LifeLink Foundation

A not-for-profit corporation dedicated to serve patients in need of transplantation therapy.

01 MAY -7 A9:59 8297

May 4, 2001

Dockets Management Branch
HFA-305, Room 1061
Food and Drug Administration
5630 Fishers Lane
Rockville, MD 20852

Docket No. 97N-484P – Current Good Tissue Practice for Manufacturers of Human Cellular and Tissue-Based Products; Inspection and Enforcement

Dear Sir/Madam:

Thank you for the opportunity to comment on the proposed rule for Good Tissue Practices. The LifeLink Foundation Tissue Bank believes the proposed rule will form the basis for appropriate regulation of tissue procurement and processing practices. In particular, we support one important aspect of the draft regulation, Section 1271.220(c), the prohibition on pooling tissue for processing. Because we believe so strongly that pooling should be prohibited, and that no waivers or exceptions should be allowed, we are submitting this comment to provide information supporting the prohibition of pooling in the proposed regulation.

To our knowledge, only one tissue bank in the United States, Regeneration Technologies, Inc. (RTI), utilizes pooling in human tissue processing. RTI is located in Florida, and has recently been the subject of attention by Florida's Agency for Health Care Administration (AHCA), which has cited RTI for its failure to comply with Florida tissue-banking regulations and track individual donor tissue to individual recipients because of RTI's pooling process. (Citation letter attached as exhibit "A"). RTI is currently applying for a waiver of this Florida regulation.

More importantly, responding to concerns about processing accidents, unknown diseases, and Transmissible Spongiform Encephalitis diseases, (most particularly "Mad Cow Disease", or Bovine Spongiform Encephalitis), the Florida Organ and Tissue Procurement and Transplantation Advisory Board, a Board created by Florida Statutes to advise the State of Florida about appropriate legislation related to tissue banking, among

97N-484P C10

409 Bayshore Boulevard, *(entrance on De Leon Street)* Tampa, Florida 33606 ◆ 813-253-2640 ◆ 1-800-262-5775 ◆ Facsimile 813-251-1819 ◆ www.lifelinkfound.org
LifeLink of Florida LifeLink of Georgia LifeLink of Southwest Florida LifeLink of Puerto Rico
LifeLink Tissue Bank LifeLink Transplantation Immunology Laboratory

other things, recently voted to recommend the prohibition of tissue pooling in the State of Florida (letter from the Florida Organ and Tissue Procurement and Transplantation Advisory Board to the Florida Secretary of Health attached as Exhibit "B").

Dr. Charles Wright, Associate Medical Director of the LifeLink Foundation Tissue Bank, wrote a letter to the Florida Agency for Health Care Administration after visiting RTI's facility, concluding that the risk of transmission of "Mad Cow" type diseases and other unknown diseases was too great to allow the number of donors processed at one time to increase from one to one-hundred, as RTI currently does. (Letter from Charles Wright to the Florida Agency for Health Care Administration attached as Exhibit "C").

Additionally, an overview of issues relating to pooling, and why pooling should be prohibited, have been outlined in LifeLink Foundation's Objection to RTI's request for Waiver in Florida (attached as Exhibit "D").

RTI's President, Mr. Grooms, stated before the Florida Organ and Tissue Procurement and Transplantation Advisory Board that he believed RTI would receive an exemption from the FDA prohibition on pooling, if it becomes part of the final rule, or that the prohibition on pooling would not be incorporated into the final rule. As stated, herein, LifeLink Foundation strongly believes that the scientific evidence, and the risk of exponentially increasing the number of recipients who might be exposed to dangerous pathogens in the case of a processing accident, the discovery of a new pathogen, or exposure to Transmissable Spongiform Encephalitis, clearly mandate that there is no reason to remove the current draft prohibition on pooling, or to consider an exemption. This is particularly so if the only reason to do so is to increase revenues or profits for the stockholders of any entity which might pool tissue.

Thank you for the opportunity to comment.

Sincerely,

Dana L. Shires

Dana L. Shires, M.D., F.A.C.P.
Chairman/Chief Executive Officer, LifeLink Foundation, Inc.
Medical Director, LifeLink Tissue Bank

EXHIBIT A

STATE OF FLORIDA
AHCA
AGENCY FOR HEALTH CARE ADMINISTRATION

JEB BUSH, GOVERNOR

February 26, 2001

C. Randall Mills, Ph.D.
Regeneration Technologies, Inc.
One Innovation Drive
Alachua, Florida 32615

Dear Dr. Mills:

The Agency has reviewed the information presented in your letter of December 8, 2000 regarding Regeneration Technologies Inc.'s (RTI) compliance with the requirements of Chapter 59A-1, Florida Administrative Code (F.A.C.). Rule 59A-1.005(1)(b)4, F.A.C. requires that tissues from each individual donor will have one unique identification number that will identify each donor's tissue from retrieval through distribution and utilization. Further, as we discussed during our meeting on January 30, 2001, Rule 59A-1005(45), F.A.C. makes clear that each tissue sample must contain tissue from only one donor. A sanitizing method that permits multiple donor tissues to be processed simultaneously could meet that requirement only if it allowed the tissue from each donor to remain identifiable. Your statement that RTI's process enables you only to trace tissue components back to a lot number representing more than one donor demonstrates that your procedures are in violation of the rule.

There are a number of other provisions of the rule with which RTI cannot comply to the extent that it cannot trace donated tissue to a particular donor or recipient. Rule 59A-1.005(1)(b)5, F.A.C., requires that all information regarding a particular donor be provided to the transplant surgeon on request. The recall and look-back procedures required by Rule 59A.1005(14) and (15), F.A.C., respectively, depend upon the ability to trace transplanted tissue from an individual donor to each individual recipient. Should the need arise, RTI cannot comply with these rules as written.

These violations subject RTI to administrative action to enforce compliance with the rules under Rule 59A-1.012(1)(d), F.A.C. The possible penalties include, but are not limited to, an administrative fine not to exceed $500 per day per violation, suspension, or revocation

of the tissue bank certificate issued to Regeneration Technologies, Inc. However, the Agency is in receipt of your petition for a waiver pursuant to § 120.542, F.S. At this time, the Agency does not contemplate enforcement action regarding the above violations pending consideration of the petition for rule waiver or variance, and timely provision of all information required to make a determination. However, should safety violations arise, the Agency will not delay administrative action based upon the pendency of a petition for waiver.

Our preliminary review of the petition indicates that additional information or alternative procedures will be needed. If, after reading this notice you wish to add anything as an addendum to your request, please feel free to do so and provide it to us as soon as possible. We anticipate completion of the review within thirty days of filing, *i.e.*, by March 4, 2001. Upon completion of the review, the Agency will notify you of the additional items required. Should you have any questions, please contact Michelle Oxman in the General Counsel's office at (850) 922-8854.

Sincerely,

Elizabeth Dudek, Acting Deputy Secretary
Division of Managed Care and Health Quality

cc: Julie Gallagher, General Counsel
Michelle Oxman, General Counsel's Office
John Gilroy, General Counsel's Office
Jeffrey Gregg, Chief, Health Facility Regulation
Mary Loepp, Hospital & Outpatient Services Unit

EXHIBIT B

March 16, 2001

Ruben King-Shaw, Secretary
Agency for Health Care Administration
2727 Mahan Drive, Mail Stop 1
Tallahassee, FL 32308

Dear Secretary King-Shaw:

The Organ and Tissue Procurement and Transplantation Advisory Board is authorized under Chapter 381.6023, Florida Statutes (F.S.). One of the purposes of the Board is to make recommendations to the Agency regarding "changes to the laws...and administrative rules or procedures required to assure that the statewide organ and tissue procurement and transplantation system will be able to function smoothly, effectively, and efficiently..."

Recent developments in the tissue bank industry that have been brought to the attention of the Board are a cause of concern regarding the safety of one processing technique used to sterilize tissue. Strict guidelines have been developed to ensure that any tissue distributed for transplantation has been adequately screened to eliminate the transmission of communicable diseases and cross-contamination. Traditionally, and because transplantation involves donor-recipient interaction, tissues from each donor are processed separately to ensure the highest degree of safety. However, recently tissue pooling which was previously deemed unsuitable procedure has re-emerged because of some new technological developments. This means simultaneous processing of tissues and including in the same container tissue from multiple donors. When tissue is pooled in this manner, it becomes impossible to track contaminated tissue back to the original donor. It also would make it impossible to avoid transplanting tissues from Rh Negative donors into women of childbearing age.

The American Association of Tissue Banks (AATB) is the professional accrediting organization that sets standards for tissue banking. Accreditation by the AATB is voluntary. The AATB prohibits its accredited facilities from pooling tissues.

The Food & Drug Administration (FDA) issued draft tissue bank regulations on January 8, 2001, which will also prohibit tissue pooling when they are finalized. All tissue banks are required to comply with FDA regulations.

No sterilization technique is fool proof. New and emerging diseases, such as prion diseases, may not be caught by the currently recognized sterilization processes used. Simultaneously processing tissues from multiple donors will increase the risk of cross-contamination to transplant recipients. If contaminated tissue is transplanted, a recall or notification of surgeons who used tissue from that donor would be very difficult.

The Advisory Board recently voted to recommend to the Agency that Ch. 59A-1, Florida Administrative Code, be revised to prohibit tissue pooling by any Florida certified tissue bank. This revision would be added when the rule is next updated.

In addition, the Board feels this matter is of sufficient importance in ensuring transplant safety to all individuals that the Board is now formally requesting the Agency to appoint an independent and well-qualified panel to investigate the details of the process being used with tissue pooling. This study should include the safety and efficacy of tissue pooling and new and emerging diseases. The Board is also requesting that the findings of the panel be reported back to the Board. It is anticipated that such a study may indicate additional revisions that need to be made to the regulations governing organ and tissue procurement agencies.

