Stem Cells and Development

DOI: 10.1089/scd.2017.0009


# Human neural stem cell therapy for chronic ischemic stroke: charting progress from laboratory to patients

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## Abstract

Chronic disability after stroke represents a major unmet neurological need. ReNeuron's development of a human neural stem cell (hNSC) therapy for chronic disability after stroke is progressing through early clinical studies. A Phase I trial has recently been published, showing no safety concerns and some promising signs of efficacy. A single arm Phase II multicenter trial in patients with stable upper limb paresis has recently completed recruitment. The hNSCs administrated are from a manufactured, conditionally immortalized hNSC line (ReNeuron's CTX0E03 or CTX), generated with c-*myc*ER$^{TAM}$ technology. This technology has enabled CTX to be manufactured at large scale under cGMP conditions, ensuring sufficient supply to meets demands of research, clinical development and eventually the market. CTX has key pro-angiogenic, pro-neurogenic and immunomodulatory characteristics that are mechanistically important in functional recovery post stroke. This review covers the progress of CTX cell therapy from its laboratory origins to the clinic, concluding with a look in to the later stage clinical future.

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# 1. Introduction

## 1.1 Stem cells and stroke

The past decade has seen a rise in the number of stem cell-derived therapies targeting ischemic stroke in preclinical and early clinical studies. Corroborated by numerous scientific reports, the therapeutic benefits of stem cells include an extension of the time window for drug intervention, improvement of neurological deficits, reduction of infarct volume, pro-regenerative cerebral reorganization, mitigation of post-stroke neuro-inflammation and tissue restoration, all of which depend on the time after infarct, cell type used and route of administration [1-3]. The wide range of effects observed for stem cell therapies demonstrates that functional recovery after stroke occurs via multiple mechanisms rather than a single target [4-6]. Research indicates that the mode of action may depend on the stem cell type and other key factors including infarct size and location, mode of intervention and timing post stroke [6-8]. Thus, some understanding of the cellular, molecular, and biochemical events involved in the mode of action of a stem cell type is a prerequisite to improve and optimize its therapeutic benefits.

Our 2012 review of cell therapy in stroke showed the wide variety of cell types used preclinically and clinically in stroke treatment research [1]. Mesenchymal cells (MSCs) of multiple origin and phenotype are most commonly employed in the literature and mainly applied systemically in high doses in acute stroke settings, because of their non-engraftment and potent “drug-like” biological activity. Neural stem cells (NSCs), by contrast, are multipotent cells derived from developing or adult brain tissue or differentiated from pluripotent cells such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) in culture. These stem cells have both capacity for engraftment and neural cell differentiation as well as potent biological activity and are delivered intracerebrally in smaller volumes and cell doses, we believe more suitable in patients presenting with pre-existing chronic,

stable disability. There is a growing number of hNSC-derived therapies currently in preclinical development for ischemic stroke (see Table 1). Leading these therapies, ReNeuron's CTX0E03 cell line (CTX) has been evaluated in a first-in-human, single-center trial in patients with moderate to severe disability, 6 months to 5 years after ischemic stroke [9]. Currently a Phase II stroke trial in patients with upper limb disability, 3-12 months' post-stroke is underway across multiple sites in the UK (clinicaltrials.gov NCT02117635). In this review, we summarize nearly 15 years of research behind the CTX line and discuss its mode of action together with implications for therapeutic potential in stroke disability.

Table 1 about here

## 2. Technical development of hNSC therapy products for CNS indications

### 2.1 Cell transplantation

Progress in a cell therapy approach for CNS indications is dependent on product safety and efficacy and a manufacturing strategy to provide a product of consistent quality and supply to meet the demands of experimental and clinical research and the future commercial market. Early studies demonstrated that transplanted primary fetal neural cells can survive and exert positive effects in animal models of neurological indications including ischemic stroke damage [10-12]. Preclinical and clinical studies established anecdotal proof of concept data for their therapeutic efficacy and tested the surgical feasibility of safe CNS implantation. However, the use of primary cells from donated fetal brain tissue is impractical as a widely available therapeutic strategy. Procurement of tissues is limited by supply and the quality (i.e. purity and homogeneity of cells) is variable, producing inconsistent clinical results [13].

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### 2.2 Use of neural stem cells

The use of human neural stem or neural progenitor cell lines is a practical alternative to primary cell implants for brain repair. Stem cells can be expanded in culture, with potential to deliver treatment at scale. Human neural stem cells (hNSCs) can be expanded in defined media supported by growth factors, such as epidermal growth factor (EGF) and fibroblast growth factor (FGF-2) and cultured as monolayers or free-floating neurospheres [14]. At the time of CTX's derivation, hNSCs were most efficiently isolated from fetal brain tissue. However, equivalent cell types can now be obtained from ESC and iPSC origins.

Some limitations for using hNSCs have surfaced, including their limited expandability in culture and issues related to genetic/phenotypic instability [15-17]. Neurospheres, for example, may contain mixed populations of stem cells and progenitors at differing stages of development, which may prevent the expansion of a homogenous cell line and limit their clinical potential [18]. In contrast, genetically immortalized NSCs, using genes for transcription factors such as Myc, have proved to be highly effective in extending the life span of hNSCs in vitro and maintaining a stable genotype and phenotype [19,20]. Long term cell expansion with associated karyotype stability is a feature of Myc immortalization [17,21]. Moreover, current reports indicate that the *myc* gene, may be a 'stemness' gene which drives rapid proliferation while maintaining multipotent capability of stem cells [22].

### 2.3 c-mycERTAM conditional immortalization technology

ReNeuron generated CTX a genetically stable, conditionally immortalized, clonal hNSC line, using its proprietary c-*myc*ER$^{TAM}$ stem cell expansion technology (see Figure 1) [10,18,23]. CTX is genetically modified by insertion of a single copy of the c-*myc* gene fused with a modified murine estrogen receptor (ER) [18,23,24]. The transgene expresses a recombinant protein (c-MycER$^{TAM}$), which is present as an inactive monomer in the cytosol of the cell [24]. The activity of the recombinant

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fusion protein is controlled exclusively by the addition of the 4-hydroxytamoxifen (4-OHT) to the cell culture media. 4-OHT binds to the modified receptor and causes the protein to dimerize. The protein dimer translocates to the nucleus where c-Myc acts as a transcription factor to maintain cell division in the presence of growth factors in the media. The c-Myc protein enables cell cloning and increased cell proliferation as well as extending stable growth of cells by upregulation of telomerase activity and other c-Myc targets. In the absence of 4-OHT and the mitogenic growth factors in the media, both in vitro and after cell implantation, the c-MycER$^{TAM}$ fusion protein remains in the cytoplasm and is inactivated. Under these conditions, the cells undergo growth arrest and can differentiate into neurons and glial cells in vitro and in vivo [23]. The CTX cell line in culture remains dependent on mitogenic growth factors for significant proliferative capacity and the presence of 4-OHT to enhance cell growth to permit exponential proliferation. Telomerase activity, which supports karyotype stability across repeated cell doublings, is also 4-OHT dose-dependent [23]. In CTX in vitro, the c-MycER protein is gradually down-regulated upon growth arrest and neural differentiation. The c-*myc*ER gene is epigenetically silenced by methylation of the CPG islands of the promoter element of the construct, at least one week following cell implantation in stroke rats [25].

