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# Progress Towards Establishing ES/iPS Cell-Based Clinical Translation

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## Abstract

**Purpose of Review—**Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are pluripotent and therefore capable of differentiating into different cell types and tissues. However, their clinical potential so far has not been sufficiently probed. The major obstacle is the lack of protocols that allow efficient derivation of clinical grade cells or tissues. This review will address these questions and discuss the current state of the field.

**Recent Findings—**I will address some of the ongoing clinical trials using stem cell-derived retinal pigment epithelial (RPE) cells, cardiomyocytes, neurons and attempts to establish insulin producing cells (IPCs) for the treatment of type 1 diabetes.

**Summary—**Are we there yet? The answer is clearly no. Progress in the different organs and tissues that are being generated is quite variable. Clearly there has been more success in the derivation of RPE cells, neuronal cells and cardiomyocytes than in any other tissues or organs. The derivation of IPCs and that of definitive hematopoietic progenitor cells in humans remains a challenge. Having said that the progress already made with other tissues is an encouraging sign that we may eventually see progress across the board.

### Keywords

iPS cells; ES cells; cardiomyocytes; insulin producing cells

## Introduction

The discovery of human ES cells by Thomson and colleagues ushered in a new period of medical discovery in humans that potentially could give us unprecedented tools to improved management of disease (1). This discovery was preceded by decades long of hard work by several groups that hoped to establish human ES cells. In contrast, mouse ES cells had already been discovered 17 years earlier. They form the basis for modern day developmental biology and mouse genetics (2, 3). Human ES cells stirred a lot of controversy and lawsuits in the US for ethical and religious reasons. In fact there was so much controversy that in

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political campaigns stem cells became one of the leading topics that garnered a lot of interest among voters. Several lawsuits were brought up for several years bringing federally funded research to a scratching halt. Fortunately, Yamanaka discovered the so called induced pluripotent stem (iPS) cells. These cells are a result of converting somatic cells into pluripotent stem cells using the so called 4 factors, Oct4, Klf4, c-Myc and Sox2(4, 5). The beauty of this protocol is that it works well in both mice and in humans despite its low efficiency.

The major advantage of iPS cells over ES cells is that iPS cells can be individualized. Dermal fibroblasts from any patient can be differentiated into iPS cells and then differentiated into any given cells that the patient may require. Because the cells are patient-derived, there is no concern about immunological rejection. However, as we have described before, iPS cell-derived hematopoietic progenitor cells poorly express MHC antigens. They poorly express class I antigens and do not express class II antigens. The lack of class I antigens on ES cell-derived hematopoietic progenitor cells makes the cells vulnerable to NK cells in vivo. This appears to be true particularly in the mouse (6–8). In the mouse the derivation of hematopoietic cells from ES cells has been well established by us and other (6, 9). In humans, till now it has been difficult to derive definitive hematopoietic progenitor cells. There are epigenetic differences between iPS cells and ES cells which regulate the ability of these cells to differentiate. These epigenetic differences clearly determine the differentiation capabilities of these pluripotent stem cells. A better understanding of these factors will enable improved differentiation of human pluripotent stem cells.

A major challenge in iPS cell biology is the establishment of cell lines that have no viral integration. There is concern that virally established cell lines might form tumors in humans. One approach that has been pursued is the use of minicircles. A minicircle DNA is a vector type that is free of bacterial DNA and capable of high expression in cells. This approach allows the generation of transgene-free iPS cells from adult human cells (10). Compared to plasmids, minicircle vectors benefit from higher transfection efficiencies and longer ectopic expression owing to their lower activation of exogenous silencing mechanisms and thus may be an ideal strategy for generating iPS cells (11, 12). Nonviral and nonintegrating viral methods for generating iPS cells using adenovirus (13), plasmids (14) or excision of reprogramming factors using Cre-loxP (15, 16), or piggy BAC transposition (15) have been reported, but they suffer from low reprogramming efficiencies (<0.003%) and may leave behind residual vector sequences. Additional methods in generating iPS cells free of viral vectors have been reported. For example, proteins have been used but are very inefficient (17). Thus, the ideal method for generating iPS cells still needs to be established. What really is needed are approaches that are easy to use, non-integrating and highly efficient at differentiating into somatic cells. Although most of the methods that have been probed so far use fibroblasts, ideally we need methods that allow reprogramming of all types of somatic cells.

