Current status and perspectives on stem cell-based therapies undergoing clinical trials for regenerative medicine: case studies

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Background: Apart from haematopoietic stem cell transplantation for haematological disorders many stem cell-based therapies are experimental. However, with only 12 years between human embryonic stem cell isolation and the first clinical trial, development of stem cell products for regenerative medicine has been rapid and numerous clinical trials have begun to investigate their therapeutic potential.

Source of data: This review summarizes key clinical trial data, current and future perspectives on stem cell-based products undergoing clinical trials, based on literature search and author research.

Areas of agreement: It is widely recognized that the ability to stimulate stem cell differentiation into specialized cells for use as cellular therapies will revolutionize health care and offer major hope for numerous diseases for which there are limited or no therapeutic options.

Areas of controversy: Stem cell-based products are unique and cover a large range of disorders to be treated; therefore, there is significant potential for variation in cell source, type, processing manipulation, the bioprocessing approach and scalability, the cost and purity of manufacture, final product quality and mode of action. As such there are gaps in regulatory and manufacturing frameworks and technologies, only a small number of products are currently within late phase clinical trials and few products have achieved commercialization.

Growing points: Recent developments are encouraging acceleration through the difficulties encountered en route to clinical trials and commercialization of stem cell therapies.

Areas timely for developing research: The field is growing year on year with the first clinical trial using induced pluripotent stem cells anticipated by end 2013.

Keywords: stem cell-based therapy/regenerative medicine/clinical trial/regulation/commercialisation

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Introduction

Regenerative medicine (RM) is fast becoming the next major innovation in health care targeted at the repair or replacement of damaged or diseased human cells, tissues or organs to restore normal function.\(^1,2\) Historically, tissue repair utilizing principles of cell culture application has been around since the late 1930s\(^3\) and the clinical use of bone marrow-derived or haematopoietic stem cells for treatment of haematological disorders has been around since the late 1950s,\(^3\) with the first successful bone marrow transplantations in the late 1960s.\(^4,5\) Since the late 1990s there has been significant expansion in basic and clinical research in the isolation, generation and application of multiple types of therapeutically useful stem cells. The rapid advancement can be illustrated by the progression of human embryonic stem cells (hESCs) into clinical trials (Fig. 1); first isolated in 1998\(^6\) hESCs have the ability to self-renew indefinitely and retain the potential to differentiate into every cell type (pluripotency).\(^7–9\) Twelve years later, in 2010, clearance was granted in the USA for clinical trials on hESC-derived therapies for treatment of thoracic spinal cord injury (SCI) and two types of macular degeneration.\(^10–12\) Additionally, as hESC generation requires the destruction of human embryo’s raising controversy and ethical concern,\(^9,13\) alternative sources of pluripotent stem cells without the destruction of embryonic tissue have since been developed.\(^13,14\) Induced pluripotent stem cell (iPSC) technology encompasses the reprogramming of adult differentiated cells to pluripotency with equivalent potency and differentiability to that of hESCs, and offers great potential for advances in disease modelling and generation of therapeutic cell types.\(^14\) However, there are numerous limitations on the clinical utility of retroviral reprogrammed iPSCs with regard to efficiency of generation rates, heterogeneity, potential for mutation, in vivo tumour formation and immune responses.\(^15,16\) Enormous recent research effort has developed alternative reprogramming strategies and human leukocyte antigen (HLA) matching to mitigate many of the safety and immunogenicity risks in a push to rapidly approach the clinic in the near future.\(^15,17,18\)

There are a large number of stem cell therapy products in clinical development and the global industry was estimated to have annual commercial sales of over $1 billion in 2011 with growth to $20 billion by 2025.\(^19\) Maturation of the market shift to commercial products is expected in the next 5–10 years with a series of therapies in the clinical pipeline for cardiovascular conditions,\(^20\) cancer,\(^21\) diabetes\(^22\) and genetic diseases.\(^23\) The challenges surrounding commercial viability and clinical uptake are related to difficulties in establishing clinical utility and cost-effectiveness. Key barriers relate to important aspects of the translation process of developing a cell therapy product beyond the primary focus of successfully reaching...
‘first-in-man’ clinical targets towards later phase trials and commercial endpoints. This requires combined efficient quality bioprocessing and scale-up approaches in order to realize the ‘one-to-many’ translation process for scaled production at a price affordable to society.24,25

This review gives an overview of the clinical and regulatory pathway for stem cell-based therapies, provides a breakdown of the clinical trial landscape and highlights key case studies as the RM industry matures towards a commercial market. Our focus is on stem cell therapies, defined as any treatment for a medical condition that employs viable human stem cells at its core, encompassing both autologous (patient-derived cells) and
allogeneic (donor-derived cells) therapies, including ESCs, iPSCs and adult stem cells.¹⁹

