Cell Replacement Therapies: Is It Time to Reprogram?

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Abstract

Hematopoietic stem cell transplantations have become a very successful therapeutic approach to treat otherwise life-threatening blood disorders. It is thought that stem cell transplantation may also become a feasible treatment option for many non-blood-related diseases. So far, however, the limited availability of human leukocyte antigen-matched donors has hindered development of some cell replacement therapies. The Nobel-prize rewarded finding that pluripotency can be induced in somatic cells via expression of a few transcription factors has led to a revolution in stem cell biology. The possibility to change the fate of somatic cells by expressing key transcription factors has been used not only to generate pluripotent stem cells, but also for directly converting somatic cells into fully differentiated cells of another lineage or more committed progenitor cells. These approaches offer the prospect of generating cell types with a specific genotype *de novo*, which would circumvent the problems associated with allogeneic cell transplantations. This technology has generated a plethora of new disease-specific research efforts, from studying disease pathogenesis to therapeutic interventions. Here we will discuss the opportunities in this booming field of cell biology and summarize how the scientists in the Netherlands have joined efforts in one area to exploit the new technology.

Introduction

IN CONSIDERING CELL REPLACEMENT THERAPY for any specific conditions, the first questions that arise are: What cell types should be replaced? How can we generate them, and how should they be delivered to the body? Depending on the application, many millions of cells may be required to restore function in the afflicted tissue. The exact number of cells depends on size of the endogenous cell population, complexity of the tissue, turnover rate of the tissue, and the number that has already been lost. Replenishing differentiated cells in high-turnover tissues by transplanting postmitotic cells would be like rearranging the deck chairs on the *Titanic*. The preferred donor cells for cell therapy of high-turnover organs would not be differentiated cells but rather those cells that normally maintain tissue homeostasis in the adult body.

The cells that protect our body from tissue exhaustion are the adult (or somatic) stem cells. Adult stem cells reside in a specific microenvironment (the so-called niche), which helps them to maintain their undifferentiated state and at the same time allows them to give rise to the differentiated (or specialized) cells of a tissue when necessary. If the progeny belongs to one, two, or many cell types, they are designated as unipotent, bipotent, or multipotent, respectively. Stem

cells exhibit a self-renewal mode in which they show the capacity to generate identical daughter stem cells. This feature allows adult stem cells to maintain tissue homeostasis during the lifespan of the organism, and in principle renders the stem cell population expandable *in vitro*.

With this in mind, it is remarkable that the first and, up to now, only commonly applied curative cell replacement therapy, namely, bone marrow transplantation (BMT), was pioneered without awareness of the existence of stem cells. BMT was developed immediately after World War II by a small group of scientists trying to understand and treat radiation sickness. Radiation sickness, which was sometimes called the “bone marrow syndrome,” was responsible for many deaths in the first weeks to months after the nuclear bomb attacks on the Japanese cities of Nagasaki and Hiroshima. After more than 15 years of research, the first patients with bone marrow failure were successfully treated by the infusion of bone marrow from identical twins (Robins and Noyes, 1961). Unfortunately, effective transplantations of allogeneic donor material turned out to be far less successful.

Mismatching has a high probability to yield graft versus host disease (GvHD), where host cells are attacked by donor-derived T lymphocytes. Once a few of the human leukocyte antigens (HLA) that are most relevant for a successful

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transplantation were identified by Van Rood and colleagues (1966), among others, and HLA “typing” became feasible, it did not take long before therapeutically successful allogeneic transplantations were reported by the groups of Good and van Bekkum (Gatti *et al.*, 1968; De Koning *et al.*, 1969). In general, survival rates are predominantly determined by the extent of HLA matching. A good match increases the take rate of the graft and reduces the chance of developing GvHD. Currently, main hematopoietic stem cell (HSC) transplantation centers select allogeneic donors on the basis of HLA A, B, and DR (6/6 match). Nowadays, many patients suffering from blood disorders, such as primary immune deficiencies and different forms of leukemia, can be successfully treated thanks to HSC transplantation, allogeneic in particular. Survival rates are high, averaging ~70% for allogeneic transplantations, and could be even higher if fully matched donor cells were readily available. Worldwide, HSC registries have been established but suitable donor material is still scarce and the lack is expected to grow as populations become melting pots of ethnic backgrounds in which novel mixtures of HLA haplotypes are generated.

