Frequently asked questions concerning the 14 June 2001 *Nature* paper from Dr. Claudio Soto and colleagues (Gabriela P. Saborio, Bruno Permanne & Claudio Soto, "Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding"; Nature; June 14 2001; 411: 810-813.)

1. What are prions?

Prions, or abnormal prion proteins, are the infectious agents associated with fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs) – so named because brain tissue develops holes, taking on a sponge-like appearance, in the final stage of the disease. In animals, TSEs include bovine spongiform encephalopathy (BSE or "mad cow" disease) and scrapie. TSEs afflicting humans include standard forms of Creutzfeldt-Jakob disease and a new variant (vCJD), kuru, Gerstmann-Straussler and fatal familial insomnia.

2. How much is known about the disease mechanism of prions?

So-called "normal" prion proteins (PrP^{C}), those with a normal molecular shape, are naturally occurring and are attached to the surface of many cell types in the body. Their function is still unknown. Prions become pathological when their shape changes. Once they have taken on an abnormal shape, these "abnormal" prion proteins (PrP^{Sc}) have the ability to replicate themselves. They convert normal prion proteins into abnormal prion proteins, which in turn convert other normal prion proteins, causing a slow but relentless chain reaction. Over time, aggregates of these abnormal prion proteins form plaques in the brain, causing degeneration of tissue and neuronal death, which leads to the disease symptoms.

3. Are prions similar to bacteria or viruses?

Abnormal prion proteins have a completely different mode of infection from bacteria or viruses, and are very stable. Many methods of killing bacteria and viruses are ineffective against prions, which can resist heat up to 100°C, chemical disinfectants, and radiation. They decompose biologically very slowly

4. What caused the current epidemic of BSE?

The recent epidemic of bovine spongiform encephalopathy, which peaked in 1992, was caused by feeding cattle protein supplements containing meat and other byproducts from animals infected with BSE. Ingesting one gram of

contaminated material — the size of a peppercorn — is sufficient to cause infection in cattle. See WHO Fact Sheet No. 113 on causes and symptoms of BSE www.who.int/inf-fs/en/fact113.html

5. Is variant Creutzfeldt-Jakob disease caused by eating meat from cattle infected with BSE?

There is strong evidence linking variant Creutzfeldt-Jakob disease with meat and other byproducts from cattle infected with BSE. See WHO Fact Sheet No. 180 on causes and symptoms of variant Creutzfeldt-Jakob disease: www.who.int/inf-fs/en/fact180.html

6. How long do these diseases take to manifest themselves?

There is a long incubation period in vivo. Cattle can be infected with BSE for 5-7 years without showing clinical symptoms. In humans, nvCJD can take 10-20 years to manifest itself. Once clinical symptoms appear in humans, life expectancy is usually six months to two years. Therefore to treat these diseases early, one needs a diagnostic tool.

7. How many people have been infected with vCJD?

The first confirmed case of vCJD was reported in the UK in 1995. Today, there are about 100 confirmed cases in the UK, Ireland and France. Because of the long incubation period of vCJD and uncertainty about the date when infections started, it is difficult to know where we are today on the time curve of the disease. Estimates on the total number of people infected vary from a few hundred to 250,000, according to a recent UK government study. See the latest monthly statistics on incidences of vCJD from the UK Department of Health at www.doh.gov.uk/cjd/stats/jun01.html

8. What medical therapies are available to treat prion-related diseases?

Currently, there are no known therapies and so these diseases are fatal.

9. Do diagnostic tests exist for BSE?

At present, there are a number of companies making diagnostic tests for BSE, including Prionics (Switzerland), Bio-Rad (US, UK), Enfer Scientific (Ireland)). All of the current diagnostic tests are performed post mortem on brain tissue from infected cattle. There are currently no tests available for live animals.

10. According to these tests, is beef on the market today free from BSE?

Current tests, which are performed on the brain tissue of animals before they are butchered, are not able to detect BSE in animals younger than 30 months of age or those recently infected, due to the low number of abnormal prion proteins in the early stage of the disease. Efforts thus far have focused on improving the speed and sensitivity of the tests, but the limiting factor remains the inability to detect small amounts of the abnormal prion proteins in brain tissue samples.

11. Are there diagnostic tests for vCJD?

Clinical symptoms can be observed at a late stage in the disease. But currently, the only definite confirmation of vCJD is post mortem analysis of brain tissue. A number of companies have made statements of strategic intent on developing blood screening tests for vCJD patients, including Biorad, Bayer, Roche and Ortho Johnson & Johnson.