Clinical and regulatory pathway overview

Regulatory pathway

Numerous cell therapy trials are being carried out throughout Europe, the USA and the rest of the world. The regulatory framework for development and compliance procedures for production and commercialization has progressed alongside industry establishment to provide guidance and legislation on the assessment of safety, quality, purity, potency and efficacy utilizing experience from conventional pharmaceuticals and blood banks.²⁶ However, the framework for regulating such products is far more complex than for conventional molecular therapies, where the final product is distinct from cell culture bioprocessing, because the cell is the active therapeutic agent. Therefore, the whole bioprocess is critical to the integrity of the product and involves numerous biological input materials to manufacture a complex viable product that constantly changes in response to its environment.²⁵ Additionally, the therapeutically attractive self-renewal and lineage differentiation features of stem cells also pose potentially detrimental safety risks including uncontrolled cell proliferation or tumorigenicity (hESC, iPSC) and undesirable cell differentiation. Stem cell products therefore inherently do not lend themselves to a ‘one-size-fits-all’ concept of product development and regulation. The journey from laboratory bench to patient bedside as an approved reimbursed product was recently systematically reviewed by the UK House of Lords Science and Technology Committee. Following this enquiry, the RM Report, published in July 2013, echoes the above complexities and makes recommendations on strategies for regulatory streamlining, funding mechanisms, NHS adoption and reimbursement to ensure the UK delivers in the RM sector.²⁷

It is widely recognized across the industry that the issues surrounding cell therapy development and regulation are critical and strategic plans are in progress to address them.²⁸ As the regulatory framework continues to evolve increased regulatory certainty will enable cost-effectiveness improvements on multiple levels (cost of goods, manufacturing technologies and reimbursement policies); drive improvements in product characterization and performance specification; and ensure early clinical end-user collaboration to establish clinical utility, cost-effectiveness and enable timely post-approval clinical adoption. Acceleration of pre-clinical therapies towards clinical trials is widely supported, for example, the California Institute of Regenerative Medicine (CIRM) have invested over
$220 million in collaborative funding for this purpose\textsuperscript{10} and more recently in 2012, the UK Technology Strategy Board invested over £50 million to establish the Cell Therapy Catapult; a world-leading technology and innovation centre of excellence designed to grow the UK cell therapy industry.\textsuperscript{19}

Stem cell-based therapies are assessed on an individual product basis and most products are subject to clinical assessment for market approval.\textsuperscript{29,30} There are a number of guideline documents with bearing on cell-based therapies produced by the European Medicines Agency (EMA), the US Food and Drug Administration (FDA), the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the British Standards Institution (BSI).\textsuperscript{19} Critical guides produced by the BSI include PAS 83:2012 ‘Developing Human Cells for Clinical Applications in the European Union and the United States of America’, PAS 93:2011 ‘Charactenization of Human Cells for Clinical Applications’, and the recently revised PAS 84:2012 ‘Cell Therapy and Regenerative Medicine Glossary’.\textsuperscript{19} These include specific regulatory guidelines for the collection, process, evaluation, preservation, storage and distribution of cell products for human application to ensure safe and reliable administration of cells and tissues for clinical use.\textsuperscript{31} Additionally, the glossary encourages the use of common and current terminology for effective communication among key stakeholders. Different regulatory bodies around the world have their own specific systems and pathways for approval. In Europe, cell-based products are regulated as Advanced Therapy Medicinal Products (ATMPs) as defined in Directive 2001/83/EC\textsuperscript{32} and include gene therapies, somatic cell therapies and tissue engineered products. In the UK, the Medicines and Healthcare Products Regulatory Agency (MHRA) is responsible for authorizing clinical trials and inspecting and issuing the manufacturer’s license for an Investigational Medicinal Product. Europe has a centralized authorization procedure for ATMP marketing authorization applications. This is comprising draft opinion formation on product quality, safety and efficacy by the EMAs Committee for Advanced Therapies (CAT) that includes expert representatives from all EU member states, medical personnel and patient associations. Followed by submission for final approval to the EMA’s Committee for Medicinal Products for Human Use.\textsuperscript{33,34} Additionally, in the UK, there are two controversial exemption schemes with narrowed criteria that allow the non-routine supply of unlicensed ATMPs not subject to European Commission marketing authorization.\textsuperscript{33} Regulatory approval of cellular-based products around the world is growing, from 2001 to 2009 there were no product approvals, whereas from 2009 to 2012 eight approvals were made.\textsuperscript{35} There are several commercially distributed cellular products in the USA and select European countries,
primarily including autologous chondrocyte therapies for cartilage repair and skin substitutes using cultured epidermal cells for wound repair.\textsuperscript{35} Two such products, Carticel and Epicel, produced by Genzyme (Cambridge, MA, USA) are marketed in the USA and Europe.\textsuperscript{35} Other examples include amnion-derived wound coverings (BioDfactor, BioDfence, Nucel, US only) and adult human bone allografts (US; PureGen, EU; Allostem).\textsuperscript{35} However, specifically regarding stem cell therapies, the ground breaking first regulatory approval of a manufactured stem cell therapy occurred in May 2012. The product, Prochymal\textsuperscript{\textregistered} (remestemcel-L), is a first-in-class allogeneic stem cell therapy developed and manufactured by Osiris Therapeutics, Inc. (Columbia, MD, USA), and was approved for market authorization in Canada and New Zealand for the treatment of graft versus host disease (GvHD) in children.\textsuperscript{36}