Besides HSC transplantation, organ/tissue transplantation is successfully used in the clinic to treat a number of disorders. A full HLA match (6/6) is far less crucial for organ/tissue transplantation compared with HSC transplantation. Nevertheless, the extent of matching is important for transplantation success. Patients transplanted with allogeneic material always receive immunosuppressive medication, either applied locally or systemically, even if the transplant concerns immune privileged sites like the cornea of the eye. Immunosuppression influences the quality of life after transplantation, and more importantly may cause deleterious side effects, for example glaucoma and cataract in cornea transplantations. Organ/tissue transplants are rather efficient as survival of the graft ranges from 65% to 90% and 50% to 75% after 5 and 10 years, respectively (Transplantation Survival in the Netherlands, 2014). In spite of the fairly loose HLA matching criteria for organ/tissue transplants, transplantations are hampered by the limited availability of suitable donor material. Only a small part of tissues/organs used for transplantations comes from healthy living donors, and the majority is derived from deceased individuals.

Other Diseases Could Benefit from Cell Transplantation

From the above, it is evident that several diseases can be treated by cell replacement therapies, but it is anticipated that the list of treatable disorders will be extended in the near future (Table 1). Evidence is accumulating that other diseases like lysosomal storage disorders (Wynn, 2011; Aiuti *et al.*, 2013; Biffi *et al.*, 2013), autoimmune diseases (Tyndall, 2011), and skin diseases such as epidermolysis bullosa (Wagner *et al.*, 2010) may also be treatable by BMT. In addition, animal cell replacement strategies employing other cell types have already shown promise for the treatment of a number of diseases that so far have been untreatable other than with organ or tissue transplantation (Table 1).

Expandable Endogenous Sources of Transplantable Cells

Expandable sources of somatic stem cells may provide part of the solution to the donor-scarcity problem. Recent

advances in the expansion of somatic stem cells, like intestinal stem cells or salivary gland stem cells in self-organizing organoids outside the body, are very promising with respect to future transplantations of autologous somatic stem cells (Sato and Clevers, 2013). However, it is difficult to isolate certain tissue-specific stem cells, like neural stem cells (NSCs), which require an invasive biopsy of the brain. For other stem cells, like HSCs, the isolation is rather straightforward, but these cannot be expanded sufficiently in culture. Some patients are also less suitable as donors of endogenous stem cells, because the disease is genetically inherited, or there is a risk of transplanting pathogenic cells in the case of cancer or viral infections. For these reasons, researchers are investigating stem cells that could provide an alternative and non-lineage-restricted source of transplantable cells, eliminating excessively long transplantation waiting lists, and increasing the success rate of transplants.

Pluripotent Stem Cells as an Alternative Cell Source

Cells that would fit the criteria of being “universal” stem cells, applicable for all conditions that could be beneficial for transplantation, are pluripotent stem cells (PSCs). The best-known pluripotent cells are embryonic stem cells (ESCs). These cells are derived from the inner cell mass of a blastocyst-stage embryo in which they represent a transient population of cells. Once isolated they exhibit an extensive, presumably indefinite, self-renewal capacity *in vitro* without losing the ability to give rise to all (200 or so) cell types of the human body. A resident population of PSCs only exists in the early blastocyst-stage embryo, however, meaning that there are no options for isolating endogenous PSCs for autologous therapy. A possible alternative to endogenous PSCs would be banked ESCs that could be selected for HLA matching as required. Presently, more than 1000 human ESC lines have been derived and documented worldwide. Although this number is not sufficient to serve as a common registry, it could have been a good starting point if the lines had been derived according to current good manufacturing practices (cGMP) conditions and pre-selected on haplotype.

For tissue transplantations, it was estimated that a cell bank of 150 ESC lines derived from donors would provide less than 20% of the U.K. population with HLA-A-, HLA-B-, and HLA-DR-matched material (Taylor *et al.*, 2005; Okita *et al.*, 2011). In a less diverse population as that of Japan, 50 homozygous ESC lines were estimated to be required to aid approximately 73% of the Japanese population with a high-resolution HLA-matched transplant (Okita *et al.*, 2011). Yet, derivation of human ESC lines is surrounded by ethical dilemmas resulting from the destruction of human embryos during their generation. Many countries do not allow research or therapy development based on human ESCs for this reason. The breakthrough for PSC research into autologous cells for transplantation came from two Japanese researchers, Yamanaka and Takahashi, who found that, through the ectopic expression of four transcription factors (OCT4, KLF4, SOX2, and cMYC), the epigenetic program in human fibroblasts changes to one that very closely resembles that of ESCs (Takahashi *et al.*, 2007). These induced PSCs (iPSCs) can be created in this way from any individual, allowing banking of iPSCs with a specific haplotype and the generation of autologous cells from nonaffected tissue.