Figure 1 about here

**2.4 CTX cell line generation, banking, and manufacturing of drug product**

The CTX cell line was originally selected following in vitro and in vivo screening of several cell lines based on its stable NSC phenotype, survival and differentiation into relevant cell lineages in vivo [10,18,23]. The CTX cell line is clonal, expands rapidly in culture and has a normal karyotype [23]. The approach we have followed is to progress cell expansion/manufacturing through a cell banking process in line with procedures that have been used to manufacture biologicals such as recombinant proteins or antibodies [10]. This approach can allow a sustainable

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supply of standardized material for preclinical safety studies and potentially all the way to marketing authorization. To achieve this goal, CTX cell lines were generated to make 'master' and 'working' cell banks of frozen vials of cells from which reproducible clinical lots of drug substance and drug product batches can be derived, as required. The resultant CTX "Drug Product" (CTX-DP) is composed of CTX cells at a passage of ≤37 [23]. Clinical release criteria include measures of sterility, purity (cell number, cell viability) and a number of other tests of identity, stability and potency required for clinical product release or for information as requested by regulatory authorities (see Table 2). The active DP is a fresh or frozen suspension of living cells formulated in a proprietary excipient (Hypothermosol FRS (Biolife Solutions, Bothell, WA)) suitable for intracranial administration using stereotaxic surgical techniques. CTX-DP may be stored at 4° to 25°C for extended periods (hours to days). Currently, the CTX clinical DP is an "off the shelf" cryopreserved product in a solvent-free excipient (US Patent 9265795) with a shelf life of many months.

Table 2 about here

Initiating the strategy of cell banking of the CTX cell line early in its developmental program has ensured that all pivotal preclinical safety and efficacy studies were conducted using the DP material equivalent to that used in ongoing or subsequent clinical trials. Importantly, CTX will not need to be re-derived, as sufficient vials are available at every level of the manufacturing process to enable potentially limitless manufacture. Robotic automation of the CTX manufacturing process has also been demonstrated [26], further validating the potential of this cell line to be efficiently and safely scaled at a reasonable cost of goods.

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## 3. Preclinical studies of hNSCs in chronic ischemic stroke

For clinical translation of any cell therapy product, regulatory authorities require evidence of therapeutic efficacy in animal models, together with extensive data to support product quality (cell characteristics and quality assurance regarding cells, their manipulation and manufacturing), and in vivo safety in acute and long term toxicology and tumorigenicity studies. Critical issues related to cellular therapeutic strategy, impacting patient selection (such as age, anatomic location and size of infarct), functional targets, timing of transplantation, dose of cells, site and route for delivery and requirement for immunosuppression, should be assessed in preclinical models wherever possible prior to clinical translation. The most widely used, characterized and validated animal model of ischemic stroke is the middle cerebral artery occlusion (MCAo) in the rat [27,28]. The MCAo lesion in this model demonstrates the same ischemic territory (i.e. basal ganglia and sensorimotor cortex) as the human brain following ischemic stroke. This animal model exhibits the same core functional deficits such as unilateral paresis, sensory dysfunction and visuospatial neglect. However, it does not reproduce the heterogeneity of human stroke. The MCAo model permits investigation of key aspects of stroke pathophysiology to examine potential therapeutic agents with minimal sample size. Its use and reproducibility across laboratories has accrued sufficient evidence of potential efficacy to justify clinical development.

### 3.1 Toxicology and in vivo safety

Standard toxicology designs, using functional observational batteries, hematology and necropsy endpoints, can be used to assess acute and long term product safety. Evaluation of exogenous cell survival, proliferation, migration, and differentiation of implanted cells is necessary for both tumorigenicity and biodistribution assessments. Full in vivo biodistribution analysis requires the use of validated stem cell-specific markers and methods, such as immunohistochemistry, in situ

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hybridization or quantitative PCR to detect implanted cells. These studies require pilot feasibility and validation studies and suitable controls to eliminate false positives. For tumorigenicity studies these methods are implemented to confirm the cellular origin of any forming tumors.

Stem cell-derived therapy products that are implanted, including hNSCs, require extensive safety testing. CTX safety has been evaluated across a battery of preclinical studies. Following intracerebral implantation of CTX in MCAo rats, non-human primates and NOD SCID mice, general safety was assessed by a functional observation battery of tests for up to 12 months. No CTX-related adverse events were reported in any of these studies. Both the cell dose and volume of implant were well tolerated in all studies conducted [ReNeuron Internal Report; [29]].

In terms of tumorigenicity, cell therapy products need thorough evaluation. Long-term safety evaluation requires investigation of tumorigenic potential in large cohorts of animals using the clinical route of administration. For these studies, it is usual to implant cells into immunodeficient strains of mice using large numbers to give the best chance of cell survival and to maximise the chance of tumor formation. The duration of studies will vary depending on the survival time of the cells, which can range from about 3 months for non-engrafting cell types to 12 months or longer for cells that survive well in vivo. For CTX long term survival required for in-life tumorigenicity studies was only achievable in the MCAo model. In this system CTX cells at 12 months' post implantation were not proliferative and showed no evidence of tumor formation. In addition, long term (9 months) treatment of CTX implanted MCAo animals with tamoxifen had no impact on CTX cell survival and proliferation, with no CTX-related reports of tumor formation [ReNeuron Internal Report; [29]].

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Neoplasm formation has never been observed with CTX cells across multiple preclinical studies. This information is nonetheless important in assessing the inherent risk of using a genetically modified cell line for clinical applications. Additionally, because of the presence of the c-*myc* gene and its single site retroviral insertion, further CTX implantation studies were undertaken to demonstrate c-*myc*ER gene transcript and protein down-regulation and epigenetic silencing in vivo as described above [25].

Studies in vitro have also confirmed that re-exposure of growth arrested/differentiated cells to 4-OHT does not return differentiated CTX cells to a proliferative state [ReNeuron Internal Report; [29]]. Further, CTX cells do not proliferate when exposed to endogenous steroid hormones showing that activation of the c-*myc*ER$^{TAM}$ technology is specific for the 4-OHT ligand. Together, these data support the view that CTX is safe and does not present a tumor risk following implantation into the brain.

### 3.2 In vivo efficacy

A validated MCAo rat model of ischemic stroke was used for non-clinical CTX efficacy studies [30-32]. This model is well characterized in terms of sensorimotor and cognitive dysfunctions associated with region-specific stroke damage [28]. For CTX evaluation rats were transplanted 3-4 weeks' post-occlusion, permitting prior recovery from acute phase neurological dysfunction and establishment of steady-state sensorimotor deficits.

Three MCAo studies have demonstrated long-term improvements in sensorimotor function following intracerebral CTX implantation. In the first study, animals were treated with methylprednisolone for 2 weeks after cell administration and cyclosporine A for the duration of the study. Transplantation of CTX cells into the striatum in this study caused statistically significant improvements in both

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sensorimotor function and gross motor asymmetry at 6-12 weeks post implantation [23]. In a second study, CTX delivered adjacent to the infarcted region demonstrated a cell dose-response effect [33]. Again, animals were treated with methylprednisolone and cyclosporine A; however, for this study, treatment was only given for the first 2 weeks after cell administration. Recovery in sensorimotor function deficits (bilateral asymmetry test in the mid- and high-dose groups and the rotameter test after amphetamine exposure in the high-dose group) were found in the CTX implanted groups compared with the vehicle group [33]. In-life functional improvements correlated with cell dose; however, there were no statistically significant correlations between surviving CTX cell numbers and test performances.

In the third study, intraparenchymal (but not intraventricular) implantation of CTX cells in the rat MCAo model improved sensorimotor dysfunction (bilateral asymmetry test) and motor deficits (foot-fault test, rotameter) [34] . Importantly, analyses based on lesion topology (striatal versus striatal plus cortical damage) revealed a significantly greater (approximately 80%) functional improvement in animals with a stroke confined to the striatum [34]. Cell survival 3 months' post-implant was positively correlated with infarct size, but there was no correlation between cell survival and motor improvement.