**Clinical pathway**

During clinical application for human use, the regulatory principles applied to conventional molecular therapies are also applied to stem-cell-based products. However, a more thorough approach regarding technical specificity is required particularly for product quality, safety and efficacy.\textsuperscript{26} Clinical trial design and performance must be in accordance with Good Clinical Practise (GCP) and associated regulatory Directives.\textsuperscript{26} Resolution in the choice of valid endpoints that provide useful analogues for clinical benefit is critical. Due consideration must be given to patient age and stage of disease, plus any comorbidities or complications that arise from the disease, as the complex biology of other clinical factors cannot be sufficiently replicated in experimental model organisms.\textsuperscript{37} Assessment of stem cell therapy clinical studies involves evaluation of several factors including cell source or donor eligibility, cell isolation, culture and differentiation methods, analytical test design to evaluate identity, purity and potency, and immunological risk management. Safety concerns surround product characterization, potency characterization, defining the mechanism of action, undesirable \textit{in vivo} cell differentiation, undesirable cell migration, uncontrolled proliferation or tumourigenicity, immunogenicity, graft versus host reactions and undesirable interactions with tissues, drugs or devices. Products with well-defined distinguishable phenotypic characteristics that serve as analogues of desired function are rare, e.g. dark pigment morphological cell phenotype for retinal pigmented epithelial cell differentiation.\textsuperscript{38} Commonly used bone marrow-mononuclear cell (BM-MNC) products and umbilical cord blood products are heterogeneous cell populations, and for mesenchymal stromal cell products, the rudimentary capacity to adhere to tissue culture plastic remains the standard \textit{in vitro} selection process.

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\textsuperscript{78} E. Ratcliffe \textit{et al.}
Careful interpretation of clinical trial data aids establishment of cell types with defined dosage and delivery methods.\textsuperscript{37} Clinical development requires several stages: preclinical phase, clinical trial phases (0/I/II/III) and post-marketing surveillance phase (IV). A database of UK preclinical research estimated to be within 2 years of reaching the clinic was recently published by the Cell Therapy Catapult.\textsuperscript{19} Preclinical testing in animal models provides insight into product behaviour, dosing levels to be used clinically, administration, mode of action, efficacy and toxicity.\textsuperscript{37} However, prediction of human toxicity or immune reaction is complex and current animal models are not necessarily predictive of performance in the humans. Currently, there is great research effort focussed on producing stem cell tools and applications to enable greater prediction with regard to toxicity studies and with focus on the 3Rs to reduce the amount of preclinical animal testing required.\textsuperscript{39} For example, in 2011, the US Defense Advanced Research Projects Agency and National Institutes of Health (NIH) announced the allocation of $140 million for a 5-year project in coordination with the FDA to develop the human physiome on a chip to predict drug and vaccine toxicity.\textsuperscript{40} During preclinical development, it is also necessary to define product administration including defining the administrative time window for transplanting cells at the appropriate stage of the disease. Precise cell delivery strategies are required as efficacy is dependent upon efficient engraftment, homing, survival and function of the cells at the site of injury or disease. Critically, precise and accurate purity of manufacture of the cell type of interest in sufficient quantity for effective dosing underpins the preclinical and clinical research.\textsuperscript{25} Cell therapies for clinical trials must be produced aseptically according to Good Manufacturing Practise (GMP) guidelines within clean room environments that are classified in accordance with air quality.\textsuperscript{26} The entire manufacturing process is carried out under quality-controlled GMP-validated conditions and the robustness of the manufacturing process ensures product consistency and reproducibility with minimal risk of contamination.\textsuperscript{25,26} Phase (Ph) 0 trials are often substituted for Ph I* trials and are defined as the first-in-man experiments; a small number of subjects are administered a single subtherapeutic dose of the therapy to gather preliminary data on the pharmacodynamics and pharmacokinetics.\textsuperscript{41} No efficacy data are achieved by virtue of the fact that a subtherapeutic dose is administered. Ph I trials evaluate the safety (pharmacovigilance), tolerance, pharmacokinetics and pharmacodynamics of the therapy. In contrast to Ph 0/I trials for conventional molecular therapies, stem cell therapies cannot be given to healthy volunteers to demonstrate safety and feasibility. Therefore, cell therapies are investigated in a small group of patients with an untreatable disease to demonstrate safety and feasibility while making observations on efficacy. Once a safe dose has been determined
Ph II clinical trials are performed on patients with the disease of interest to further evaluate safety, efficacy and define the therapeutic range. The pivotal trials are Ph III trials as they are designed to create the conditions required for scaled normal use of the therapy and consider available therapeutic alternatives. Finally, Ph IV trials comprise additional efficacy and refined pharmacovigilance studies to detect long-term efficacy, side effects, morbidity and mortality rates; they are the post-marketing studies.

