**α-cell makeover for diabetes therapy**

Mostafa Bakhti1-3 and Heiko Lickert1-4

*1Institute of Diabetes and Regeneration Research, Helmholtz Zentrum München, Neuherberg, Germany*

*2Institute of Stem Cell Research, Helmholtz Zentrum München, Neuherberg, Germany*

*3German Center for Diabetes Research (DZD),* *Neuherberg, Germany*

*4Technical University of Munich, Medical Faculty, Munich, Germany*

Corresponding author: [heiko.lickert@helmholtz-muenchen.de](mailto:heiko.lickert@helmholtz-muenchen.de)

**Regeneration of insulin-producing β-cells would greatly improve diabetes therapy. Furuyama et al now provide evidence that human α-cells can be partially converted into** **β-cells, which normalize blood glucose in a diabetic mouse model and evade autoimmunity.**

Diabetes mellitus is a world-wide epidemic with steadily increasing patient numbers. The two major forms of diabetes develop when either insulin-producing β-cells in the pancreas are destroyed due to autoimmunity (type 1 diabetes; T1D) or progressively fail due to glucolipotoxicity and insulin resistance (type 2 diabetes; T2D). T1D and late stage T2D patients require daily insulin injections for survival, but even the best current treatments do not prevent hypo- and hyperglycemic excursion associated with micro- and macro-vascular complications. This urges for the development of novel therapies that stop or reverse disease progression1,2,3. The regeneration or replacement of dysfunctional or lost β-cells can restore glucose-dependent insulin secretion, tightly regulate blood glucose and therefore prevent secondary complications. β-cell regeneration would be an ideal approach as it does not involve invasive islet transplantation and is applicable to a wide range of diabetic patients. However, the routes and mechanisms of *in vivo* β-cell regeneration need to be better investigated. Currently, inducing proliferation of residual β-cells, redifferentiation of dedifferentiated β-cells and conversion of non-β-cells to insulin-producing cells are among the most promising avenues. The conversion of α- and δ-cells into insulin-producing β-cells has been previously pioneered by the Herrera laboratory in the mouse model4,5, however, if islet cell plasticity can be harnessed in normal and diabetic human islets is unknown.

Now, Furuyama et al6reports in *nature* on the conversion of human non-β-cells into functional insulin-producing cells. The authors established a highly efficient *in vitro* model system (Figure 1). Islets from non-diabetic or T2D patients were isolated, dissociated and flow sorted to obtain single islet cell subtypes that resulted in almost pure α- and γ-cell fractions. Using adenoviral vectors, they transduced more than ~99% of α- and γ-cells with the two β-cell transcription factors (TFs) *PDX1* and *MAFA*7as well as the green fluorescent protein (GFP) reporter gene to trace the transduced cells. The ectopic expression of these TFs forces human α-cells to partially convert into glucose-responsive insulin-producing cells after *in vitro* aggregation into pseudoislets. Furthermore, the pseudoislets exhibited glucose-stimulated insulin secretion (GSIS), which was enhanced in the presence of endothelial (HUVECs) and mesenchymal (MSCs) cells likely providing paracrine niche factors. When converted human α-cell pseudoislets from several non-diabetic or T2D donors were transplanted into β-cell ablated diabetic mice, they exhibited functional GSIS and ameliorated hyperglycemia. Detailed transcriptomic and proteomic analyses of the partially reprogramed cells *in vitro* and/or after transplantation revealed that these cells acquire a hybrid molecular phenotype with an α/β-cell gene signature, in which cells preserve some α-cell features, while partially acquiring a β-cell program. Whereas these hybrid cells expressed many genes involved in insulin synthesis and secretion, they still expressed several essential genes specific for the α-cell fate, such as the key TF, ARX. Despite the hybrid features, the converted cells, however, were stable up to 6 months *in vivo*, and exhibited hypo-immunogenic features when exposed to T-cells from T1D patients *in vitro* (Figure 1).

Altogether, Furuyama et al provide the first direct evidence on how one can utilize human islet cell plasticity to reprogram non-β- into β-like cells. They showed the conversion of human α- and γ-cells into β-cells that together with previous work4,5, suggests that almost all non-β-islet cells have the potential to become β-cells. Among non-β-cells, α-cells might be the best candidate for reprograming towards β-cells, not only to generate insulin-producing cells, but also to reduce α-cell hyperplasia and hyperglucagonemia in T1D. Notably, the rate of cell conversion was efficiently enhanced upon cell aggregation, stressing the importance of the islet three-dimensional (3D) architecture to harness islet cell plasticity8. Despite showing GSIS, the converted cells still expressed an α-cell signature and represented a hybrid phenotype, indicating that the two TFs used in this study are not sufficient to fully transdifferentiate α- into β-cells. On the other hand, partially converted hybrid cells might be desired, as they seem to evade the immune system. This is a particular interesting observation and might become relevant for the treatment of autoimmune T1D, where one needs to resolve autoimmunity and protect and/or regenerate lost β-cell mass. Alternatively, reprograming of dedifferentiated β-cells to enforce a redifferentiation program would be another option, especially in T2D. In this scenario, β-cell regeneration will be achieved without manipulating non-β-islet cells, which would better maintain normal islet physiology. Therefore, different strategies for partial or complete reprogramming into β-cells might provide personalized therapies for various forms of diabetes.

