Applicant: Dr Ian Wilmut

Title of the project: Derivation of human embryo stem cells by cell nuclear replacement for technology development and the study of Motor Neuron Disease

Area of research
This proposal falls into 3 categories contemplated in the Act:

1- to increase the knowledge about the causes of congenital diseases;
2- to increase knowledge about serious disease;
3- to enable any such knowledge to be applied in the developing of treatments for serious diseases.

Importance
This proposal, if successful, will contribute with tools for the understanding of the origins of Motor Neuron Disease (MND). 5000 people in the UK and 25,000 to 30,000 in the US are affected by this devastating disorder. The causes of neuronal death are not known. The applicant is proposing to take somatic cells taken from a patient suffering from MND and de-differentiated them into embryonic stem (ES) cells using cell nuclear replacement (CNR) techniques. ES cells will be later differentiated into motor neurons. Such motor neurons can be study in a direct attempt to determine the aetiology of the disease. These cells will be shared with the scientific community and new treatments for MND will be likely tested on them.

Originality
This application is original. No such work has been published before. There are many aspect of this proposal that make it very appealing beyond MND. Among them, the use of crypreserved oocytes as cytosol donors for CNR. Developing new protocols using this kind of eggs will dramatically impact the field of reproductive and regenerative medicine.

Justification
MND animal models are deficient and currently there are no *in vitro* systems to study this disease. The proposal is justified in detail in the application. This reviewer agrees with such justification.

Creation of embryos for research
Currently there is only one method to regenerate terminally differentiated cells such as motor neurons and/or cardio-myocytes, CNR. CNR has been tested in animal models extensively and recently in human cells (Hwang et al, Science 2004). The applicants will be creating embryos by CNR from patients with MND only to generate ES cells. The applicant explicitly mentions that these embryos will not be used for human reproduction.

Methodology
The methodology is adequate and explained in detail. The applicant has described the latest protocols that, if applied, will maximize the use of human eggs and therefore minimize the creation of human embryos for research.

Analysis of the results
Endpoints are clearly stated. Statistical analysis to be used is not mentioned.

Duration
3 years. This is adequate.

Applicant
Outstanding. The applicant and his group of collaborators is one of the most exquisite teams ever assemble to perform this work.

verall Assessment
the applicant is able to complete this work, great contributions to the field of MND will be ade.

uestions to the applicant:
n their previous work they obtained 61 fresh eggs from the New Royal Infirmary of Edinburgh. 8 were used. 3 are unaccounted. Please explain the fate of these 3 eggs.
low epigenetics will affect the outcome of this work?
n page 28 the applicant says that only human embryos will be used when they have at least alf the normal number of cells a human embryo has. 1st What it is consider 'normal' number of ells? And 2nd What method will be used for counting?
Applicant will use embryo culture medium that is currently been used in IVF clinics. They should consider using medium optimized for nuclear transfer human embryos as the one recently described by Hwang et al Science 2004.

Signature: [redacted]

Date: 11.2.04

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UMAN FERTILISATION & EMBRYOLOGY AUTHORITY

PEER REVIEW FORM (New Applications)

Applicant: IAN WILMUT AND PAUL DE SOUSA

Centre number: N/A

Research project number: R0158

Title of project: DERIVATION OF HUMAN EMBRYO STEM CELLS BY CELL NUCLEUS REPLACEMENT FOR TECHNOLOGY DEVELOPMENT AND THE STUDY OF MND

Referee: [redacted]

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Position held / area of work: [redacted]

1. THE AREA OF RESEARCH

Do the proposals fall into one of the categories of research listed in the Act? If so, which one? .(You may tick more than one box)

a. to promote advances in the treatment of infertility ☐

b. to increase knowledge about the causes of congenital disease ☑

c. to increase knowledge about the causes of miscarriage ☐

d. to develop more effective techniques of contraception ☐

e. to develop methods for detecting the presence of gene or chromosome abnormalities in embryos before implantation ☐

f. i to increase knowledge about the development of embryos ☐

ii to increase knowledge about serious disease ☑

iii to enable any such knowledge to be applied in developing treatments for serious disease ☑

2. IMPORTANCE

Do you consider that the proposals address important issues in the advancement of knowledge or treatment of infertility? ~~YES~~ / (NO)

Please state your reasons:

The objectives of the proposal related to generation of models for study of neurodegenerative disorders, specifically motor neurone disease.

