Stem cells for stroke: translating animal models into clinical treatment

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The intracerebral implantation of 2 million human neural stem cells from ReNeuron's CTX0E03 conditionally immortal, clonal cell line into a disabled stroke patient in Glasgow on 13 November 2010 represented the culmination of over 7 years of research and development of a unique stem cell product. It was also the realization of a vision of a fully scalable and good manufacturing practice (GMP) manufactured stem cell therapy for a genuine unmet medical need shared by the founders and the early investors over 13 years previously. At that time, the scientific, regulatory, financial and clinical hurdles to clinical translation of stem cell therapies directly administered into the damaged brain for neurological disorders were unknown. Thus, it has transpired that 13 years of scientific translation from laboratory proof-of-principle to the clinic was not atypical in the field. The Glasgow Pilot Investigation of Stem Cells in Stroke (PISCES) trial is the first fully regulated stem cell therapy trial in the UK and possibly the first fully regulated stem cell therapy trial for a major (in terms of patient numbers) neurological indication anywhere [101].

When we were developing our technologies at the Institute of Psychiatry, King's College London in the early 1990s, the groundbreaking field of neural stem cell (NSC) biology was in its ascendancy. Sharing many characteristics of hematopoietic stem cells (HSCs), NSCs are self-sustaining multipotent cells present throughout most of the mammalian lifespan, located in identifiable supportive neural niches and able to respond to environmental stimuli to proliferate and differentiate [1]. In contrast to HSCs, NSCs can be readily isolated and expanded in bulk culture, either as 'neurosphere' suspensions [2] or adherent cultures [3]. The early interest in the translational science of cultured NSCs was

on neural development and its disorders [4], not on repair of brain degeneration in the adult or elderly. Where treatment was envisaged, the focus was directed to pharmacology-based approaches, particularly Parkinson's disease models of dopamine denervation and its motor consequences [5]. However, in spite of many successful studies with primary dopaminergic neurons, functional, engraftable dopamine neurons derived from expanded stem cells remain elusive [6].

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Leading up to our *in vivo* work with NSCs, our group had been centered on animal models linking memory disorders with, on the one hand, their pharmacologic modulators, particularly the forebrain cholinergic system and, on the other hand, key neural circuits, in particular the hippocampal system. We focused on the mechanisms by which primary fetal tissue transplants in rodents could restore lost functions. In respect to hippocampal function, we had built on the anatomical work of Raisman and colleagues who demonstrated that implants of cells isolated by means of microdissections of late fetal rat hippocampal CA1 and CA3 cell fields soon after they developmentally segregate (embryonic days [E] 18–19) would only structurally integrate into lesioned hippocampus by forming normal laminar connections in lesioned cell fields if they were homotypic (i.e., CA1 grafts into lesioned CA1) [7].

Using a global ischemia four-vessel occlusion model (4VO) in rats, we, like others, were able to obtain selective loss of CA1 pyramidal cells in

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the hippocampus [8]. This lesion reliably produced discrete deficits in spatial learning and working memory [9], for example, in the Morris water maze [10,11]. Implants of primary cell suspensions isolated from E18–19 CA1 were able to restore water maze memory function, whereas similar implants of fetal CA3 were ineffective, in spite of equivalent survival of the two cell-type grafts [12,13].

Such cell-type specificity of functional repair therefore represented a stringent test of the ability of multipotent NSCs to respond appropriately to local brain conditions and correctly differentiate into the right kind of cells to restore function. We were able to demonstrate that implants of MHP36 cells, a conditionally immortalized NSC line from murine fetal hippocampus, could restore memory function in 4VO mice as well as primary CA1 cell grafts [14]. The cells were found aligned in the CA1 layer formerly occupied by the pyramidal cells. Moreover, we were able to extend this result to demonstrate restoration by MHP36 cells of conditional object discrimination learning and memory in marmosets after excitotoxic CA1 lesions, with recovery equivalent to that observed with primary fetal marmoset CA1 grafts, again with surviving cells occupying the lesioned CA1 field [15].

“We were able to demonstrate that implants of MHP36 cells ... could restore memory function in (four-vessel occlusion model) mice as well as primary CA1 cell grafts.”

Therefore, the evidence showed that a prototype NSC line, of clonal origin and essentially with infinite expansion capacity could produce, after intracerebral implantation in both rodents and primates, a clear-cut restoration of complex cognitive function along with plausible evidence that the surviving cells were adopting a disposition consistent with cellular replacement, namely alignment in the areas associated with pathological cell loss. This scenario, if it could be translated with human-derived, clinical quality NSCs to patients with ischemic pathology, represented the goal that ReNeuron set out to achieve.

As ReNeuron, the start-up company, focused on development of the ‘Product’ (i.e., humanizing the murine cell line technology to generate the candidate clinical products with the requisite quality, manufacturability, purity and biological potency), several scientific and technological barriers were crossed. The key milestone was the licensing of the c-MycER conditional

fusion-protein technology [16] that delivered the stable clonal cell line that we had struggled to find using the old mouse cell-line technology. In addition, we began to focus more on stroke disability in preclinical studies.

Clinically, stroke disability is a treatment dead-end. At least 50% of all stroke survivors will suffer with a syndrome that covers the entire spectrum of neurological deficits. Rehabilitation therapy can help stroke survivors for the first 3 months or so after the event, but the plateau of recovery seldom takes the patient to an independent quality of life [17]. After long hospital stays, a very large number of these patients are in the community and reliant on full-time care through family or state institutions. We considered this target for stem cell therapy an immensely challenging, but potentially very rewarding, goal.