### 3.3 Key factors affecting neurological recovery and therapeutic efficacy

Brain environment alters dramatically and dynamically after the initial insult of stroke and presents in varying anatomical regions at any given time point. Therefore, the timing and mode of administration are of clinical relevance in terms of different types of cell therapies and mode of action. In the clinical scenario, early systemic delivery of cell therapy (within 36 hours after the stroke event) is aimed as a neuroprotective strategy to reduce the cascade of injury and limit brain tissue loss. But restoration of cerebral blood flow (CBF) beyond a critical time cannot rescue irreversibly damaged brain cells, which leads to long term disability

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observed in stroke survivors. In this setting, cell therapy administered weeks, months and years post stroke targets the functional recovery of stable or deteriorating neural systems. This chronic stage is of interest to the majority of hNSC therapies currently in development (Table 1). During this period, the therapeutic opportunity is 'brain repair'' including neurogenesis, angiogenesis, growth factor secretion, engraftment and cell differentiation [33,35-40].

The majority of hNSC experimental stroke studies have used stereotaxic intracerebral delivery in order to place the cells in close proximity to the lesion and increase their survival (Table 1) [23,25,33,34,36-39,41-45]. Hicks, et al. [37] determined time-related CTX cell survival in the striatum of naive mice following implantation and found that the substantial and rapid reduction of the implanted CTX cells by day 7 was due to apoptosis. In the MCAo model, CTX cells implanted 4 weeks' post lesion were observed in 37% (10/27) of the treated cohort at 12 weeks' post implantation using immunofluorescence methods. Cell survival was not influenced by dose concentration (22% (2/9) low-dose ($4.5x10^2$ cells), 56% (5/9) medium-dose ($4.5x10^3$ cells), and 33% (3/9) of high-dose animals ($4.5 x10^5$ cells) in this study) [33]. In another MCAo study quantification of CTX survival at 1 and 4 weeks post implantation by Alu qPCR showed the presence of CTX cells in all grafted brains which ranged between 6.3% and 39.8% of the total cells administered [25]. These findings are consistent with other reports of the loss or short term survival of implanted hNSCs [40,42,46]. Further, a time course study following the fate of CTX cells post implantation into both ipsilateral and contralateral striatum of MCAo rats demonstrated a significant loss of implanted cells between 2 and 7 days' post implantation. At time points beyond 7 days and up to 6 months after implantation, CTX cells demonstrated better survival in the lesioned compared with the non-lesioned hemisphere [ReNeuron internal report].

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### 3.4 Implantation site and lesion topology

The extent to which the site of implantation and host environment influence the fate of hNSCs after intracerebral transplantation also needs consideration. Appropriate placement of cells is an important factor that is of clinical relevance affecting therapeutic outcome. The post-stroke brain manifests specific microenvironments that can influence implantation efficacy. Two contrasting sites - intracerebroventricular (ICV) and the peri-infarct environments - were compared for implantation efficacy [34]. The ICV environment is similar to the lesion environment as it contains cerebrospinal fluid (CSF) throughout. However, it lacks both a microvascular blood supply and extracellular matrix to support the integration and survival of implanted cells: an ICV injection of CTX cells did not result in any improvement. In contrast, the peri-infarct environment provides an extracellular matrix together with ischemia induced changes such as increased vasculature, neuronal loss and gliosis. When implanted into peri-infarct tissue, CTX cells survived and gradual improvements in sensorimotor dysfunctions (bilateral asymmetry test) and motor deficits (foot fault test and rotameter) were noted between 4 and 12 weeks' post implantation [34].

Lesion topology indicated that rats with stroke damage confined to the striatum recovered dysfunction to control levels following striatal CTX cell implantation. Animals with striatal lesions showed a more substantial improvement (83%) with CTX cell implantation, compared to animals with striatal and cortical lesions (48% improvement) [34].

## 4. Mechanisms of action of hNSCs in ischemic stroke

### 4.1 Cellular infarct reconstruction

An understanding of the cellular, molecular, and biochemical events involved in mechanisms of action of cell therapy is essential to improve and optimize its

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therapeutic benefit. Two main NSC-based strategies for the mechanism of action have been explored in animal models. The first strategy is via the replacement of neurons to potentially reconstruct and repair the stroke damaged neural circuitry using intracerebral implanted NSCs or stimulation of neurogenesis through endogenous NSCs [47,48]. In the second strategy, NSCs upon delivery to the brain (intracerebral, intravenous (IV) or intra-arterial (IA)) act via paracrine mechanisms to promote immunomodulatory and, neuroprotective mechanisms, endogenous neurogenesis and angiogenesis [37,49]. A recent proposal of a “biobridge” by the Borlongan laboratory, using a Notch-modified mesenchymal stromal cell (MSC) line, links both strategies and suggests that the therapeutic benefit is derived via a combination of direct cell transplantation and changes to the microenvironment via paracrine actions of administered cells [50]. In models of traumatic brain injury, the administered cells aid in the creation of the biobridge using MMP-dense signals. Importantly, upon completion of the biobridge, the administered cells defer the repair process to endogenous neurogenic cells [51]. As the newly generated host cells replace the implanted cells, the implanted cells die, leaving only endogenous cells to maintain the bridge between the SVZ and the injured site [50]. The role of the biobridge in stroke models using intracerebrally implanted hNSCs warrants further investigation.

### 4.2 Possibility for direct cell replacement as a mechanism of repair

Direct cell replacement as a mechanism for repair post stroke has been to a degree discounted as therapeutic benefits cannot be attributed solely to implanted cell survival and/or differentiation [37,52]. As presented in section 3.3 above, studies using CTX have consistently reported variable incidence of cell survival (1% to 58%) following implantation. This variability may be accounted for by sampling limitations, the method used to detect and quantify the cells and the site and timing

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of administration post-occlusion. The peri-infarct environment has been shown to favor implanted cell survival over non-ischemic or infarct environments [34,45,53].

Stroemer, et al. [33] observed that the majority of the surviving CTX cells were found in or close to the intracranial injection site. Any migration of implanted cells was restricted to the lesioned striatum ipsilateral to implantation [33]. Studies using other hNSCs have also consistently reported cell migration to ischemic regions, following administration via various routes such as intracranial implantation [42], IV [54], or IA [55]. Intracerebrally implanted hNSCs were reported to have migrated up to 1.2mm in the lesioned hemispheres compared to 0.2mm in naive rat brain [45]. In another study delaying implantation (48 hours to 6 weeks after ischemic insult) did not affect the magnitude of migration, neuronal differentiation and proliferation of the implanted hNSCs [42]. Further, surviving hNSCs displayed a wide spectrum of fates ranging from 78% remaining in an immature state [41] weeks after implantation, to unquantified numbers of differentiated neurons forming synapses with host cells [56].

Characterization of CTX phenotype post intracerebral implantation showed expression of vascular specific markers, CD31, CD62, and Flt4, and the oligodendroglial marker, Olig2 in a small proportion of surviving CTX cells in the MCAo brain [33]. Smith, et al. [34], however, reported that 3 months after implantation into stroke brains, almost 20% of CTX cells were glial fibrillary acidic protein positive (GFAP+) astrocytes, and less than 2% expressed the neuronal marker, FOX3.

In summary, studies of implanted CTX have shown variable and limited long term survival rates in MCAo rats; of these, only a small percentage of implanted cells expressed neural markers [33,36]. These results do not support the hypothesis that

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stem cell therapeutic effects can be solely attributed to neuronal replacement and are consistent with data from other studies [4-6,38].