TABLE 1. NONHEMATOPOIETIC DISEASES THAT ARE GOOD CANDIDATES TO BE TREATED BY PLURIPOTENT STEM CELL—BASED CELL REPLACEMENT THERAPY

<i>Organ affected</i>	<i>Disease</i>	<i>Cell type</i>	<i>Reference</i>
Bone/cartilage	Congenital defects Trauma/osteoarthritis/ osteoporosis	Mesenchymal stem cells Mesenchymal stem cells	(Horwitz <i>et al.</i> , 1999) (Taiani <i>et al.</i> , 2014)
Cardiovascular	Heart failure	Cardiomyocytes	(Chong <i>et al.</i> , 2014)
Central nervous system	Spinal cord injuries	Oligodendrocyte progenitor cells	(Keirstead <i>et al.</i> , 2005)
	Parkinson	NSCs/dopaminergic neurons	(Kriks <i>et al.</i> , 2011)
	Amyotrophic lateral sclerosis	NSCs/motorneurons	(Teng <i>et al.</i> , 2012)
	Multiple sclerosis ^a	NSCs/oligodendrocyte progenitors	(Huang and Franklin, 2012)
	Spinal cord muscular atrophy	NSCs/motorneurons	(Corti <i>et al.</i> , 2010)
	Pelizaeus–Merzbacher	NPCs/NSCs	(Gupta <i>et al.</i> , 2012; Uchida <i>et al.</i> , 2012)
Ear	Hearing loss	Hair cells/NPCs/spiral ganglion neurons	(Nishimura <i>et al.</i> , 2012)
Eye	Retina degeneration	RPE/photoreceptors	(Schwartz <i>et al.</i> , 2012; Barber <i>et al.</i> , 2013)
	Cornea injuries	Limbal stem cells	(Kolli <i>et al.</i> , 2010)
Liver	Acute liver failure	Immature liver cells	(Takebe <i>et al.</i> , 2013)
	Chronic liver disease	Liver cells	(Pietrosi <i>et al.</i> , 2014)
	Liver-based metabolic disease	HSCs	(Prasad and Kurtzberg, 2008)
Muscle	Muscular dystrophy	Myogenic progenitors/ mesangioblasts	(Darabi <i>et al.</i> , 2012; Filareto <i>et al.</i> , 2013)/ (Tedesco <i>et al.</i> , 2012)
Pancreas	Type I diabetes ^a	Beta cell	(Rezania <i>et al.</i> , 2012; Kirk <i>et al.</i> , 2014)
Skin	Skin injuries	Epidermal stem cells	(Lough <i>et al.</i> , 2014)
	Epidermolysis bullosa	Epidermal stem cells	(Mavilio <i>et al.</i> , 2006)
Thymus	DiGeorge	Thymus epithelial cellsBone marrow	(Markert <i>et al.</i> , 1999; Land <i>et al.</i> , 2007; Sun <i>et al.</i> , 2013)
	Autoimmune diseases	HSCs	(Tyndall, 2011)
	<i>Diseases treated by long-term secretion of factors by transplanted cells</i>		
	Epidermolysis bullosa ^b	Bone marrow	(Wagner <i>et al.</i> , 2010)
	Metabolic disorders	HSCs	(Wynn, 2011)

HSCs, hematopoietic stem cells; NPC, neural progenitor cells; NSCs, neural stem cells; RPE, retinal pigment epithelial cells. References in bold indicate proof-of-concept or clinical trials using embryonic stem cells as cell source.

^aAutoimmune disease.

^bIt is unclear whether the amelioration is mediated only by secretion of factors or by additional cell replacement.

Since the original discovery, the list of other factors that can induce pluripotency has increased, as have the somatic cell sources that can be reprogrammed. Sources commonly used to isolate cells for reprogramming include peripheral blood, skin, and dental pulp (Dambrot *et al.*, 2013).

