ISSUE SUMMARY
TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES
ADVISORY COMMITTEE MEETING
September 18, 2006
Gaithersburg, MD

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

Issue: **FDA seeks the advice of the Committee whether standardized methods and assessment criteria are feasible and appropriate for determining TSE clearance in the manufacturing processes for plasma-derived FVIII products.**

Background and Rationale:

TSE clearance in plasma products has been studied by many manufacturers on a voluntary basis. At the February 20, 2003 TSEAC meeting, the Committee provided advice to FDA in support of labeling claims for TSE clearance where manufacturers have undertaken clearance studies using TSE spiking agents. To date, FDA has approved labeling claims for TSE clearance for two IGIV products, and one Antithrombin III product. Typically, sponsors submitting labeling claims are asked for the following information:

- Characterization and titration of spiking agent
- Accurately scaled-down processes (including use of actual production intermediates)
- Robust and reproducible experiments
- Well-characterized assay(s) for TSE infectivity
- Estimated logs clearance of TSE by processing steps (log reduction factor, LRF)
- Demonstration of mass balance for the spike.
- Demonstration, where feasible, that at least two clearance steps are orthogonal

In evaluating TSE clearance studies, FDA is interested in 1) effect of steps which might alter the context or physical state of TSE-agent-containing material that could impact removal by a subsequent step; and 2) the rationale for use of specific animal models and spiking preparations.

FDA is now considering development of recommendations for TSE clearance studies that encourage the use of standard validation methods. Standardized studies have not been recommended to date, in part because there has not been definition of optimal and most relevant methodologies. Scientific issues with respect to TSE clearance studies include the following:

- What are the most relevant spiking materials (e.g. spiking materials that most accurately represent the form of infectivity in blood)?

- What TSE agent strain(s) and animal models should be used?
- How should standard spiking materials be prepared?
- What assays best detect and quantify infectivity (e.g. bioassays or binding assays)?
- Might any of the processes used to manufacture plasma derivatives alter TSE agent properties and, if so, what are the implications for clearance studies and their interpretation?

FDA seeks the Committee's advice on the feasibility and scientific value of adopting standardized methods to assess TSE clearance in manufacturing. Additionally we seek the Committee's advice on whether a minimum TSE agent reduction factor might serve as an appropriate standard for demonstrating vCJD safety of the products. We also seek advice on the use of this clearance information in product labeling.

Discussion:

1. Current Status of TSE Clearance Study Methods

Spiking studies versus studies of endogenously infected blood. Studies using extracts of TSE-infected brain spiked into plasma or into manufacturing intermediates can achieve the high inputs of infectivity ($>10^6$ 50%-infectious doses [ID_{50}] per gm of material) needed to demonstrate a substantial $\log_{10}$-reduction factor similar to what has usually supported the conclusion that a process reliably results in significant clearance of an infectious agent. However, it is possible that the TSE agent in infected brain tissues might be in a physical form—mainly in large aggregates and contaminated with lipids—that is not representative of the agent in blood. Unfortunately, the physical nature of the TSE infectious agent in blood is not well understood or characterized. If the TSE agent in blood is poorly aggregated, it may be more difficult to remove via nanofiltration or depth filtration than published studies using brain homogenates as spiking material suggest (1). Small, soluble forms of infectivity can be derived from brain homogenates (1,2) but, to date, few clearance studies using these preparations as spiking agents have been published (2).

Blood of animals with natural or experimental TSEs contains small amounts of infectious agent in a form that probably resembles that of TSE infectivity in blood of humans with variant Creutzfeldt-Jakob disease (vCJD), thus offering a highly relevant model for assessing clearance of the agent from plasma derivatives or process intermediates. However, the low titers of infectivity likely to be present in human blood[1] make it difficult to measure meaningful reductions without employing very large numbers of rodents. Although the recent demonstration, by transfusion studies, that both BSE and scrapie agents are present in blood of infected sheep (4) offers a possible method for improved studies using large volumes of endogenously infected blood or blood derivatives, the amounts of infectivity in sheep blood are not yet known, and logistics for conducting studies with farm animals are currently much more difficult than for studies

[1] Infectivity ranges from 2 ID to a maximum of about 30 ID/ml of blood in scrapie-infected hamsters, and similar ranges have been observed in blood of mice infected with BSE (7) or vCJD agents (8).

with rodents. Such difficulties include herd management, availability of locations for infected animals, experimental scale, time (incubation periods), and containment.

One strategy to address concerns about the relevance of brain-derived or other tissue-derived spiking materials to blood infectivity would be to conduct a series of studies in sequence. Initial studies would be spiking studies in which a high-titered TSE-infected brain preparation is added to plasma or to a manufacturing intermediate (in volumes small enough not to alter the matrix). Results of those studies could then be used to design studies with endogenously TSE-infected animal blood. The latter studies would be limited in feasible number and power, but would have the advantage of likely being more relevant to TSE-infected human blood. Failure to confirm the clearance noted in spiking studies in clearance studies using endogenously infected blood, with its much lower level of infectivity, would be a highly significant finding.

Tissue spikes. The highest titers of TSE infectivity have been found in brains of rodents (particularly in hamsters infected with scrapie (11)). For that reason, brain tissue suspensions or extracts have been used as TSE spiking materials. Some have suggested that various brain extracts—microsomal fractions, "caveoli-like domains" [CLDs], partially purified protease-resistant prion protein [PrP^{TSE},also designated PrP^{Sc} or PrP^{res}], or a relatively non-aggregated infective preparation more realistically model the form of infectivity found in blood (1, 5, 6) than do crude tissue homogenates. However, except for a relatively insoluble membrane-free PrP^{TSE}, which was generally more effectively cleared by precipitations, the other three spiking materials had similar LRF for each of several clearance steps (6). Available evidence does not support a preference for microsomal fractions or CLDs over clarified crude brain homogenates as spiking materials, nor is there any convincing basis for predicting which preparation most faithfully models the form of TSE infectivity in blood.

