CJD Incidents Panel
Teleconference to discuss SNBTS issue
1st February 2011
Action notes

Present

Mr David Pryer (Chairman, CJD Incidents Panel)
Dr Jillian Cooper, NIBSC, HPA
Professor James Hope, Veterinary Laboratories Agency
Professor Jean Manson, The Roslin Institute, University of Edinburgh
Dr Simon Mead, National Prion Clinic and MRC Prion Unit
Dr Philip Minor, NIBSC, HPA
Dr Chris Prowse, Research & Development Director, Scottish National Blood Transfusion Service
Professor Marc Turner, Medical Director, Scottish National Blood Transfusion Service
Dr Mark Head, National CJD Surveillance Unit, School of Molecular & Clinical Medicine, University of Edinburgh (Professor Richard Knight, NCDSU Director, was also present with Dr Head during the teleconference)
HPA CJD Section Secretariat
Nicky Connor
Helen Janecek
Victoria Hall

1 Introduction

The Scottish National Blood Transfusion Service had requested advice from the Panel concerning the actions which should be taken in relation to four donors implicated in the unexpected finding of a positive PMCA test during the development of a confirmatory assay for future screening of blood donations. At its 20th January meeting the Panel had decided that further information was required from other experts, including members of the CJD Resource Centre Oversight Committee (ROC), about the interpretation of the assay result before it would be possible to issue advice.

The objectives of the teleconference were to:

- Enable members of ROC to discuss the assay methodology and results with the SNBTS and NCJDSU scientists.
- Discuss what further work should be taken to help members of ROC interpret the assay results.
- Ensure that appropriate independent scientific expertise was available to the Panel to enable it to respond to the SNBTS request for advice.

2 Discussion

PMCA Assay

The initial precipitation step of the PMCA assay had been improved since being tested previously on the blinded panel provided by ROC. This initial blinded run had revealed issues with sensitivity of the assay, and some lesser

issues with false positive results. The modified assay had been rerun on an un-blinded panel, with better results.

Sample anonymisation had been broken in order to further investigate the reactive sample. SNBTS planned to run the assay on additional archived samples from the four donors.

Participants in the teleconference expressed their concerns about the ethics of testing patient-identifiable samples without specific consent. SNBTS reported that they had decided to do this because of the blood services' ethical and legal responsibility to take a highly precautionary stance in protecting the safety of the blood supply.

SNBTS explained that the blood of a donor with a repeat reactive assay cannot continue to be used even if (as here) the scientific and clinical significance is highly uncertain, and the donor must be deferred and informed of the reason for the deferral.

Extra samples were available from original donation date, as well as for some other donation dates. The first set of tests had been undertaken in the SNBTS laboratory and repeated in the NCJDSU laboratory; the second set of tests (on the archived samples) would be carried out using the same methodology, with certain steps carried out at the NCJDSU laboratory and others performed at the SNBTS laboratory. This was because of staffing shortages at SNBTS and technical issues with equipment. Governance issues aside, it was **agreed** that there were several possible explanations for the observed positive result from one sample; testing of archival samples from the same individual(s) might help determine which of these explanations was most likely to be correct. The data from these tests would be available by the end of February.

It was **agreed** that the modified assay would also be run again on a new blinded panel. However this work, involving two sets of 24 samples, would take some months. Professor Manson **agreed** to see if she could provide help with staffing.

An additional possibility would be for the four archived donor samples to be tested using another assay, such as the one being developed by the MRC Prion Unit. However, Dr Mead reported that the Prion Unit assay had only been developed using whole blood samples, while the donor samples were buffy coat. Dr Mead **agreed** to discuss the possibility of using the MRC Prion Unit assay with Professor John Collinge and Dr Graham Jackson.

Since the PMCA assay had been tested in a newly commissioned SNBTS laboratory, it was unlikely, although still possible, that the reactive result was due to contamination. It was **agreed** that this issue would be thoroughly investigated by undertaking a detailed audit of each step of the testing process. This would require input from key staff members, currently on sick leave.

Inoculation into mice

In April 2010, once the repeat reactive test result had been obtained, the amplicon from the reactive sample was inoculated into 22 homozygous (MM)

transgenic mice (tg650), with 22 positive control mice; and 15 negative control mice. The original plan had been to sacrifice 10 of each group in April 2011. However, when eight of the mice died of inter-current illness, their spleens were investigated with the following results:

- Three of the four dead mice inoculated with the index donation amplicon had PrP^{res} immunoreactivity in their spleens.
- Both of the two positive controls tested positive for PrP^{res} immunoreactivity.
- Both of the two negative controls tested negative for PrP^{res} immunoreactivity.

It was **agreed** that no mice should be sacrificed ahead of schedule, and that the experiment should run its course. (These transgenic mice have an average lifespan of 300 to 500 days.)

Genotype and sequencing

The sample with a reactive PMCA result had been codon 129 genotyped and found to be MM homozygous.

It was **agreed** that the donors would not be investigated for mutations associated with inherited prion disease, as it would be unethical to carry out this work without the donors' explicit consent. [1]

3 Next steps

It was **agreed** that the results of the next round of testing would be discussed in early March 2011 at a CJD Incidents Panel subgroup with a view to issuing advice to SNBTS on the management of the four implicated donors. The Panel Secretariat would consult the teleconference participants on which other experts should be invited to the subgroup.

4 Agreed actions

1. SNBTS to send results of un-blinded panel for modified PMCA assay to CJD Resource Centre Oversight Committee.
2. SNBTS and NCJDSU laboratories to run tests on the archived samples from the four relevant donors using the modified PMCA assay.
3. SNBTS to arrange further assay runs on blinded panels provided by ROC.
4. Professor Manson to explore feasibility of providing help with staffing to SNBTS.

[1] Following the meeting it was reported that the sample relating to the reactive PMCA had also been sequenced (with a normal result). It was confirmed by SNBTS that no further genotyping would be undertaken. SNBTS confirmed that an investigation was being undertaken into the genetic sequencing of this linked sample without specific donor consent for this activity.

5. Dr Mead to discuss with Professor John Collinge and Dr Graham Jackson feasibility of using the MRC Prion Unit assay on samples from the reactive donor.
6. SNBTS to investigate the possibility of contamination by undertaking detailed audit of each step of the testing process.
7. SNBTS to complete experiment on inoculated mice.
8. Panel Secretariat to organise Panel subgroup meeting.