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

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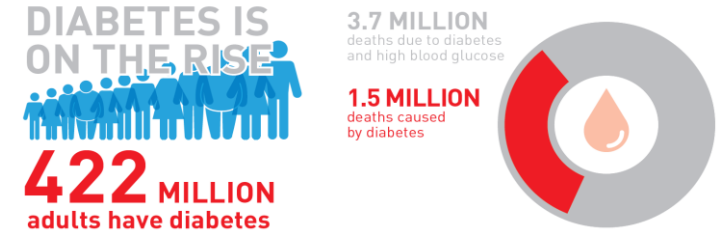
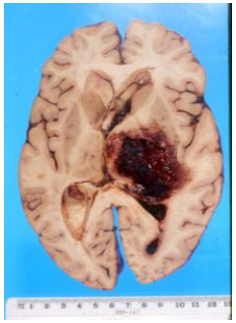
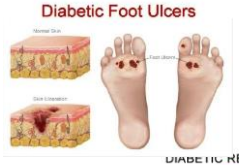
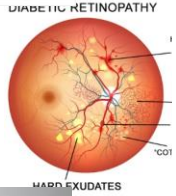
How can we cure diabetes?



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





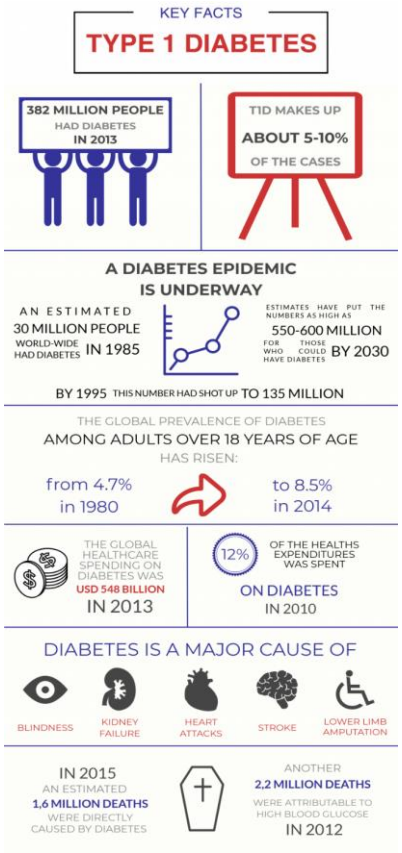


Main types of diabetes

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



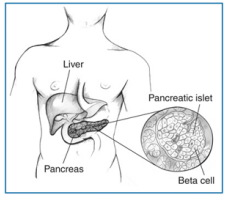
The boy on the picture, Leonard Thompson received his first insulin injection in Toronto, Ontario, on 11 January 1922, at 14 years of age - exactly 100 years ago. The boy who weighted just 24 kg, displayed allergic reaction following first injection due to apparent impurity. Scientists worked day and night on purifying the extract even further, and Leonard was given a second injection on 23 January 1922. This time it was a complete success and Leonard's blood sugar levels become near-normal, with no obvious side effects. Thompson showed signs of improved health and went on to live 13 more years taking doses of insulin, eventually dying of pneumonia at age 26. Insulin saved Leonard's life and countless others over the last century. Until insulin was made clinically available, a diagnosis of Type 1 diabetes (T1DM) was a death sentence. Such an incredibly important milestone for so many, that has saved so many lives! But, there's still so much more to do, and scientists are committed to keep building on this legacy, to keep innovating and driving change for people living with T1DM.
#insulin100