### 4.3 Host tissue responses

Following the implantation of CTX cells, there was a significant increase in cell proliferation in the host brains, which may have partly contributed to post-occlusion repair and protection of the penumbra and led to enhanced compensatory networks arising elsewhere [33,36]. The reason that implanted hNSCs generally increase endogenous neurogenesis may be due to the production of certain growth factors or repression of inflammation and apoptosis [57,58]. Human NSCs can express several neurotrophic factors including EGF, FGFs, glial cell line-derived neurotrophic factor 1 alpha (GDNF), sonic hedgehog (Shh), stromal cell-derived factor 1 alpha (SDF1-alpha) and VEGF [59-61]. Also, it has been shown that implanted hNSCs can repress the expression of caspase-3 and up-regulate the activity of heat shock protein 27 (HSP27) to reduce apoptosis [58]. These paracrine activities of NSCs provide a microenvironment that is more favorable to host cells and thus leads to proliferation of endogenous NSCs and suppression of apoptosis.

### 4.4 Immunomodulatory /Anti-inflammatory effect

The SVZ is an essential source of new cells in the developing brain and remnants of these zones are known to persist into adulthood. In the adult rodent brain, the SVZ contains proliferating progenitor cells and relatively quiescent neural stem cells [62]. Following MCAo, there is a significant decrease in the number of Ki67 positive cells in the SVZ, indicating a reduction in cell division and a likely reduction in neurogenesis and gliagenesis. Implantation of CTX into MCAo-lesion brains significantly restored host proliferation in the neurogenic SVZ [33]. These findings are in agreement with reports using other hNSCs [49,63]. Ekdahl, et al. [63] also attributed the decreased neurogenesis to inflammation; however, increased proliferation in the SVZ has also been noted at relatively early time points following

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injury in the adult brain [48,64,65]. The Stroemer et al. [33] study suggested that at 3 months' post stroke, an inflammatory mechanism may operate to suppress cell proliferation and that an anti-inflammatory influence may be exerted by CTX at an early stage post-implantation to counteract the effects of MCAo. Although the phenotype of the newly-born cells was not determined in that study; it is highly likely that these cells in the SVZ would have differentiated into neurons, astrocytes and oligodendrocytes [66]. Horie, et al. [67], using hNSCs from neurosphere cultures showed fewer ionizing calcium-binding adaptor molecule 1 (Iba-1) positive monocytes/macrophages 1-2 weeks' post implantation. Interestingly, this immunomodulatory effect was blocked by the anti-VEGF antibody Avastin, indicating that VEGF was important in hNSC immunomodulation.

Human MSCs have been shown to have a profound inhibitory effect on T-cell proliferation and cytotoxicity [68]. There is also evidence that hNSC modulation of macrophage/microglia and endothelial cells occurs in the ischemic lesion area [69]. The underlying mechanisms of the immunosuppressive effect of CTX and other hNSCs involve several soluble molecules such as nitric oxide, the enzyme indoleamine 2,3-dioxygenase (IDO), transforming growth factor-$\beta$1 (TGF-$\beta$1), hepatocyte growth factor (HGF), interleukin-(IL-)10, IL-6, and soluble human leukocyte antigen (HLA)-G5, and are only partly understood [70-72]. The enzyme IDO is also activated during inflammation and drives towards conditions that favor immune suppression and tolerance [73]. High levels of IDO protein in the brain have been linked to neurological disorders. Repression of IDO appears to improve these diseases. When CTX cells were treated with interferon-ɣ, they dramatically upregulated IDO mRNA and protein expression [74]. A group of naturally occurring flavonoid phytochemicals were found to potently repress the CTX-IDO activity, independently of gene expression and protein translation, suggesting a mechanism to attenuate the IDO activity without damaging the hNSCs.

In ischemic situations, there is a large increase in necrotic cells: their lysis and resulting content release leads to inflammatory macrophage (M1) responses (CD68+, CD80+, CCR7+) which are pro-inflammatory/tissue destructive and counterproductive in an ischemic injury repair setting [75]. In contrast, in general tissue homeostasis, the removal of apoptotic cells enables tissue remodelling and repair. It is believed that uptake of apoptotic cells leads to polarization of the macrophages towards the M2, CD63+/CD168+, anti-inflammatory, tissue constructive type which secrete cytokines such as IL-10, IL-4, IL-13 and prostaglandin E2 in the local environment and promote wound repair [75,76].

Post implantation, a substantial proportion of the CTX cells undergoes rapid apoptosis over a period of 24-72 hours with an exponential decline in cell numbers. In vivo, apoptotic cells are removed rapidly (within minutes) by tissue macrophages and bystander cells. Engulfment of apoptotic cells by macrophages promotes M2 polarization resulting in tissue repair and angiogenesis. Furthermore, anerobic conditions lead to hypoxia-inducible factor-1 (HIF-1) activation in macrophages. This, in combination with an M2 macrophage phenotype, up-regulates pro-vascularization factors (e.g. PIGF, VEGF) and endothelial chemoattractants. In vitro studies indicate that addition of viable CTX cells to U937 cells in culture promote them to a M2 phenotype showing expression of CD206 and IL-10 release (see Figure 2).

Figure 2 about here

Therefore, it could be predicted that M2 polarization of macrophages, particularly in the presence of hypoxia in vivo, underlies an important component of the therapeutic benefit of injecting CTX cells directly into tissues.

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### 4.5 Microglia and stimulation of endogenous neurogenesis

The hypothesis that hNSC-mediated recovery in ischemic stroke conditions involves the neuroprotective action of microglial populations and the recruitment of a proliferative neuroblast population has gathered considerable support [36,43,77,78].

It has recently been shown in humans that neurogenesis arising from the SVZ is seen not only in olfactory bulb, but more prominently in striatal regions [79]. Hassani, et al. [36] reported evidence for a pool of endogenous proliferating cells in the rat striatum consisting of neuroblasts and microglial cells, which were generated in response to MCAo and implantation of CTX hNSCs. There was no effect of stroke on the ventral SVZ and although there was an effect of stroke on the presence of proliferating cells in the dorsal SVZ, there was no CTX effect in this region. There were changes to the proliferative population in the striatum in response to stroke which were enhanced by CTX implantation. CTX enhanced microglial responses at both 1 and 4 weeks' post CTX treatment in MCAo. In contrast, a significant increase in the presence of dividing neuroblasts was only afforded by CTX implantation in MCAo brain at 4 weeks' post implantation. These data suggest that the primary effect of CTX may be by modulation of the microglia response. It is possible that paracrine factors secreted by the microglia promote recruitment of dividing neuroblasts or have a neuroprotective action to support and maintain the neuroblast population and contribute to the therapeutic effect of CTX.

### 4.6 Evidence for angiogenesis, tubule formation and neovascularization

Angiogenesis contributes to the functional recovery of the ischemic region with increased collateral circulation to repair damaged vasculature [80,81]. Implanted CTX cells were also found to increase cerebral blood flow (CBF) on the lesioned side in MCAo-lesioned rats [82]. PET studies indicate that there is a significant ischemic penumbra in humans, and that reversibly ischemic tissue may persist for

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much longer than initial experiments in animal models have suggested [83]. The functional recovery of the penumbra is the most clinically relevant target and successful treatment would be aided by a local increase in CBF with the restoration of transport of soluble factors to the site of injury.