## Cardiac treatment with stem cell-derived cardiomyocytes

The generation of cardiomyocytes from pluripotent stem cells is relatively well established. In fact if pluripotent stem cells are left alone in culture, they will spontaneously differentiate

into cardiomyocytes. However, they still require purification from non-differentiated or partially differentiated ES or iPS cells in order to avoid the formation of teratomas. Studies have been tried whereby the patient's own bone marrow cells were either delivered intravenously or directly into cardiac tissue after cardiac infarction. None of these studies showed any measurable benefit to cardiac function (18–21). The difference between the potential use of ES or iPS cell derived cardiomyocytes to bone marrow cells is that stem cell derived cardiomyocytes possess angiogenic properties, which improve tissue repair and perfusion. After cardiac infarction, the damaged tissue requires revascularization which may not be feasible with bone marrow-derived mesenchymal stem cells. To be able to design appropriate clinical trials, there is a requirement for carefully planned preclinical trials preferably in large animals such as the pig or sheep. In fact one of the first studies in a large animal model was published several years ago after cardiac infarction in sheep (21). Mouse ES cells were differentiated into cardiomyocytes and transplanted in infarcted sheep. Left ventricular ejection fraction improved in the treated sheep, whereas it deteriorated in the control group. However, recent protocols have significantly improved the efficiency of differentiating iPS cells into cardiomyocytes (22). Functional studies need to be carried out in large animals that are closer to humans rather than in mice.

A recent study in rats showed that human ES cell-derived cardiomyocytes engraft but do not alter cardiac remodeling after chronic infarction in rats (23). The lessons learnt in previous clinical studies with bone marrow cells seem to suggest that lack of improved vasculogenesis after bone marrow transplantation in patients with cardiac infarction was not helpful in cardiac repair. One major advantage of cardiomyocytes generated from pluripotent stem cells is that they can initiate new vascularization in infarcted hearts. This is an important property that promotes repair of damaged tissues.

## Neurological diseases

A huge advantage on the use of stem cells is that they can be manipulated to form any desired cells. Two obvious examples of neurological disease that could benefit from stem cell therapy are spinal cord injuries and Parkinson's disease. Indeed the commercial company Geron initiated a clinical trial in 2010. This trial drew a lot of attention and was heralded as the first of its kind. A year later the trial was stopped. The reasons for the shutdown are still unclear to the scientific community. However, there are reports that mice that were treated with the neuronal product developed cysts leading to the shutdown of the trial. Unfortunately the data have not been published yet. Independent of whether the study had any promising data at all, it is important to make these data public so that we can learn about the possible problems that we might need to solve in the use of pluripotent stem cells. Clinical trials in pluripotent stem cells will take a while to establish in general because the derivation of any somatic cells from stem cells is still in its infancy. The challenges are ensuring that the final product to be used clinically is not contaminated with non-differentiated or partially differentiated pluripotent stem cells which could induce teratoma formation. In addition, there is a need for long-term animal studies to ensure safety of the cells.

An alternative approach that could benefit patients suffering from neurological diseases is deriving stem cells from diseased patients and generating neurons which can then be used for drug screening. Kevin Eggan at Harvard did exactly that with ALS cells (24). They generated iPS cells from ALS patients and differentiated them into neurons. Using a panel of drugs, they were able to identify a drug that is used in epileptic patients which shows promise in ALS. Drug discovery using iPS cell-derived cells could ultimately allow for individualized drug testing that can lead to more effective therapies.