Since the February 2 meeting of the Advisory Board where this matter was discussed, I have learned that Regeneration Technologies International (RTI) has sent a letter to AHCA requesting a waiver on this matter of tissue pooling. To my knowledge, AHCA has not yet drafted a response to this request.

If you would like to discuss the possibilities of developing such a panel or need additional information, please feel free to contact me. I can be reached at (1-800) 329-7000, extension 4323.

Sincerely

Mary Anne Taylor, M.S., Chair
Organ and Tissue Procurement and
Transplantation Advisory Board

Cc: Members of Advisory Board
Mr. Sam Power
JoAnn Linch

EXHIBIT C

LifeLink Foundation

A not-for-profit corporation dedicated to serve patients in need of transplantation therapy.

January 24, 2001

Ms. Jo Ann Linch, M.S.W.
Health Services & Facilities Consultant
Bureau of Health Facility Compliance
Agency For Health Care Administration
2727 Mahan Drive
Bldg. 1, Room 252
Tallahassee, Florida 32308

Dear Ms Linch,

I will not be able to attend the meeting of the Advisory Board on February 2, 2001. By this letter I hope to make my position clear, that as a member of the Florida Organ and Tissue Procurement and Transplantation Advisory Board and a Medical Director of the LifeLink Tissue Bank, I am against the practice of batch processing of tissue. I am convinced that batch processing by any currently described technique cannot be relied on to provide safe tissue and assure the public that there is no cross contamination. I am concerned about HIV, HCV and other defined pathogens, but I have a greater concern for the poorly defined Prion diseases or other yet unrevealed pathogens. Even now with our current techniques, we can not know that tissue is 100% safe. If batch processing is allowed, the potential for cross contamination and the amplification of the spread of contamination is a distinct possibility. More importantly, the ability to trace each donor directly to each recipient, and each recipient to each donor is of vital importance with these diseases. This ability would be lost with batch processing.

It is significant that the proposed new FDA Tissue Banking Regulations, published at Federal Register: January 8, 2001 (Volume 66, Number 5), prohibit batch processing, and require direct donor to recipient tracing.

Also, I have read the "Technical Monograph BioCleanse Tissue Processing System: Biological Safety" by C. Randal Mills, Ph.D. and Michael R. Roberts, M.A. (March 30, 2000). It has extensive documentation of the purported ability of BioCleanse to clear standard pathogens. No mention is made of Prions. The authors do not acknowledge the unknown. Many members of the Advisory Board have lived through the evolution of new previously unknown infectious agents (HIV, HVC, lime disease). To presume that

409 Bayshore Boulevard. *(entrance on De Leon Street)* Tampa. Florida 33606 ◆ 813-253-2640 ◆ 1-800-262-5775 ◆ Facsimile 813-251-1819 ◆ www.lifelinkfound.org
LifeLink of Florida LifeLink of Georgia LifeLink of Southwest Florida LifeLink of Puerto Rico
LifeLink Tissue Bank LifeLink Transplantation Immunology Laboratory

process can protect against a biologic unknown borders on arrogance. A prohibition on batch processing and a requirement of direct donor to recipient tracing would provide protection against spreading Prions and unknown pathogens.

I have attached a short summary of the biology of Prions and their diseases. This is an important area which should not be ignored. I did not note any testing relevant to Prions in the RTI document.

Regeneration Technologies is offering technologies to the arena of tissue transplanting. Their quest for safer tissue is admirable. A process like BioCleanse may be proven to make tissue safer at some time in the future. Unfortunately, their dependence on batch processing eclipses any potential benefit. The Advisory Board has gone on record in the past opposed to batch processing of tissue and I vote that we continue this stance.

If this document does not suffice as my vote on this matter, I give my proxy vote to Dr. Metzger, or in his absence Mr. John Campbell, to cast in compliance with this document.

Sincerely,

Charles E. Wright, M.D.
Associate Medical Director,
LifeLink Tissue Bank

cc: Members of Florida Organ and Tissue Procurement and Transplantation Advisory Board
Ms. Laura Branker

UpToDate®
Vol. 8 No. 3

Prions

BIOLOGY OF PRIONS
- Prion protein
- Biosynthesis of PrPC
- Conversion of PrPC to PrPSc
- Transport of PrPSc to the nervous system
- Neurotoxicity of prion protein

GENETICS OF HUMAN PRION DISEASES
- Creutzfeldt-Jakob disease
- Gerstmann-Straussler-Scheinker syndrome
- Fatal familial insomnia

Kenneth L Tyler, MD

Prions

Kenneth L Tyler, MD
Feb 17, 2000

Prion diseases are neurodegenerative diseases that have long incubation periods and progress inexorably once clinical symptoms appear. Five human prion diseases are currently recognized: kuru, Creutzfeldt-Jakob disease (CJD), variant Creutzfeldt-Jakob disease (vCJD also known as new variant CJD), Gerstmann-Straussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) [1]. Bovine spongiform encephalopathy (BSE), one of a number of prion infections affecting animals, focus more widespread public attention on these diseases with its possible link to vCJD [2,3].

These human prion diseases share certain common neuropathologic features including neuronal loss, proliferation of glial cells, absence of an inflammatory response, and the presence of small vacuoles within the neuropil which produces a spongiform appearance. Current evidence indicates that prion diseases are associated with the accumulation of an abnormal form of a host cell protein, designated the prion protein (PrP) [4].

The biology of prions will be reviewed here. The clinical manifestations, genetics, and diagnosis of prion diseases are discussed separately. (See "Diseases of the central nervous system caused by prions-I").

BIOLOGY OF PRIONS – Dr. Stanley Prusiner coined the term "prion" in 1982 which he defined as a small infectious pathogen containing protein but apparently lacking nucleic acid [5]. The prion protein (PrP) is the critical component of these agents and may, in fact, be its exclusive constituent.

One of the characteristic features of prions is their resistance to a number of normal decontaminating procedures. These pathogens are resistant to processes affecting nucleic acids such as hydrolyis or shearing [6]. However, agents which digest, denature or modify proteins do have activity against prions [4]. The prion protein purified from the brains of scrapie-infected animals (PrPSc) can be inactivated by prolonged autoclaving (at 121°C and 15 psi for 4.5 h), immersion in 1N NaOH (for 30 min, repeat three times), or in concentrated (>3 M) solutions of guanidine thiocyanate [7]. However, certain cautions prevail; it appears that inadequate autoclaving can establish heat resistant subpopulations which fail to diminish with a further cycle of autoclaving [8]. Stainless steel instruments also may retain infectivity even after treatment with 10 percent formaldehyde [9].

Prion protein – Scrapie prions have been used as a model for prion diseases. PrPSc is a conformational isomer of PrPC, a glycoprotein found in the brains of normal animals [10]. The normal function of PrPC is unknown. PrPC exists primarily in an alpha helical conformation, while PrPSc is beta helical and appears to result from a yet uncharacterized conformational alteration in PrPC [11,12]. The resistance of PrPSc to digestion with proteases and its tendency to polymerize into scrapie-associated fibrils or prion rods differentiates PrPSC from PrPC [13,14]. The hydrophobicity of this protein, which may in turn affect aggregation, and its beta-sheet conformation may play a role in neurotoxicity [15].

Biosynthesis of PrPC – A key step during the biosynthesis of PrPC involves modification of both the amino and carboxy terminals with the addition of a phosphatidylinositol glycolipid which serves to anchor the protein to the cell surface [16,17]. PrPC can be detected attached to the plasma membrane of neurons [18] and may be concentrated at synaptic membranes [19]. In addition, PrPC also has transmembrane domains, indicating that it spans the cellular cytoplasmic membrane. Surface PrPC is degraded after endocytosis in acidic vesicles, although some protein may recycle to the cell surface [20]. Secreted forms of PrPC also occur [21].

Conversion of PrPC to PrPSc – In contrast to PrPC, PrPSc accumulates within cells and does not normally appear on the cell surface. PrPSc is found predominantly in cytoplasmic vacuoles and secondary lysosomes [22]. Conversion of PrPC to PrPSc may occur in caveolae-like membranous domains [23].

Studies with mice either devoid of PrPC or with abnormal isoforms indicate that host PrPC must be present for the development of prion disease. Prion diseases appear to result from accumulation of abnormal isoforms of the PrP which is dependent upon conversion of normal PrPC into PrPSc [24,25]. This conversion appears to be the result of a conformational change in PrPC, rather than a chemical modification.

One group developed a peptide, iPrP13 which can break a beta-sheet conformation [26]. This peptide was able to reduce the protease resistance of PrPSc and to delay the onset of symptoms in transmission experiments in mice. There may be another as yet unidentified host factor, designated protein X, which may bind to the carboxy terminus of PrPC then interact with a site near the N-terminus to effect a conformational change [27].

How the first molecule of PrPSc appears in the host remains a mystery, but the initial appearance, which may be de novo, probably triggers the replication of PrPSc. This process has been compared to crystallization in solution where a single seed crystal serves as a nidus [3]. It is hypothesized that the initiating PrPSc molecule is derived from an exogenous source in sporadic and iatrogenic prion diseases, while mutations are invariably detected within the PRNP gene in familial forms [29]. These mutations could destabilize PrPC which might lead to spontaneous conversion to PrPSc.

Transport of PrPSc to the nervous system – Transport of PrPSc to the nervous system, once it appears in the host, occurs via axons [30]. Previous investigations suggested that the predominant mechanism was by slow axoplasmic transport [31]. However, several studies now provide data that rapid anterograde axonal transport also occurs [32,33]. In one of these reports, a specific isoform of the protein was transported via this route in a hamster model compared to several other isoforms found within neural tissues which appeared to arrive by a slower transport mechanism [33].