Current therapeutic options to treat diabetes

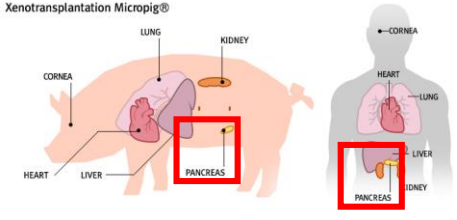
- Insulin injections

- Pancreas transplantation

- Pancreatic islet transplantation

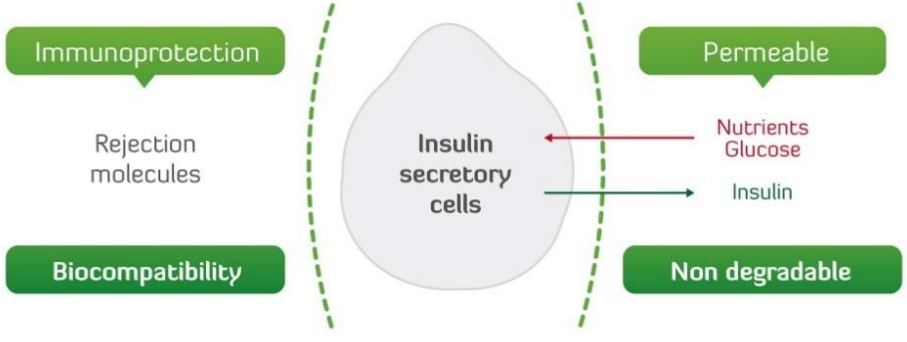


- xenotransplantation

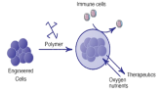
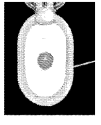


New and efficient alternative treatment by stem cell-derived REGENERATIVE therapy

BioArtificial Pancreas (BAP)



Bioartificial pancreas (BAP) technology competition

	Defymed	Sernova Corp	BetaO2 Technologies	ViaCyte Regenerating Health	sigilon	semma Therapeutics
Products	**MailPan®**	**CellPouch®**	**β-air®**	**Encaptra®**	**Afibromer™**	**"Cell housing"**
Development status	Late preclinical trials	New Phase I/ IIa started in 2019	Phase I/ IIa (negative)	New Phase I/ IIa started in 2017	Preclinical trials	Preclinical trials
Implantation site	EP	SC	EP	SC	IP	IP
Immuno-protection	✓	✗	✓	✗	✓	✓
Safety	- ● ● ● ● +	- ● ● ● ● +	- ● ● ● ● +	- ● ● ● ● +	- ● ● ● ● +	- ● ● ● ● +
Efficacy	- ● ● ● ● +	- ● ● ● ● +	- ● ● ● ● +	- ● ● ● ● +	- ● ● ● ● +	- ● ● ● ● +
						

- ***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

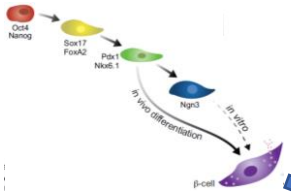
- Nanotechnologies for imaging cellular transplants and regenerative processes in vivo in a pig model for type 1 diabetes treatment
- Collaboration of Hungarian, German, French, Belgian companies and universities
- Coordinator: Andras Dinnyes /**BioTalentum Ltd**
- Budget: **7 M EUR** /5 years

Partners:

Structure of the project

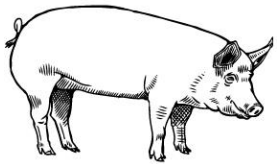
integration of Nano- and Biotechnology for Beta-cell and Islet Transplantation

Beta-cell sources



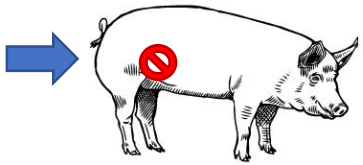
hiPSC-derived beta-cells

or



pig xeno-islets

BioArtificial Pancreas **MAILPAN® device**



Genetically modified humanised preclinical diabetic pig model (MIDY)