## Age-related Macular Degeneration

Age-related macular degeneration (AMD) is the leading cause of vision loss in older adults. It is caused by the death of photoreceptor cells in the macular area of the neural retina. Loss of the retinal pigment epithelium is the cause of AMD. Thus, this disease is an excellent candidate for stem cell-based therapies. In addition, the eye is immune privileged and likely to be protected from inflammation. RPE are tightly connected by adherens and tight junctions, which are essential for the role of the RPE as a component of the blood: retina barrier. The generation of RPE cells from ES cells was first described in primates (25). Several methods have now been described that are being used in different labs to generate RPE cells (26). Recently a clinical trial was initiated to treat patients suffering from AMD with human ES cell-derived RPE cells. Two studies were initiated to establish the safety and tolerability of subretinal transplantation of human ES cell-derived RPE cells in patients with Stargardt's macular dystrophy and dry age-related macular degeneration. RPE were greater than 99 % pure, a prerequisite for clinical trials due to the danger of transplanting non-differentiated or partially differentiated ES cells which potentially could form teratomas. Expression of pluripotent cell markers was significantly downregulated in the RPE cells, however expression of RPE markers PAX6, RPE65, bestrophin and MITF was very high. Although this study was very small, only 2 patients have been reported so far, the study was successful in that the vision was significantly improved in both patients. None of the patients developed teratomas or hyperproliferation of the cells.

More recently an iPS cell based clinical trial has just been initiated at the RIKEN in Kobe, Japan and headed by Dr. Takahashi. Six patients are lined up for treatment and so far one patient has already been transplanted. Unfortunately no details are yet available as no data have been published yet. We anticipate seeing more of these studies in the near future as more differentiated cells are becoming available.

Although the number of patients so far treated is small, there have been so far no issues with tumor formation or hyperproliferation, tumorigenicity, ectopic tissue formation or apparent rejection (27). Thus, progress being made is very promising as more studies become available. More importantly, it will become more exciting to establish stem cell-based therapies as we take advantage of iPS cells. Although the eye is immune privileged, autologous cells as a source of RPE are preferable as rejection can be completely avoided.

## Stem cell-derived insulin producing cells

Type 1 diabetes (T1D) is a disease caused by the degeneration of pancreatic β-cells. This condition can be cured by the replacement of the β-cells, usually in the form of cadaveric β-

cells. However, there is a chronic shortage of cadaveric organs. With the advent of pluripotent stem cells, it has now become feasible to generate β-cells from either ES or iPS cells. The caveat is that with existing methods, it is still difficult to generate high amounts of mature β-cells. None of the existing protocols has so far been able to generate β-cells that are glucose responsive in vitro (28–33). However, when these cells are transplanted, they seem to mature in vivo into glucose responsive cells. The reasons why stem cell-generated β-cells are initially non-glucose responsive could be epigenetic. For example, our own studies appear to suggest that treating iPS cells with demethylating agents significantly improves their ability to differentiate into insulin producing cells. Time will tell whether we can further improve on the existing methods to generate therapeutic grade cells for the management of degenerative diseases. To our knowledge there are no clinical trials yet on stem cell-derived insulin producing cells. A central problem that appears to have been resolved is the specification of the pancreatic endoderm and its discrimination from the hepatic endoderm. Our current protocol now allows the enrichment of the pancreatic endoderm to over 95 %. We anticipate that the yield of the pancreatic precursor cells which express Pdx1 will be a lot highr than previously published. These cells should generate mature IPCs.

## Conclusion

In each disease type discussed, there has been much progress made, towards achieving cells that are suitable for clinical use. Differentiation of stem cells into transplantable progenies is a huge challenge with a different unique set of challenges for each tissue. Developmental studies in mice have been highly beneficial despite apparent differences to human cell development. We need to appreciate these differences and exploit them for the establishment of improved protocols.

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**Key points**

- Establishment of clinical grade somatic cells from pluripotent stem cells is feasible
- There is a need for improved differentiation protocols that allow efficient derivation of cells to be used clinically
- Cardiomyocytes, RPE cells and neurons, that are derived from ES or iPS cells are already being used in clinical trials