Human embryonic and induced pluripotent stem cells in clinical trials

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Abstract

Background: Human embryonic and induced pluripotent stem cells (hESC and hiPSC) have tremendous potential for clinical implementation. In spite of all hurdles and controversy, clinical trials in treatment of spinal cord injury, macular degeneration of retina, type 1 diabetes and heart failure are already ongoing.

Sources of data: ClinicalTrials.gov database, International Clinical Trials Registry Platform, PubMed and press releases and websites of companies and institutions working on hESC- and iPSC-based cellular therapy.

Areas of agreement: The initial results from multiple clinical trials demonstrate that hESC-based therapies are safe and promising.

Areas of controversy: Are iPSC cells safe in the clinical application? Is there a room for both hESC and iPSC in the future clinical applications?

Growing points: Increasing number of new clinical trials.

Areas timely for developing research: Development of hESC- and/or iPSC-based cellular therapy for other diseases.

Key words: human embryonic stem cells (hESC), human induced pluripotent stem cells (hiPSC), macular degeneration, spinal cord injury, diabetes, heart repair

Introduction

Since they were isolated for the first time,1 human embryonic stem cells (hESC) remain at the centre of controversy. Whereas scientists clearly have seen their potential in the treatment of debilitating disease, the regulators and public became widely divided, from
being very supportive to seeking a regulatory ban on hESC research. In spite of all the obstacles, the hESC-based therapies moved to clinical trials very quickly. Only 12 years later, the first patient has been treated with hESC-based cellular therapy. Just to remind you, the first antibody was commercially produced in 1978, and the first antibody-based treatment was approved in 1998. It took 20 years to reach that step and we knew a lot about antibodies already. About hESC we did not know anything. ‘The new kid on the block’, human induced pluripotent stem cells (hiPSC) seemed to hold even more potential than hESC.2-4

The review article is aiming to provide an overview of the studies conducted in human subjects using hESC- or iPSC-based cellular therapies in treatment of diseases (Table 1).

## Spinal cord injury

**Geron**

Geron (CA, USA; [www.geron.com](http://www.geron.com)) is considered to be the pioneer in the business of hESC and regenerative medicine. For years Geron opened new areas of research and development and took a lead in bringing hESC towards therapeutic application. The company exclusively licensed fundamental hESC-related patents from Wisconsin Alumni Research Foundation and learned how to manufacture several different types of functional cells derived from hESC. In collaboration with researchers at the University of California, Irvine, Geron had shown that in animal models hESC-derived oligodendrocyte progenitor cells (GRNOPC1) could improve functional locomotor behaviour after implantation in the injury site 7 days after injury. Histological analysis also provided evidence for the engraftment and function of these cells.5 The reliability and reproducibility of their differentiation protocols for these specific hESC-derived GRNOPC1 cells were probably behind the decision to embark on a risky task: repair of spinal cord injury.

After the completion of extensive animal toxicology testing, including 24 separate studies in rats and mice, that required more than five billion GRNOPC1 cells, Geron filed a 21 000 page Investigational New Drug (IND) application with the FDA containing data from the animal and *in vitro* testing of the cells to ensure the highest possible degree of

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safety of the product before initiating human clinical trials. In January 2009, Geron received FDA clearance to begin the world’s first human clinical trial of a hES cell-based therapy. On news of the FDA approval, stocks jumped from $4.81 to $8.05 (68%). However, a few weeks later, when the company realized that its worldwide license for its stem cell technology was not valid in Europe, shares plummeted 16.7%, from $1.30 down to $6.47 in midday trading in New York. The same month a group in Israel reported that following neural stem cell transplantation in Russia, the unexpected development of a multifocal brain tumour in the recipient. Although this was nothing to do with Geron’s trial, it caused their stock value to drop further. In August 2009 Geron announced that its IND had been placed on clinical hold by the FDA pending the agency’s review of new nonclinical animal study data submitted by the company. In this study, the injected animals developed a higher frequency of microscopic cysts in the regenerating injury site. A year later, on July 30, 2010, the FDA notified the company that the clinical hold had been lifted and the Phase I clinical trial of GRNOPC1 in patients with acute spinal cord injury could proceed.