Clinical trial landscape

There are several publicly available database sources recording stem cell therapy clinical trial activity around the world and due to differences in definitions, data recorded and areas covered can be difficult to establish an accurate and up-to-date picture of the clinical trial landscape for the field. Currently, >4000 stem cell-based therapies are registered globally with the US NIH clinical trials database and the greatest activity is within North America (60%), Europe (23%) and East Asia (8%). Regarding the US landscape, earlier this year the Medicines in Development: Biologics report was published which lists all industrial sponsored biologics in clinical trials or under review by the FDA. Of the 69 reported cell therapies, 57 were registered as stem cell therapies. In the UK, the Cell Therapy Catapult verified 34 cell therapy clinical trials as of April 2013, of which 21 are stem cell therapies and 81% are autologous; 95% are early phase (Ph I/II or II) and 62% are academically sponsored. The primary clinical applications are cardiovascular and neurology, followed by blood, ophthalmology and gastroenterology indications. There are a diverse range of cell types being studied, predominantly bone marrow-derived cells (CD34+ stem cells, BM-MNCs Mesenchymal stem cells-MSCs). Online Supplementary data, Table S1 provides details of individual stem cell-based therapy clinical trials compiled from the Medicines in Development: Biologics report and the Cell Therapy Catapult database. Figure 2 displays data from the supplementary table to provide an easily digestible overview of these clinical trials. The charts show the clinical trials by phase of trial, type of therapy and disease indication, and demonstrate the nuances that arise depending on the subset of data used. >80% of these trials under review by the US FDA and UK MHRA are in early phases, with a near even split of autologous and allogeneic therapies. Major disease indications are cardiovascular and neurology, followed by oncology, immunology and bone/cartilage indications. Recently, the Catapult UK database was compared with a wider European analysis of 318 EU ATMP trials (EudraCT) of which 250 were cell-based products. There were similarities and agreement in several areas including predominant disease indications, cell type.
breakdown and prevailing early phase trials.\textsuperscript{19,45} Additionally, clinical trial sponsorship by academia or charitable organizations predominated but across Europe commercial sponsorship was proportionally greater (40\%), with small- and medium-sized enterprises ascending.\textsuperscript{45} Commercial sponsorship was most prevalent for gene therapies, or orphan drug status products for rare diseases.\textsuperscript{45}

The rise of on-line social networking services has also led to the utilization of these tools for publicizing, tracking and sharing information regarding stem cell therapy’s clinical trial data. For example, since 2011 ‘twitter.com’ has been used to publicize the registration of international clinical trials with annual reports on the findings.\textsuperscript{46} The 2012 report details 226 cell therapy trials and shows agreement with trends described above: predominant academic or government sponsorship (77\%), prevailing autologous therapies (60\%), diverse cell types (36), and prevalence of MSCs (62\% of 123 defined stem cell trials).\textsuperscript{47} There were minor nuances in major indication breakdown (29\% cancer, 15\% neurology, 13\% musculoskeletal and 11\% cardiovascular), and a more striking difference in the

\textbf{Fig. 2} Clinical trial landscape overview charts. Data compiled from US and European sources including therapies currently under review by the US FDA and UK MHRA regulatory bodies (refer to online Supplementary data, Table S1 for details of individual trials). (A) Bar chart displaying the percentage breakdown of trials by clinical phase and showing >80\% are within early phases. (B) Pie chart displaying the percentage breakdown of autologous and allogeneic therapies. (C) Pie chart displaying the percentage breakdown of therapies by disease indication.

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number of trials by region with Asia dominating (36%), and North America (28%) and Europe (24%) following. The number of trials by country showed the USA with the highest proportion (32%), followed by China (22%) and then Spain (9%). The reasons for the differences observed could be related to inclusion of clinical trial data from area-specific databases that are not registered on US or EU databases, or increases in reporting and registering of clinical trials as a means of publicizing commercial activity. Additionally, there have been significant rises in RM stem cell-based research in China in recent years with apparent translation into the clinic. However in January 2012, China’s Ministry of Health ordered the suspension of all clinical trials with unapproved stem cell treatments pending further development of the Chinese regulatory framework. Currently, it is unclear what impact this suspension has made or whether it will be reflected by a decrease in the number of registered trials in 2013. A similar announcement in 2009 ordering hospitals and clinics to obtain approval or face closure is reported to have not made significant impact according to stem cell ethics researchers from the RIKEN Center for Developmental Biology (Kobe, Japan).

Clinical trial case studies

Autologous therapies

Many investigators have isolated stem cells from the patient’s own bone marrow for the treatment of heart failure and cardiovascular diseases. These studies set out to treat the parenchymal loss that underlies the development and progression of chronic heart failure and the cells demonstrate excellent clinical safety, with no risk of rejection and no requirement for immunosuppression. However, the results of these studies show an extremely varied cardiac repair outcome from patient to patient and it is recognized that further optimization is required. Work is continuing in this area, for example in February 2012 Baxter International, Inc. (Deerfield, IL, USA) launched a Ph II trial (RENEW study) to evaluate safety and efficacy of its adult autologous therapy for treatment of chronic myocardial ischaemia. The product comprises collection and processing of patient bone marrow to isolate CD34+ stem cells, which are then delivered back into the patient heart via 10 intramyocardial injections. The study aims to enrol 450 patients to evaluate the ability of the therapy to improve exercise capacity 12 months post-treatment, as well as reduction of angina and safety; currently, the trial is still enrolling.

