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In Translation

Stem cell-derived β cells go in monkeys

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Du et al. transplanted β cells derived from pluripotent stem cells in diabetic monkeys for the first time, as an intermediate stage toward clinical translation. They observed benefits unfolding over months but also observed immune rejection of the grafts by 5–6 months.

Insulin-therapy is the first-line treatment for type 1 diabetes (T1D) but imperfectly replaces the function of the β cells lost to autoimmune destruction and inadequately mimics metabolic regulation. The glycemia of certain patients is very difficult to control, and since 1999 a treatment method consisting in transplanting pancreatic β cells from deceased donors into the liver of T1D patients—the Edmonton Protocol—was adopted throughout the world (Shapiro et al., 2000). The islet graft helps regain glycemic control and prevents or delays diabetes-associated complications. However, critical human donor organ shortage is limiting and has driven research into alternative cell sources such as the production of β cells from pluripotent stem cells (Nair et al., 2020).

Since 2006, stepwise differentiation protocols inspired from development have been applied to human pluripotent stem cells (hPSCs) (D'Amour et al., 2006). Transplantation into mice was then introduced to test their function (Kroon et al., 2008). Mice have remained the gold standard to test pluripotent stem cell-derived β cells, creating a big gap from mice to human in clinical trials (Ramzy et al., 2021; Shapiro et al., 2021).

In a recent article in *Nature Medicine*, Du and colleagues have now transplanted β cells derived from pluripotent stem cells in non-human primates (Du et al., 2022). While Hongkui Deng's lab built on their previous work and proposed updated protocols to reprogram induced pluripotent stem cells (iPSCs) chemically and to generate β cells from hPSCs, the transplantation in rhesus macaques (*Macaca mulatta*) is the key novelty of their recent work (Figure 1).

Using a new optimized chemical reprograming, they first derived human iPSC (hiPSC) lines. They also improved previously published protocols to produce β cells, notably combining two small molecules: ISX9, a NeuroD1 inducer, and the Wnt inhibitor Wnt-C59 at the endocrine precursor stage (Figure 1). They could eventually produce islet-like cell clusters that contained a high proportion of β cells, α-like cells secreting glucagon, and δ-like cells secreting somatostatin. The efficiency was remarkably high, reaching 80%–90% endocrine cells at the end of the protocol, and was applicable across several lines. Analyses showed that the hPSC-derived islets had similar gene expression levels to those of human islets, except for a few differences and with the limitation that their reference islets appeared of mixed quality. In the last 10 years, the quality control of endocrine cells produced *in vitro* has increased tremendously. In this article, the authors could show glucose-induced insulin secretion in both static and dynamic assays, calcium signaling, and electron microscopy, in comparison with human islets (Figure 1). They further demonstrated the glucose regulation of glucagon production by α cells. The study showed high islet quality and state-of-the-art characterization. However, another recent study sets new standards with a deep functional characterization to further probe the quality of *in-vitro*-produced islets (Balboa et al., 2022). In this study further maturation was promoted by omitting the commonly used ALK5 inhibitor and adding an anti-proliferative aurora kinase inhibitor; both applied for several weeks, leading to important improvements in functionality and islets exhibiting a balanced proportion of α and β cells. In addition to the previous tests, islet responses to escalating doses of glucose, biphasic insulin release, patch-clamp analysis of the conductance of ion channels, exocytosis, and a deep metabolic characterization were applied, revealing that even the best *in-vitro*-produced hPSC-derived β cells have glycolytic and mitochondrial glucose metabolism differences with islets. Should we worry about it if the cells further develop functionally after transplantation? The question remains open as a similar functional characterization after transplantation *in vivo* remains to be done.

Having established the state-of-the-art quality of their *in-vitro*-produced islets, Du et al. transplanted them into the kidney capsule of adult mice made diabetic by Streptozotocin (STZ) injection. The islets further matured and became vascularized and more similar to islet cells, as determined by single-cell sequencing at 10 weeks post transplantation (Du et al., 2022). They reversed diabetes and restored fasting euglycemia, glucose-responsive C-peptide secretion was observed, and body weight was increased (Figure 1). Up to 48 weeks, immunofluorescence staining showed that the grafts still contained β cells, and no sign of tumorigenesis was detected. Moreover, they developed a cryopreservation protocol