The first patient was treated at the Shepherd Center in Atlanta in October 2010. Data on the first two patients with neurologically complete American Spinal Injury Association (ASIA) impairment scale grade A thoracic spinal cord injuries were presented at the two conferences in June 2011. According to Geron’s press release, these patients received GRNOPC1 at a dose of 2 million cells, 7–14 days post-injury, delivered by injection into the lesion site between T3 and T10, using a specially designed syringe-positioning device. None had either surgical complication during or after procedure, nor adverse events related to the injection procedures or to GRNOPC1. Low-dose tacrolimus was given for temporary immune suppression from the time of injection for 46 days, at which point the dose was tapered and withdrawn completely at 60 days. Initial analyses showed no evidence of immune responses to GRNOPC1 through until Day 90, which includes the 30-day period after complete withdrawal of immune-suppression. There was also no evidence of cavitation in the spinal cord at the injury site on MRI through until Day 180. Following this report, Geron received clearance from the FDA to expand eligibility criteria to include patients with injuries down to T11. In addition, the FDA approved that the current 30-day period between subjects in the trial could be reduced to 10 days. In October 2011, safety data on four patients presented at two symposia showed that the results were fulfilling all expectations.

So, why then was Geron switching gears and closed the promising GRNOPC1 clinical trial only a month later, in November 2011, and ostensibly giving up on their stem cell programme, which was one of the most innovative in the world? The answer is simple: money. Although the prime goal of the clinical trial was safety, public expectations were driven by a Geron’s short movie of a rat with a spinal cord injury that could walk after being treated with GRNOPC1. No matter how many times the Company emphasized that their trial was specifically only about safety, almost everyone expected that the treated patients would walk shortly after the GRNOPC1 injection. Of course, this did not happen and Geron’s stock started to slide. With each new patient enrolled and each day that passed it became clear that there would be no miracle, and from January till September 2011 shares dropped nearly 60%.

As a medium size enterprise, Geron did not have readily available resources to bring a potential drug from bench to bedside. For example, they conducted in total 28 animal studies, involving >3500 rodents and pigs. In the recent economic climate, without government or Big Pharma wading in to help cope with enormous regulatory and manufacturing costs, small and medium enterprises are incapable of bringing a new drug to market. The fact that even if injection of GRNOPC1 cells could make people walk, the costs would leave no room for profit, did not help Geron’s case. In order to survive, lack of investment and support forced the company to do the only sensible thing: temporarily close their stem cell programme. Their action has brought to light ethical and social questions about prematurely ended trials and compromising the contract between participants and trial sponsor.
In total, they treated five patients 21–32 year-old, four males and one female. All participants received $2 \times 10^6$ GRNOPC1 cells within 2 weeks of injury. None of them developed antibodies or cellular immune response to GRNOPC1 through a year post treatment even though some had complete HLA mismatch with GRNOPC1; the closes match was 5 out of 10 alleles. The treatment per se caused no serious adverse events. However, no motor or sensory neurological changes were observed.

**Asterias**

In January 2013, Geron entered into Asset Contribution Agreement with BioTime (CA, USA; www.biotimeinc.com) and its subsidiary Asterias Biotherapeutics (http://asteriasbiotherapeutics.com). The transactions were closed 10 months later with Geron contributing to Asterias both intellectual property and tangible assets related to its discontinued hESC programmes. Asterias received multiple lots of OPC1 cells used in the clinical trial as well as multiple lots of hESC manufacturing cell banks from which additional lots of OPC1 cells could be made.

In May 2014, Asterias received USD 14.3 million as a strategic partnership award from the California Institute for Regenerative Medicine (CIRM) to reinitiate clinical development of hESC-derived OPC1 cells, now renamed AST-OPC1, in a dose-escalating trial. Additional USD 13 million was raised shortly after in an equity financing. With enough money on the account and a green light from the US FDA, Asterias has initiated a Phase 1/2a open-label, single-arm dose-escalation clinical trial and the first patient was treated in Atlanta in June 2015. With the third patient treated in Chicago in August 2015, the trial finalized initial low-dose ($2 \times 10^6$ cells) safety cohort. The study, conducted at a total of up to eight centres in the United States, will test three sequential escalating doses with highest being $20 \times 10^6$ cells. The AST-OPC1 cells will be administered in 13 patients with sub-acute, C-5 to C-7, neurologically complete cervical spinal cord injury. These patients are quadriplegic, have lost all sensation and movement below their injury site. AST-OPC1 will be administered 14–30 days post-injury. Additional information on this study, including trial sites, can be found at www.clinicaltrials.gov (ID: NCT02302157) and at the SCiStar Study Website www.scistarstudy.com