Other investigators have gone further in that they have not just isolated and expanded autologous cells, they have stimulated them towards a particular cell lineage using specific biologics. In early 2013, Bartunek et al.
published the first-in-man application of lineage-guided stem cells for targeted regeneration of a failing organ; specifically cardiopoietic stem cell therapy in heart failure. The multicentre C-Cure® Ph II clinical trial with lineage-specified biologics was conducted by investigators at the Mayo Clinic Centre for Regenerative Medicine (Rochester, Minnesota, USA) for Cardio 3 BioSciences (C3BS, Mont-Saint-Guibert, Belgium). The prospective, randomized, open study was conducted in patients with stable heart failure of ischaemic origin. The trial was a parallel two-arm study, where recruited patients received standard-of-care or standard-of-care plus lineage-specific stem cells. The primary endpoint was feasibility and safety over 2 years and secondary endpoints included cardiac structure and function measurements as well as global clinical performance 6 months post-treatment. Bone marrow harvested from patient iliac crest underwent GMP processing to isolate and expand bone marrow-derived MSCs, which were then exposed to a cardiogenic cocktail to drive lineage specificity. Cells were centrally manufactured at a single accredited GMP facility. To establish cell viability immediately prior to administration, product doses were packaged and transported under specific conditions, ensuring transplantation within 72 h of derivation, and parallel cell aliquots were maintained under identical conditions at the core manufacturing facility for reference. Product release criteria included cell yield, a pre-specified does range, purity, identity, homogeneity and sterility. Cardiopoietic lineage-specific stem cells were achieved for each patient with a sufficient dose attained in 75% of cases, for which the cell therapy was injected endoventricularly, under electromechanical guidance. Cell delivery was well tolerated, there was no evidence of systemic toxicity and no patients were discontinued from the study due to adverse reactions. During the 2-year follow-up period, no adverse event was recorded as a definite or probable link to the cell therapy. Cardiac function tests showed the left ventricular ejection fraction increased 7% in patients receiving the cell therapy, while it was unchanged in the control group. This was associated with a decrease in left ventricular end systolic volume. Patients that received the cell therapy also achieved increased distances in the 6 min walk test and gave a higher clinical score. On the basis of these Ph II trial outcomes, C3BS has initiated a European Ph III trial of C-Cure® to be delivered to patients using a proprietary catheter called C-Cath® for treatment of congestive heart failure. This trial, termed CHART1, is the first Ph III trial using organ-specified cells for the treatment of ischaemic heart failure and will recruit ~240 patients with chronic advanced symptomatic heart failure. Further to this, on 10 June 2013, C3BS announced treatment of its first patient. However, more recently the validity of the Phase II results were called into question by Francis et al. who identified numerous errors in the Phase II publication. The errors included inconsistencies in patient enrolment numbers, errors in percentage calculations and discrepancies in
randomization. A conflict of interest of one author was not disclosed and discrepancies between trial registration details and details reported in the paper were not acknowledged, for example there was a change to the method used to measure cardiac function from a radionuclide-derived ejection fraction measurement to a more subjective echocardiographic-derived ejection fraction measurement.\textsuperscript{59,60}

Another autologous therapy in Ph II/III trials for treatment of congestive heart failure is Bioheart, Inc.’s (Sunrise, FL, USA) MyoCell\textsuperscript{®} product, derived from a biopsy of patient thigh muscle; myoblasts are isolated, expanded and injected back into the patients’ heart scar tissue to improve cardiac function. Bioheart also have a next generation version of this product called MyoCell\textsuperscript{®} SDF-1, where the myoblasts are modified to express angiogenic proteins for enhanced angiogenesis, and this is currently within Ph I clinical trials.\textsuperscript{43,61}

Another type of stem cell therapy adopting the lineage-specific guided method is NurOwn\textsuperscript{™} from Brainstorm Cell Therapeutics, Inc. (New York, NY, USA) for the treatment of amyotrophic lateral sclerosis (ALS) or motor neurone disease that causes denervation of the muscle and muscle atrophy. The product that has completed a Ph I/II trial comprises autologous MSCs differentiated to express neurotrophic factors (MSC-NTF) that promote motor neuron survival and have been shown to have beneficial therapeutic effects in pre-clinical models of motor nerve damage.\textsuperscript{62} The interventional, non-randomized study was designed to establish MSC-NTF safety and tolerability.\textsuperscript{63} ALS patients with disease duration of <2 years and with at least 60% forced vital capacity (FVC, an indicator of respiratory function) were included in the trial and some key exclusion criteria included respiratory dependence, and electromyography (EMG)-confirmed slow nerve conduction velocities.\textsuperscript{62} Prior to receiving the therapy, 12 patients were followed in parallel for 3 months to determine whether they had early- or late-stage ALS (ALSFRS-R scale). Six early-stage ALS patients were administered autologous MSC-NTF cells via intramuscular injections at 24 separate sites in the biceps and triceps. Six late-stage ALS patients were administered a single intrathecal injection of autologous MSC-NTF cells directly into the spinal canal. Patients were monitored for 6 months post-treatment, with monthly measurements including ALSFRS-R scoring, FVC, physical and neurological examination, muscle bulk, muscle circumference and concomitant drug use. As well as twice-monthly measurements of EMG, standard blood analyses, immunology and urinalysis.\textsuperscript{62} The Ph I/II primary safety evaluation and tolerability endpoints were achieved with no observation of serious treatment-related adverse events. Secondary efficacy endpoint measurement showed a decrease in disease progression as evidenced by ALSFRS-R score and FVC in the late-stage, intrathecally injected patients suggesting a trend toward possible disease stabilisation.\textsuperscript{62,63} Further to this, in January 2013, the Israeli Ministry of Health
approved acceleration of the NurOwn™ study to Ph IIa to establish the effects of increasing dosages.64