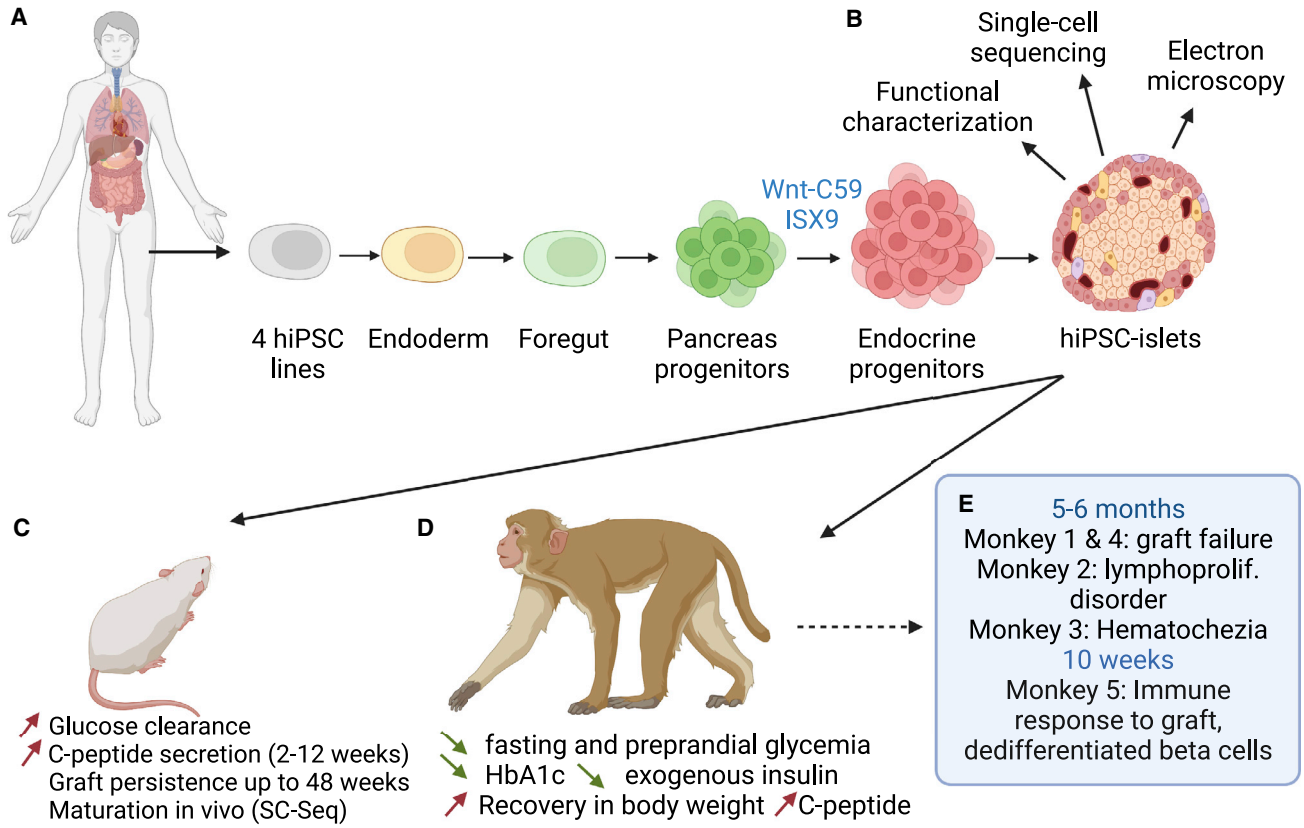

Figure 1. Graphic summary of Du et al. (2022)
(A) Chemical reprogramming of four hiPSC lines from adipose-tissue fibroblasts and subsequent differentiation.
(B) Quality control of β cells by single-cell sequencing (SC-Seq) and QPCR, electron microscopy, and histology, as well as by functional test.
(C) Transplantation in the kidney capsule of diabetic immunosuppressed mouse models shows further maturation *in vivo* by single-cell sequencing.
(D) Transplantation in the liver of diabetic macaques shows amelioration of symptoms.
(E) Further *in vitro* functional maturation is an alternative or additional option.

enabling the recovery of above 50% initial cells, which will be critical for future clinical applications.

As a step between mouse models and human trials, the authors proposed transplantation in rhesus macaques. Macaques have previously been used as models for islet transplantation (Ludwig et al., 2017). This enabled them to follow a surgical procedure that is currently used in islet transplantation in humans and follow up for 6 months. The procedure consisted of introducing hiPSC-derived islets intraportally in monkeys made diabetic by STZ treatment. All macaques showed improvement in diabetic symptoms within a few days after transplantation and a stabilization within 4–5 weeks (Figure 1). The number of individual animals treated remains small, as a total of five monkeys were treated, especially considering that each individual showed a different response. Despite this, common observations were made: both fasting and pre-prandial blood levels of glucose decreased. The requirements for exogenous insulin were lowered and even the most severely diabetic macaque showed less severe fluctuations of blood glucose levels. Most notably, HbA_{1c} levels, which measure chronic glucose toxicity, decreased by 25% at 3 months after transplantation.

While being promising, the study also showed signs of decreased graft function occurring gradually after 2 months, with eventual graft failure within 6 months. In autopsied monkeys, most grafted cells had been eliminated by this time point and signs of immune-mediated graft loss were detected. Severe adverse effects, possibly related to the strong immunosuppression needed for xenografts, developed in two macaques. No evidence of malignancy was detected, but 6 months represents a relatively short time frame when compared to the scale of a human life.

STZ is commonly used in diabetes transplant models in rodents but also in higher animals (Ludwig et al., 2017). In this study, STZ seemed to have important side effects. Liver injury following intraportal islet transplantation was observed and required specific treatment. This is not common in human islet transplantation and might be caused by STZ rather than by the islet infusion.

The strong immunosuppression used in this study goes beyond today's commonly used protocols in human islet transplantation and might be a relevant confounder with respect to functional outcome. Notably, the strong immunosuppressant regimen is known to promote bone marrow depletion, which would likely affect blood HbA_{1c} levels, an important readout of the study. Other long-term indicators such as serum

fructosamine would be useful. A control group consisting of adequate quality human islets would have helped interpret the study results.

Taken together, stem cell-based therapies open great opportunities for a functional cure for patients with T1D and even extended indications. However, many obstacles still exist with respect to efficacy and—most importantly at this early phase—to safety aspects. Therefore, preclinical models that assess and refine translation to a human application are very valuable, and the work presented by Du et al. (2022) is an important and insightful contribution. It also triggers numerous questions for the community. What can we expect from an animal model? Is there a perfect model or should we consider several? Will the model have predictive value for human therapy and justify using these animals and resources?

DECLARATION OF INTERESTS

The authors declare no competing interests.

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