The lymphoreticular system may play a critical role in the initiation of some prion diseases [34]. Studies in mice indicate that for some prion diseases acquired by inoculation, a period of replication in lymphoreticular tissue is required. This would be expected for certain types of exogenously aquired prion diseases such as iatrogenic CJD, kuru, and perhaps vCJD, but might not occur in sporadic and genetic forms of prion diseases.

Neurotoxicity of prion protein – PrPSc appears to be neurotoxic; accumulation of this protein or fragments of it in neurons leads to apoptosis and cell death [35,36]. As an example, the PrP fragment containing amino acids 106-126 induces death of hippocampal neurons following exposure in vitro [35]. However, PrPC must be present for this effect to occur; PrP106-126 does not destroy neurons in mice which do not express PrPC [36].

GENETICS OF HUMAN PRION DISEASES – The gene encoding PrP ("PRNP") in humans is located on the short arm of chromosome 20 [37]. A strong link was established between mutations in the PRNP gene and forms of prion disease with a familial predisposition (fCJD, GSS, FFI). More than 20 different mutations have been identified [28,38]. Some experts have advocated classifying prion diseases based upon the responsible mutation rather than the traditional classifications such as fCJD or GSS since sometimes a single mutation produces different clinical phenotypes in different individuals or families. As an example, the D178N mutation, in which asparagine substitutes for aspartic acid in codon 178, occurs in families with FFI, fCJD, and GSS [37]. A large English and Irish kindred has been described containing individuals diagnosed with a variety of conditions including CJD, vCJD, and GSS [39]. However, when the PRNP gene was examined, all affected individuals had a valine for alanine substitution at codon 117 regardless of the clinical diagnosis.

The phenotype of a particular mutation may be influenced by the nature of the amino acids present at codon 129. Codon 129 of the PRNP gene is a polymorphic codon; normal individuals have either valine or methionine at that site. However, since the PRNP gene is on an autosomal dominant gene, there are two copies, and individuals can be homozygous or heterozygous at this site. Patients with the D178N mutation who are homozygous for valine at codon 129 appear to develop CJD, while those who are homozygous for

methionine tend to have FFI [40,41]. Despite these patterns, the clinical expression of individual mutations can vary even between affected members of the same family.

Creutzfeldt-Jakob disease – In fCJD the most common mutation is a substitution of lysine for glutamine in codon 200, which has been observed in regions ranging from Libya to Chile [42,43]. One study described a differing presentation of this syndrome with codon 129 phenotype changes [44]. When the mutant codon 200 was linked to a valine at codon 129, PrP deposits were observed in the cerebellum and the prion protein was resistant to type 2 protease, neither of which have been described with methionine at codon 129.

As noted above, the D178N mutation occurs in fCJD. A substitution of isoleucine for valine in codon 210 has also been noted in fCJD [45].

Unlike fCJD, sCJD and iCJD are not associated with PRNP gene mutations. However, even in these forms of CJD and vCJD, phenotyping at codon 129 appears to affect susceptibility and perhaps expression of the clinical illness. While 51 percent of the general population are heterozygous at codon 129, for example, all cases of vCJD and 85 to 95 percent of individuals with sCJD have been found to be homozygous at this codon [46]. In a separate report, five of seven patients who developed iCJD after receiving human cadaveric growth hormone were homozygous at codon 129 [47].

One group has proposed a molecular classification scheme for sCJD based upon codon 129 polymorphism and characterization of the properties of PrPSc which was used to evaluate 300 sCJD patients [48]. As examples, a pattern of type 1 PrPSc plus at least one methionine at codon 129 was demonstrated in 70 percent while type 2 PrPSc plus codon 129 homozygous or heterozygous for valine was present in 25 percent and associated with ataxia.

Gerstmann-Straussler-Scheinker syndrome – All GSS kindreds investigated to date have PRNP gene mutations of which P102L is the most common and the one described in the descendants of the original family described by Gerstmann, Straussler, and Scheinker [49,50]. Other reported mutations include P105L, A117V, Y145STOP, Q217R, and E219L [38,51]. As noted above, patients with some of these mutations have been clinically classified as fCJD or FFI rather than GSS.

GSS exhibits a large degree of phenotypic heterogeneity. This may be partially due to differences in the underlying PRNP mutation. GSS patients with the P102L mutation, for example, may have more prominent cerebellar features [49], while dementia may be a more prominent feature in patients with A117V, Y145STOP, and F198S mutations. Polymorphism at codon 129 may also play a modulating role in the manifestations of GSS in patients with the P102L mutation [52,53]. However, varied clinical expression of these diseases both between and within affected families with the same gene mutations suggests that other unidentified factors probably also are influential [53,54].

Fatal familial insomnia – As noted above, the D178N mutation has been the predominant mutation found in nearly all families with FFI [55]. This mutation also occurs in fCJD. It appears that patients with this mutation who are homozygous for methionine at codon 129 develop an FFI-like clinical syndrome whereas those homozygous for valine develop fCJD [40,41]. Heterozygosity at codon 129 may prolong the duration and slow the temporal progression of FFI.

References

1. Haywood, AM. Transmissible spongiform encephalopathies. N Engl J Med 1997; 337:1821.
2. O'Brien, C. Mad cow disease. Scant data cause widespread concern [news]. Science 1996; 271:1798.
3. Collinge, J. Variant Creutzfeldt-Jakob disease. Lancet 1999; 354:317.
4. Prusiner, SB. The prion hypothesis. In: Prions, Prusiner, SB, McKinley, MP (Eds), Academic Press, San Diego, 1987. p. 17.
5. Prusiner, SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216:136.
6. Bellinger-Kawahara, C, Diener, TO, McKinley, MP, et al. Purified scrapie prions resist inactivation by procedures that hydrolyze, modify, or shear nucleic acids. Virology 1987; 160:271.
7. Manuelidis, L. Decontamination of Creutzfeldt-Jakob disease and other transmissible agents. J Neurovirol 1997; 3:62.
8. Taylor, DM, Fernie, K, McConnell, I, Steele, PJ. Observations on thermostable subpopulations of the unconventional agents that cause transmissible degenerative encephalopathies. Vet Microbiol 1998; 64:33.
9. Zobeley, E, Flechsig, E, Cozzio, A, et al. Infectivity of scrapie prions bound to a stainless steel surface. Mol Med 1999; 5:240.
10. Harris, DA. Cellular biology of prion diseases. Clin Microbiol Rev 1999; 12:429.
11. Downing, DT, Lazo, ND. Molecular modelling indicates that the pathological conformations of prion proteins might be beta-helical. Biochem J 1999; 343 Pt 2:453.
12. Harrison, PM, Chan, HS, Prusiner, SB, Cohen, FE. Thermodynamics of model prions and its implications for the problem of prion protein folding. J Mol Biol 1999; 286:593.
13. Prusiner, SB, McKinley, MP, Bowman, KA, et al. Scrapie prions aggregate to form amyloid-like birefringent rods. Cell 1983; 35:349.
14. Merz, PA, Kascsak, RJ, Rubenstein, R, et al. Antisera to scrapie-associated fibril protein and prion protein decorate scrapie-associated fibrils. J Virol 1987; 61:42.
15. Jobling, MF, Stewart, LR, White, AR, et al. The hydrophobic core sequence modulates the neurotoxic and secondary structure properties of the prion peptide 106-126. J Neurochem 1999; 73:1557.
16. Stahl, N, Borchelt, DR, Hsiao, K, Prusiner, SB. Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 1987; 51:229.
17. Harris, DA, Huber, MT, van Dijken, P, et al. Processing of a cellular prion protein: identification of N- and C-terminal cleavage sites. Biochemistry 1993; 32:1009.
18. Narwa, R, Harris, DA. Prion proteins carrying pathogenic mutations are resistant to phospholipase cleavage of their glycolipid anchors. Biochemistry 1999; 38:8770.
19. Herms, J, Tings, T, Gall, S, et al. Evidence of presynaptic location and function of the prion protein. J Neurosci 1999; 19:8866.
20. Shyng, SL, Huber, MT, Harris, DA. A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. J Biol Chem 1993; 268:15922.
21. Hay, B, Prusiner, SB, Lingappa, VR. Evidence for a secretory form of the cellular prion protein. Biochemistry 1987; 26:8110.
22. Borchelt, DR, Taraboulos, A, Prusiner, SB. Evidence for synthesis of scrapie prion proteins in the endocytic pathway. J Biol Chem 1992; 267:16188.
23. Vey, M, Pilkuhn, S, Wille, H, et al. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. Proc Natl Acad Sci U S A 1996; 93:14945.
24. Bueler, H, Aguzzi, A, Sailer, A, et al. Mice devoid of PrP are resistant to scrapie. Cell 1993; 73:1339.
25. Bueler, H, Fischer, M, Lang, Y, et al. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 1992; 356:577.
26. Soto, C, Kascsak, RJ, Saborio, GP, et al. Reversion of prion protein conformational changes by synthetic ß-sheet breaker peptides. Lancet 2000; 355:192.
27. Kaneko, K, Zulianello, L, Scott, M, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc Natl Acad Sci U S A 1997; 94:10069.
28. Prusiner, SB. Prions. In: Fields Virology. 3rd Ed Fields, BN, Knipe, DM, Howley, PM (Eds), Lippincott-Raven, New York, 1996. p.2901.
29. Cohen, FE, Prusiner, SB. Pathologic conformations of prion proteins. Annu Rev Biochem 1998; 67:793.
30. Kimberlin, RH, Walker, CA. Pathogenesis of mouse scrapie: Evidence for neural spread of infection to the CNS. J Gen Virol 1980; 51:183.
31. Fraser, H, Dickinson, AG. Targeting of scrapie lesions and spread of agent via the retino-tectal projection. Brain Res 1985; 346:32.
32. Borchelt, DR, Koliatsos, VE, Guarnieri, M, et al. Rapid anterograde axonal transport of the cellular prion glycoprotein in the peripheral and central nervous systems. J Biol Chem 1994; 269:14711.
33. Rodolfo, K, Hassig, R, Moya, KL, et al. A novel cellular prion protein isoform present in rapid anterograde axonal transport. Neuroreport 1999; 10:3639.
34. Klein, MA, Frigg, R, Flechsig, E, et al. A crucial role for B cells in neuroinvasive scrapie. Nature 1997; 390:687.
35. Forloni, G, Angeretti, N, Chiesa, R, et al. Neurotoxicity of a prion protein fragment. Nature 1993; 362:543.
36. Brown, DR, Schmidt, B, Kretzschmar, HA. Role of microglia and host prion protein in neurotoxicity of a prion protein fragment. Nature 1996; 380:345.
37. Sparkes, RS, Simon, M, Cohn, VH, et al. Assignment of the human and mouse prion protein genes to homologous chromosomes. Proc Natl Acad Sci U S A 1986; 83:7358.
38. Gajdusek, DC. Infectious amyloids: Subacute spongiform encephalopathies as transmissible cerebral amyloidoses.

