October 25, 2006

Dear Senator Humphries,

Response to request of Senator Humphries, Chair of Senate Committee, to provide comments on the document provided by Foursight associates to the Committee on Tuesday, October 24.

Thank you for the opportunity to speak with the Committee. In response to your request, I provide below my comments on the documents presented to the Committee. I have addressed the main points as I see them, having insufficient time to address many other points of detail. I have taken th liberty also of including some comments on two scientific papers that have appeared in Nature journals in the last few days, which may have been brought to the Committee's attention.

The letter from Foursight Associates contains little specific material, but is provided in support of an accompanying evaluation of the science, prepared by Dr Nicholas Gough, of Nick Gough and Associates.

Foursight Associates. **September 12, 2006**

1. One specific question addressed by Foursight concerned immune rejection, where they comment that "*..if transplant rejection is the biggest single concern then this is where the extraordinary legislatively-constrained technology of SCNT comes into its own*". Immune rejection is not the biggest single problem, but only one of several major concerns.

2. Page 1 – 2, " the field of stem cell transplantation faces three serious technical hurdles."
   These hurdles receive scant attention in the document , and some are so great that long continued animal research is necessary to resolve them. In (b) it is conceded only that there is still much to be learned about the mixture of specialised growth factors which will be required in order to guide the ES cells appropriately through differentiation pathways. This can , and should, obviously be done under existing legislation, both in animal ES cells and in human ES cells under licence. It is not in any way an argument for SCNT.
   In (c) the potential of ES cells to develop into cancers is mentioned, with the added clause "*even on rare occasions*". There is no basis on which to suggest that this is a rare complication of ES cell transplantation. This can occur in as many as 25% of transplanted animals.
   A further serious hurdle not mentioned is the problem of abnormalities in genetic programming following SCNT. It certainly is a major problem with embryos that are allowed to proceed through development (reproductive cloning), and it will take much further work to determine whether ES cell lines vary in gene expression also because of programming abnormalities and epigenetic effects. This work must also obviously be carried out under existing legislation.

3. These correspondents make only general statements without supporting evidence, but express their strong support of the views expressed by Dr Gough in his report.

Dr Nicholas Gough,
Nick Gough & Associates. September 8, 2006.

Key recent advances in human embryonic stem cell research.

Much of the introductory portion of this literature review describes research on the culture conditions that are most suitable for the propagation of human ES cells, the progress made towards culturing them in conditions free of animal cell feeder layers, and the search to identify growth factors etc that might facilitate proliferation and differentiation. This work is of course mandatory, aimed at developing cells lines grown under GMP-compliant growth conditions. This is an essential preliminary to all that needs to be done to understand the behaviour of ES cells, how they can be influenced to change their nature in culture, how stable these changes are, whether their genetic controls are normal or disturbed, and what needs to be done to them to abolish the tumour formation problem. None of this work presents an argument for the need for availability of SCNT, and all of it can and should be continued under existing Australian legislation. It might be noted that on Page 11-12, the report states *"..it should be recognised that cellular differentiation mechanisms are yet to be fully understood and therefore the ability to produce a range of different mature cell types is still at a relatively early stage"*.

1. Mention is made of the problem of genomic instability (page 9-10), and later (page 17-18) reference to 2 papers (Refs 100, 101) that showed that mouse ES cells derived from normally fertilised blastocysts were indistinguishable from those derived by SCNT. Given the very evident problem of abnormal genetic programming that is a feature of reproductive cloning of animals, proof that programming and epigenetic effects do not occur in SCNT-derived cell lines will require much more work than this, including especially study of expression of a very much wider array of genes, including studies in clonally derived ES lines in prolonged culture, and studies in multiple clones from the same original source.

2. The suggestion is made (top page 16) that there is a need for generation of ES cell lines from individuals with specific genotypes for dissection of complex multigenic diseases - those listed are Alzheimer's, motor neuron disease, and others of unknown cause or origin. This is an exceptionally ambitious claim in the case of diseases of late onset and variable penetrance, and especially since it still must be suspected that there are indeed genetic programming errors commonly occurring as a result of SCNT - despite the claims referred to in my preceding paragraph.
   Whether SCNT-derived ES cells can be made use of in such studies of disease mechanisms is indeed an important question - one that can, and should, be investigated in animal models in the first instance, to determine whether it is in any way feasible.

