Development of a human neural stem cell line for use in recovery from disability after stroke

Paul Stroemer¹, Andrew Hope¹, Sara Patel¹, Kenny Pollock¹, John Sinden¹

¹ReNeuron Ltd. 10 Nugent Rd, Guildford, Surrey UK GU2 7AF

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1. ABSTRACT

A clonal human neural stem cell line (ReN001) has been developed for clinical use in the treatment of stable disability after stroke. This cell line has been conditionally immortalized using the fusion transgene c-mycERTAM to allow controlled expansion when cultured in the presence of 4-hydroxytamoxifen. The cell line has been banked and fully characterized to assure there is genetic stability and no phenotypic drift with extended passages. In vivo studies determined the ability of the cell line to survive after implantation into damaged brain and its efficacy in the reduction of chronic behavioural dysfunction after implantation into a rodent model of stroke damage. A further study was conducted in this model and a dose-dependent effect was observed on behavioural recovery. No safety or toxicity issues were identified in in vivo studies with this cell line, which made REN001 a strong candidate for one of the first cell-based IND applications to be submitted to the Food and Drug Administration in the United States for consideration for the treatment of stroke in humans.

2. REVIEW

Therapeutic treatment for cerebral ischemia at either an acute stage or for chronic behavioural deficits has presented a great clinical challenge. As the population of the industrialized world ages, the burden of care for stroke survivors is expected to expand. There has been a wide range of approaches trialed to protect the brain after stroke (1,2). Unfortunately there is only one approved medication to reduce stroke damage, tissue plasminogen activator (TPA). There are limitations in this therapeutic approach as the time window for treatment is limited and many patients have a delay between stoke onset and presenting to the clinic. There has been a recent thrust to treat stroke patients after the initial onset of damage with the use of physical therapy combined with pharmaceutical agents to try and promote recovery from behavioural dysfunction. Many of the agents that are being tested for their efficacy in promoting recovery are already in use for other indications. However, at this point in time there are no clinically proven agents for promotion of behavioural recovery after stroke (3,4).
Stem cells have been heralded as a potential therapy for a number of CNS diseases (1). There has been a paradigm of the use of stem cells for direct replacement of damaged areas of the brain, for example, as dopaminergic neurons in Parkinson’s disease (5,6). Other indications are more problematic as the amounts of tissue lost may not be practically replaced using current surgical methods. Mechanisms of recovery are also not well understood in the plasticity of the brain and in the “rewiring” of circuitry between the cortex and the striatum after ischaemic damage. Every stroke is slightly different in the amount of tissue damaged and in the location of damage. The amounts of stem cells that would be adequate to replace damaged tissue are staggering. However, the total dosage of stem cells that would be efficacious in promoting recovery may be much lower (7,8).Regardless to the final dosage of stem cells, the establishment of cell lines that could be grown in quantities sufficient to be taken into the clinic presents a number of difficulties in ethics, expansion and in testing. The initial source of stem cells leads to ethical questions. Embryonic stem cells can be derived from tissue produced by in vitro fertilization. Some embryos are destroyed if they are not implanted or designated for use. The current funding status in the United States has led to limited use and development of embryonic stem cells.

Adult tissue can be readily sourced from patients. There have been experimental treatments employing autologous implantations of stem cells that have been expanded from a patient (9). However, there is a difficulty in the consistent use of autologous stem cells for implantation as the adult cells have an increased time of expansion and a greater chance of damaged DNA. Some tissue may not supply adequate numbers of cells. Another source of stem cells is the use of foetal tissue. Previous studies of transplants of ventral mesencephalalum tissue for Parkinson’s disease treatment have demonstrated efficacy in relieving dyskinetias in transplant patients (4,5). An advantage of using foetal tissue is that stem cells are being tied to a specific fate (e.g. neuronal stem cells differentiate into neurons, astrocytes or oligodendrocytes). Cells can be derived from tissues in specific periods of development and regions of the brain, which may lead to greater consistency in differentiation. Whereas the Parkinson’s studies have required multiple foetuses for each patient, we have derived numerous stem cell lines from relatively few foetal tissues.