In: Fields Virology. 3rd Ed Fields, BN, Knipe, DM, Howley, PM (Eds), Lippincott-Raven, New York, 1996, p.2851.
39. Mallucci, GR, Campbell, TA, Dickinson, A, et al. Inherited prion disease with an alanine to valine mutation at codon 117 in the prion protein gene. Brain 1999; 122 (Pt 10):1823.
40. McLean, CA, Storey, E, Gardner, RJ, et al. The D178N (cis-129M) "fatal familial insomnia" mutation associated with diverse clinicopathologic phenotypes in an Australian kindred. Neurology 1997; 49:552.
41. Gambetti, P. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: A tale of two diseases with the same genetic mutation. Curr Top Microbiol Immunol 1996; 207:19.
42. Meiner, Z, Gabizon, R, Prusiner, SB. Familial Creutzfeldt-Jakob disease. Codon 200 prion disease in Libyan Jews. Medicine (Baltimore) 1997; 76:227.
43. Brown, P, Galvez, S, Goldfarb, LG, et al. Familial Creutzfeldt-Jakob disease in Chile is associated with the codon 200 mutation of the PRNP amyloid precursor gene on chromosome 20. J Neurol Sci 1992; 112:65.
44. Hainfellner, JA, Parchi, P, Kitamoto, T, et al. A novel phenotype in familial Creutzfeldt-Jakob disease: Prion protein gene E200K mutation coupled with valine at codon 129 and type 2 protease-resistant prion protein. Ann Neurol 1999; 45:812.
45. Prusiner, SB. Prion diseases and the BSE crisis. Science 1997; 278:245.
46. Palmer, MS, Dryden, AJ, Hughes, JT, Collinge, J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease [published erratum appears in Nature 1991 Aug 8;352(6335):547]. Nature 1991; 352:340.
47. Collinge, J, Palmer, MS, Dryden, AJ. Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. Lancet 1991; 337:1441.
48. Parchi, P, Giese, A, Capellari, S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46:224.
49. Kretzschmar, HA, Kufer, P, Riethmuller, G, et al. Prion protein mutation at codon 102 in an Italian family with Gerstmann-Straussler-Scheinker syndrome. Neurology 1992; 42:809.
50. Hainfellner, JA, Brantner-Inthaler, S, Cervenakova, L, et al. The original Gerstmann-Straussler-Scheinker family of Austria: divergent clinicopathological phenotypes but constant PrP genotype. Brain Pathol 1995; 5:201.
51. Collinge, J. Inherited prion diseases. Adv Neurol 1993; 61:155.
52. Young, K, Jones, CK, Piccardo, P, et al. Gerstmann-Straussler-Scheinker disease with mutation at codon 102 and methionine at codon 129 of PRNP in previously unreported patients. Neurology 1995; 45:1127.
53. Barbanti, P, Fabbrini, G, Salvatore, M, et al. Polymorphism at codon 129 or codon 219 of PRNP and clinical heterogeneity in a previously unreported family with Gerstmann-Straussler-Scheinker disease (PrP-P102L mutation). Neurology 1996; 47:734.
54. Tanaka, Y, Minematsu, K, Moriyasu, H, et al. A Japanese family with a variant of Gerstmann-Straussler-Scheinker disease. J Neurol Neurosurg Psychiatry 1997; 62:454.
55. Medori, R, Tritschler, HJ, LeBlanc, A, et al. Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. N Engl J Med 1992; 326:444.

EXHIBIT D

LifeLink Foundation

A not-for-profit corporation dedicated to serve patients in need of transplantation therapy

March 22, 2001

Mr. Sam Powers
Senior Clerk
Agency for Health Care Administration
2727 Mahan Drive
Building 3, Room 3426
Tallahassee, Florida 32308

Dear Mr. Powers:

This is being sent to you as the strong objection (comment) of LifeLink Foundation, Inc. to the Waiver request of Regeneration Technologies, Inc. (RTI) pursuant to Fl Stat. 120.542.

RTI has requested a waiver from a Florida regulation, 59A-1.005(45)(a), which is in place to protect the citizens of Florida from being infected with disease from tissue transplants. To our knowledge, no other tissue bank in the state of Florida, nor in the United States, "Batch" processes tissue as RTI does, subjecting recipients of this tissue to the possible diseases of up to 100 tissue donors, with no way to identify which of those donors may have been the source of this disease. The increase in risk is exponential – instead of a possible contamination of 50 tissue grafts, RTI risks exposing 5,000 patients, as noted by the scientific analyses of Tissue banking and transplant specialists Dr. Charles Wright (attached as Exhibit "A"), Dr. Theodore Malinin (on record with AHCA from his testimony at the Florida Statewide Organ and Tissue Advisory Board meeting), and Dr. Josh Miller (by letter, on file with AHCA). Each of these Florida based specialists has firmly denounced the RTI practice, and voted to force RTI to stop batch processing and to comport with other Florida tissue banking regulations on this matter, which they have never done, as your Department's Citation of RTI of February 26, 2001 notes. In fact, RTI cannot comply with these regulations if it continues to batch process tissue. (See letter from AHCA to RTI, attached as Exhibit "B", and letter from Mary-Ann Taylor, Chair of the Advisory Board, to Secretary of Health Rubin King-Shaw, attached as Exhibit "C").

Although there is disagreement as to whether current Florida regulations specifically prohibit pooling of tissue, there is no disagreement that the current regulations require a tracking and "look-back" process which RTI cannot and does not

3409 Bayshore Boulevard, *(entrance on De Leon Street)* Tampa, Florida 33606 ◆ 813-253-2640 ◆ 1-800-262-5775 ◆ Facsimile 813-251-1819 ◆ www.lifelinkfound.org
LifeLink of Florida LifeLink of Georgia LifeLink of Southwest Florida LifeLink of Puerto Rico
LifeLink Tissue Bank LifeLink Transplantation Immunology Laboratory

comply with, as the State has found. This tracking and "look-back" process is specifically in the regulations to protect the health and safety of human tissue recipients.

We are in absolute accord with the vote of the Florida Statewide Organ and Tissue Advisory Board, which voted at its meeting of February 2, 2001 to prohibit the pooling of tissue in the state of Florida, although RTI is already in violation, and has been in violation, of the tracking and safety regulations. In fact, we note that RTI has requested a waiver from Rule 59A-1.005(45)(a), F.A.C., but the AHCA Letter of Citation notes that RTI also currently violates 59A-1.005(1)(b)5, F.A.C, 59A-1.005(14), F.A.C, 59A-1.005(15), F.A.C. and 59A-1.1005(1)(b)4, F.A.C. RTI has not applied for a waiver from these other regulations, to LifeLink's knowledge.

RTI has been in violation of this large number of Florida regulations designed to protect the safety of patients in Florida for years, as noted below. AHCA has finally cited RTI for these violations by letter of February 26, 2001 (attached as Exhibit "B"), but has taken no enforcement action pending the waiver process. However, the Florida Statewide Organ and Tissue Procurement Advisory Board, in its meeting of February 2, 2001 approved a motion to prohibit batch processing (pooling of tissue) in Florida. Similarly, The Federal Food and Drug Administration (FDA), in its newly published draft regulations, expressly prohibits "batch" processing of tissue. The FDA grants no waiver to RTI, and RTI's suggestion in its waiver request to the contrary is a blatant misrepresentation. As Jamie Grooms of RTI stated in front of the Florida Statewide Organ and Tissue Advisory Board, RTI has not received a waiver, only a letter from the FDA stating that RTI's Biocleanse process was validated.

PLEASE NOTE: this objection has nothing to do with Biocleanse. It is an objection to pooling or batch processing of tissue. The issues are not connected. Every other tissue bank has a valid process in place for removing bacteria and other contaminants from tissue. This does not mean it is worth the risk to batch process.

As argued strongly by Dr. Wright, Dr. Malinin, and Dr. Miller, all of whom are Florida-based tissue banking and /or transplantation experts, the greatest risk of allowing pooling is with unknown diseases, and with diseases caused by prions, or "Mad Cow" diseases, known as Transmissable Spongiform Encephalitis (TSE) diseases. We cannot test for, treat, or cure these diseases, which have been transmitted to humans from donated human tissue (human dura mater imported from Europe has transmitted CJD disease to patients in the United States. CJD, along with vCJD disease, is the human version of TSE or "Mad Cow" disease. This transmission occurred because of batch processing in Europe!).

The CDC has recognized the real possibility of transmitting these diseases by tissue donation, by prohibiting tissue donation in the United States from persons who have been to England within six months of the donation. This is because of the real possibility of a person being infected with "Mad Cow" disease from eating infected beef. Further, as of March 22, 2001, the first case of suspected outbreak of Scrapie, the equivalent of "Mad Cow" disease in sheep, was reported in the United States (See attached newspaper report at Exhibit "D"). Clearly, TSE diseases caused by prions are increasing in epidemic proportions worldwide. 90 deaths in Britain have occurred to date. A number of deaths have occurred in the United States.