Pivotal Ph III withdrawal: a cautionary tale

In April 2013, Aastrom Biosciences, Inc. (Ann Arbor, MI, USA) announced the termination of the Phase III clinical study of ixmyelocel-T as a treatment for Critical Limb Ischaemia (CLI), which had been granted Fast Track status from the FDA.65 Aastrom is now focussing on ixmyelocel-T as a treatment for dilated cardiomyopathy (DCM) that has completed a Phase II clinical trial and has received Orphan Drug Status from the FDA, as the clinical development may require smaller studies with lower costs and a shorter path to regulatory approval.65 Ixmyelocel-T is an autologous BM-MNC product, manufactured by Aastrom under GMP conditions at an annual capacity of 3000 patients per year. The product comprises the isolation of lymphocytes and granulocytes (5x reduced) and the isolation and 12-day expansion of monocytes and macrophages (expanded 200x), haematopoietic progenitors and stromal cells (50x expanded CD90+ cells).66 Cells are administered to the patient either intramuscularly for treatment of CLI, or through endocardial catheter injections for treatment of DCM, within 72 h of manufacture.43 Poor trial design and strategic business considerations appear to be the reasons for the termination of the CLI Ph III trial.67 Recruitment commenced in May 2012 and the target for enrolment was 594 patients, but by March 2013 only 40 patients were enrolled.43,67 The initial enrolment target would have been defined from the Ph II trial results to attain significant statistical power between placebo and therapeutic-treated patient groups but the lack of enrolment suggests that either the inclusion criteria were too rigorous or there was an overestimation of the number of eligible patients.67 In late 2012, Aastrom67,68 proposed a plan to accelerate enrolment, including widening the enrolment criteria and increasing the number of trial sites. However, it is likely that the implementation of these strategic changes could not be performed rapidly, effectively or without large financial losses. Additionally, taking the step to widen the inclusion criteria potentially increases the risk of not achieving the trial primary endpoint as a heterogeneous patient population may dilute therapy efficacy.67 Prior to the termination of the trial, the company consistently reported quarterly net losses68 without any news regarding significant investment. Additionally, a potential investment deal between Takeda Pharmaceutical Company Ltd. (Osaka, Japan) and Aastrom regarding the commercialization of Ixmyelocel-T in CLI did not work out.69 Further to this, the market for commercial gene- and cell-therapeutic products for CLI is becoming increasingly competitive. For example, Harvest Technologies Co. (Plymouth, MA, USA) has a
point-of-care centrifugation device (SmartPReP2®) designed to concentrate the buffy coat from whole blood or bone marrow that is currently undergoing Ph III trials in the USA, clinical trials in Asia and the device has been marketed for CLI in Europe.43,70,71 Point-of-care devices are considered more attractive due to the associated low costs, convenience of use and relative similar efficacy. It is estimated that such devices will have a potential market cost of $10 000, whereas the potential cost of ixmyelocel-T is estimated at $40 000, therefore, ixmyelocel-T would have to demonstrate a significantly improved efficacy profile in order to compete.67