12. What is the focus of Dr. Soto's work on prions as described in *Nature*?

The 14 June 2001 *Nature* paper (www.nature.com) describes a procedure for amplifying, or increasing, minute amounts of abnormal prions in vitro to levels high enough to be detected with current testing methods. This procedure mimics the replication of abnormal prions in the body in a "fast forward" mode — compressing years of real time into a few hours in the lab. With this new procedure, combined with currently available diagnostic tests, BSE in cattle or various forms of Creutzfeldt-Jakob disease in humans could be detected at a much earlier stage.

13. What is the process for speeding up prion replication in the lab?

The process is called Protein Misfolding Cyclic Amplification. A minute amount of abnormal prion proteins is added to a healthy tissue sample and incubated for a set time. Ultrasound ("sonication") is then used to break up the aggregated abnormal proteins into smaller components, followed by another period of incubation in which new combinations, or aggregates, are formed. With each cycle of incubation and sonication, the number of abnormal prion proteins increases exponentially, vastly increasing the speed at which abnormal prion proteins are able to transform the shape, or "infect," the normal prion proteins. A comparable cyclic process for amplifying minute amounts of DNA, called polymerase chain reaction (PCR), has become an everyday working tool in hospitals, research labs and police laboratories.

14. Why does the Nature paper of Dr. Soto and his team on prions represent a scientific breakthrough?

The work done by the Serono scientists marks the first time that the abnormal prion protein has been cultivated in vitro, with a greater efficiency than the conversion process that has been postulated to occur in vivo with prion diseases such as BSE and CJD.

As a result of this research, there is now a very sensitive assay (test) that helps to understand the underlying biology of prions, to identify other factors that may be responsible for the abnormal prion protein conversion, and to identify potential novel drug targets for prion diseases.

15. Are there other potential breakthroughs to be expected from Dr. Soto's ongoing work?

Preliminary experiments suggest in vivo that the abnormal prion protein is infectious. If this data is confirmed, it would strongly support the hypothesis for which Stanley Prusiner won the Nobel Prize in 1997. Dr Soto's team are currently conducting confirmatory experiments that will be published in due course.

Preliminary experiments using this procedure also suggest that the abnormal prion protein can be detected in the blood of animals. Once again, confirmatory experiments are on-going.

16. What practical applications will this research have?

The prion amplification process has enormous potential in allowing current diagnostic tools to detect BSE and vCJD at a much earlier stage. Today, it is not possible to detect abnormal prion proteins in cattle younger than 30 months of age or those recently infected, complicating efforts to keep the food chain BSE-free by testing dead animals prior to release of the meat for sale. Although clinical symptoms are observable in humans, the only conclusive proof of vCJD is an autopsy of brain tissue. Dr. Soto's research opens the door to detecting the disease at a much earlier stage using blood or tissue samples from dead and living animals or humans. The process also has potential for detecting other diseases characterized by protein misfolding, such as Alzheimer's.

17. What commercial implications would this research have for Serono?

Serono is currently exploring the opportunities of collaboration or licensing agreements with leaders in the field of diagnostics for both animal or human applications using brain tissue, other tissue types or blood samples. Serono has already moved to patent proprietary knowledge and processes resulting from Dr. Soto's research. For an overview of Serono's current therapeutic areas and business performance, refer to www.serono.com

18. How soon could a diagnostic test for vCJD be available on the market?

It is too early to speculate on the commercial availability of this technology.

19. How does Dr. Soto's research on prions relate to his work in Alzheimer's disease?

Alzheimer's disease and prion diseases follow the same biological principles. In Alzheimer's disease, a different (amyloid) protein takes on an abnormal shape, adopting a form called a beta sheet — the same form taken on by abnormal prion proteins in vCJD and BSE. Over time, aggregates of these abnormal proteins form plaques that damage brain tissue, causing the symptoms of Alzheimer's disease.

In a *Lancet* article published in January 2000 (full reference….), Dr. Soto and his colleagues described a therapeutic use for a piece of a protein, or peptide, that was engineered in Serono's labs. This peptide, a "beta sheet breaker," reversed the abnormal shape of prion proteins in scrapie-infected mice, converting them back to their normal state. This approach significantly delayed the onset of clinical symptoms in the mice, raising the possibility of new therapies for diseases caused by protein misfolding, or abnormal shapes, including Alzheimer's.

20. Are Serono's efforts focused primarily on diagnostic applications or medical therapies?

Serono is working on multiple tracks simultaneously. Diagnostic applications in detecting vCJD at an earlier stage also have potential applications in Alzheimer's disease. Therapeutic advances with beta sheet breakers in Alzheimer's could also be applied to vCJD. Because of similar biological principles in diseases caused by abnormally shaped proteins, breakthroughs could find many potential applications.