Also, RTI's system increases risks of transmission of ordinary pathogens if there should be a system failure. There have already been a number of recalls of human tissue. However, in each individual donor, these recalls have been limited to approximately 50 pieces of transplantable tissue. If such a recall were to occur with RTI's process, up to 5,000 pieces of tissue would have to be recalled, with no way to reliably track which donor of the 100 processed together may have been responsible for the problem.

LifeLink objects strongly to the State of Florida granting a waiver to RTI, a for-profit agency which has been in violation of the established Florida tissue banking regulations for a number of years, and which does not comply with the American Association of Tissue Banks Standards prohibiting pooling, nor the proposed FDA regulations prohibiting pooling, nor the formal position of the Florida Statewide Organ and Tissue Advisory Board to prohibit pooling, to allow them to continue to profit from a violation of the regulation by exposing unsuspecting patients to a greater risk of infection. Simply because RTI has made millions of dollars in the last few years on a for-profit basis by violating the Florida regulations is no reason to allow them to continue to do so.

We will be closely monitoring this issue, and will appreciate any opportunity to amplify our comments.

Sincerely,

John R. Campbell, P.A., J.D.
Executive Vice President

cc: Ms. Jo Ann Linch, Health Services and Facilities Consultant
Ms. Michelle Oxman, General Counsel's Office
Mr. John Gilroy, General Counsel's Office
Mr. Jeffery Gregg, Chief, Health Facility Regulation
Ms. Mary Loepp, Hospital & Outpatient Services Unit

LifeLink Foundation

A not-for-profit corporation dedicated to service patients in need of transplantation therapy.

January 24, 2001

Ms. Jo Ann Linch, M.S.W.
Health Services & Facilities Consultant
Bureau of Health Facility Compliance
Agency For Health Care Administration
2727 Mahan Drive
Bldg. 1, Room 252
Tallahassee, Florida 32308

Dear Ms Linch,

I will not be able to attend the meeting of the Advisory Board on February 2, 2001. By this letter I hope to make my position clear, that as a member of the Florida Organ and Tissue Procurement and Transplantation Advisory Board and a Medical Director of the LifeLink Tissue Bank, I am against the practice of batch processing of tissue. I am convinced that batch processing by any currently described technique cannot be relied on to provide safe tissue and assure the public that there is no cross contamination. I am concerned about HIV, HCV and other defined pathogens, but I have a greater concern for the poorly defined Prion diseases or other yet unrevealed pathogens. Even now with our current techniques, we can not know that tissue is 100% safe. If batch processing is allowed, the potential for cross contamination and the amplification of the spread of contamination is a distinct possibility. More importantly, the ability to trace each donor directly to each recipient, and each recipient to each donor is of vital importance with these diseases. This ability would be lost with batch processing.

It is significant that the proposed new FDA Tissue Banking Regulations, published at Federal Register: January 8, 2001 (Volume 66, Number 5), prohibit batch processing, and require direct donor to recipient tracing.

Also, I have read the "Technical Monograph BioCleanse Tissue Processing System: Biological Safety" by C. Randal Mills, Ph.D. and Michael R. Roberts, M.A. (March 30, 2000). It has extensive documentation of the purported ability of BioCleanse to clear standard pathogens. No mention is made of Prions. The authors do not acknowledge the unknown. Many members of the Advisory Board have lived through the evolution of new previously unknown infectious agents (HIV, HVC, lime disease). To presume that

409 Bayshore Boulevard. *(entrance on De Leon Street)* Tampa. Florida 33606 ◆ 813-253-2640 ◆ 1-800-262-5775 ◆ Facsimile 813-251-1819 ◆ www.lifelinkfound.org
LifeLink of Florida LifeLink of Georgia LifeLink of Southwest Florida LifeLink of Puerto Rico
LifeLink Tissue Bank LifeLink Transplantation Immunology Laboratory

EXHIBIT A

process can protect against a biologic unknown borders on arrogance. A prohibition on batch processing and a requirement of direct donor to recipient tracing would provide protection against spreading Prions and unknown pathogens.

I have attached a short summary of the biology of Prions and their diseases. This is an important area which should not be ignored. I did not note any testing relevant to Prions in the RTI document.

Regeneration Technologies is offering technologies to the arena of tissue transplanting. Their quest for safer tissue is admirable. A process like BioCleanse may be proven to make tissue safer at some time in the future. Unfortunately, their dependence on batch processing eclipses any potential benefit. The Advisory Board has gone on record in the past opposed to batch processing of tissue and I vote that we continue this stance.

If this document does not suffice as my vote on this matter, I give my proxy vote to Dr. Metzger, or in his absence Mr. John Campbell, to cast in compliance with this document.

Sincerely,

Charles E. Wright, M.D.
Associate Medical Director,
LifeLink Tissue Bank

cc: Members of Florida Organ and Tissue Procurement and Transplantation Advisory Board
Ms. Laura Branker

UpToDate®
Vol. 8 No. 3

Prions

BIOLOGY OF PRIONS
- Prion protein
- Biosynthesis of PrPC
- Conversion of PrPC to PrPSc
- Transport of PrPSc to the nervous system
- Neurotoxicity of prion protein

GENETICS OF HUMAN PRION DISEASES
- Creutzfeldt-Jakob disease
- Gerstmann-Straussler-Scheinker syndrome
- Fatal familial insomnia

Kenneth L Tyler, MD

Prions

Kenneth L Tyler, MD
Feb 17, 2000

Prion diseases are neurodegenerative diseases that have long incubation periods and progress inexorably once clinical symptoms appear. Five human prion diseases are currently recognized: kuru, Creutzfeldt-Jakob disease (CJD), variant Creutzfeldt-Jakob disease (vCJD also known as new variant CJD), Gerstmann-Straussler-Scheinker syndrome (GSS), and fatal familial insomnia (FFI) [1]. Bovine spongiform encephalopathy (BSE), one of a number of prion infections affecting animals, focus more widespread public attention on these diseases with its possible link to vCJD [2,3].

These human prion diseases share certain common neuropathologic features including neuronal loss, proliferation of glial cells, absence of an inflammatory response, and the presence of small vacuoles within the neuropil which produces a spongiform appearance. Current evidence indicates that prion diseases are associated with the accumulation of an abnormal form of a host cell protein, designated the prion protein (PrP) [4].

The biology of prions will be reviewed here. The clinical manifestations, genetics, and diagnosis of prion diseases are discussed separately. (See "Diseases of the central nervous system caused by prions-I").

BIOLOGY OF PRIONS – Dr. Stanley Prusiner coined the term "prion" in 1982 which he defined as a small infectious pathogen containing protein but apparently lacking nucleic acid [5]. The prion protein (PrP) is the critical component of these agents and may, in fact, be its exclusive constituent.

One of the characteristic features of prions is their resistance to a number of normal decontaminating procedures. These pathogens are resistant to processes affecting nucleic acids such as hydrolyis or shearing [6]. However, agents which digest, denature or modify proteins do have activity against prions [4]. The prion protein purified from the brains of scrapie-infected animals (PrPSc) can be inactivated by prolonged autoclaving (at 121°C and 15 psi for 4.5 h), immersion in 1N NaOH (for 30 min, repeat three times), or in concentrated (>3 M) solutions of guanidine thiocyanate [7]. However, certain cautions prevail; it appears that inadequate autoclaving can establish heat resistant subpopulations which fail to diminish with a further cycle of autoclaving [8]. Stainless steel instruments also may retain infectivity even after treatment with 10 percent formaldehyde [9].

Prion protein – Scrapie prions have been used as a model for prion diseases. PrPSc is a conformational isomer of PrPC, a glycoprotein found in the brains of normal animals [10]. The normal function of PrPC is unknown. PrPC exists primarily in an alpha helical conformation, while PrPSc is beta helical and appears to result from a yet uncharacterized conformational alteration in PrPC [11,12]. The resistance of PrPSc to digestion with proteases and its tendency to polymerize into scrapie-associated fibrils or prion rods differentiates PrPSC from PrPC [13,14]. The hydrophobicity of this protein, which may in turn affect aggregation, and its beta-sheet conformation may play a role in neurotoxicity [15].

Biosynthesis of PrPC – A key step during the biosynthesis of PrPC involves modification of both the amino and carboxy terminals with the addition of a phosphatidylinositol glycolipid which serves to anchor the protein to the cell surface [16,17]. PrPC can be detected attached to the plasma membrane of neurons [18] and may be concentrated at synaptic membranes [19]. In addition, PrPC also has transmembrane domains, indicating that it spans the cellular cytoplasmic membrane. Surface PrPC is degraded after endocytosis in acidic vesicles, although some protein may recycle to the cell surface [20]. Secreted forms of PrPC also occur [21].

Conversion of PrPC to PrPSc – In contrast to PrPC, PrPSc accumulates within cells and does not normally appear on the cell surface. PrPSc is found predominantly in cytoplasmic vacuoles and secondary lysosomes [22]. Conversion of PrPC to PrPSc may occur in caveolae-like membranous domains [23].

Studies with mice either devoid of PrPC or with abnormal isoforms indicate that host PrPC must be present for the development of prion disease. Prion diseases appear to result from accumulation of abnormal isoforms of the PrP which is dependent upon conversion of normal PrPC into PrPSc [24,25]. This conversion appears to be the result of a conformational change in PrPC, rather than a chemical modification.

One group developed a peptide, iPrP13 which can break a beta-sheet conformation [26]. This peptide was able to reduce the protease resistance of PrPSc and to delay the onset of symptoms in transmission experiments in mice. There may be another as yet unidentified host factor, designated protein X, which may bind to the carboxy terminus of PrPC then interact with a site near the N-terminus to effect a conformational change [27].

