthcare settings.

In addition, the usual paradigm for ensuring blood availability in response to local disasters (i.e., hurricanes) may not be available under severe pandemic scenarios. In local disasters, interregional transfer of blood from unaffected to affected areas has been an effective strategy. However, in a more severe pandemic scenario, international, national, and regional outbreaks may occur simultaneously and a pandemic wave may last for months. Therefore, advanced planning is reasonable to prepare for the possible need to mitigate the effects of a more severe pandemic and to help ensure that blood is available in affected areas

Standard precautions for avoidance of contact with respiratory secretions may help to reduce the transmission of pandemic (H1N1) 2009 virus in blood and plasma collection establishments. The CDC has issued recommendations for infection control in the

community[5], places of business[6], and in health care settings[7]. CDC also has issued "Interim Infection Control Guidance on 2009 H1N1 Influenza for Personnel at Blood and Plasma Collection Facilities."[8] We recognize the importance of the CDC recommendations for infection control in blood and plasma collection establishments.

## III. RECOMMENDATIONS

FDA, in communication with DHHS Office of Public Health and Science, CDC, and the AABB Interorganizational Task Force on Pandemic Influenza and the Blood Supply, monitors blood availability closely. Similarly, we anticipate that you will maintain close communications with your hospital customers to anticipate demand for blood and blood components.

While shortages are not forecast at present, we are reminding you of regulatory pathways and providing regulatory clarification that may be helpful to you both in dealing with the current outbreak and in continuing to stay prepared.

We will continue to review any new scientific information about the potential risk of transfusion transmission of pandemic (H1N1) 2009 virus. We also will monitor closely the impact of the pandemic on blood availability. As our knowledge base grows, we may revise the recommendations in this guidance document as appropriate.

### A. Training of Back-Up Personnel

Under 21 CFR 211.25 and 21 CFR 606.20, personnel performing critical functions in blood establishments must be adequate in number, educational background, training and experience, including professional training as necessary, or combination thereof, to assure competent performance of their assigned functions. Given the unknown extent of the disease caused by pandemic (H1N1) 2009 virus, we recommend that you have adequate back-up personnel, in the event of anticipatable personnel shortages. We further recommend that where possible, more than one back-up person should be trained for each critical function. Any such back-up personnel should be trained pursuant to your existing training program. We also recommend that as provided in your training program, you document this training and/or re-training.

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[5] http://www.cdc.gov/h1n1flu/guidance/exclusion.htm, (Accessed Nov. 2, 2009).
[6] http://www.cdc.gov/h1n1flu/business/guidance, (Accessed Nov. 2, 2009).
[7] http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm, (Accessed Nov. 2, 2009).
[8] http://www.cdc.gov/h1n1flu/guidance/blood_facilities.htm.

## B. Blood Donor Suitability, Donor Deferral and Product Management

*Blood Donor Suitability*

In general, a donor medical history is obtained at the time of blood collection. However, under 21 CFR 640.3(a) and 21 CFR 640.63(a), the suitability of a donor as a source of Whole Blood or Source Plasma, must be made on the *day of collection* from the donor. These regulations do not explicitly define the term *day of collection*. Occasionally, donor's responses to the donor questions presented before collection are found to be incomplete upon review by the blood establishment. You may clarify a donor's response to the donor history questionnaire or obtain omitted responses to questions within 24 hours of the collection.

*Blood Donor Deferral*

- Under current FDA regulations, blood donors must be in good health, as indicated in part by normal temperature and free of acute respiratory diseases on the day of collection (21 CFR 640.3(a), (b)(1) and (4) and 21 CFR 640.63(a), (c)(1) and (7)).

- Available data do not currently support donor deferral for exposure to or contact with a person who has confirmed or probable pandemic (H1N1) 2009 influenza or influenza-like symptoms.

- To ensure donors are in good health on the day of donation as required under 21 CFR 640.3(b) and 21 CFR 640.63(c), donors with a confirmed or probable case of pandemic (H1N1) 2009 virus infection should be deferred until at least 24 hours after they are free of fever without the use of fever reducing medications[9] and they are otherwise asymptomatic.

- Available data do not support the deferral of donors following vaccination with live attenuated influenza vaccines (LAIV) or inactivated influenza vaccines against pandemic (H1N1) 2009 virus or for prophylactic use of the antiviral medications oseltamivir (Tamiflu) and zanamivir (Relenza). However, consistent with the recommendation above, donors taking anti-viral medications for confirmed or probable pandemic (H1N1) 2009 virus infection should be deferred until at least 24 hours after they are free of fever without the use of fever reducing medications[10] and they are otherwise asymptomatic.

[9] A daily dose of pediatric aspirin (81 mg) is not considered fever-reducing medication.
[10] A daily dose of pediatric aspirin (81 mg) is not considered fever-reducing medication.

*Blood Product Management*

The recommendations in this section apply to donations of Whole Blood and blood components intended for transfusion. This section does not apply to blood components intended for further manufacture (recovered plasma, Source Plasma, Source Leukocytes) since validation studies have shown that viruses with similar characteristics to pandemic (H1N1) 2009 virus are effectively inactivated and/or removed during manufacturing of plasma derivatives.

