Regenerative medicine to cure Type 1 diabetes: progress globally and in the iNanoBIT H2020 project

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How can we cure diabetes?

www.who.int/mediacentre/factsheets/fs312/en/

Main types of diabetes

**TYPE 1 DIABETES**
Body does not produce enough insulin

**TYPE 2 DIABETES**
Body produces insulin but can’t use it well

**GESTATIONAL DIABETES**
A temporary condition in pregnancy

www.who.int/mediacentre/factsheets/fs312/en/
Current therapeutic options to treat diabetes

• Insulin injections
• Pancreas transplantation

• Pancreatic islet transplantation
• xenotransplantation

New and efficient alternative treatment by stem cell-derived REGENERATIVE therapy

BioArtificial Pancreas (BAP)
Bioartificial pancreas (BAP) technology competition

<table>
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<tr>
<th>Products</th>
<th>MailPan®</th>
<th>CellPouch®</th>
<th>β-air®</th>
<th>Encaptra®</th>
<th>Afibromer™</th>
<th>“Cell housing”</th>
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<tbody>
<tr>
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<td>Late preclinical trials</td>
<td>New Phase I/IIa started in 2019</td>
<td>Phase I/IIa (negative)</td>
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<td>Preclinical trials</td>
<td>Preclinical trials</td>
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<td>SC</td>
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<td>✔</td>
<td>✔</td>
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</table>

- **2015** Sanofi - **Evotec** $329M Diabetes Beta Cell Therapy Collaboration (2017 milestone 3M)
- **2017** Ely Lilly - **Sigilon Therapeutics** (Cambridge MA) $473M T1D, stem cell project
- **2019** Vertex Pharmaceuticals: - **Semma Therapeutics** for $950M
The iNanoBIT project

**integration of Nano- and Biotechnology for Beta-cell and Islet Transplantation**

- Nanotechnologies for imaging cellular transplants and regenerative processes in vivo in a pig model for type 1 diabetes treatment
- Collaboration of Hungarian, German, French, Belgian companies and universities
- Coordinator: Andras Dinnyes /BioTalentum Ltd
- Budget: **7 M EUR /5 years**

**Partners:**

- BioTalentum
- University of Bicocca
- Mediso
- Defymed
- LMU
- UCL
- BBS NanoTech
- iThera Medical
integration of Nano- and Biotechnology for Beta-cell and Islet Transplantation

Structure of the project

BioArtificial Pancreas MAILPAN® device

Genetically modified humanised preclinical diabetic pig model (MIDY)

Nano-marker material development

Optoacoustic imaging

SPECT/CT

H2020-NMBP-2016-2017, NMBP-15-2017- Integration of Nano- and Biotechnology for beta-cell and islet Transplantation- Project No.: 760986
Supply of porcine neonatal islet-like cell clusters (NICCs) INS-LEA29Y transgenic pigs as donors for islet XT

Klymiuk et al., Diabetes 61, 1527-1532 (2012)

H2020-NMBP-2016-2017, NMBP-15-2017- Integration of Nano- and Biotechnology for beta-cell and islet Transplantation- Project No.: 760986
INS-eGFP transgenic pigs for islet studies

*Fluorescent-based cell sorting*
for omics analyses of beta cells
to study
beta-cell differentiation and maturation
in pig pancreas

*In vitro imaging*
e.g. NPI maturation during culture

*In vitro maturation of NPI*

*Improved in vitro maturation protocols*

*In vivo differentiation and maturation of beta cells*
→ Identification of pathways

*Long-term in vivo imaging*
to study in vivo e.g.
XTx engraftment
beta-cell maturation
NPI-XTx mass expansion

Optimized islet xenografts
- Reduced XTx islet mass needed
- Improved engraftment
- Improved *in vivo* maturation
- Improved reversal of diabetes
- Improved long-term survival
Correlation between XTx islet mass and function feasible *in vivo*