Human NSCs release VEGF in vitro [84], known in vivo to restore CBF by promoting new blood vessel growth [85,86]. Previous studies which monitored blood flow in the lesioned hemisphere using laser Doppler measurements post-occlusion demonstrated a return of CBF to baseline levels 12-14 days after a transient MCAo and MSCs implantation [87]. However, Ulrich, et al. [88] followed CBF for 3 and 6 weeks after permanent MCAo and did not see a complete return to normal levels. Eve, et al. [82], after examining both hemispheres, noted a difference in CBF between the left and right sides and a shifting in direction in a visual oscillatory fashion over time in control, sham-occluded, rats. This change in dominance between the left and right CBF suggested a natural oscillation of CBF in normal animals. In occluded animals, the laser Doppler measurements of CBF showed consistent left side dominance. By 3 months after implantation, the CBF was observed to have improved in the lesioned hemisphere of the CTX-treated group compared with the untreated stroke rats and demonstrated a similar oscillatory nature as that seen in sham-occluded animals [82].

These findings support a regulated pro-angiogenic activity for CTX as one of its modes of action. Over the past years, several paracrine factors that play important roles in angiogenesis have been studied for their role in the angiogenic process, including VEGFA, basic FGF, and EGF [89,90]. Basic FGF and EGF promote angiogenesis by a direct effect on endothelial cells and indirectly by the upregulation of endothelial cell VEGFA [91]. VEGFA is an angiogenic growth factor, potent mitogen and survival factor specific to endothelial cells [92,93], which has also demonstrated neuroprotective effects against ischemic injury [94-98]. CTX

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appears to perform a dual role in the promotion of angiogenesis in vitro: first, by the release of paracrine factors, (VEGFA, EGF, bFGF, ANGPT1, TGFb1, HIF-1a and ANGPT2); second, by direct physical cell interaction. In vitro, CTX treatment showed an increase in total tubule length formation compared with untreated controls [37].

In vivo, both MCAo and naive mouse brains demonstrated a clear increase in new host blood vessel formation with CTX implantation using immunohistochemistry analysis (an antibody raised against VWF, an endothelial cell marker) [37]. In addition, BrdU/CD31 labelling in naive mouse brain confirmed enhanced recruitment of proliferating endothelial progenitor cells and accelerated neo-vessel formation following CTX treatment. Human NSCs were found in precise association with blood vessels suggesting the establishment of a “neurovascular niche” [37]. Within this type of niche, it is proposed that neural progenitor cells lie in close proximity to endothelial cells to induce angiogenesis and neurogenesis. Furthermore, endothelial cells can secrete soluble factors that regulate neuronal differentiation [69,99-101]. Moreover, CTX may support this effect by the local delivery of growth factors, including VEGF. The study data demonstrated that angiogenesis and neurogenesis may be coupled processes similar to that observed in a co-culture system employing neural progenitors and activated endothelial cells [81]. CTX can interact with vascular endothelial cells specifically in areas of vascular remodelling, sprouting, and angiogenesis and ultimately aid repair (see Figure 3). These therapeutic benefits of CTX have also been demonstrated in ischemic muscles in the animal model of hind limb ischemia [102] and formed the basis for a UK clinical trial of CTX DP in lower limb peripheral arterial disease (NCT01916369), currently underway.

Figure 3 about here

## 5. Recent clinical experience

Of the more than 50 stem cell trials in ischemic stroke registered on the clinicaltrials.gov database, only two are investigating hNSCs, both CTX. The CTX Pilot Investigation of Human Neural Stem Cells in Chronic Ischemic Stroke Patients (PISCES) Phase I (NCT01151124) has completed and the locked trial data to 2 years has been published [9]. A follow-on Phase II efficacy trial (NCT02117635) has now completed patient recruitment.

### 5.1 PISCES first-in human trial

Translation of CTX stem cell therapy from the laboratory to the first-in-human trial in the UK has been approached with caution and based on rigorous preclinical studies in rats with chronic stroke, which showed improvements in behavioral outcomes and indicated long term safety [33]. The PISCES trial was designed as an open dose-escalation study, in which men aged 60 years or older with stable chronic stroke received single doses of 2 million, 5 million, 10 million, or 20 million CTX-DP cells by stereotactic ipsilateral putamen injection [9]. The primary objective of the trial was to assess the safety and tolerability of intracerebral transplantation of CTX and second, to observe neurological and functional outcomes over the 24 months after treatment. There were no cell-related adverse events. Serious adverse events (SAEs) related to the procedure were noted from imaging in 4 of 11 patients, but none were symptomatic, a finding consistent with general safety data for brain stereotactic procedures [103,104]. No incidence of seizures was recorded, unlike previous trials in which teratocarcinoma-derived neuronal cells [105,106] and fetal porcine cells [107] were implanted to treat stroke.

The secondary endpoints monitored were exploratory indices of efficacy, using modified Rankin Scale (mRS), NIH stroke scale (NIHSS), Ashworth Scale for upper and lower limb spasticity and Barthel Index of activities of daily living (BI). After CTX-DP implantation, statistically significant improvements were seen over time in

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NIHSS, and non-significant improvements in summed arm and leg Ashworth scale and BI scores [9]. Disability, as measured by the mRS, at 12 months was unchanged in 7 of 11 patients and improved by one grade in four. At 24 months, disability was unchanged in 7 patients, worsened by two grades in one, and improved by one grade in three. Patient reported overall health status had improved by a median of 18 points (IQR –5 to 30) at 12 months compared with baseline. Functional magnetic resonance imaging (fMRI) data were also collected pre- and post-treatment to identify potential biomarkers of change in neurological function in the brains of the treated patients. Some longitudinal changes in motor activation fMRI were seen, consistent with the improvements in neurological measures.

Due to the small number of patients treated in the trial, their heterogeneity as a study population and the open label, single arm study design, it is not possible to draw reliable conclusions about the effects of CTX cell implantation on neurological or functional recovery. However, it was possible to note that despite selection of chronic, stable patients at late stages after stroke, most showed some improvement across several indices of function [9]. Whether attributable to cell implantation or to other factors, such as extra medical attention, change in this patient group suggests that trials of interventions are worthwhile late after stroke, when recovery is not generally believed to be attainable. The researchers also described anecdotal accounts of reduced spasticity, minor return of finger movement at phalangeal joints, and improved visual perception and better bed-to-chair transfers, which were supported by the changes in health-related quality of life, activities of daily living and neurological impairment [9].

In this first trial, only men were enrolled given the early nature of stem cell clinical research and the lack of reproductive toxicology outcomes for stem cells of any origin and in particular for CTX, in which a tamoxifen metabolite analogue receptor is used in vitro to control cell manufacture [25]. Subsequent studies, however, are

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not limited to men as further preclinical studies did not show any tumor formation or increased CTX proliferation in male and female stroke rats chronically treated with tamoxifen.

Patients were not given immunosuppressive therapy because non-clinical studies with CTX showed no evidence that cell survival and efficacy required immunosuppression [33]. Additionally, in vitro studies for MHC class II (DR) and MHC class I (A, B, and C) showed low protein expression for CTX [9]. Further, immunosuppressive therapy heightens the risk of infection after stroke, which is independently associated with poor outcomes. The putamen was chosen as the site for implantation based on preclinical data which defined it as the closest intact subcortical neuronal cluster to the lesion and for its preference to white-matter, in which injections can cause further axonal injury related to increased pressure. We selected CTX-DP doses by scaling up from those that were efficacious in rats, and the ascending dose design (from 2 to 20 million cells) allowed cautious incremental increases after safety review. All patients received short and long term safety reviews and will be followed life-long for cancer.

In parallel, SanBio Inc in the US is undertaking a clinical development program with a *Notch-1* modified MSC line, SB623 in similar chronically disabled stroke patients. Recently, the Phase I outcomes from a US multicentre trial were reported [108] and showed similar improvements in NIHSS over time, with other measures employed in that study also showing some improvements. No SAEs were attributable to the cell therapy [109,110].