Nowadays, multiple techniques are used to induce pluripotency. Some of these leave undesirable genetic footprints behind, particularly relevant in looking forward to therapeutic applications but also in current disease modeling applications. More recent nonintegrating methods include mRNA transfection, episomal and Sendai virus vector-based transductions, and the use of chemical compounds. Initially, iPSC generation also required human or mouse feeder cells and media containing xenogeneic ingredients that are not compliant with clinical application. However, the multiplicity of potential applications has accelerated research in this area tremen-

dously, and reagents are now available to derive and maintain iPSCs under cGMP conditions using chemically defined, xeno-free media (Ross *et al.*, 2010; Chen *et al.*, 2011).

Current Status of PSC-Derived Cell Transplantations

The possibility of generating autologous PSCs has opened new avenues for research on human diseases. Patient-specific pluripotent cells enable construction of disease models mimicking human disease pathogenesis, and allow generation of autologous cells for future cell replacement therapies. A hint on these prospects for cell replacement strategies comes from studies using human ESCs. On the basis of promising results achieved in animal experiments, three applications based on human ESC-derived cells have made it into clinical trials. One is to study the repair of

spinal cord injuries through the injection of PSC-derived neural progenitor cells (Keirstead *et al.*, 2005). This trial was put on hold after the company, Geron, redirected their strategies away from stem cell-based therapies; however, another company may relaunch the phase 1 study soon. The other studies involve macular degeneration, a progressive blinding disease caused by loss of retinal pigment epithelial (RPE) cells and concurrently photoreceptor cells in the macular part of the retina.

Human ESC-derived RPE cells have been transplanted subretinally into patients. First results from phase 1 safety and feasibility studies were encouraging enough to accelerate this approach into a combined phase 2b/3 study of efficacy (Schwartz *et al.*, 2012). Last year, a similar clinical trial for iPSCs-derived RPE cells was initiated in Japan.

Proof-of-concept experiments in animal models have demonstrated that other cell replacement therapies may also enter the clinical trial stage (Table 1). Building on the original studies performed by Swedish researchers who transplanted fetal brain material in the midbrain region of Parkinson patients to replace the dying neurons in the substantia nigra (Lindvall and Bjorklund, 2004), Studer and colleagues published encouraging data on the improvements of amphetamine-induced rotation behavior, forelimb use, and akinesia after transplantation of human ESC-derived midbrain dopaminergic neurons in rodents (Kriks *et al.*, 2011). Researchers have also been able to generate thymic epithelial progenitor-like cells from human ESCs that could be helpful for DiGeorge syndrome patients who lack T cells because of the absence of a thymus. Upon transplantation under the kidney capsule of mice that do not have a thymus, the transplanted cells instructed T-cell development, indicating that they can replace the thymus epithelium (Sun *et al.*, 2013). Also, diabetic patients could benefit from PSC-derived cells as diabetes was reversed within 3 months in animals transplanted with encapsulated ESC-derived beta cell progenitor cells (Rezania *et al.*, 2012; Kirk *et al.*, 2014). Finally, a Japanese group of researchers showed that very small human iPSC-derived liver buds, which were transplanted into the mesenterium of mice, rescued the liver-failure-mediated drug-induced lethality (Takebe *et al.*, 2013).

Hurdles

It presently appears that PSCs may eventually live up to expectations, but a number of hurdles still have to be overcome to exploit PSCs to their full potential (Fig. 1). One of the main barriers has been the development of defined protocols for the differentiation of PSCs into specific cell lineages. Protocols for the differentiation of a wide variety of ectodermal, mesodermal, and endodermal cell derivatives are available, but published protocols often lack details and robustness, turning proper differentiation sometimes into a form of art. At the moment, there is a huge commitment to research worldwide to develop differentiation protocols that are more robust. In addition, considering the number of cell types in a human body, the list of cell types that have been generated from PSCs *in vitro* is still limited. For example, the differentiation of human PSCs into HSC-like cells that are able to functionally repopulate the bone marrow of patients has not been achieved. As this would mean a major breakthrough for HSC-based transplantations (van Bekkum and Mikkers, 2012), many researchers, including ourselves, have tried but failed to generate functional HSCs from PSCs. However, recently it was demonstrated that human PSCs are able to generate HSC-like cells in a teratoma model, where PSCs are co-injected with hematopoiesis-supporting stroma cells into an immune-deficient mouse (Amabile *et al.*, 2013; Suzuki *et al.*, 2013).