How the first molecule of PrPSc appears in the host remains a mystery, but the initial appearance, which may be de novo, probably triggers the replication of PrPSc. This process has been compared to crystallization in solution where a single seed crystal serves as a nidus [3]. It is hypothesized that the initiating PrPSc molecule is derived from an exogenous source in sporadic and iatrogenic prion diseases, while mutations are invariably detected within the PRNP gene in familial forms [29]. These mutations could destabilize PrPC which might lead to spontaneous conversion to PrPSc.

Transport of PrPSc to the nervous system – Transport of PrPSc to the nervous system, once it appears in the host, occurs via axons [30]. Previous investigations suggested that the predominant mechanism was by slow axoplasmic transport [31]. However, several studies now provide data that rapid anterograde axonal transport also occurs [32,33]. In one of these reports, a specific isoform of the protein was transported via this route in a hamster model compared to several other isoforms found within neural tissues which appeared to arrive by a slower transport mechanism [33].

The lymphoreticular system may play a critical role in the initiation of some prion diseases [34]. Studies in mice indicate that for some prion diseases acquired by inoculation, a period of replication in lymphoreticular tissue is required. This would be expected for certain types of exogenously aquired prion diseases such as iatrogenic CJD, kuru, and perhaps vCJD, but might not occur in sporadic and genetic forms of prion diseases.

Neurotoxicity of prion protein – PrPSc appears to be neurotoxic; accumulation of this protein or fragments of it in neurons leads to apoptosis and cell death [35,36]. As an example, the PrP fragment containing amino acids 106-126 induces death of hippocampal neurons following exposure in vitro [35]. However, PrPC must be present for this effect to occur; PrP106-126 does not destroy neurons in mice which do not express PrPC [36].

GENETICS OF HUMAN PRION DISEASES – The gene encoding PrP ("PRNP") in humans is located on the short arm of chromosome 20 [37]. A strong link was established between mutations in the PRNP gene and forms of prion disease with a familial predisposition (fCJD, GSS, FFI). More than 20 different mutations have been identified [28,38]. Some experts have advocated classifying prion diseases based upon the responsible mutation rather than the traditional classifications such as fCJD or GSS since sometimes a single mutation produces different clinical phenotypes in different individuals or families. As an example, the D178N mutation, in which asparagine substitutes for aspartic acid in codon 178, occurs in families with FFI, fCJD, and GSS [37]. A large English and Irish kindred has been described containing individuals diagnosed with a variety of conditions including CJD, vCJD, and GSS [39]. However, when the PRNP gene was examined, all affected individuals had a valine for alanine substitution at codon 117 regardless of the clinical diagnosis.

The phenotype of a particular mutation may be influenced by the nature of the amino acids present at codon 129. Codon 129 of the PRNP gene is a polymorphic codon; normal individuals have either valine or methionine at that site. However, since the PRNP gene is on an autosomal dominant gene, there are two copies, and individuals can be homozygous or heterozygous at this site. Patients with the D178N mutation who are homozygous for valine at codon 129 appear to develop CJD, while those who are homozygous for

methionine tend to have FFI [40,41]. Despite these patterns, the clinical expression of individual mutations can vary even between affected members of the same family.

Creutzfeldt-Jakob disease – In fCJD the most common mutation is a substitution of lysine for glutamine in codon 200, which has been observed in regions ranging from Libya to Chile [42,43]. One study described a differing presentation of this syndrome with codon 129 phenotype changes [44]. When the mutant codon 200 was linked to a valine at codon 129, PrP deposits were observed in the cerebellum and the prion protein was resistant to type 2 protease, neither of which have been described with methionine at codon 129.

As noted above, the D178N mutation occurs in fCJD. A substitution of isoleucine for valine in codon 210 has also been noted in fCJD [45].

Unlike fCJD, sCJD and iCJD are not associated with PRNP gene mutations. However, even in these forms of CJD and vCJD, phenotyping at codon 129 appears to affect susceptibility and perhaps expression of the clinical illness. While 51 percent of the general population are heterozygous at codon 129, for example, all cases of vCJD and 85 to 95 percent of individuals with sCJD have been found to be homozygous at this codon [46]. In a separate report, five of seven patients who developed iCJD after receiving human cadaveric growth hormone were homozygous at codon 129 [47].

One group has proposed a molecular classification scheme for sCJD based upon codon 129 polymorphism and characterization of the properties of PrPSc which was used to evaluate 300 sCJD patients [48]. As examples, a pattern of type 1 PrPSc plus at least one methionine at codon 129 was demonstrated in 70 percent while type 2 PrPSc plus codon 129 homozygous or heterozygous for valine was present in 25 percent and associated with ataxia.

Gerstmann-Straussler-Scheinker syndrome – All GSS kindreds investigated to date have PRNP gene mutations of which P102L is the most common and the one described in the descendants of the original family described by Gerstmann, Straussler, and Scheinker [49,50]. Other reported mutations include P105L, A117V, Y145STOP, Q217R, and E219L [38,51]. As noted above, patients with some of these mutations have been clinically classified as fCJD or FFI rather than GSS.

GSS exhibits a large degree of phenotypic heterogeneity. This may be partially due to differences in the underlying PRNP mutation. GSS patients with the P102L mutation, for example, may have more prominent cerebellar features [49], while dementia may be a more prominent feature in patients with A117V, Y145STOP, and F198S mutations. Polymorphism at codon 129 may also play a modulating role in the manifestations of GSS in patients with the P102L mutation [52,53]. However, varied clinical expression of these diseases both between and within affected families with the same gene mutations suggests that other unidentified factors probably also are influential [53,54].

Fatal familial insomnia – As noted above, the D178N mutation has been the predominant mutation found in nearly all families with FFI [55]. This mutation also occurs in fCJD. It appears that patients with this mutation who are homozygous for methionine at codon 129 develop an FFI-like clinical syndrome whereas those homozygous for valine develop fCJD [40,41]. Heterozygosity at codon 129 may prolong the duration and slow the temporal progression of FFI.

References

1. Haywood, AM. Transmissible spongiform encephalopathies. N Engl J Med 1997; 337:1821.
2. O'Brien, C. Mad cow disease. Scant data cause widespread concern [news]. Science 1996; 271:1798.
3. Collinge, J. Variant Creutzfeldt-Jakob disease. Lancet 1999; 354:317.
4. Prusiner, SB. The prion hypothesis. In: Prions, Prusiner, SB, McKinley, MP (Eds), Academic Press, San Diego, 1987, p. 17.
5. Prusiner, SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 216:136.
6. Bellinger-Kawahara, C, Diener, TO, McKinley, MP, et al. Purified scrapie prions resist inactivation by procedures that hydrolyze, modify, or shear nucleic acids. Virology 1987; 160:271.
7. Manuelidis, L. Decontamination of Creutzfeldt-Jakob disease and other transmissible agents. J Neurovirol 1997; 3:62.
8. Taylor, DM, Fernie, K, McConnell, I, Steele, PJ. Observations on thermostable subpopulations of the unconventional agents that cause transmissible degenerative encephalopathies. Vet Microbiol 1998; 64:33.
9. Zobeley, E, Flechsig, E, Cozzio, A, et al. Infectivity of scrapie prions bound to a stainless steel surface. Mol Med 1999; 5:240.
10. Harris, DA. Cellular biology of prion diseases. Clin Microbiol Rev 1999; 12:429.
11. Downing, DT, Lazo, ND. Molecular modelling indicates that the pathological conformations of prion proteins might be beta-helical. Biochem J 1999; 343 Pt 2:453.
12. Harrison, PM, Chan, HS, Prusiner, SB, Cohen, FE. Thermodynamics of model prions and its implications for the problem of prion protein folding. J Mol Biol 1999; 286:593.
13. Prusiner, SB, McKinley, MP, Bowman, KA, et al. Scrapie prions aggregate to form amyloid-like birefringent rods. Cell 1983; 35:349.
14. Merz, PA, Kascsak, RJ, Rubenstein, R, et al. Antisera to scrapie-associated fibril protein and prion protein decorate scrapie-associated fibrils. J Virol 1987; 61:42.
15. Jobling, MF, Stewart, LR, White, AR, et al. The hydrophobic core sequence modulates the neurotoxic and secondary structure properties of the prion peptide 106-126. J Neurochem 1999; 73:1557.
16. Stahl, N, Borchelt, DR, Hsiao, K, Prusiner, SB. Scrapie prion protein contains a phosphatidylinositol glycolipid. Cell 1987; 51:229.
17. Harris, DA, Huber, MT, van Dijken, P, et al. Processing of a cellular prion protein: identification of N- and C-terminal cleavage sites. Biochemistry 1993; 32:1009.
18. Narwa, R, Harris, DA. Prion proteins carrying pathogenic mutations are resistant to phospholipase cleavage of their glycolipid anchors. Biochemistry 1999; 38:8770.
19. Herms, J, Tings, T, Gall, S, et al. Evidence of presynaptic location and function of the prion protein. J Neurosci 1999; 19:8866.
20. Shyng, SL, Huber, MT, Harris, DA. A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. J Biol Chem 1993; 268:15922.
21. Hay, B, Prusiner, SB, Lingappa, VR. Evidence for a secretory form of the cellular prion protein. Biochemistry 1987; 26:8110.
22. Borchelt, DR, Taraboulos, A, Prusiner, SB. Evidence for synthesis of scrapie prion proteins in the endocytic pathway. J Biol Chem 1992; 267:16188.
23. Vey, M, Pilkuhn, S, Wille, H, et al. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. Proc Natl Acad Sci U S A 1996; 93:14945.
24. Bueler, H, Aguzzi, A, Sailer, A, et al. Mice devoid of PrP are resistant to scrapie. Cell 1993; 73:1339.
25. Bueler, H, Fischer, M, Lang, Y, et al. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 1992; 356:577.
26. Soto, C, Kascsak, RJ, Saborio, GP, et al. Reversion of prion protein conformational changes by synthetic ß-sheet breaker peptides. Lancet 2000; 355:192.
27. Kaneko, K, Zulianello, L, Scott, M, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc Natl Acad Sci U S A 1997; 94:10069.
28. Prusiner, SB. Prions. In: Fields Virology. 3rd Ed Fields, BN, Knipe, DM, Howley, PM (Eds), Lippincott-Raven, New York, 1996, p.2901.
29. Cohen, FE, Prusiner, SB. Pathologic conformations of prion proteins. Annu Rev Biochem 1998; 67:793.
30. Kimberlin, RH, Walker, CA. Pathogenesis of mouse scrapie: Evidence for neural spread of infection to the CNS. J Gen Virol 1980; 51:183.
31. Fraser, H, Dickinson, AG. Targeting of scrapie lesions and spread of agent via the retino-tectal projection. Brain Res 1985; 346:32.
32. Borchelt, DR, Koliatsos, VE, Guarnieri, M, et al. Rapid anterograde axonal transport of the cellular prion glycoprotein in the peripheral and central nervous systems. J Biol Chem 1994; 269:14711.
33. Rodolfo, K, Hassig, R, Moya, KL, et al. A novel cellular prion protein isoform present in rapid anterograde axonal transport. Neuroreport 1999; 10:3639.
34. Klein, MA, Frigg, R, Flechsig, E, et al. A crucial role for B cells in neuroinvasive scrapie. Nature 1997; 390:687.
35. Forloni, G, Angeretti, N, Chiesa, R, et al. Neurotoxicity of a prion protein fragment. Nature 1993; 362:543.
36. Brown, DR, Schmidt, B, Kretzschmar, HA. Role of microglia and host prion protein in neurotoxicity of a prion protein fragment. Nature 1996; 380:345.
37. Sparkes, RS, Simon, M, Cohn, VH, et al. Assignment of the human and mouse prion protein genes to homologous chromosomes. Proc Natl Acad Sci U S A 1986; 83:7358.
38. Gajdusek, DC. Infectious amyloids: Subacute spongiform encephalopathies as transmissible cerebral amyloidoses.