**Allogeneic therapies**

**World’s first hESC clinical trial and current status of hESC trials**

As mentioned previously, a pioneering Ph I clinical trial using hESCs began in 2010 (Fig. 1). The multicentre trial was initiated by Geron Co. (Menlo Park, CA, USA) and used their allogeneic GRNOPC1 hESC-derived oligodendrocyte progenitor cells for treatment of SCI.72 These cells demonstrated re-myelination and nerve growth stimulating properties in pre-clinical studies and the Ph I trial aimed to enrol 10 patients with neurologically complete, sub-acute traumatic SCI. Major inclusion criteria were a single spinal cord lesion, ‘complete’ classified injury (no motor or sensory function preserved in the sacral segments, ASIA scale) and preserved neurological level from T-3 through T-11 thoracic vertebral levels. Major exclusion criteria included penetrating trauma being the cause of the SCI, traumatic spinal cord transection or laceration, any injury, pre-existing condition or an inability to communicate that could interfere with neurological examination performance, significant organ damage or systemic disease posing an unacceptable risk for surgery or immunosuppression, or history of any malignancy.43 Patients were treated between 7 and 14 days post-injury with an injection of $2 \times 10^6$ GRNOPC1 cells administered during elective surgery. The primary clinical endpoint was safety, as measured by the frequency and severity of adverse events within a year of cell administration related to either the cells or administration procedure. The secondary endpoint was measurement of neurological function assessed by sensory scores and lower extremity motor scores (ISNCSCI examinations).43 However, in November 2011, after treating four patients, Geron announced that it was halting the SCI trial activity with immediate effect, and cited cost and regulatory issues as the cause of the decision.73 Geron also has two experimental cancer therapies (Imetelstat and GRN1005) in Ph II trials and narrowing their focus to oncology therapeutics enabled reallocation of funds to ensure sufficient financial resources were in place to reach important near-term value inflection points without the necessity of
raising additional capital. Geron continued to monitor the four GRNOPC1-treated SCI patients, and reported data show that while there are no signs of SCI improvement in these patients, there have also been no signs of any significant safety problems and treatment appears to have been well tolerated with no substantial adverse events.\textsuperscript{73,74} In early January 2013, Geron announced entering into an Asset Contribution Agreement with BioTime, Inc. and its subsidiary BioTime Acquisition Corporation (Alameda, CA, USA) for the acquisition of Geron’s intellectual property (patents and patent applications), cell lines and assets related to its hESC programmes, including the Ph I SCI trial, by 30 September 2013.\textsuperscript{75}

Although it is anticipated that BioTime will resecure Geron’s hESC programme, currently Geron’s withdrawal leaves Advanced Cell Technology (ACT, Santa Monica, CA, USA) as the only company currently conducting clinical trials involving hESCs.\textsuperscript{73} ACT’s hESC-derived retinal pigmented epithelial (RPE) cell therapy is targeted at the treatment of macular degeneration. Specifically, the most common paediatric macular degenerative disease, Stargardt’s macular dystrophy (SMD), and the leading cause of blindness in the developed world, dry age-related macular degeneration (AMD).\textsuperscript{76} Currently, there are no therapeutic options available for these diseases and ACT’s product encompasses sub-retinal transplantation of RPE cells to the central visual area of the retina: the relatively small (~1 mm) macular and fovea area of high visual acuity. In 2011, ACT began enrolling patients into three Ph I/II open-label, non-randomized, multicentre prospective clinical trials in the USA and Europe to determine safety and tolerability of hESC-RPE transplantation. Each trial aims to enrol 16 patients with 5 cohorts of varying vision. In brief, the major inclusion and exclusion criteria for patient enrolment were the presence of central visual loss and end-stage disease, the absence of other clinically significant ophthalmic pathologies, no history of any malignancy and the absence of risks for surgery or systemic immunosuppression.\textsuperscript{73,76}

ACT’s allogeneic product is derived from hESC line MA09. RPE cells are manufactured via expansion of GMP-grade hESC-MA09 on mitomycin-inactivated murine embryonic fibroblasts, followed by formation of embryoid bodies and cell outgrowth to isolate pigmented RPE cellular patches. RPE cells are then purified (99% purity), expanded for two further passages and cryopreserved for clinical use. Due to \textit{ex vivo} exposure to murine cells, they are classified as a xenotransplantation product.\textsuperscript{76} Characterization occurs both in-process and after thawing and product formulation. During surgery, reconstituted product is injected through a cannula into the subretinal space of the macular region of the patients’ eye and low-dose tacrolimus and mycophenolate mofetil are used for the immunosuppression regimen.\textsuperscript{73,76} In 2012, short-term follow-up data 4 months post-treatment with a low RPE cell dose ($5 \times 10^4$) in two patients
was released and showed favourable results. In terms of safety, retinal detachment, hyper-proliferation, abnormal growth, intraocular inflammation and teratoma formation were not observed. Functional visual acuity improvements were observed in both patients: one with AMD and one with SMD. The AMD patient showed the greatest improvement, progressing from baseline 20/500 vision (20/20 being perfect vision) and recognition of 21 letters in the visual acuity chart to 20/320 vision and recognition of 28 letters, which remained stable 6 weeks post-transplantation. The SMD patient baseline central vision progressed from recognition of hand motion only to counting fingers and recognition of 1 letter in the visual acuity chart within 2 weeks post-transplantation, to stable recognition of 5 letters and 20/800 vision by 4 weeks. Subjective improvement in colour vision, contrast and dark adaptation was also noted in the treated eye. Safety was the primary endpoint of the Ph I/II studies and for the duration of the 4 month follow-up the lowest product dose appeared to be both safe and effective. Further to this, in May 2013, a press release stated that the vision of a trial patient had improved from 20/400 to 20/40 vision following treatment. Taken together and given the progressive nature of both diseases that lack alternative therapeutic options, these preliminary results are highly encouraging.