The cell types generated from iPSCs often look phenotypically identical to their endogenous counterparts, but it remains to be confirmed whether iPSC-derived cells are functionally equivalent to similar ESC-derived progeny, or to the endogenous cells. For example, midbrain dopaminergic neurons generated from mouse iPSCs differ from endogenous dopaminergic neurons in the expression of key neuronal genes (Roessler *et al.*, 2014). If functional repopulating HSCs could be derived from iPSCs, a study could be undertaken to study safety of iPSCs in a high-turnover compartment. This is crucial since iPSCs, irrespective of the method by which they were generated, can contain genomic alterations, presumably because of stress during the reprogramming phase. Copy number variations as well as exonic mutations have been found frequently (Gore

FIG. 1. Hurdles related to iPSC-based replacement therapies. The generation of clinical-grade iPSCs is feasible using nonintegrating reprogramming methods, but multiple other hurdles have only been partially overcome or have not been thoroughly investigated. Colors indicate the extent of the obstacles (green, feasible; red, problematic). Gray boxes depict issues that have remained largely unaddressed. iPSCs, induced pluripotent stem cells.

et al., 2011; Hussein *et al.*, 2011; Laurent *et al.*, 2011). This may not be a major issue for the transplantation of post-mitotic cells, like DA neurons, but is of concern when transplanting rapidly dividing stem/progenitor cells to repair high-turnover tissue. The genetic differences induced by the reprogramming have also been linked to iPSCs being immunogenic (Zhao *et al.*, 2011), which could compromise their therapeutic potential.

Recent studies have attenuated this problem as the immunogenicity is predominantly evoked by undifferentiated iPSCs and to a lesser extent by iPSC derivatives (Araki *et al.*, 2013; Guha *et al.*, 2013). The fact that immunogenicity is only induced by iPSCs would be good news for transplantation protocols. Transplantation of only a few PSCs can in principle lead to the formation of a benign tumor containing derivatives of all germ layers. Efficient removal of undifferentiated cells is therefore a prerequisite for cell therapies based on PSC derivatives. Immunogenicity would decrease the chance of forming teratomas. Also, isolation of the iPSC derivatives on basis of surface markers before transplantation would further decrease the chance at teratomas. Unfortunately, unique surface markers have not been identified for all the cell types that could be useful in future iPSC-based transplantation. Alternatively, purging human PSCs from mixed populations before

transplantation using specific compounds (Smith *et al.*, 2012; Ben-David *et al.*, 2013) or viruses (Mikkers, unpublished) may minimize the risk of teratoma formation.

Finally, it is essential that the transplanted iPSC derivatives integrate into the existing cellular networks. iPSC-derived HSCs should migrate to and remain in the stem cell niche of the bone marrow to prevent HSC exhaustion, and fully differentiated neurons should establish the right projections in the nervous system. Exceptions are cell therapies based on the secretion of factors that the patients lack, as in metabolic diseases.

iPSC-Based Gene Therapy

If iPSCs are used as a source of cells for autologous replacement therapies, genetic defects present in the iPSCs will need to be corrected. This can be realized by the heterologous expression of a correct copy of the disease gene from a randomly inserted lentiviral vector or transposon, from an expression vector integrated in a well-characterized locus (safe harbor) in the host cell genome, or from non-integrating large-capacity vectors (Fig. 2). Alternatively, at least one of the abnormal alleles can be replaced by a normal allele by homologous recombination (HR). A potential

FIG. 2. Restoration of congenital defects for the use of autologous cells. The defect in autologous cells can be restored by adding another copy into the genome (*gray shading*), or by repairing at least one of the mutant alleles. The extra copy can be delivered through different strategies, such as lentiviral vectors, transposons, human artificial chromosomes, or locus-specific insertions. Here, only restorations by lentiviral vectors and by a designed insertion into a safe-harbor locus are depicted. Double-strand break-mediated homologous recombination (HR) using targeting vectors can be exploited for tailored delivery of the extra copy, or repair of at least one of the mutant alleles. Nowadays, higher HR efficiencies are achieved with site-specific nucleases such as zinc finger nucleases (ZFN), TAL effector nucleases (TALENs), or clustered regularly interspaced short palindromic repeats (CRISPR) with CRISPR-associated proteins (CAS).

downside of integrating systems is that the inserted DNA sequence may affect endogenous gene expression, a process called insertional mutagenesis, possibly creating proneness to tumor formation. In the past, gamma-retroviral vectors have caused leukemia in a number of treated SCID-X1 patients (Biasco *et al.*, 2012).