**Immunotherapy vaccine for lung cancer**

In addition to OPC1 and spinal cord injury, Geron contributed to Asterias all the documents related to antigen-presenting dendritic cells GRNVAC1 and GRNVAC2. GRNVAC2, now called AST-VAC2, is hESC-derived cancer vaccine designed to stimulate patients’ immune systems to attack telomerase, a protein that is expressed in majority of cancers but is rarely expressed in normal adult cells (http://www.businesswire.com/news/home/20140911006326/en/BioTime-Subsidiary-Asterias-Biotherapeutics-Cancer-Research-UK#.VbR2Y3iyXTR). The vaccine was developed following successful early phase clinical trials of a similar, autologous vaccine GRNVAC1, now called AST-VAC1. GRNVAC1 were autologous mature dendritic cells transfected with mRNA encoding human telomerase reverse transcriptase (hTERT) and a portion of the lysosome-associated membrane protein LAMP-1. Dendritic cell-based telomerase immunotherapy GRNVAC1 showed telomerase-specific immune responses in 55% of acute myeloid leukaemia and 95% of prostate cancer patients. In September 2014, Asterias teamed up with the UK charity Cancer Research UK and its development and commercialization arm Cancer Research Technology to bring AST-VAC2 into clinical trials in patients with non-small lung cancer.

**Macular degeneration of retina**

Ocata (former Advanced Cell Technologies)

Ocata Therapeutics (MA, USA; https://www.ocata.com) reported derivation of retinal pigment epithelial (RPE) cells from hESC more than 10 years ago. At that time, the report did not catch much attention and nobody could predict that this would be actually the seminal paper for what is today considered the first hESC-based therapy that successfully demonstrated clinical benefits in clinical trials. Two years later, the group showed reproducible generation
of RPE cells from 18 different hESC lines. Furthermore, RPE cells derived from one of the hESC lines were tested in animal model of retinal disease—the Royal College of Surgeons rat, in which photoreceptor loss is caused by a defect in the adjacent retinal pigment epithelium. The improvement in visual performance of treated rats was stunning and in November 2009, the company filed an IND with the US FDA to treat patients with Stargardt’s macular dystrophy. The US FDA cleared application a year later, in November 2010. The IND to treat dry age-related macular degeneration (AMD), was cleared by the FDA in January 2011. Shortly after, in July 2011, the first two patients, a 77-year-old woman with dry AMD and a 27-year-old woman with Stargardt’s macular dystrophy were enrolled at the Jules Stein Eye Institute at the University of California, Los Angeles, and about 50,000 RPE cells were administered into one eye of each patient. Additional information on these studies can be found at www.clinicaltrials.gov (ID: NCT01344993, NCT01345006).

About the same time, in June 2011, EMEA granted orphan medicinal product designation to their hESC-derived RPE cells and in January 2012, the first European patient suffering from Stargardt’s macular degeneration was treated at the Moorfields Eye Hospital in London. Additional information on this study can be found at www.clinicaltrials.gov (ID: NCT01469832).

Each of the three studies will enrol 16 patients in five cohorts: four low vision cohorts will contain three patients each, whereas one better vision cohort will contain four patients. In the low vision cohorts, the patients will be transplanted with 50,000 (cohort 1), 100,000 (cohort 2), 150,000 (cohort 3) or 200,000 (cohort 4) hESC-derived RPE cells, whereas the cohort with better vision patients (cohort 2a) was transplanted with 100,000 cells. Each cohort will be enrolled sequentially, with the exception of the better vision cohort, which may be enrolled in parallel with the other cohorts.

Although the results are preliminary and the number of treated patients is still too small, the initial data are encouraging. Among 18 patients treated so far, visual acuity improved in ten, remained the same in seven and worsen in one. In addition, it seemed that in dry AMD the improvement in visual acuity has a dose trend—more cells injected, better vision. This had not been observed in Stargardt’s macular degeneration patients. Immunosuppression has been given to the participants in the trials for total of 13 weeks, one week before the treatment and 12 weeks after. Regardless, no signs of rejection were evident in follow up.

The company also reported the data from two Asian patients with dry AMD and two with Stargardt’s macular degeneration and one year and follow up. No serious safety issues were noted and visual acuity improved in three patients, whereas in one remained stable. Asian patients may carry different risk alleles for retinal disorders and the report suggested that hESC-derived RPE cells seemed to be safe for them too.

Long-term follow up of 15 years for both dry AMD and Stargardt’s macular dystrophy trials in the USA are registered as separate studies at www.clinicaltrials.gov, NCT02463344 and NCT02445612 respectively. The first visit of extended follow up will be 12 months post-injection. In addition, Ocata is embarking on a trial in patients with geographic atrophy secondary to myopic macular degeneration (ID: NCT02122159 at www.clinicaltrials.gov).

The first patient with dry AMD has been enrolled in the Phase 2 clinical trial in September 2015.