In: Fields Virology. 3rd Ed Fields, BN, Knipe, DM, Howley, PM (Eds), Lippincott-Raven, New York, 1996, p.2851.
39. Mallucci, GR, Campbell, TA, Dickinson, A, et al. Inherited prion disease with an alanine to valine mutation at codon 117 in the prion protein gene. Brain 1999; 122 (Pt 10):1823.
40. McLean, CA, Storey, E, Gardner, RJ, et al. The D178N (cis-129M) "fatal familial insomnia" mutation associated with diverse clinicopathologic phenotypes in an Australian kindred. Neurology 1997; 49:552.
41. Gambetti, P. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: A tale of two diseases with the same genetic mutation. Curr Top Microbiol Immunol 1996; 207:19.
42. Meiner, Z, Gabizon, R, Prusiner, SB. Familial Creutzfeldt-Jakob disease. Codon 200 prion disease in Libyan Jews. Medicine (Baltimore) 1997; 76:227.
43. Brown, P, Galvez, S, Goldfarb, LG, et al. Familial Creutzfeldt-Jakob disease in Chile is associated with the codon 200 mutation of the PRNP amyloid precursor gene on chromosome 20. J Neurol Sci 1992; 112:65.
44. Hainfellner, JA, Parchi, P, Kitamoto, T, et al. A novel phenotype in familial Creutzfeldt-Jakob disease: Prion protein gene E200K mutation coupled with valine at codon 129 and type 2 protease-resistant prion protein. Ann Neurol 1999; 45:812.
45. Prusiner, SB. Prion diseases and the BSE crisis. Science 1997; 278:245.
46. Palmer, MS, Dryden, AJ, Hughes, JT, Collinge, J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease [published erratum appears in Nature 1991 Aug 8;352(6335):547]. Nature 1991; 352:340.
47. Collinge, J, Palmer, MS, Dryden, AJ. Genetic predisposition to iatrogenic Creutzfeldt-Jakob disease. Lancet 1991; 337:1441.
48. Parchi, P, Giese, A, Capellari, S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46:224.
49. Kretzschmar, HA, Kufer, P, Riethmuller, G, et al. Prion protein mutation at codon 102 in an Italian family with Gerstmann-Straussler-Scheinker syndrome. Neurology 1992; 42:809.
50. Hainfellner, JA, Brantner-Inthaler, S, Cervenakova, L, et al. The original Gerstmann-Straussler-Scheinker family of Austria: divergent clinicopathological phenotypes but constant PrP genotype. Brain Pathol 1995; 5:201.
51. Collinge, J. Inherited prion diseases. Adv Neurol 1993; 61:155.
52. Young, K, Jones, CK, Piccardo, P, et al. Gerstmann-Straussler-Scheinker disease with mutation at codon 102 and methionine at codon 129 of PRNP in previously unreported patients. Neurology 1995; 45:1127.
53. Barbanti, P, Fabbrini, G, Salvatore, M, et al. Polymorphism at codon 129 or codon 219 of PRNP and clinical heterogeneity in a previously unreported family with Gerstmann-Straussler-Scheinker disease (PrP-P102L mutation). Neurology 1996; 47:734.
54. Tanaka, Y, Minematsu, K, Moriyasu, H, et al. A Japanese family with a variant of Gerstmann-Straussler-Scheinker disease. J Neurol Neurosurg Psychiatry 1997; 62:454.
55. Medori, R, Tritschler, HJ, LeBlanc, A, et al. Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. N Engl J Med 1992; 326:444.

STATE OF FLORIDA
AHCA
AGENCY FOR HEALTH CARE ADMINISTRATION

JEB BUSH, GOVERNOR

February 26, 2001

C. Randall Mills, Ph.D.
Regeneration Technologies, Inc.
One Innovation Drive
Alachua, Florida 32615

Dear Dr. Mills:

The Agency has reviewed the information presented in your letter of December 8, 2000 regarding Regeneration Technologies Inc.'s (RTI) compliance with the requirements of Chapter 59A-1, Florida Administrative Code (F.A.C.). Rule 59A-1.005(1)(b)4, F.A.C. requires that tissues from each individual donor will have one unique identification number that will identify each donor's tissue from retrieval through distribution and utilization. Further, as we discussed during our meeting on January 30, 2001, Rule 59A-1005(45), F.A.C. makes clear that each tissue sample must contain tissue from only one donor. A sanitizing method that permits multiple donor tissues to be processed simultaneously could meet that requirement only if it allowed the tissue from each donor to remain identifiable. Your statement that RTI's process enables you only to trace tissue components back to a lot number representing more than one donor demonstrates that your procedures are in violation of the rule.

There are a number of other provisions of the rule with which RTI cannot comply to the extent that it cannot trace donated tissue to a particular donor or recipient. Rule 59A-1.005(1)(b)5, F.A.C., requires that all information regarding a particular donor be provided to the transplant surgeon on request. The recall and look-back procedures required by Rule 59A.1005(14) and (15), F.A.C., respectively, depend upon the ability to trace transplanted tissue from an individual donor to each individual recipient. Should the need arise, RTI cannot comply with these rules as written.

These violations subject RTI to administrative action to enforce compliance with the rules under Rule 59A-1.012(1)(d), F.A.C. The possible penalties include, but are not limited to, an administrative fine not to exceed $500 per day per violation, suspension, or revocation

of the tissue bank certificate issued to Regeneration Technologies, Inc. However, the Agency is in receipt of your petition for a waiver pursuant to § 120.542, F.S. At this time, the Agency does not contemplate enforcement action regarding the above violations pending consideration of the petition for rule waiver or variance, and timely provision of all information required to make a determination. However, should safety violations arise, the Agency will not delay administrative action based upon the pendency of a petition for waiver.

Our preliminary review of the petition indicates that additional information or alternative procedures will be needed. If, after reading this notice you wish to add anything as an addendum to your request, please feel free to do so and provide it to us as soon as possible. We anticipate completion of the review within thirty days of filing, *i.e.*, by March 4, 2001. Upon completion of the review, the Agency will notify you of the additional items required. Should you have any questions, please contact Michelle Oxman in the General Counsel's office at (850) 922-8854.

Sincerely,

Elizabeth Dudek, Acting Deputy Secretary
Division of Managed Care and Health Quality

cc: Julie Gallagher, General Counsel
Michelle Oxman, General Counsel's Office
John Gilroy, General Counsel's Office
Jeffrey Gregg, Chief, Health Facility Regulation
Mary Loepp, Hospital & Outpatient Services Unit

March 16, 2001

Ruben King-Shaw, Secretary
Agency for Health Care Administration
2727 Mahan Drive, Mail Stop 1
Tallahassee, FL 32308

Dear Secretary King-Shaw:

The Organ and Tissue Procurement and Transplantation Advisory Board is authorized under Chapter 381.6023, Florida Statutes (F.S.). One of the purposes of the Board is to make recommendations to the Agency regarding "changes to the laws...and administrative rules or procedures required to assure that the statewide organ and tissue procurement and transplantation system will be able to function smoothly, effectively, and efficiently..."

Recent developments in the tissue bank industry that have been brought to the attention of the Board are a cause of concern regarding the safety of one processing technique used to sterilize tissue. Strict guidelines have been developed to ensure that any tissue distributed for transplantation has been adequately screened to eliminate the transmission of communicable diseases and cross-contamination. Traditionally, and because transplantation involves donor-recipient interaction, tissues from each donor are processed separately to ensure the highest degree of safety. However, recently tissue pooling which was previously deemed unsuitable procedure has re-emerged because of some new technological developments. This means simultaneous processing of tissues and including in the same container tissue from multiple donors. When tissue is pooled in this manner, it becomes impossible to track contaminated tissue back to the original donor. It also would make it impossible to avoid transplanting tissues from Rh Negative donors into women of childbearing age.