### 5.2 PISCES Phase II efficacy trial

Based on early demonstration of safety and feasibility in the Phase I trial, PISCES Phase II is following on, in order to assess the safety and efficacy of intracerebral CTX-DP in patients with paresis of an arm following an ischemic MCA stroke. The

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trial design is a UK multi-center, open label, single arm, non-comparative design, administering a single dose of 20 million cells 3 months' post-ischemic stroke with follow-up over 12 months. Eligible patients, men or women aged 40 years or older, who have no useful function of the paretic arm for a minimum of 3 months to a maximum of 12 months after the ischemic stroke qualify to enrol into a minimum cohort of 21 patients. The design will determine if a sufficient proportion of patients experience recovery of function with CTX-DP at a dose level of 20 million cells (maximum dose from the Phase I) to justify a subsequent larger prospectively randomized efficacy study. Endpoint measures will include recovery of useful upper limb movement based on, for example, changes in the Action Research Arm Test (ARAT) [111], as well as established neurological and disability scales, such as NIHSS, a more objective version of mRS, Rankin Focused Assessment and BI at 3, 6 and 12 months' post treatment.

## 6. Future clinical aspects

The PISCES Phase I trial results demonstrated the feasibility of intracerebrally administered CTX-DP therapy and safety in elderly severely disabled men with chronic stroke. Due to its open trial design and the small number and heterogeneity of patients treated, it is not possible to draw reliable conclusion about the effects of hNSC therapy on neurological or functional recovery; although improvements were noted despite the selection of chronic, stable patients at late stages after stroke [9].

Phase II studies address methodologies and investigate biological evidence of activity to progress to pivotal trial data. The STEPS 3 guidelines recommend a Phase II study design which, along with standard measures of functional disability and activities of daily life, include domain-specific end points assessing the recovery of sensory, motor, visual, and cognitive functions using validated measures [112]. A desirable goal for a stroke disabled patient would be to achieve a level of functional independence that would enable them to return home and

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reintegrate into community life as fully as possible. Hemiparesis and motor recovery have been the most studied of all stroke impairments, affecting as many as 88% of patients acutely post stroke. A systematic review of 58 studies confirms the most important predictive factor for upper limb recovery following stroke is the initial severity of motor impairment or function [113]. The prognosis for return of useful hand function is unfavorable when upper arm paralysis is complete at onset or grasp strength is not measurable by 4 weeks, with most recovery taking place in the first 3 months with little further recovery thereafter. This suggests that 3 months is the earliest time point for an invasive stem cell intervention.

The determination of clinical efficacy requires an appropriately designed and controlled study. However, appropriate controls for a randomized trial are likely to be a compromise in some respects. Historical controls are not valid, and concurrent controls who undergo only conventional medical and rehabilitation therapies are also sub-optimal, since they are not exposed to the invasive procedures and the potent placebo effect of “stem cell therapy” [114]. While essential scientifically, placebo (or ‘sham’) neurosurgery is of uncertain acceptability to patients, and the need for sham neurosurgery has been challenged by patient groups [115]. Patient acceptability of the design of a proposed trial is essential to ensure recruitment, and rigid adherence to conventional parallel-group randomized controlled trial designs is difficult at the early stages of efficacy testing, since patients who accept the risks of surgery and experimental treatment are frequently unwilling to consider the possibility that they will be randomly allocated to a placebo control group [116]. The reward-risk balance for neurosurgical administration may also necessitate the use of clinically (not just statistically) significant endpoints in a relatively small number of patients which poses a major challenge.

The ability to adjust for concomitant rehabilitation therapy in both active and control arms represents a difficulty in trial design. It is also possible that rehabilitation

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training is a necessary facilitatory factor for regenerative effects of cell therapies to be seen, although animal models of non-specific physical therapies suggest that negative interactions are also possible. Implementation of standardized therapy as part of a clinical trial is difficult when dealing with what is traditionally a highly individualized and time-limited intervention that varies widely across healthcare systems.

## Disclosure statement

JS, CH, PS and RC are employees of and stock and/or option holders in ReNeuron. IV received financial support from ReNeuron for her assistance in drafting and preparing the manuscript for publication.

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# Table 1

**Examples of human neural stem cell lines in preclinical models of ischemic stroke, their reported modes of action and behavioral endpoints observed**

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| **Source of hNSCs** | **Administration of cell therapy (Timing/ / Mode / Site)** | **Putative modes of action** | **Effects on behavioral outcomes (MCAo rat model)** |
|---|---|---|---|
| *Conditionally immortalized fetal cortical cell line product CTX [23,25,33,34,37]* | 4 weeks' post MCAo / IC / ipsilateral peri-infarct (right putamen) | Some engraftment and mainly astrocyte differentiation<br>Paracrine effect: secretion of paracrine factors, such as vascular endothelial growth factor (VEGF); Enhanced angiogenesis: improvement in CBF, new blood vessels formation<br>Enhanced neurogenesis<br>Immunomodulation; microglial effect; anti-inflammatory | Significant improvement:<br>• Bilateral asymmetry test [23,33,34]<br>• Rotameter test [23,33,34]<br>• Foot fault test [34]<br>No effect:<br>• Whiskers reflex [33]<br>• Water maze test [34] |
| *Immortalized human fetal NSC line HB1.F3 [38]* | 24 hours' post MCAo / IC / SVZ | Reduced infarct volume<br>Activated proliferation and differentiation of | Significant improvement:<br>• modified Neurologic |

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| | | | |
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| | | endogenous neurogenesis to produce mature neuron-like cells<br>Enhanced angiogenesis | Severity Score [38] |
| *Human cortical NPCs [41,67]* | 7 days' post MCAo / IC / ipsilateral cortex [45] | Increases dendritic plasticity in both the ipsi- and contralesional cortex 3-5 weeks' post implantation<br>Increased corticocortical, corticostriatal, corticothalamic and corticospinal axonal rewiring from the contralesional side; with the transcallosal and corticospinal axonal sprouting<br>Rescued axonal transport, which is critical for both proper axonal function and axonal sprouting<br>Identified VEGF, | Significant improvement:<br>• Vibraissae-elicited forelimb placing test [41]<br>• Cylinder test [41]<br>• Postural reflex test [41]<br>• Whiskers stimulation test [67]<br>No effect:<br>• Elevated body swing test [41] |

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| | | thrombospondins 1 and 2, and slit as mediators partially responsible for stem cell-induced effects on dendritic sprouting, axonal plasticity and axonal transport | |
| *hESC-NSCs*<br>*BG01 [39]* | 14 days' post distal MCAo / IC / SVZ or SGZ | Increased neurogenesis (Dcx expression) in ipsilateral SVZ, but not in contralateral or SGZ | Significant improvement:<br>• Cylinder test [117]; in young (3 month) rats [118]<br>• Adhesive removal test [117]<br>• Y maze [117]<br>• Elevated body swing test in older (24 months) rats [118]<br>No effect:<br>• Elevated body swing in young (3 months) rats [118] |

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| | | | |
|---|---|---|---|
| | | | • Cylinder test in older (24 months) rats [118] |
| *Human fetal striatal NSC [40]* | 48 hours' & 6 weeks' post MCAo / IC / right striatum | Mainly through paracrine effect, as cell survival and differentiation even dosing at optimal timing (1 week) was inadequate for cell replacement<br>Time window for intervention before 17 to 18 days after ischemia, avoiding maximal activation of microglia in response to stroke | Not reported |
| *Human striatal neurospheres [44]* | 2 days' or 3 weeks' MCAo / IC / contralateral striatum | Promoted striatal neurogenesis<br>Suppression of striatal inflammation | Significant improvement:<br>• Stepping test (left forelimb: forehand & backhand) [44]<br>• Cylinder test [44] |

| | | | |
|---|---|---|---|
| | | | No effect:<br>• Stepping test (right forelimb: forehand & backhand) [44] |