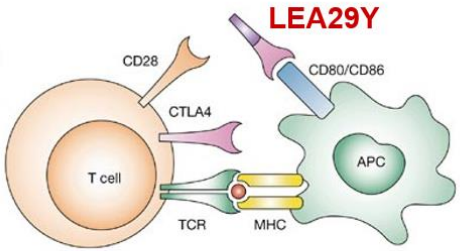
Monitoring

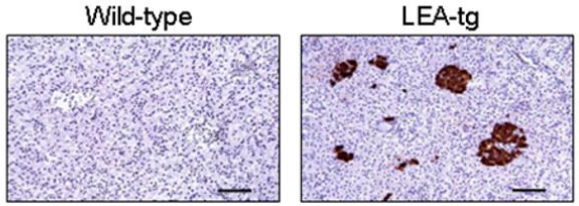
Optoacustic imaging

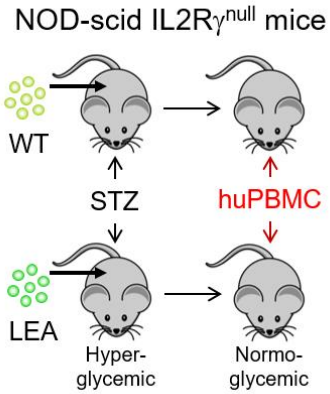
SPECT/CT

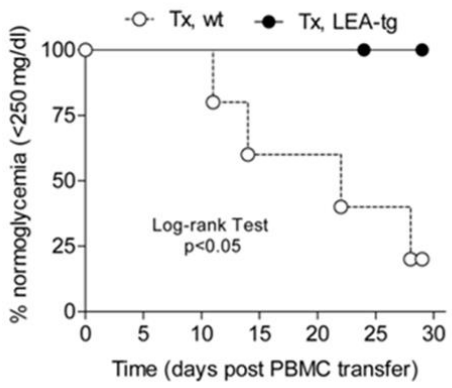
Nano-marker material development

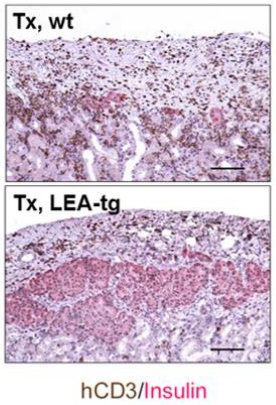
Supply of porcine neonatal islet-like cell clusters (NICCs)
INS-LEA29Y transgenic pigs as donors for islet XT







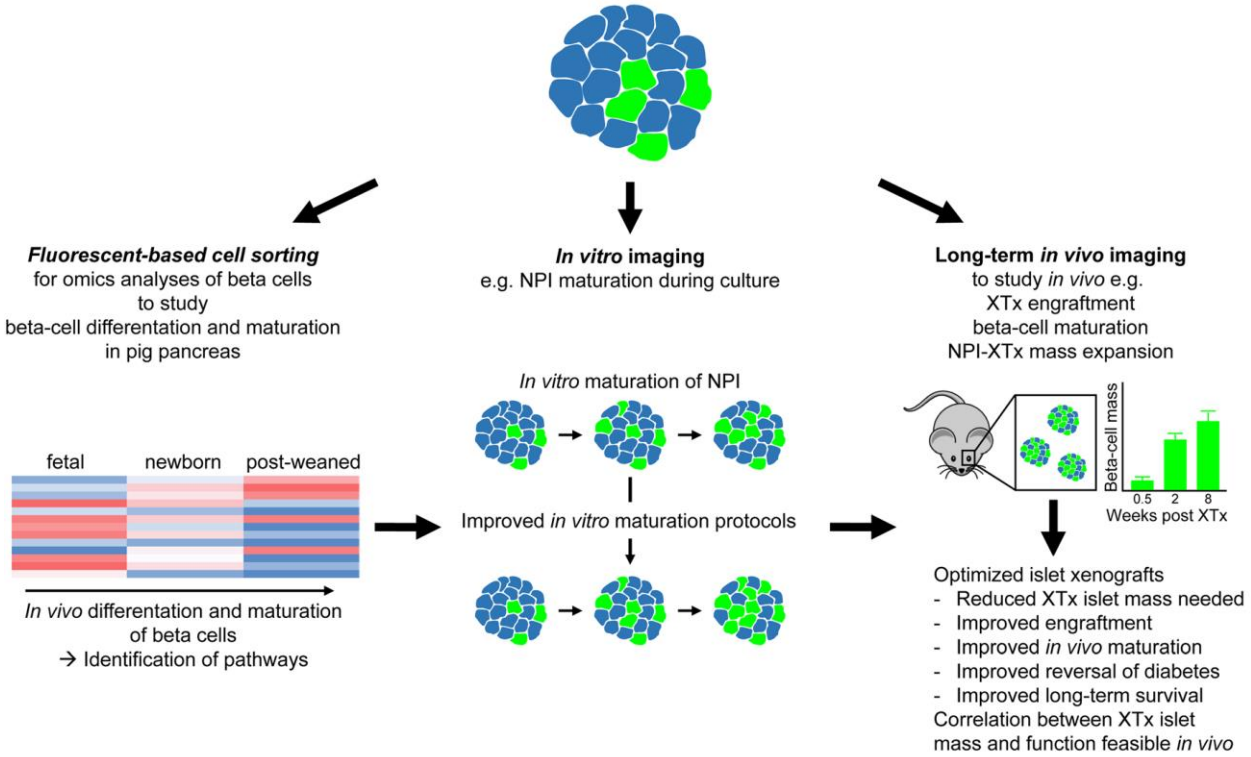




Klymiuk et al., Diabetes 61, 1527-1532 (2012)

