Editorial

Cardiac regeneration using HLA-matched induced pluripotent stem cells—no monkey business, but still a long and winding road ahead

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The discovery that somatic cells such as skin fibroblasts can be reprogrammed to induced pluripotent stem cells (iPSCs), which are capable of differentiating to any somatic cell type (1-3), has sparked considerable interest in the field of cardiology. Human iPSC-derived cardiomyocytes are now widely used to investigate pathomechanisms of cardiac diseases and to evaluate cardiac actions of drugs (4,5). However, the other great promise of iPSC technology in cardiology is that these cells, which unquestionably have the potential of differentiating to cardiomyocytes, may be used to replace myocardium lost due to infarction or regenerate failing hearts in non-ischemic cardiomyopathies.

Several important hurdles have to be overcome before an iPSC-based regenerative therapy of human heart failure patients is feasible. In a recent study, Shiba and colleagues (6) addressed some of these issues using a non-human primate model of myocardial infarction.

The first issue addressed by the study is the possibility of immunological rejection of the transplanted cells. In theory, iPSC technology offers the possibility of autologous transplantation: somatic cells (e.g., skin fibroblasts or blood mononuclear cells) from a cardiac patient could be reprogrammed to iPSCs, which could be then differentiated to cardiomyocytes for transplantation into the same patient. However, it is questionable whether such a strategy will be feasible for both technical and economic reasons. The process from the generation of iPSCs to the production of a sufficient number of cardiomyocytes for transplantation takes several months, limiting the utility of this strategy for acute diseases. Moreover, if the cells are to be transplanted into a human patient, high quality standards will have to be met, including, but not limited to, genomic sequencing to exclude genomic aberrations and exclusion of tumorigenic potential of the cells. All measures undertaken to ensure safety of the cells will require additional cost and time. It should be also noted that the differentiation protocols used to generate cardiomyocytes from iPSCs typically have to be optimized for every iPSC line, which may be of particular importance for human transplantation studies requiring a huge amount of highly-pure cardiomyocytes.

Therefore, an alternative approach to avoid immunological graft rejection might be to use immunologically-matched allogenous iPSC lines. It has been estimated that, given careful selection of donors homozygous for common HLA types, a limited number of pluripotent stem cell lines would be sufficient to generate HLA-matched grafts for a majority of patients (7).

This concept of allogenous transplantation of HLA-matched iPSC-derived cardiomyocytes was investigated by Shiba and co-authors (6) using Filipino cynomolgus monkeys as a model system. They generated iPSCs from an animal homozygous in MHC-class I and II regions. Using previously-described differentiation and purification protocols, they were able to generate large amounts (4×10^8

cells for one transplantation experiment) of iPSC-derived cardiomyocytes. The cell preparations had a reasonably-high purity (84% of the cells expressing cardiac troponin T) and could be cryopreserved and thawed prior to transplantation.

In ten animals in which either of the MHC haplotypes were identical to the donor iPSC line, myocardial infarction was induced by ligating the left anterior descending coronary artery for 3 hours, followed by re-perfusion. After 14 days, five of the animals received an injection of the iPSC-derived cardiomyocytes into the infarct and border zone. The control group consisted of five animals in which the same injection procedure was performed using only vehicle, but no cells. All animals received an immunosuppressive regimen with methylprednisolone and tacrolimus. After 12 weeks of follow-up, during which repetitive investigations of cardiac function and Holter ECG recordings were performed, the animals were sacrificed and their hearts were physiologically and histologically investigated.

There was no evidence for acute graft rejection in any of the animals. By contrast, when the iPSC-derived cardiomyocytes were injected into hearts of HLA-mismatched animals treated with the same immunosuppressive regimen, a severe graft rejection was evident 4 weeks after transplantation.

In the transplantation group, partial remuscularization of the scar region by the transplanted cardiomyocytes was evident, amounting to roughly 16% of the scar area. Left-ventricular ejection fraction, measured by computed tomography, was significantly higher in the transplantation group than in the control group after 4 and 12 weeks. However, there was no significant difference in the plasma levels of the heart failure biomarker BNP.