**Abbreviations:** CBF, cerebral blood flow; Dcx, doublecortin; hESC, human embryonic stem cell; IC, intracerebral; IS, intrastriatal; MCAo, middle cerebral artery occlusion; NPC, neural progenitor cell; hNSC, human neural stem cell; SGZ, subgranular zone of dentate gyrus; SVZ, subventricular zone; VEGF, vascular endothelial growth factor

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Table 2: Identity, stability and potency tests that are employed to characterize CTX cell banks and/or drug product (for Phase II trial)

| **TEST** | **OUTCOME** |
|---|---|
| PCR Sequencing of cDNA | Sequence of insert conforms to transgene identity. No insertions, deletions or mutations from expected sequence |
| Determination of Flanking Nucleotide Sequence | Consistent with published sequence |
| PCR across integration site | PCR across integration site confirms cell line identity |
| Karyology | Comparable with published normal chromosome, male XY |
| Viability and growth | ≥ 70% viability on recovery. Viable cell numbers at least double within 7 days |
| c-mycER$^{TAM}$ gene copy number (PCR) | Modal ~ 1 (range 0.87 – 3.46) |
| Phenotypic marker (Nestin) | At least 95% of cells are Nestin positive |
| Position, sequence and indication of number of integrated target gene by Fluorescent *In Situ* Hybridization (FISH) | Chromosomal (Chr 13) localization of integrated c-mycER$^{TAM}$ sequences |
| Potency | Cell dose-dependent IL10 production in co-culture with U937 monocyte cell line |
| Neural Differentiation | Upregulation of Tub-β3, GFAP and GAL-C marker expression by Q-PCR following seeding into Alvatex® 3-dimensional cell matrix |

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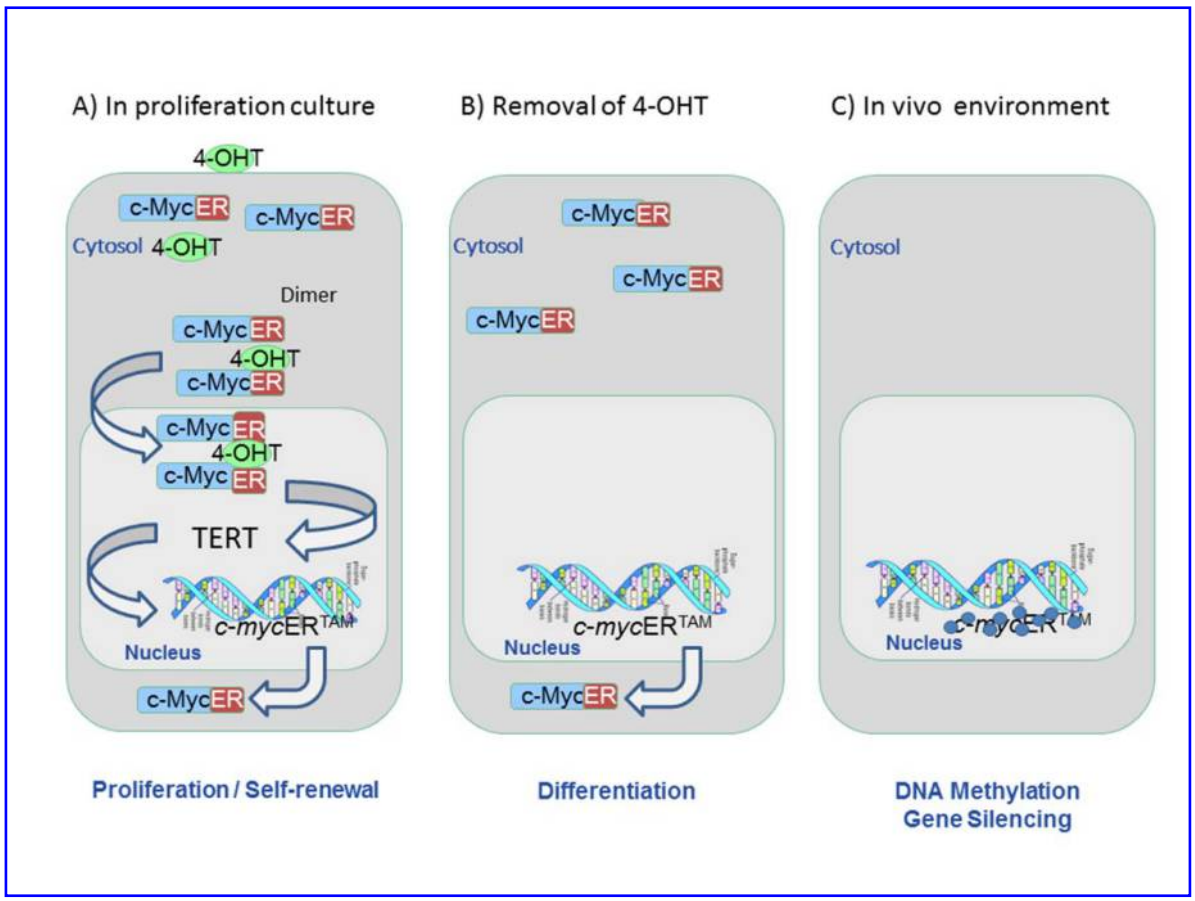


Figure 1: Conditional growth of CTX0E03 is dependent upon the presence of 4-hydroxytamoxifen

A) Proliferation: The conditional immortalizing gene c-*myc*ER$^{TAM}$ generates a fusion protein of c-Myc and a hormone receptor (ER) that is regulated by 4-hydroxytamoxifen (4-OHT). In the presence of 4-OHT, the fusion protein (c-MycER) forms a dimer that translocates into the cell nucleus. Once in the nucleus, the dimer c-MycER activates the cell cycle and regulates the transcription of Telomerase Reverse Transcriptase (TERT), which controls long-term cell division with genetic stability. B) Removal of 4-OHT: When 4-OHT is removed from the cell media, the fusion proteins no longer form dimers and remain in the cytoplasm. Cell division is markedly reduced. Cells then begin to differentiate into neural phenotypes. C) In vivo environment: When cells are implanted into the brain, the c-*myc*ER$^{TAM}$ gene is

effectively “silenced” within 7 days by methylation of the promoter sequence. Thus, the fusion protein is no longer expressed.



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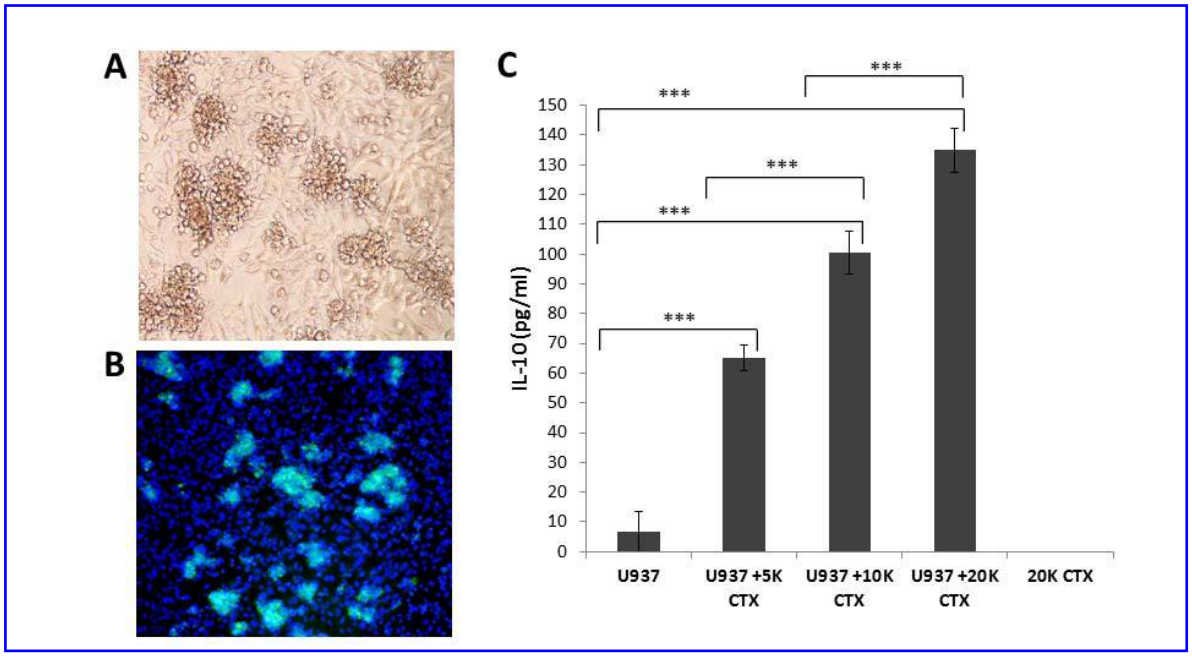