- Upon receipt of post donation information about a donor with confirmed or probable pandemic (H1N1) 2009 disease or influenza like illness within 48 hours after the donation, the Medical Director should evaluate the safety of the previously donated products consistent with existing Standard Operating Procedures (SOPs).

### C. Changes to an Approved Application

As provided under 21 CFR 601.12(c)(5), we have determined that the following changes to an approved application for licensed blood establishments may be submitted as a "Supplement-Changes Being Effected".

- Use of a different outside test lab, provided the test lab is registered with FDA and has been performing donor testing.
- Implementation of self-administered donor history questionnaires, provided you follow the critical control points described in FDA's "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (July 2003), and the submission contains the content recommended for all self-administered procedures and computer assisted interactive procedures outlined in the same guidance.

The recommendations set forth above supersede the recommendations in FDA's "Guidance for Industry: Changes to an Approved Application: Biological Products: Human Blood and Blood Components Intended for Transfusion or for Further Manufacture" (July 2001) at section IV.C and FDA's "Guidance for Industry: Streamlining the Donor Interview Process: Recommendations for Self-Administered Questionnaires" (July 2003) at section IV.A, respectively (in both of these guidances, we previously had determined that these changes would require a "Supplement – Changes Being Effected in 30 Days").

## IV. BIOLOGIC PRODUCT DEVIATION AND FATALITY REPORTING

Licensed manufacturers, unlicensed registered blood establishments, and transfusion services are subject to reporting requirements with respect to the reporting of product deviations under

21 CFR 606.171. Blood establishments are not expected to submit biological product deviation reports for post-donation information related to pandemic (H1N1) 2009 virus. If a complication of blood transfusion results in the fatality of a recipient, blood establishments must report the fatality to FDA as soon as possible (21 CFR 606.170(b)).

## V. COLLECTION AND USE OF CONVALESCENT PLASMA

Plasma obtained after recovery from an acute infection (convalescent plasma) generally contains highly-specific antibodies directed at the infectious agent, and has theoretical potential to serve as a therapeutic product. In consideration that circumstances could arise where vaccines and antiviral drugs might not be sufficiently available, or where a patient is not responding to approved therapies, transfusion of convalescent plasma has been discussed as a possible empirical treatment during an influenza pandemic. (Ref. 7-8)

In July 2009, the WHO Blood Regulators Network issued a position paper[11] on the collection and use of convalescent plasma as an element in pandemic influenza planning. This paper recommends that scientific studies on the feasibility and medical effectiveness of the collection and use of convalescent plasma, and possibly fractionated immunoglobulins, should be explored through clinical trials. FDA encourages the development of new, safe and effective therapies for influenza. Because of its experimental nature, collection and administration of convalescent plasma should be conducted only under an Investigational New Drug Application. Blood establishments that intend to manufacture convalescent plasma should contact FDA to discuss their plans.

## VI. IMPLEMENTATION

This guidance has been issued for comment purposes only.

[11] http://www.who.int/bloodproducts/brn/BRNPosition-ConvPlasma10July09.pdf, (Accessed Nov. 2, 2009).

## VII. REFERENCES

1. Naficy K., "Human Influenza Infection with Proved Viremia, Report of a Case". N Engl J Med. 1963. 31; 269:964-6.

2. Ritova VV, Schastnyi EI, Ratushkina LS, Shuster IY., "Investigation of the incidence of influenza A viraemia caused by virus strains circulating among children in 1968 – 1977". J Hyg Epidemiol Microbiol Immunol. 1979; 23(1):35-41.

3. Khakpour M, Saidi A, Naficy K., "Proved viraemia in Asian influenza (Hong Kong variant) during incubation period". British Medical Journal 1969:4, 208-209.

4. Roberts JT and Roberts GT., "Postsplenectomy sepsis due to influenzal viremia and pneumococcemia". Can Med Assoc J. 1976 September 4; 115(5): 435–437.

5. Stanley ED and Jackson GG., "Viremia in Asian Influenza". Trans Assoc Phys. 1966: 79: 376-7.

6. Alford RH, Kasel, JA, Gerone PJ, Knight V., "Human influenza resulting from aerosol inhalation". Proc. Soc. Exp. Biol. Med. 1966: 122:800-4.

7. Zhou B, Zhong N, Gong Y., "Treatment with convalescent plasma for influenza A (H5N1) infection". NEJM, 2007; 357(14):1450-1

8. Luke TC, Kilbane EM, Jackson JL, Hoffman SL., "Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment?" Ann Intern Med 2006; 145:599-609.