The American Association of Tissue Banks (AATB) is the professional accrediting organization that sets standards for tissue banking. Accreditation by the AATB is voluntary. The AATB prohibits its accredited facilities from pooling tissues.

The Food & Drug Administration (FDA) issued draft tissue bank regulations on January 8, 2001, which will also prohibit tissue pooling when they are finalized. All tissue banks are required to comply with FDA regulations.

EXHIBIT C

No sterilization technique is fool proof. New and emerging diseases, such as prion diseases, may not be caught by the currently recognized sterilization processes used. Simultaneously processing tissues from multiple donors will increase the risk of cross-contamination to transplant recipients. If contaminated tissue is transplanted, a recall or notification of surgeons who used tissue from that donor would be very difficult.

The Advisory Board recently voted to recommend to the Agency that Ch. 59A-1, Florida Administrative Code, be revised to prohibit tissue pooling by any Florida certified tissue bank. This revision would be added when the rule is next updated.

In addition, the Board feels this matter is of sufficient importance in ensuring transplant safety to all individuals that the Board is now formally requesting the Agency to appoint an independent and well-qualified panel to investigate the details of the process being used with tissue pooling. This study should include the safety and efficacy of tissue pooling and new and emerging diseases. The Board is also requesting that the findings of the panel be reported back to the Board. It is anticipated that such a study may indicate additional revisions that need to be made to the regulations governing organ and tissue procurement agencies.

Since the February 2 meeting of the Advisory Board where this matter was discussed, I have learned that Regeneration Technologies International (RTI) has sent a letter to AHCA requesting a waiver on this matter of tissue pooling. To my knowledge, AHCA has not yet drafted a response to this request.

If you would like to discuss the possibilities of developing such a panel or need additional information, please feel free to contact me. I can be reached at (1-800) 329-7000, extension 4323.

Sincerely

Mary Anne Taylor, M.S., Chair
Organ and Tissue Procurement and
Transplantation Advisory Board

Cc: Members of Advisory Board
Mr. Sam Power
JoAnn Linch

Mar 22, 2001 - 12:24 AM

Disease fears hit home

STEPHANIE SIMON
of the Los Angeles Times

Federal officials on Wednesday seized a Vermont farmer's flock of sheep over suspicions that some of the animals may be infected with mad cow disease and could pose a threat to livestock nationwide.

It was the first time that the U.S. government has confiscated livestock as a precaution against mad cow disease. Some two dozen federal agents converged on a farm in Greensboro, Vt., at dawn to load 233 sheep and lambs onto trailers. Security agents will escort the animals to a lab in Ames, Iowa, where they will be killed and tested.

The U.S. Department of Agriculture plans to seize a second flock of 140 sheep in East Warren, Vt., within the next few weeks.

It will take at least two years - and possibly much longer - before pathologists can determine for sure whether the animals harbor mad cow disease or a related illness, scrapie, that is relatively common in sheep and cannot be transmitted to humans.

Given the uncertain science, the flocks' owners have bitterly protested the seizure as premature.

But USDA veterinarian Linda Detwiler insisted that the agency ``had no choice but to take decisive action. ... We needed to take those sheep.''

Mad cow disease, or bovine spongiform encephalopathy, has never been detected in the United States. Yet it has devastated Europe, spreading from Britain to France, Italy, Germany and beyond. Nearly 100 people have died of a human version believed to be transmitted through tainted meat products.

Even if the Vermont sheep do have mad cow disease, the chances of them infecting humans or other livestock are extremely low.

Some 50 lambs from the two suspect flocks were sold to local residents for meat several years ago. And cheese made from the ewes' milk continues to be marketed in Vermont and some neighboring states. But mad cow disease is not known to spread to humans through milk products, or through the cuts of meat that commonly are eaten.

Instead, the disease seems to concentrate in brain and spinal tissue. So the most likely scenario for the Vermont sheep to infect the food chain would be if they were slaughtered, ground up and fed to other livestock. Federal law bans this practice. And the Vermont sheep have been under quarantine since 1998, their every movement monitored by federal inspectors.

Given all those controls, ``the risks [the sheep pose] to both animal and human health are very small,'' said George Gray, who has studied mad cow disease as director of the Harvard University's center for risk assessment. Nonetheless, he acknowledged, ``you can construct a chain of possibles'' leading to widespread contamination.

``It's impossible to say never,'' he said. ``It's impossible to say it couldn't happen.''

And the longer the Vermont sheep grazed their pastures, the more nervous USDA officials got, Detwiler said. ``It just increases your chances of something getting

EXHIBIT D

officials got, Detwiler said. ``It just increases your chances of something getting away, the longer [the animals] are out there.''

From the owners' perspective, there is nothing to ``get away.''

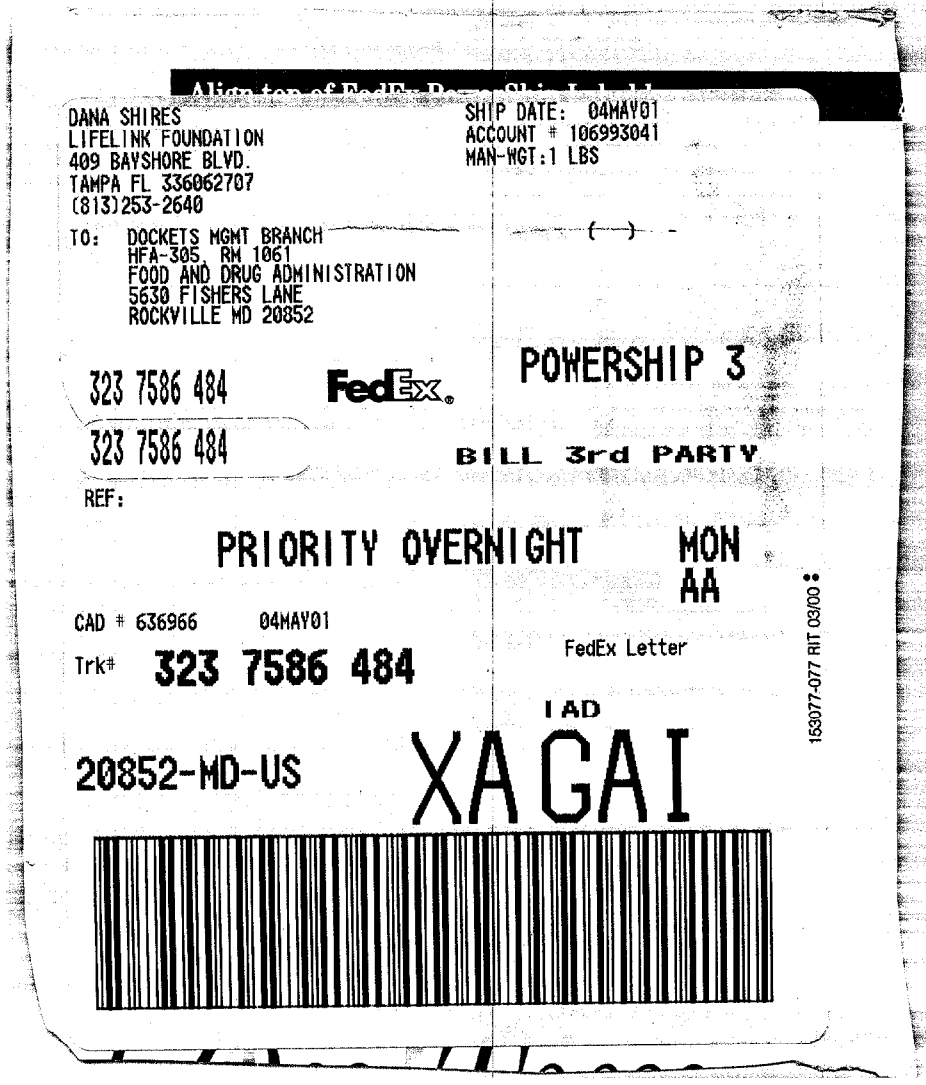
They are convinced that their animals are healthy - or at worst have scrapie, which crops up in several dozen sheep across the United States each year.

Scrapie shares certain characteristics with mad cow disease. Both illnesses can incubate for years before attacking an animal's central nervous system. Both show up with the same awful symptoms: animals slipping, falling, scraping against fences and, eventually dying - their brains having turned spongy. But scrapie has been around in sheep for 250 years and has never, as far as scientists can tell, jumped the species barrier to infect humans. Mad cow disease has, with devastating results.

The USDA maintains that the Vermont sheep - which were imported from Europe in 1996 - could have been exposed to feed tainted with mad cow disease before they came to the United States. There has never been a documented case of a farm sheep coming down with mad cow. But scientists have proved in the lab that it is possible.

The USDA has promised to pay the owners of the sheep fair market value.

Concerned about the mad cow and foot-and-mouth outbreaks? Go to TBO.comrelated articles in our multimedia report.



DANA SHIRES
LIFELINK FOUNDATION
409 BAYSHORE BLVD.
TAMPA FL 336062707
(813)253-2640

SHIP DATE: 04MAY01
ACCOUNT # 106993041
MAN-WGT:1 LBS

TO: DOCKETS MGMT BRANCH
HFA-305, RM 1061
FOOD AND DRUG ADMINISTRATION
5630 FISHERS LANE
ROCKVILLE MD 20852

323 7586 484

FedEx

POWERSHIP 3

323 7586 484

BILL 3rd PARTY

REF:

PRIORITY OVERNIGHT MON
AA

CAD # 636966 04MAY01

Trk# 323 7586 484

FedEx Letter

163077-077 RIT 03/00

IAD

20852-MD-US XA GAI