Current lentiviral vectors and other integrating approaches appear to be much safer, but the risk of insertional mutagenesis remains an issue. Consequently, the number of integrated vector copies should be kept to a minimum and the promoter driving the expression of the gene should not be too strong. In addition, the cargo of some delivery systems is restricted; for example, lentiviral vectors have a cargo size of around 9 kb, excluding the treatment of diseases originating from defects in very large genes. Besides these bottlenecks, inserted foreign DNA sequences can be subjected to silencing. For example, lentiviral vectors are, depending on the promoter, efficiently silenced in undifferentiated human PSCs or upon the differentiation of human PSCs (Xia *et al.*, 2007; Norrman *et al.*, 2010).

A promising but underexplored alternative method is the use of human artificial chromosomes, which can be efficiently maintained in mouse iPSCs (Kazuki *et al.*, 2010). Another alternative is based on site-specific modification through HR. In contrast to HSCs, iPSCs are well suited to be repaired by currently available strategies of HR. iPSCs are easily transfected and, more importantly, can be expanded *in vitro*, a feature required to select for the rare clones that have undergone HR. The ORF can be knocked into a locus that is safe and maintains the expression of the ORF. A commonly suggested safe-harbor region is the *AAVS1* locus on chromosome 19 (DeKolver *et al.*, 2010). However, targeting the endogenous locus is mostly preferred because this guarantees transcription properties identical to those of the endogenous gene. Until recently, HR was challenging in iPSCs but recent advances in the induction of site-specific double-strand breaks (DSB) have improved HR efficiencies (for review, see Gaj *et al.*, 2013). Nevertheless, there is room for improvement in delivery and expression of the ZN-finger nucleases, TAL effector nucleases, or the CRISPR/CAS9 factors that induce the

DSB. Moreover, improving the delivery of the targeting DNA and reducing the off-target effects are important factors to enhance the applicability of these genome-editing techniques.

Alternative Reprogramming Routes

The remarkable Japanese discovery that a small subset of transcription factors is sufficient to completely change the epigenome in somatic cells made researchers realize that it should be possible to convert somatic cells into tissue-specific stem/progenitor cells, or into fully differentiated, postmitotic cells by certain factors. Indeed, numerous examples of direct conversion have been reported to date, and this list of direct conversions is still expanding. In general, it is now thought that if the right combination of transcription factors is expressed in cells, and an appropriate supportive (micro-) environment is provided, somatic cells readily acquire the epigenetic and transcription status of the predestined cell type. Successful transcription factor combinations have included the original “Yamanaka” factors or tissue-specific transcription factors with or without specific miRNAs. Rodent fibroblasts have thus been converted into beta-cells, cardiomyocyte-like cells, hepatocyte-like cells, NSCs, and neurons (Miki *et al.*, 2013).

Very recently, functional induced HSCs were even generated *in vivo* by the expression of a combination of eight factors in monocytes or pre-B cells (Riddell *et al.*, 2014). Direct conversion of human cells is a little more demanding, seemingly requiring additional factors. Nevertheless, neurons (Qiang *et al.*, 2011; Yoo *et al.*, 2011) and cardiomyocyte-like cells (Fu *et al.*, 2013; Nam *et al.*, 2013) have been generated from human adult fibroblasts. Although directly converted cells have potential for cell regenerative purposes, iPSC-based therapy looks more realistic at the moment. Current direct conversion methods are very inefficient, leading to heterogeneous mixtures of the parental cells with cells that have been reprogrammed to a varying degree (Miki *et al.*, 2013). In addition, the effect of direct conversion on the genomic stability and integrity remains to be investigated.