3. Proof of the concept that ES cells can be used with prolonged safety and efficacy in experimental models of disease is required. The Gough report produces in "Proof of concept – Animal models" (page 17 – 18) the two papers that are most commonly brought forward in support of this. Neither of these comes close to establishing a case that human SCNT should be supported. The report also adds a further paper, recently published, illustrating the use of SCNT-derived cells in the study of cancer.

   (i) The Barberi 2003 paper (ref 91) compared SCNT and normally derived ES cells in the treatment of Parkinson's disease induced experimentally in the mouse, They found no difference between the two types of ES cells in efficacy, perhaps because the brain is relatively immune privileged. The experiment consisted only of 6 mice per group, and did not continue for sufficient time to exclude tumour formation, which has been virtually inevitable in other, similar rodent experiments. Relevant to this, I must note that in the last few days there appeared in Nature Medicine (online, October 22, Roy et al) a paper describing transplantation into rats of human ES cells which had been enriched in the ability to change into dopaminergic cells (for use in Parkinson's Disease) by co-culturing with immortalised astrocytes (other nerve cells). Transplantation resulted in beneficial effects on symptoms and signs in rats with induced Parkinson's, but the dramatic consequences were that the cells were phenotypically unstable, not maintaining the dopaminergic properties, and subject to persistent and uncontrolled cell division, due to overgrowth of partially differentiated cells.

   (ii) The Rideout et al 2002 paper (same ref used twice, 92 & 102) is often quoted as a "proof of concept " of therapeutic cloning, the authors having claimed this in the Discussion section of the paper. This is not the case, but the Gough report regards it as a "*landmark paper"*.
   The work was as follows - ES cells from a mouse with a genetic immune defect were repaired by replacing with a normal gene. The repaired ES cells were used as a transplant - thus, a combination of gene therapy and therapeutic cloning. This did not repair the genetic defect in these mice, as suggested (page 17 Gough report), but "*partially restored normal function to the immune system*" (page 18, para 2). So the authors used a second approach, in which a new mouse was made by SCNT, using the genetically repaired ES cells. Adult bone marrow-derived cells from the cloned mice were then used to treat affected mice. That is not therapeutic cloning. Thus these authors clearly failed to carry out therapeutic cloning by their own definition, and there is no basis for claiming this work as "proof of concept".

   (iii) Reference is made on page 18 to two recent cell biology studies (refs 103, 104), showing that transplantation of cancer cell nuclei into enucleated mouse eggs has the possibility of revealing

valuable information in cancer cell biology. That might well be so, and the work in these papers is extremely interesting and well done. The way forward from these early observations though is to continue studying mouse models before claiming that such work provides an argument for human SCNT lines to be developed in a similar way. Among the very many questions to be addressed would be how any results are influenced by abnormalities of genetic programming, quite apart from the genetic abnormalities introduced with the cancer cell. What would be controls for such experiments? How could anything meaningful be done without carrying the embryos on well beyond 14 days? It would require a great deal of work to make a compelling argument that such work should be carried out by manufacturing human embryos by SCNT.

4. On pages 11-12 the report lists a number of recent studies investigating the behaviour of human ES cells (refs 61 –69). None of these papers provides evidence supporting the need for development of SCNT-derived human ES cell lines. Detailed refutation could be supplied if requested.

5. It is possible that the Committee might have had drawn to its attention a paper currently in Nature Biotechnology (online October 19), reporting the development from human ES cells of insulin-producing cells that also produce other hormones expected of the pancreatic beta cell. This is also a useful technical development, The cells do not respond to glucose by secreting insulin though, and as the authors suggest, they appear to represent a fetal stage of pancreatic islet cell development. Such an approach could be the starting point to try and develop fully mature insulin-producing cells. The stability both in vitro and in vivo will need to be established, as well as the questions of tumour development and genetic stability resolved by appropriate studies.

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