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**研究報告の概要**

多くの生物種に影響を及ぼしている致死的な神経組織障害である伝染性海綿状脳症（TSEs）において、病原性の重要因子は宿主にコードされている正常型プリオン蛋白（$PrP^C$）の異常型アイソフォーム（$PrP^{Sc}$）の蓄積である。$PrP^C$から$PrP^{Sc}$への変換についての正確な機序は理解されていないが、宿主$PrP^C$発現はプリオン伝播に効果的な感染性プリオンの産生の必要条件であることは明らかである。哺乳類のTSEsについては多くの研究が実施されているが、魚におけるTSEの病原性についてほとんどわかっていない。
ここで私達は、BSE感染ウシ又はスクレイピー感染ヒツジより調製された脳ホモジネートを経口投与されたヨーロッパヘダイは臨床症状を現わさなかったが、投与2年後に採取されたヨーロッパヘダイの脳は神経変性の徴候と抗タイPrP抗体が上昇し陽性に反応する沈着物の蓄積を示したことを提示する。
非感染動物由来の脳を投与されたコントロール群はそのような徴候を示さなかった。
注目すべきことに、TSE感染脳ホモジネートよりもBSE感染物質を投与された魚に多数のプロテアーゼK抵抗性の沈着物が急速かつ広範囲に現れた。これらのプラーク様凝集はコンゴ好染性と偏光下における複屈折を示し、アミロイド様成分と一致した。プリオン、特にBSEを投与された魚の脳における神経変性と異常な沈着物は公衆衛生上の潜在的リスクに関する懸念を増大させる。
魚の養殖はヒトや他の動物種に対する高タンパク栄養源を供給する経済的で重要な産業であり、感染性哺乳動物$PrP^{Sc}$に汚染されている養殖魚におけるプリオン病発生の予測は気がかりであり、更なる評価が必要である。

**使用上の注意記載状況・その他参考事項等**

代表としてテタノブリンIH静注250単位の記載を示す。
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 |
|---|---|
| BSE感染ウシ及びスクレイピー感染ヒツジの脳ホモジネートを経口投与されたヨーロッパヘダイの脳は神経変性と抗タイPrP抗体に反応する沈着物の蓄積を示し、公衆衛生上の潜在的リスクに関する懸念を増大させる可能性があるとする報告である。<br>血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。 | 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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# Evaluation of the Possible Transmission of BSE and Scrapie to Gilthead Sea Bream (*Sparus aurata*)

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## Abstract

Transmissible spongiform encephalopathies (TSEs), a group of fatal neurodegenerative disorders affecting many species, the key event in disease pathogenesis is the accumulation of an abnormal conformational isoform (PrP[Sc]) of the host-encoded cellular prion protein (PrP[C]). While the precise mechanism of PrP[C] to PrP[Sc] conversion is not understood, it is clear that host PrP[C] expression is a prerequisite for efficient TSE propagation. Although there have been many studies on TSEs in mammalian species, little is known about TSE pathogenesis in fish. Here we show that while gilthead sea bream (*Sparus aurata*) orally challenged with brain homogenates prepared either from a BSE-infected cow or from scrapie-infected sheep developed no clinical prion disease, the brains of TSE-fed fish sampled two years after challenge did show signs of neurodegeneration and accumulation of deposits that reacted positively with antibodies raised against sea bream PrP. The control groups fed with brain from uninfected animals showed no such signs. Remarkably, the deposits developed much more rapidly and extensively in fish challenged with BSE-infected material than in the ones challenged with the scrapie-infected brain homogenate, with numerous deposits being proteinase K-resistant. These plaque-like aggregates exhibited congophilia and green birefringence in polarized light consistent with an amyloid-like component. The neurodegeneration and abnormal deposition in the brains of fish challenged with prions, especially BSE, raises concerns about the potential risk to public health. As fish are a cornerstone in economically important industry providing high protein nutrition for humans and other mammalian species, the prospect of farmed fish being co-farmed with infectious mammalian PrP[Sc], or of a prion disease developing in farmed fish is alarming and requires further evaluation.

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## Introduction

Transmissible spongiform encephalopathies or prion diseases are a group of fatal neurodegenerative disorders, including Creutzfeldt-Jakob disease (CJD), Fatal Familial Insomnia (FFI) and Gerstmann-Sträussler-Scheinker disease (GSS) in humans, scrapie in sheep and goats and bovine spongiform encephalopathy (BSE) in cattle [1].

The transmission of clinical prion diseases is limited by the so-called "species barrier" to conversion of endogenous host prion protein ($PrP^{C}$) to its abnormal, partially proteinase K-resistant conformational isoform, $PrP^{Sc}$. When high enough, this "barrier" can greatly impair or prevent potential interspecies transmissions, even under optimal conditions of dose and infection route. However, evidence of TSE replication without accompanying symptoms of clinical disease has prompted debate on the existence of asymptomatic infected individuals in an exposed population [2,3].

The identification of apparent PrP orthologues in lower vertebrates, including fish [4–16], raises the question of their susceptibility to prion diseases. While fish PrP-like sequences do not share high homology with their mammalian relatives (Table S1), they do contain several strongly conserved prion protein structural motifs [17]. Although mammalian to fish TSE transmission is considered unlikely [18], it is not certain that the species barrier would be high enough to prevent TSE transmission to fish.

The BSE epidemic has been linked to TSE-infected cattle feed [19] and the recognition of BSE in domestic cattle inevitably raised concerns about the potential risk to other ruminant and