The history of stereotactically delivered cell therapy in disabled stroke patients began with the pioneering work of Kondziolka and colleagues at Pittsburgh, who undertook the first US FDA-approved trials in the field of cell therapy for stroke treatment using neuron-like cells produced from a NTera2 teratocarcinoma cell line [18,19]. These pioneering studies were key to our being able to progress, demonstrating feasibility of the stereotactic delivery approach and good patient tolerance for the cell product. We developed a stroke-disabled rat model using middle cerebral artery occlusion, but allowing the rat to recover from acute neurological deficits for a period of 3–4 weeks prior to stereotactic NSC injection. Uniquely, the rats were followed for long durations, at least 3 months postimplant, and different behavioral tests were used assessing both sensorimotor and cognitive function and focusing on tasks that demonstrated long-term stable deficits in stroke rats. Our first studies used the MHP36 cell line, recovery of sensorimotor function was found with cell implants into the striatal area on either the infarct side or the contralateral hemisphere [20,21]. Cells were found on both sides of the brain, near the infarct and its contralateral homolog, indicating that the stem cells interact both with stroke degeneration and also contralateral reorganization. Equivalent numbers of cells injected into lateral ventricles did not produce sensorimotor performance recovery. However, improvements in spatial learning were found, not observed with striatal implants [21].

Meanwhile, the isolation, characterization and testing in animal models of a new range of clonal cell lines from different brain regions using the c-MycER technology identified the first potential clinical-grade NSC cell-line product, CTX

(a simplified version of the CTX0E03 designator) [22]. The initial proof-of-concept study demonstrated that implants of CTX could restore sensorimotor function in rats in a similar fashion to their murine predecessors, MHP36 cells [22]. We outsourced early manufacturing scale-up to generate master and working cell banks to full GMP, which we describe in detail in Pollock and Sinden [23]. Therefore, key preclinical development studies were undertaken using the 'Drug Product' level cells (i.e., taken from GMP manufactured banks [via contract manufacturing organizations or their in-house equivalents] and prepared for implantation in the same way as for patients). Parallel studies included further efficacy assessment and studies on mode of action as well as key safety analysis, including toxicity, biodistribution and, most importantly of all, tumorigenesis.

“...the remarkable capacity of (neural stem cells) to adapt their mechanisms of action...opens up...the potential to progress this cell line beyond treatment of stroke disability into a range of preclinical models of neurological disorders...”

From the regulator's perspective, the key studies in facilitating the commencement of clinical trials are safety trials. Identification of the most appropriate models to evaluate the subject of examination is paramount. Tumorigenicity models are preferably run in survival permissive strains of mice, so large population numbers can be used. Delivery, dose and other issues may require large animals, although primate studies are being discouraged as most alternatives (e.g., pigs and sheep) are now more readily available and can often be combined with efficacy evaluation studies. It is recommended, prior to major study investment, that pilot studies are run to demonstrate that the model can deliver, for example in tumorigenesis, long-term cell survival for evaluation of tumor potential. Most regulatory agencies recommend that pilot data be sent to reviewers along with plans for the main study in the context of scientific advice or pre-pre investigational new drug meetings to attain some level of agreement with the agency that you are on the right track.

Although the history of stroke pharmacology has shown that efficacy translates poorly from animal to man compared with safety, and that human dosing is almost impossible to predict from animal studies, the regulators are looking for biological activity of the cells that gives tentative

evidence of mode of action. Their assessment of the ratio of risk to benefit, which needs to be laid out in clear scientific terms by the sponsor, is critical to success, or otherwise, in proceeding to a clinical trial. Other review committees, for example federal or national advisory bodies or ethics committees will, on the other hand, focus on the data that relates to the efficacy side of the risk-benefit balance.

In our clinical trial-enabling studies in order to demonstrate clinical potential, we conducted two fully monitored efficacy studies with CTX Drug Product. The first used three different cell dose-treatment groups against vehicle and showed a dose-dependent recovery in sensorimotor function [24]. This study also demonstrated that cell-induced host angiogenesis and neurogenesis were plausible mediators of the functional recovery [24]. The second study [MODO M *ET AL.*, MANUSCRIPT IN PREPARATION] compared intrastriatal implants near to the infarct (as in the PISCES clinical trial) against intracerebroventricular implants to determine whether localized stereotaxic delivery was really required to achieve efficacy. In addition, structural MRI was performed to help guide the stereotaxic procedure and stratify animals for degree of damage, and also to monitor for any structural changes over the 3-month postimplant test period. Extensive histological analysis of the animals brains with phenotyping of the surviving cells and any host brain changes were investigated. The outcomes, so far reported, demonstrated significant reversal of sensorimotor deficits with implants near the infarct, but not when they were injected into ventricles, demonstrating again the importance of local cell delivery. The degree of recovery was greater in rats with damage restricted to striatum, rather than striatum and cortex combined [MODO M *ET AL.*, MANUSCRIPT IN PREPARATION].

In further studies, we have demonstrated that CTX has key proangiogenic and immunomodulatory characteristics that feature as mechanistically critical in the 'wound healing'-like role that NSCs play in the ischemic tissue environment [25,26] in contrast to their better known cell-replacement mechanism, as observed in the selective hippocampal lesion experiments described previously or in the case of spinal cord injury [27,28]. It is the remarkable capacity of NSCs to adapt their mechanisms of action in response to pathological changes that opens up for us the potential to progress this cell line beyond treatment of stroke disability into a range of preclinical models of neurological disorders, with variability in the extent or nature of the underlying pathology.

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101. ClinicalTrials.gov: Pilot Investigation of Stem Cells in Stroke (PISCES)
<http://clinicaltrials.gov/ct2/show/NCT01151124>