It has recently been proposed that, because they are rich in some cells similar or identical to those in blood and have a low lipid content, infected spleen tissue extracts might be a more relevant spike than brain extracts. TSE infectivity titers in spleen are generally at least 1000-fold lower than those in brain tissue and the similarity in physical form of TSE agent in spleen to infectivity in blood is hypothetical. (Furthermore, amounts of PrP^{TSE} in infected spleen are generally very low.) However, spleen extracts might be a useful addition to brain extracts under some circumstances, for example, in laboratories with very sensitive PrP^{TSE} assays. Comparative studies of TSE clearance using spleen-derived and brain-derived TSE agent spikes have not been published.

TSE assays for spiking studies. As for other clearance studies, the LRF for TSE agents is calculated by measuring the amount of agent applied (the starting titer times volume applied) and the amount remaining after treatment (titer times volume of product obtained). Two kinds of assay have been used to estimate clearance of TSE agents by plasma fractionation: tests that detect and quantify amounts of PrP^{TSE} , and *in vivo* bioassays for infectivity. Infectivity assays have been considered more reliable for two reasons: (1) With one possible exception (9), tests detecting PrP^{TSE} have been at least 1000-fold less sensitive than infectivity assays, and (2) some tissues have contained

infectivity without detectable PrP^{TSE} (10-12). Presence of PrP^{TSE} has also been described in the absence of infectivity (13). However, the ease and rapidity of detection of PrP^{TSE} and its general correlation with clearance of infectivity in published clearance studies (14-17) suggests that measurements of PrP^{TSE} should be useful as a preliminary method for evaluating clearance of TSE agents by manufacturing processes. One possible approach to minimize use of bioassays might be to test various steps in a plasma fractionation scheme individually for clearance of PrP^{TSE}, to combine the most effective steps and test for evidence of additivity in removing PrP^{TSE}, and then to confirm provisional conclusions by performing infectivity assays for the most effective single steps or combinations of steps. The steps or combinations of steps judged to be most effective might then be studied with endogenously TSE infected animal blood. Alternative *in vitro* methods may eventually become available to detect TSE infectivity; if sufficiently sensitive and well-correlated to *in vivo* infectivity, *in vitro* infectivity assays would provide a more practical and rapid approach for evaluating TSE agent clearance.

Studies of endogenously TSE infected animal blood. When clearance studies are to be performed with endogenously TSE-infected blood, it seems reasonable to evaluate only those individual plasma fractionation steps or combinations of steps found to be most effective in preliminary spiking studies. PrP^{TSE} assays have not been convincingly validated for detecting agent in blood, though preliminary reports recently suggested that some new tests might eventually achieve this goal. At the moment, clearance studies of TSE agents from blood require *in vivo* bioassays. As noted above, amounts of TSE infectivity in blood are likely to be extremely low, so a very large number of assays would be required to demonstrate clearance of infectivity by manufacturing processes. Considering the small amounts of infectivity that are present in plasma[2], should any infectivity be detected in an intermediate or in final product, the individual step or manufacturing method must be considered practically ineffective for clearing a meaningful amount of agent. Studies using blood of TSE-infected sheep or primates—which would have the advantage of allowing infectivity assays using repeated intravenous infusions of large volumes—might eventually become available but are currently not feasible for most sponsors.

Selection of TSE animal models and TSE strains. For reasons of feasibility, and because they are fairly well-characterized, hamster-adapted scrapie and several murine-adapted TSE agents are most frequently used for clearance studies. While strains differ in susceptibility to inactivation (19, 20), the few extant comparative clearance studies, all of which removed TSE agents by partitioning rather than by inactivation, have not demonstrated significant differences when measured by bioassay or Western Blot (21). Scientific evidence supporting a preference for a particular TSE strain or rodent species is lacking.

[2] A substantial proportion of total scrapie infectivity in hamster blood appears to be in the plasma (18).

2. What level of TSE clearance is likely to provide reasonable assurance of safety for pdFVIII and VWF?

In the somewhat analogous case of viral clearance validation studies, typical results accepted by FDA in support of label claims usually demonstrated at least 4 logs of clearance by each of two mechanistically dissimilar (orthogonal) steps. An additional margin of safety that assures clearance of at least 2-3 logs greater than the highest anticipated titers of viral pathogen in the starting material seems prudent. In the case of TSEs, the amount of infectivity in blood or plasma of experimentally infected animals has been estimated as 2-30 ID/ml (7, 8). The amount of infectivity in the blood of BSE-infected and scrapie-infected ruminants and in people with vCJD 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 logs, plus an additional margin of safety. Reduction of pathogens is 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 ID/ml would contain 1,600 - 24,000 infectious units (3.2 - 4.4 $\log_{10}$).[3] An appropriate 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 spite of uncertainties, a scientifically-based opinion about meaningful clearance of infectivity would provide a useful interim target to enhance 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.

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

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:
   I) Optimal spiking material and its preparation from the standpoint of relevance to blood infectivity
   II) Selection of a TSE strain and animal model
   III) TSE immunoassays for PrP^{TSE} and bioassays for infectivity
   IV) Identification of manufacturing processes that might alter TSE agent properties

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

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.

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 products with higher 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?

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