Cell Cure Neurosciences

Cell Cure Neurosciences (Israel; http://www.cellcureneurosciences.com) was funded as a subsidiary of Singapore-based ES Cell International. In 2010, through acquisition of ES Cell International, BioTime (CA, USA; www.biotimeinc.com) acquired a majority in Cell Cure. Cell Cure generated under xeno-free conditions a proprietary formulations of hESC-derived RPE cells OpRegen® and OpRegen-Plus® and it has an exclusive license agreement with Israeli pharmaceutical giant Teva Pharmaceutical Industries (http://www.tevapharm.com) to develop and commercialize the hESC-derived RPE for the treatment of dry AMD. In October 2014, Cell Cure filled an IND application with the US FDA to initiate Phase II/2a clinical trial of OpRegen in patients with
geographic atrophy, the severe type of AMD. Only a month later the US FDA gave them the green light. The clinical trial running in Hadassah Ein Kerem University Hospital in Jerusalem is now recruiting the patients. The study will enrol 15 patients, divided in four cohorts, which will, following vitrectomy, receive 50,000–500,000 RPE cells into subretinal space. Additional information on this study can be found at www.clinicaltrials.gov (ID: NCT02286089).

The US FDA has granted Fast Track designation for OpRegen in September 2015.

**The London Project to Cure Blindness**

As the result of a partnership between the Moorfields Eye Hospital (www.moorfields.nhs.uk), the University College London Institute of Ophthalmology (www.ucl.ac.uk/ioo), the National Institute for Health Research (NIHR; www.nihr.ac.uk) and Pfizer (NY, USA; www.pfizer.com) under umbrella named the London Project to Cure Blindness (www.thelondonproject.org), the first patient with wet AMD was treated in August 2015 and there have been no complications to date. The trial will recruit 10 patients in total over a period of 18 months. Each patient will be followed for a year to assess the safety and stability of the cells and whether there is an effect in restoring vision.

More recently, The London Project has secured funding to examine the use of iPSC technology for transplantation.

**RIKEN CBD**

Following a successful launching of clinical trials with hESC-derived RPE cells, Japanese researcher Masayo Takahashi from Riken Centre for Developmental Biology in Kobe (http://www.cdb.riken.jp/en/), seized the opportunity and initiated the first-in-man study with iPSC-derived RPE cells. Rigorous *in vitro* and preclinical experiments in animals were satisfactory enough for the Japanese Ministry of Health, Labour and Welfare to give the green light in July 2013 to the open-label study, designed to evaluate safety of the autologous iPSC-derived RPE cell sheets in patients with wet AMD. Enrolment started the next month. The iPSC were generated from a 4-mm skin biopsy from upper arm, differentiated into RPE cells and prepared as cell sheets for transplantation. An estimate for the entire process, Quality Control (QC) included, has been 10 months for each participant. Post-transplantation patients will be monitored each month for 6 months and then every 2 months up to one year. Follow up examinations will occur once a year for the next 3 years.

The study is planning to enrol six participants. The first three will be transplanted with the 1.3 mm × 3 mm RPE sheet, whereas the following three will receive either larger sheet or multiple smaller sheets. In case of multiple sheets, the maximum number per participant will be three. The first patient, a 70-year-old female received the treatment in September 2014. The patient did not receive any immunosuppression and 6 month later there were still no signs of rejection or other adverse effects. However, the study was temporarily halted in March 2015, when the investigators found three single nucleotide variations (SNVs) and three copy-number variants (CNVs) present in the second patient iPSC and not detectable in the original fibroblasts. Additional information on the study can be found at http://www.riken-ibri.jp/AMD/english/index.html, the International Clinical Trials Registry Platform (ICTRP; http://www.who.int/ictrp/en/) under ID: JPRN-UMIN000011929 and at the Japan Primary Registries Network (JPRN; http://www.umin.ac.jp/ctr/index.htm) under ID: UMIN000011929. The promising result and availability of the iPSC lines homozygous for HLA haplotype14–16 is taking the trial toward different direction—use of allogeneic iPSC lines.