H2020-NMBP-2016-2017, NMBP-15-2017- **I**ntegration of **Nano**- and **B**iotechnology for beta-cell and **i**slet **T**ransplantation- Project No.: 760986

INS-eGFP transgenic pigs for islet studies



Kemter et al., Diabetologia 60, 1152-1156 (2017), Kemter et al., Curr Diab Rep. 2018 Sep 18;18(11):103

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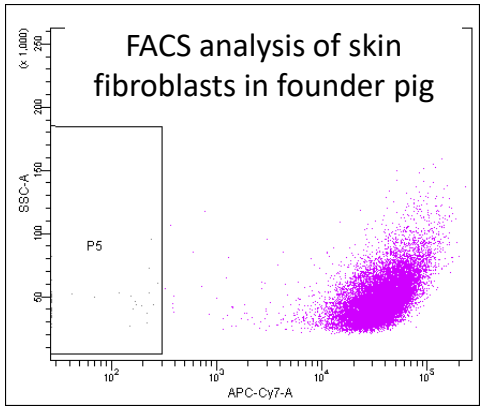
Ubiquitous reporter gene expression in iRFP transgenic pigs

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



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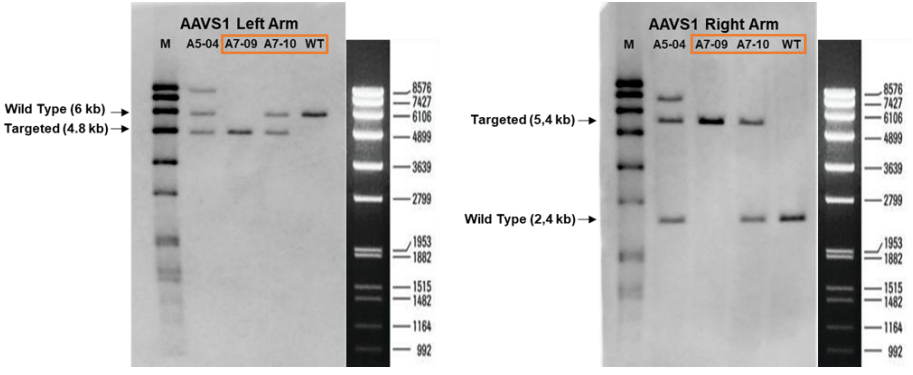
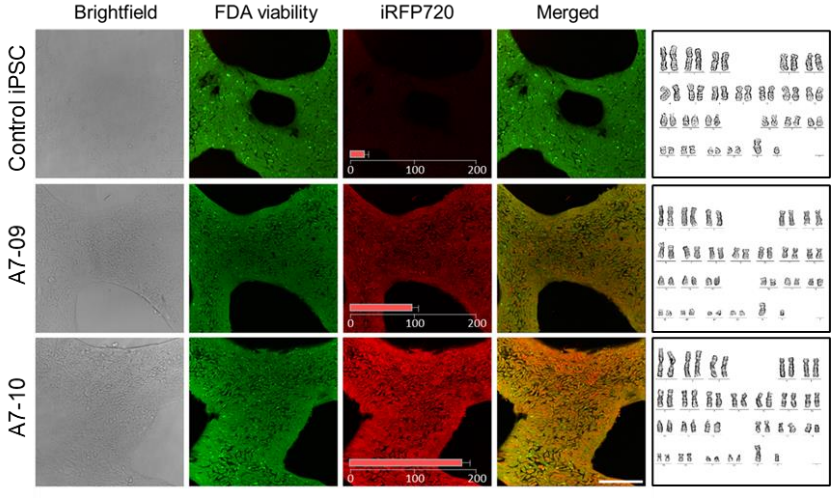
CRISPR/Cas9 targeting of human iPSCs to create iRFP720 reporter cell lines