TABLE 2. CURRENT MAIN INDUCED PLURIPOTENT STEM CELL RESEARCH INTERESTS IN THE NETHERLANDS

Center	Disease	Purpose
Amsterdam Medical Center	(Genetic) cardiac (Genetic) neural	Disease modeling/pathogenesis research Disease modeling/pathogenesis research
Erasmus University	Neural Metabolic	Disease modeling/pathogenesis research Disease modeling/pathogenesis research
Hubrecht Laboratories	Neural —	Disease modeling/pathogenesis research Pluripotency
Leiden University Medical Center	Cardiovascular Neural Hematopoietic	Disease modeling/pathogenesis research Disease modeling/pathogenesis research Disease modeling/transplantation research
Radboud University	Neural Metabolic	Disease modeling/pathogenesis research Disease modeling/pathogenesis research
Sanquin Blood Supply	Trauma	Blood transfusion
University Medical Center Groningen	Neural	Disease modeling/transplantation research
University Medical Center Utrecht	Neural	Disease modeling/pathogenesis research
VU University Amsterdam	Neural	Disease modeling/pathogenesis research

iPSC Research in the Netherlands

The Netherlands is a small country of approximately 17 million inhabitants. Nevertheless, the country harbors 12 universities and a number of other research institutes in which academic biomedical research is performed. Many of these are linked to university hospitals. This explains the Dutch interests and investments in iPSC technology. Current Dutch iPSC research interests are listed in Table 2. Several of the institutions have established iPSC core facilities to generate iPSC lines for internal and external researchers in a centralized facility and using standardized protocols. In further support of researchers, our institute recently implemented a standardized information document and informed consent form for obtaining the patient's permission for isolating cells for iPSC generation. An English translation of Leiden University Medical Center (LUMC)'s Dutch Information Document and Permission Form that was approved by the LUMC's Medical Ethical Research Committee is provided in Supplementary Document S1 (Supplementary Data are available online at www.liebertpub.com/hum). It is largely in line with similar informed consent documents used by the National Institutes of Health in the United States.

To foster collaboration and facilitate exchange of iPSC lines, the core facilities have integrated in a national iPSC consortium that has chosen to standardize the protocols for generating, maintaining, and where possible differentiating iPSCs. The development of new differentiation protocols first serves iPSC-based disease models to be set up for understanding disease pathogenesis. In addition, it may further stimulate generating protocols for drug development, drug testing, and cell replacement.

iPSC-Based SciFI

The possibility of generating organs that are haplotype identical to the patient constitutes the Holy Grail for regenerative medicine. But will it ever be possible to build complete human organs? Two approaches, both of which have currently a high degree of impossibility, may become

reality in the future. The first approach would entail the construction of autologous human organs via iPSCs. In this scenario, an interspecies chimeric animal would be created by injecting human iPSCs into blastocysts from a nonhuman species (e.g., a pig). If the cells of the blastocyst had a genetic defect that prevented the development of an organ, then the organ could only be derived from the injected iPSCs (Fig. 3). Nakauchi and coworkers have shown proof of concept for this strategy by creating rat/mouse chimeras in which the pancreas was derived from the rat cells that were injected Pdx1-deficient blastocyst (Kobayashi *et al.*, 2010). Before human-organs-produced-in-animals could become reality, many hurdles, including ethical and legislative, would have to be addressed. The second way would be 3D bioprinting, where iPSC-derived cells are printed in 3D to create an organ-like biomass. 3D molds have shown potential in steering the differentiation of human PSCs (Warmflash *et al.*, 2014), and a valve-based printer for printing human ESCs as spheroids without influencing cell viability and function has been generated (Faulkner-Jones *et al.*, 2013). However, small, specific organlike structures have yet to be printed from human PSCs.

Prospects

The application of reprogrammed cells, either iPSCs or induced tissue-specific cells, in cell replacement therapies is very appealing. They would eliminate the problem of donor availability and may enhance the success rate of replacement therapies. However, protocols for the robust differentiation or reprogramming into cells that can be functionally transplanted need to be further developed. In addition, the safety of the reprogrammed cells should be demonstrated in studies using appropriate animals models. Until that time, the concept of cell transplantations with reprogrammed cells will remain a promise. Many Dutch research groups are working in concert to ensure that we fulfill this promise. We share the ambition that the clinical impact of cellular reprogramming technology should be as large as its impact on stem cell biology research.

Genetic modification of pig cells

FIG. 3. *In vivo* organ synthesis using chimeric animals. Patient-specific human iPSCs are injected into pig blastocysts, generated from *in vitro*-expanded pig cells generated by somatic cell nuclear transfer (SCNT). Pig cells are modified in such a way that they lack the ability to develop into the organ of choice (here, pancreas). The injected blastocysts are transferred into pseudo-pregnant recipient pigs, resulting in the birth of chimeric pigs containing one organ of human origin.

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