**World’s first approved stem cell therapy**

As mentioned previously, Prochymal® (remestemcel-L), developed by Osiris Therapeutics, is the first stem cell therapy approved for use in 2012 by Health Canada and Medsafe (New Zealand’s medical regulatory agency) for the management of acute GvHD in children who fail to respond to steroids. Additionally, Prochymal® is also the first stem cell product to receive FDA Expanded Access Programme approval, enabling product availability to adults and children with life-threatening GvHD in eight countries. GvHD is a frequent transplant complication occurring in ~50% of patients and is the leading cause of transplant-related mortality. The disease is caused by immune cells in the transplanted graft recognizing the host recipient as foreign and mounting an immunological response. Severe cases are extremely painful with potential skin blistering, intestinal haemorrhage, liver failure and death in up to 80% of cases. Steroid therapy is successful in 30–50% of cases with the final therapeutic option being limited to off-label use of immunosuppressive drugs with significant toxicity and little benefit. Prochymal® comprises bone marrow-derived MSCs from healthy adult donors (aged 18–30). Large-scale expansion allows up to 10 000 doses of Prochymal® to be produced from a single donor and the product is stored frozen with a 2-year shelf life. Patient delivery encompasses a 30-min intravenous infusion per dose without the requirement for recipient type matching or immunosuppression. The Ph III randomized, double-blind, placebo-controlled trial to evaluate...
Prochymal® efficacy and safety for treatment of steroid-refractory acute GvHD was conducted at multiple sites across the world, including several US states, Australia, Canada, Italy, Switzerland and the UK. Two hundred and forty patients (aged 6 months to 70 years) received either twice weekly infusions of Prochymal® (at $2 \times 10^6$ MSCs/kg dose) or placebo over 4 weeks. In 61–64% of children with severe GvHD that were unresponsive to steroid therapy and treated with Prochymal®, a clinically meaningful response was induced by 28 days of therapy initiation. In addition to achieving this primary endpoint, the trial demonstrated statistically significant improvement in survival rates among the Prochymal®-treated group, which was most pronounced in patients with the most severe forms of GvHD. Long-term effects are being monitored via a patient registry and further evaluation of the clinical benefits will be evaluated in a case-matched confirmatory trial as a condition of approval.

Prochymal® is also being evaluated as a therapeutic in Ph III clinical trials for treatment of Crohn’s disease, for which it has also received Fast Track status from the FDA. This inflammatory disease affects the colon and small intestine causing abdominal pain, weight loss, diarrhoea and vomiting. The Ph III trial is a multicentre, placebo-controlled, randomized, double-blind study to evaluate safety and efficacy in patients with moderate-to-severe refractory Crohn’s disease. The trial aims to enrol 270 patients and administer twice weekly intravenous infusions of Prochymal® (at low $6 \times 10^8$ or high $12 \times 10^8$ dose) or placebo over 2 weeks. Further to this, Prochymal® is also being evaluated for several other disease indications including acute myocardial infarction, chronic obstructive pulmonary disease and type 1 diabetes.

Summary

In addition to the clinical trial case studies described in this review, it is with great anticipation that within the next 12 months the first iPSC products will also reach the clinic as the Japanese government drives capitalization of iPSC technology, which was first developed in Japan. Earlier this year the government invested in a significant economic stimulus package into iPSC research towards clinical acceleration. Dr Masayo Takahashi, an ophthalmologist at the RIKEN Center for Developmental Biology is leading the first-in-man trial of iPSCs for age-related macular degeneration. After receiving Health Ministry approval in July 2013, patient enrolment for a pilot study began in August 2013. The pilot study will assess the safety and feasibility of the transplantation of iPSC-derived RPE cell sheets in patients with wet AMD. The first treatment is expected in 2014 as the process to culture and validate the iPSC-derived cellular transplants takes around 10 months. Additionally in June
2013, it was reported that Professor Jun Takahashi from Kyoto University also aims to start first-in-man clinical studies of an iPSC-based treatment for Parkinson’s disease (neurodegenerative disorder) in 2014.\textsuperscript{82} In parallel to the funding surge, Japanese regulatory processes for stem cell therapies are currently being amended with the intention of streamlining to facilitate progression,\textsuperscript{79} thereby potentially increasing competitive advantage, which is likely to be cautiously observed by economists around the world.

Commercial cell therapy trials can become a very long and extremely expensive journey and a number of reasons other than potential lack of safety and efficacy can be responsible for clinical trial failure, including cost, regulatory issues, poor trial design and strategic business considerations. Traversing the regulatory framework is extremely difficult in itself, added to this the experience of others tells us that trials must be designed considering not only the statistical power to demonstrate sufficient efficacy but also considering enrolment numbers and criteria that are not overly stringent but do not also lead to heterogeneous patient populations that can dilute efficacy. Additionally, in this rapidly advancing market cell therapies must remain competitive against alternative revolutionary treatments, causing developers and investors to repeatedly reconsider their most viable products for continued market development. Historically, high attrition rates of experimental molecular therapies have been generally expected and weathered within the pharmaceutical industry, though times are changing. However, due to the enormous costs involved in clinical progression, development of a lead candidate stem cell therapy poses a much greater risk. Yet the route to market has been trodden with great success and many therapies promise to follow in the wake of Prochymal\textsuperscript{®}. There is also a highly attractive clear trend emerging that during the development of a lead stem cell therapy candidate there is huge capacity for room to manoeuvre within the relatively open market, with early developers reaping the benefits of product application for multiple disease indications as well as next generation products.

**Supplementary material**

Supplementary material is available at *BRIMED* online.

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