**Type 1 diabetes**

Viacyte (former Novocell)

Viacyte (CA, USA; www.viacyte.com), a privately held company, invested more than a decade to bring hESC-based therapy for type 1 diabetes from bench to patients with a goal of achieving long-term insulin independence without immune suppression. The company was founded in 1999 under name Novocell and merged with CyThera and Bresagen in 2004. Mimicking pancreatic organogenesis *in vitro*, they
were able to deduce a protocol to differentiate hESC into pancreatic hormone-expressing endocrine cells.\textsuperscript{17} These cells, when implanted into mice, reacted to glucose stimulation; human insulin and C-peptide were detected in sera at levels similar to those of mice transplanted with approximately 3000 human islets.\textsuperscript{18} Using surface markers, they were able to separate pancreatic hormone-expressing endocrine cells into different subtypes. Enriched pancreatic endoderm, CD142+ cells, when transplanted into mice, gave rise to all pancreatic lineages, including functional insulin-producing cells, demonstrating that they are indeed pancreatic progenitors.\textsuperscript{19} Next, the company developed manufacturing process on a scale amenable to clinical entry. The cells produced in such a way, upon implantation in mice, formed neo-pancreatic tissue sufficient to protect against streptozotocin-induced hyperglycaemia.\textsuperscript{20,21} In parallel, the company developed Encaptra\textsuperscript{®}, an encapsulating drug delivery system made from porous cell-impermeable membrane. The company filed an IND and device master file with the US FDA in July 2014. The application was approved relatively quickly, only a month later, and the first patient was implanted with hESC-derived islet replacement product candidate in October 2014 at University of California San Diego. The second site at the University of Alberta Hospitals in Edmonton in Canada was opened in July 2015.

The product candidate, called VC-01\textsuperscript{™}, is placed under patient’s skin and actually represents Encaptra-250 pouch/device filled with hESC-derived pancreatic precursor cells PEC-01\textsuperscript{™}. After surgical implantation, the cells differentiate further into fully functioning insulin-producing \(\beta\)-cells, as well as to other endocrine cell types that make up the normal human pancreatic islet. The Phase 1/2 open-label, dose-escalation study will enrol about 40 participants with a diagnosis of type 1 diabetes mellitus for at least 3 years. All the patients will be C-peptide negative and thus have essentially no ability to produce insulin. They will be divided into two cohorts. The first cohort is sub-therapeutic and each of six participants will receive two VC-01 implants, placed in the lower back, aiming to evaluate safety and tolerability. The patients in the second cohort will receive four to six dose-ranging VC-01 implants to achieve efficacy. The participants will be also implanted with sentinels, Encaptra-20 pouches, which can be periodically withdrawn from the patients and analysed. Their size is about 10-folds smaller than VC-01 implant, which is about half the size of business card (http://www.ipsccell.com/2015/03/viacyte/). Additional information on this study can be found at www.clinicaltrials.gov (ID: NCT02239354). Therapy development was supported substantially by the CIRM (www.cirm.ca.gov) and JDRF (www.jdrf.org), a global organization focused on type 1 diabetes research.

### Heart failure

#### APHP

The world-first hESC-based trial for the heart repair commenced in autumn 2014 in France under the sponsorship of the Assistance Publique – Hôpitaux de Paris (APHP; http://www.aphp.fr). The study is planning to recruit six patients who have severe left ventricular systolic dysfunction with left ventricular ejection fraction (LVEF) \(\leq 35\%\) and history of myocardial infarction, older than 6 months, with a residual akinesia involving more than 2 out of 16 contiguous segments. A fibrin patch embedding hESC-derived cardiac-committed CD15+ ISL-1+ progenitors will be transplanted into epicardium of the infarcted area and covered with an autologous pericardial flap. The flap is designed to provide nutrients to the underlying graft with the idea of improving viability of embedded cells.\textsuperscript{22} The objective of this study is to assess both the feasibility and safety of this approach. Efficacy will be assessed on the following end points: left ventricular function, viability of the grafted area, functional status and major adverse cardiovascular events.

The first patient, a 68-year-old insulin-dependent diabetic woman, with an indication for a surgical anterior myocardial revascularization, has received in addition to planned coronary artery bypass, also a fibrin patch embedded with hESC-derived cardiac-committed CD15+ ISL-1+ progenitors. At 3-month follow-up time point, the patient showed marked improvement.
Echocardiographic LVEF improved from 26 to 36%. At the time of the treatment, the patient had New York Heart Association (NYHA) Class III symptoms, whereas after the treatment the symptoms matched NYHA Class I. Furthermore, the akinetic infarct zone, under the patch, has become moderately hypokinetic. Additional information on this study can be found at www.clinicaltrials.gov (ID: NCT02057900).

**Conclusion**

In spite of significant challenges, hESC and iPSC-based cellular therapies are reaching clinical application in an extraordinary short time, especially given challenges and obstacles that at certain time seemed to be insurmountable. To fully realize their potential, first is necessary to demonstrate their safety and only then efficacy. This can be achieved only by careful planning and with adequate resources in a regulatory-friendly environment. Initial data from all trials are promising and they are likely to incite new trials for other diseases. One of the closest to the clinical trial might be Parkinson’s disease.

**References**