Obviously, the next challenge will be to translate these findings from *in vitro* and pre-clinical models into the clinic. First, the TF code that enforce complete conversion of non-β-islet cells into β-cells need to be defined using the implemented *in vitro* model and single-cell RNA sequencing analysis (Figure 1). Identification of specific TF codes designed for targeting specific islet cell subtypes would greatly enhance the efficiency of conversion into β-cells and might empower *in vivo* reprogramming. Next, the different TFs need to be targeted and delivered specifically to islet cell subtypes without causing serious side effects. This targeted delivery could be achieved by adenoviral vectors carrying cell-type specific promoters to drive TF expression9 or through antibody-mediated delivery of cargo to target cells10. The application of these techniques together with Crispr/Cas9-mediated genome and epigenome modification will facilitates gene delivery and editing procedures (Figure 1). However, such strategies should not only ensure efficient delivery and reprogramming *in vivo*, but also consider safety concerns. Overall, the study from the Herrera laboratory not only provides evidence that human islet cell plasticity can be harnessed for β-cell regeneration *in vivo*, but also suggests that direct reprogramming is a promising avenue to treat other degenerative diseases.

**Figure legend:**

**Figure 1**: Reprogramming of human - into β-cells. Using a novel *in vitro* sorting and transduction system, non-diabetic and diabetic islets are dissociated, flow sorted and islet cell subtypes are purified. α-cell transduction with β-cell transcription factors PDX1 and MAFA generates hybrid α/β-cells upon aggregation into pseudoislets, which are functional *in vitro* and restore hyperglycemia *in vivo*. Future work should focus on using this pulse-and-chase transduction system to provide the molecular programs for complete β-cell reprograming from different islet cell subtypes. When identified, such TF codes can be used to safe and efficiently target cells for β-cell reprogramming and regeneration in patients.

1. [Zhou, Q](https://www.ncbi.nlm.nih.gov/pubmed/?term=Zhou%20Q%5BAuthor%5D&cauthor=true&cauthor_uid=29769672). [Melton, D.A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Melton%20DA%5BAuthor%5D&cauthor=true&cauthor_uid=29769672). *Nature* **557**, 351-358 (2018)

2. [Aguayo-Mazzucato, C](https://www.ncbi.nlm.nih.gov/pubmed/?term=Aguayo-Mazzucato%20C%5BAuthor%5D&cauthor=true&cauthor_uid=28889951). [Bonner-Weir, S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bonner-Weir%20S%5BAuthor%5D&cauthor=true&cauthor_uid=28889951). *Cell Metab.* **27**, 57-67 (2018).

3. [Bakhti, M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Bakhti%20M%5BAuthor%5D&cauthor=true&cauthor_uid=30504925)., [Böttcher, A](https://www.ncbi.nlm.nih.gov/pubmed/?term=B%C3%B6ttcher%20A%5BAuthor%5D&cauthor=true&cauthor_uid=30504925)., [Lickert, H](https://www.ncbi.nlm.nih.gov/pubmed/?term=Lickert%20H%5BAuthor%5D&cauthor=true&cauthor_uid=30504925). [*Nat. Rev. Endocrinol.*](https://www.ncbi.nlm.nih.gov/pubmed/27585958) doi: 10.1038/s41574-018-0132-z (2018)

4. Thorel, F. *et al. Nature* **464**, 1149-54 (2010).

5. [Chera, S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Chera%20S%5BAuthor%5D&cauthor=true&cauthor_uid=25141178). *et al. Nature* **514**, 503-7 (2014).

6. Furuyama, K. *et al. Nature* **(in press)** (2019)

7. [Matsuoka, T.A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Matsuoka%20TA%5BAuthor%5D&cauthor=true&cauthor_uid=28223284). *et al.* [*Diabetes.*](https://www.ncbi.nlm.nih.gov/pubmed/28223284)  **66**, 1293-1300 (2017).

8. [Roscioni, S.S](https://www.ncbi.nlm.nih.gov/pubmed/?term=Roscioni%20SS%5BAuthor%5D&cauthor=true&cauthor_uid=27585958)., [Migliorini, A](https://www.ncbi.nlm.nih.gov/pubmed/?term=Migliorini%20A%5BAuthor%5D&cauthor=true&cauthor_uid=27585958)., [Gegg, M](https://www.ncbi.nlm.nih.gov/pubmed/?term=Gegg%20M%5BAuthor%5D&cauthor=true&cauthor_uid=27585958)., [Lickert, H](https://www.ncbi.nlm.nih.gov/pubmed/?term=Lickert%20H%5BAuthor%5D&cauthor=true&cauthor_uid=27585958). [*Nat. Rev. Endocrinol.*](https://www.ncbi.nlm.nih.gov/pubmed/27585958)  **12**, 695-709 (2016).

# 9. [Wang, Y](https://www.ncbi.nlm.nih.gov/pubmed/?term=Wang%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=29550076). *et al.* [*Mol Ther.*](https://www.ncbi.nlm.nih.gov/pubmed/29550076) 26, 1327-1342 (2018).

10. [Dorrell, C. *et al. Stem Cell Res.* **1**, 183-94 (2008).](https://www.ncbi.nlm.nih.gov/pubmed/?term=Dorrell%20C%5BAuthor%5D&cauthor=true&cauthor_uid=27399229)