Figure 2: Co-culture of CTX cells with activated U937 cells induces a concentration dependent release of IL-10 and expression of the M2 marker CD206

The assay requires cells from the mouse monocyte cell line U937, activated using the phorbol ester, Phorbol 12-myristate 13-acetate (PMA) and the plating of CTX cells on pre-laminin coated 96-well plates at three different concentrations prior to their co-culture. The co-culture is established by adding the activated U937 cells at a fixed concentration (50,000 cells per well) to the attached CTX cells and then cultured for a period of 72 hours (A). Immunocytochemical analysis of the co-cultures fixed at 72 hours using a human specific anti-CD206 monoclonal antibody detected with anti-mouse Alexa Fluor 488 conjugated secondary antibody indicates the expression of CD206 (green), a marker of M2 polarization, by U937 cells visualized against a Hoechst nuclear counter stain (blue) (B). Culture media collected from each well at 72 hours was analyzed for IL-10 concentration using a human specific ELISA (R&D Systems). The data shown are mean ± SEM, (n=3) from three independent cell samples in pg/ml; CTX cells in co-culture with U937

cells promote a statistically significant dose dependent increase in IL-10 release compared with U937 cells cultured alone in a dose dependent manner (*** p=<0.001 ANOVA) (C).



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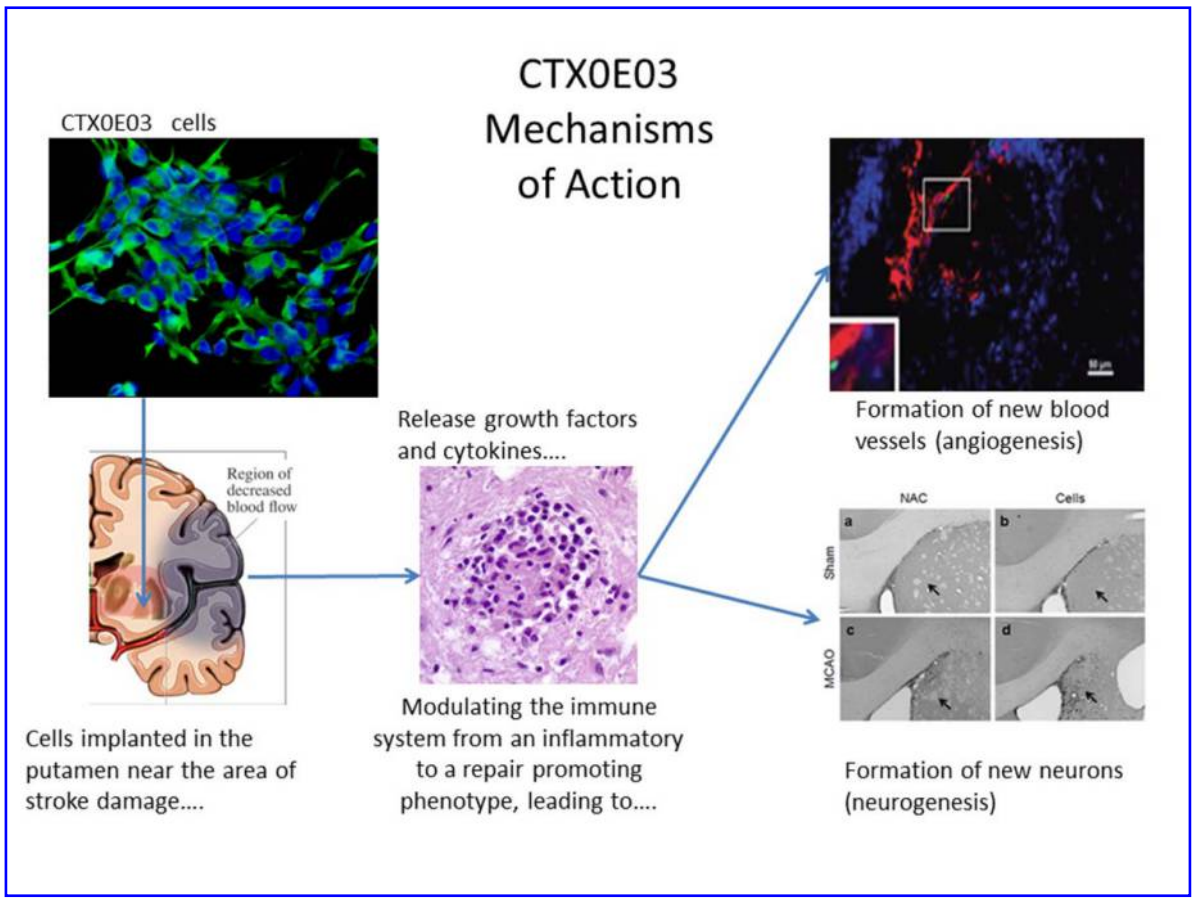


Figure 3: Summary of CTX mechanisms of action in ischemic stroke

CTX cells are stored in a frozen state and thawed just prior to use. No further processing is required prior to administration. Cells are administered via stereotaxic intracerebral injection into an area adjacent to stroke damage that has maintained blood flow (putamen).

Whereas neuronal cell replacement/engraftment was hypothesized to be the most obvious mechanism of action, the observed pharmacokinetics of CTX cells does not support this theory. CTX cells may exert their therapeutic effect by paracrine mechanisms. Upregulation of VEGFA and chemokines CCL2 and CXCL12 suggests that these may be candidate factors.

In vivo, CTX treatment promotes recruitment, proliferation and/or maintenance of host cell populations including immune and stromal cells, neural progenitor and

endothelial progenitor cell types. In vitro, CTX cells demonstrate immunomodulatory activity by promoting polarization of U937 cells from a pro-inflammatory to an alternative anti-inflammatory CD206, IL-10 producing phenotype commonly associated with tissue remodeling and repair.

Angiogenesis is promoted by CTX administration. CTX cell implantation restores von Willebrand Factor (a marker of angiogenesis) in the lesioned hemisphere to a level comparable with control (non-lesioned) tissue. Evidence of angiogenesis was demonstrated by de novo blood vessel formation and increased blood flow in the affected hemisphere in rat transient MCAo post implantation.

Analysis of treated brain sections shows that implantation of CTX cells into MCAo brain returns host cell proliferation in the subventricular zone (SVZ) to a similar level to that seen in sham-lesioned controls. CTX administration also increases the presence of proliferating microglia and neuroblasts in the striatum.



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