Kemter et al., Diabetologia 60, 1152-1156 (2017), Kemter et al., Curr Diab Rep. 2018 Sep 18;18(11):103
Ubiquitous reporter gene expression in iRFP transgenic pigs

1. Nucleofection of primary porcine kidney cells (PKCs)
2. FACS sorting of CAG-iRFP transfected PKCs
3. iRFP expressing kidney cells were used to generate embryos with SCNT, which were laparoscopically transfected into recipient sows
CRISPR/Cas9 targeting of human iPSCs to create iRFP720 reporter cell lines

A) Southern blot analysis

B) Live cell imaging - karyotyping

C) FACS analysis
Pancreatic progenitor differentiation with STEMdiff™ Pancreatic Progenitor Kit in 3D culture

1.8x10^6 cells/mL

Samples at different stages of differentiation were collected for characterization (RT-qPCR, ICC, insulin)
Pancreatic progenitor differentiation with STEMdiff™ Pancreatic Progenitor Kit in 3D culture

### Pluripotency markers

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<th>iPSC</th>
<th>Def. End.</th>
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### Definitive Endoderm markers

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### Pancreatic progenitor markers

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<td>PDX1</td>
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**Insulin content:** 8.16 µU/spheroid (day 14)

**Insulin secretion:** 1018 ± 141 µU/600 spheroids (day 14)/hour in G15
hiPSC derived pancreatic progenitor transplantation and *in vivo* maturation in mice

- Immature human beta-cell (pancreatic progenitor spheroid) implantation into non-diabetic SCID mice
- Implantation under the left kidney capsule (600 spheroids/mouse; n=3)
Follow-up of glycaemia and human c-peptide levels in sera
In vivo detection of iRFP720 expression

In vivo imaging of hiPSC-derived beta cells implanted under the left kidney capsule of immunodeficient SCID mice. Image was obtained using IVIS imaging system detecting iRFP720 fluorescence 13 months post-implantation.
Graft explantation for IHC analysis
Three functions are absolute prerequisites:

- Protecting foreign cells from the immune system
- Protecting the receiving organism from foreign cells
- Maximizing cell functionality

1) Implantation in abdominal site  
   \( T=0 \)

2) Pre-vascularization of the device  
   \( T=6-8 \) weeks

3) Injection of cells through chamber  
   \( T=2 \) months
Polyanion and polycation nanoparticles are combined and multifunctionalized with specific biological recognition motifs (targeting molecules) to selectively label beta cells and diverse contrast agents for imaging purposes. Produced nanoparticles thereby hold targeting molecules on their surface while possess imaging molecules in the core as seen on the right side.
**In vivo** MSOT analysis of iRFP expression in CAG-iRFP720 transgenic founder pigs (LMU and iThera, Munich/Germany).

- Three different regions were scanned at the belly region
- Unmixed signals were pseudocolored in yellow and overlaid to the corresponding ultrasound images
- Notably MSOT signal strengths correlate very well to the FACS results of the iRFP fibroblasts showing strongest average signals for 10105, 10106 pigs
The newly developed AnyScan TRIO system with the new multi-pinhole collimator design focusing on the pig’s liver (Mediso/Hungary)

H2020-NMBP-2016-2017, NMBP-15-2017- Integration of Nano- and Biotechnology for beta-cell and islet Transplantation- Project No.: 760986
Conclusions

- Rodent and large animal transplantation trials will allow quantifying viable islet mass and the correlation with islet function

- Animal transplantation will show if glucose/insulin control is fully restored

- Scaling up of matured human beta-cell production is a technological and financial challenge

- Vascularization of the medical device is a key issue and options to add additional islets/cells if needed

- Stably maintained iRFP reporter expression has been detected in long term in vivo transplantation experiments

H2020-NMBP-2016-2017, NMBP-15-2017- Integration of Nano- and Biotechnology for beta-cell and islet Transplantation- Project No.: 760986
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