Do you consider that the proposals address important issues in the advancement of knowledge or treatment of serious disease? (YES) / ~~NO~~

Please state your reasons:

The proposals are to use cell nuclear transfer to produce human embryos containing nuclei derived from patients with motor neuron disease (MND) of unknown cause, from which MND ES cells can be derived for study. This will include derivation of neural cells and hence of a valuable model for in vitro studies of MND. The model will be unique and will enable studies that would not be possible by other approaches at this time.

3. ORIGINALITY

Has the work proposed been carried out before or not? If so, is there justification for repeating the experiments?

The proposed work is original.

4. JUSTIFICATION

Have experiments on animal models or other types of human cells reached a point at which the use of human embryos is justified?

Yes. Comparable cell nuclear replacement experiments are reported, indicating that the technology is adequately developed for the proposed approach. proposals form part of a wider research programme into neurodegeneration diseases and builds on existing knowledge and local expertise.

Does proposed work justify need for the creation of embryos specifically for research? Could researchers feasibly utilise other avenues in order to conduct the proposed work?

The proposed utilisation of human embryos is justified as other approaches to develop models of this kind have not been developed and applied to MND. Other approaches to define the genetic and cellular basis of most of unknown aetiology are feasible, but have not yet yielded significant insights into pathogenesis.

Is the creation of human embryos by cell nuclear replacement necessary for the proposed research study? **(YES) / NO**

Please state your reasons:

The study seeks to produce a model of MND using cell nuclear replacement. These steps are an essential component of the study

Is the use of human embryonic stem cells necessary for the proposed research project, or could the same results / aims be achieved if adult stem cells were used?

Please state your reasons:

I believe that some of the objectives of the proposed research might in principle, be possible using adult stem cells. However, an integral part of the proposed work is to develop methodology for ES cell derivation from embryos following cell nuclear replacement. The prop work therefore requires use of human embryonic stem cells.

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? missing word in question

Will the creation of a stem cell line from embryos created by cell nuclear replacement using a nucleus taken a somatic cell of a patient with inherited forms of Motor Neurone Disease?

? missing word for question

Please state your reasons:

Yes, this is a specific proposal within the programme of work. The proposal is reasonable as it is probably the most realistic approach to developing an in vitro model for MND at present. The work has broader potential implications in relation to models for other genetic disorders.

METHODOLOGY

Do you consider that the objectives are clearly defined and the methods proposed are likely to yield relevant and clear results? If not, what are the problems?

In general the objectives and methods are clearly defined. It would be helpful to know whether patients with MND from whom donor cells will be taken will have defined susceptibility loci, as inferred by genetic linkage analysis. This would be advantageous as the MND phenotype may be the end result of disparate cellular processes in different genetic forms of the disorder. How many patients with inferred mutations at each susceptibility locus will be recruited? Are the investigators confident that the donors are available?

5. ANALYSIS OF RESULTS

Are the numbers of gametes/embryos to be used realistic and are the statistical methods to be used appropriate to give meaningful results? If not, can you suggest alternatives?

Details of statistical methods to be used are not detailed in the application. I do not have the expertise required to advise on whether such detail is needed and would suggest specific biostatistical advice is sought. The number of gametes to be used suggests that some statistical detail might reasonably be requested of the applicants.

6. DURATION

Is the proposed duration of study appropriate?

The proposed duration of three years is appropriate.

7. THE APPLICANT

Do you know the applicants work personally or by repute? Does the team have the necessary qualifications and ability to carry out the proposed work?

I do not know the applicants personally. By repute the applicant are considered to be international experts in the proposed area of research. They do have the qualifications and ability to carry out the work.

8. ANY OTHER COMMENTS

9. OVERALL ASSESSMENT

Please tick your recommendation of the proposed work:

___ Reject for licence, flawed in scientific or technical approach

___ Resubmit application, has potential but needs revision following feedback from reviewers

_✓_ Accept in current form

Signature: [redacted]

Date: 11.11.04

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