Another aspect crucial to cardiac regeneration using stem cell-derived cardiomyocytes that was also investigated in the study by Shiba *et al*. is the electrical integration of the transplanted cells into the host myocardium, and the closely-related issue of arrhythmogenicity. In order to augment the pump function of the heart, the transplanted cells have to electrically couple to the host myocardium, which is a prerequisite for contractions that are in synchrony with the beating heart. This requires not only the formation of gap junctions with the adjacent cardiomyocytes, but also an intracellular calcium cycling machinery that is able to generate repetitive calcium waves at physiologic heart rates. This aspect is not trivial, since *in vitro* differentiated iPSC-derived cardiomyocytes are typically immature with respect to calcium cycling (8), and the achievable beating rates are often lower than the physiological heart rates of the respective species (9). Incomplete coupling or failure to adapt to higher heart rates would not only prevent the grafted cells from improving cardiac function, but may also represent a risk for the development of arrhythmias with potentially fatal outcome.

To investigate this aspect, Shiba and colleagues chose the elegant approach of transfecting the undifferentiated iPSCs with a genetically-encoded fluorescent calcium indicator prior to differentiation to cardiomyocytes. Thus, the transplanted cardiomyocytes contained a probe that allowed optical imaging of electrical coupling with the host myocardium in *ex vivo* Langendorff preparations 12 weeks after transplantation. These experiments demonstrated that the calcium transients in the transplanted cardiomyocytes were in synchrony with the ECG, indicating 1:1 electrical coupling of the transplanted cardiomyocytes with the host myocardium. However, at higher pacing rates, it became obvious that the propagation of the calcium wave into the graft was delayed, which may be attributed to a suboptimal formation of gap junctions within the graft tissue. Such zones of delayed conduction may lay the ground for re-entry tachycardias.

Indeed, the repetitive Holter ECG recordings revealed that all animals that received iPSC-derived cardiomyocytes showed ventricular tachycardias (VTs) at some time point after transplantation. At day 14, all animals in the cell transplantation group showed sustained VTs. At later time points, the fraction of animals showing VTs decreased, but non-sustained VTs were present in one of the five animals even at the latest investigated time point (day 80 after transplantation). The authors stress that none of the animals showed any VT-related abnormal behaviour such as syncope. However, the frequent occurrence of VTs after transplantation must be considered a major safety concern for the application of this therapy to humans, given that ventricular arrhythmias are one of the most important causes of death after myocardial infarction.

Taken together, the study by Shiba and colleagues represents one important step towards translation of iPSC-based cardiac regeneration to the clinic, by showing that the combination of MHC-matched donor cardiomyocytes with a mild immunosuppressant therapy results in survival of the grafted cardiomyocytes and improvement of cardiac function without any evidence of immunological graft rejection. However, at the same time, it also highlights that many more steps are needed until such a therapy can be safely translated to human patients. Most importantly,

the issue of arrhythmogenicity has to be solved. Since the delayed conduction in the transplanted regions—a sign of immaturity of the cardiomyocytes—is a likely cause of the arrhythmias, more research into ways to improve maturation of the transplanted cardiomyocytes might we warranted. Moreover, while left-ventricular ejection fraction increased significantly after cell transplantation, there was no significant decrease in plasma BNP levels, which would be expected after a clinically-relevant improvement of heart failure. Clinical trials have shown that surrogate endpoints are often misleading, which is why hard endpoints such as mortality are normally used in clinical trials of heart failure treatments. It may, thus, be reasonable to demand that an adequately-powered study in non-human primates should demonstrate a mortality reduction by the stem cell-based treatment.

All these issues will have to be addressed in future studies before it might eventually be possible to apply this pluripotent stem cell-based regenerative approach to cardiac patients to reduce morbidity and mortality in a condition affecting millions of patients worldwide.

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Footnote

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