Stem cells derived from primary tissue have the ability to replicate in vitro. However, most stem cell populations have a limited period of replication. With increasing passages, the cell doubling time begins to increase as cells reach senescence (10,11,12). The cells begin to lose viability and begin to have altered karyotypes. The development of stem cells for clinical use requires the ability to grow cells in a scale to produce millions of doses and address issues of safety and stability (Table 1). The development of stem cells also requires a consistent cell type. Cell populations may have a mixture of cell types and the constituent cells may have different rates of cell growth. There is a potential for a population to “drift” over time, with changes in characteristics. Immortalized clonal cell lines allow for an expansion of a line for sufficient quantities for development and clinical use, however the implantation of immortalized cells would also present a potential for uncontrolled growth and tumour formation. We have addressed this issue by using a system that creates conditional immortalization. Foetal tissue was dissected into striatal and cortical regions, dissociated and transfected using a retrovirus to insert a mutated oestrogen receptor that is fused to a c-myc oncogene (13,14). The insertion of this receptor creates a cell that will replicate readily in the presence of 4-hydroxy-tamoxifen (4OHT, a minor metabolite of tamoxifen) and growth factors (EGF and FGF), but have markedly decreased growth in the absence of 4OHT. This conditional immortalization allows the creation of clonal cell lines from a single cell. The cell lines can be thoroughly characterized, expanded and taken into banks for later use.

ReNeuron generated over a hundred cell lines that could then be advanced through research and development. A screening procedure was employed that examined a number of parameters for each cell line. Amongst these were; optimal growth rates, ability to be frozen and revived, viability after harvesting, viability and plating efficiency after being extruded through a needle replicating implantation parameters. Cell lines were also subjected to a panel of molecular Phenotyping using QRTPCR. This panel established a “fingerprint” for the cell line. It was found that like fingerprints, each cell line expression pattern is unique. This fingerprint could then be used after cell banking and extended passaging to ensure that no “drift” occurred. After in vitro screening was complete, a selection of 16 cell lines was implanted into the striatum and hippocampus of rats having a Huntington’s disease lesion (striatal injection of Quinolinic acid). The cell lines were evaluated for survival shortly after implantation into the striatum and hippocampus for 2 or 5 weeks, along with differentiation into neurons and glia. Subsets of cell lines were chosen for further efficacy testing in models of Huntington’s disease and stroke (14).

Two stem cell lines, STROB05 and CTX0E03 were implanted into the striatum and cortex, bilaterally, three to four weeks after middle cerebral artery occlusion in adult male Sprague Dawley rats (Σ800,000 cells/rat)(33). The rats were evaluated in a behavioural testing battery consisting bilateral asymmetry (tape removal), turning out of a corner, and rotometer testing measuring turning bias after amphetamine intoxication before and after the onset of ischemic damage. At six weeks after implantation, the testing battery was resumed for a further 6 weeks. There was a significant improvement in the tape contact and
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removal on the affected limb in the bilateral asymmetry test in rats that had received cell implantation compared to vehicle implanted animals. There was also a significant improvement in the rotameter test in the implanted animals compared to the post occlusion test and when compared to the vehicle implanted rats. At the end of the testing battery period, the rats were tested in a Morris water maze for a further 10 days. There was evidence of dysfunction in all of the occluded groups, however there was no improvement in the water maze performance following cell implantation. While the two cell lines demonstrated efficacy in reducing behavioural dysfunction in this study, another, similar study using two other cell lines did not produce similar results. The ability to promote recovery from CNS lesions is a discrete property of a stem cell line. Development of a cell line requires efficacy testing in the desired indication with the same route of administration and formulation that is planned for the clinic. Implantation of stem cell lines had no effect on the volume of the stroke lesion. It is possible that damage in the brain was either complete or too far advanced to be affected by the delayed implantation of cells. The behavioural effects of cell implantation were therefore not the result of a reduction in lesion volume. Similarly, the implanted cells did not “fill a hole”. The volume of implantation (16µL) was dwarfed the amount of tissue that was removed as a result of damage (>200mm³). The efficacy in reducing behavioural dysfunction did not correlate to the total amount of surviving stem cells or to differentiation of cells into neurons or glia.

Current studies are investigating the mechanisms involved with stem cell therapy. Possible effects of the stem cell line in promoting recovery from behavioural dysfunction could be promotion of angiogenesis, release of growth factors, promotion of neuronal plasticity of surviving tissue. We have developed a clonal neural stem cell line that can be grown in quantities sufficient for clinical use. A thorough testing program has been evaluating cell characterization, safety and efficacy.

3. REFERENCES


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Send correspondence to: Paul Stroemer PhD, ReNeuron Ltd., 10 Nugent Rd, Guildford, Surrey, GU2 7AF, UK, Tel: 01483-302560, Fax: 014830-534864, E-mail: Paul-Stroemer@reneuron.com

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