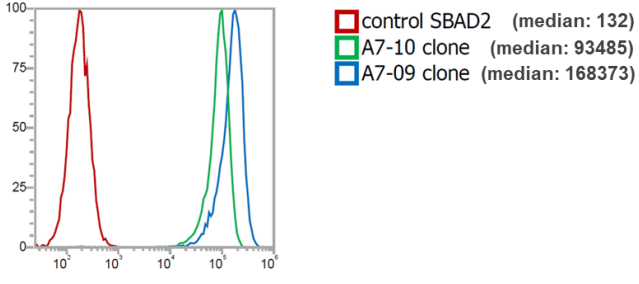
A) Southern blot analysis



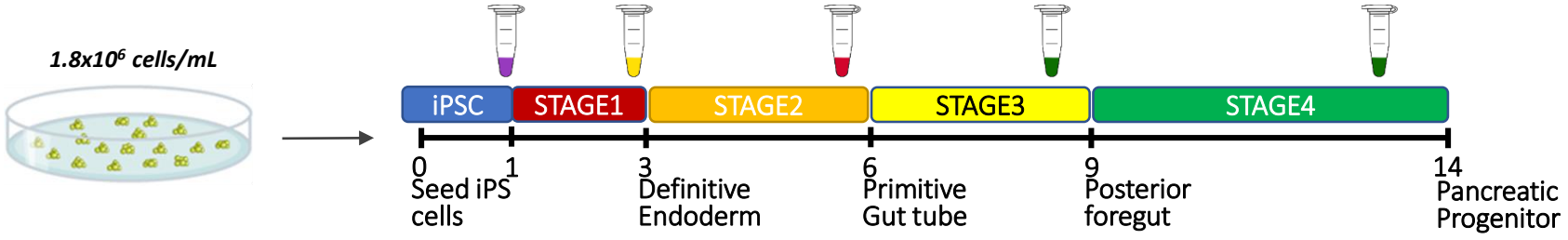
B) Live cell imaging - karyotyping

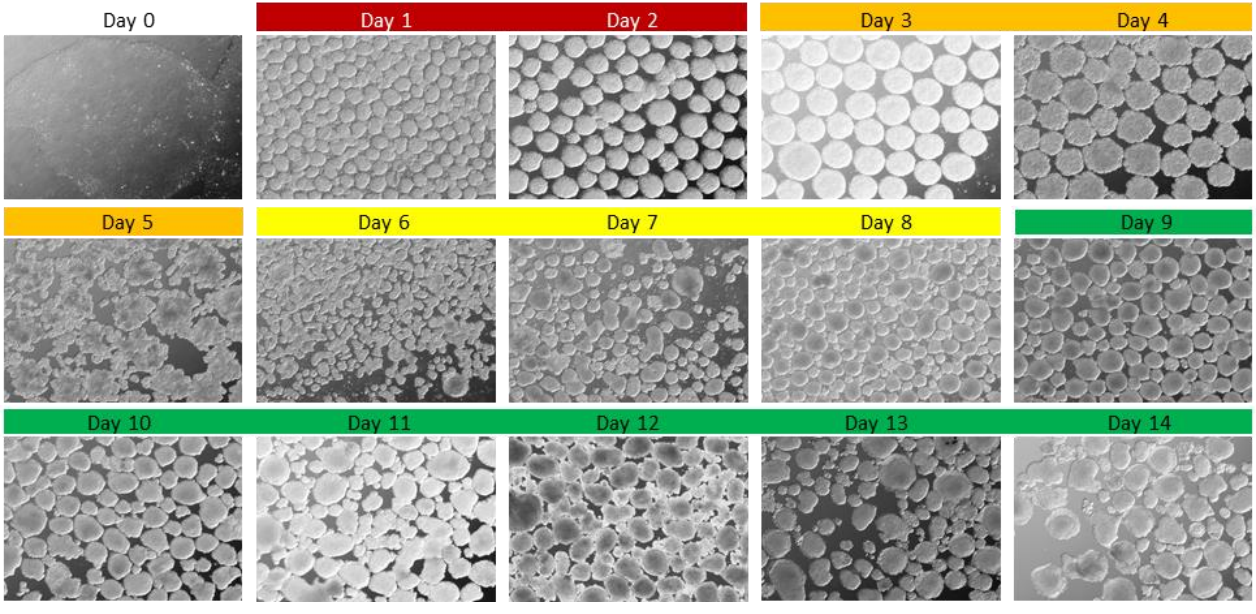


C) FACS analysis



Pancreatic progenitor differentiation with STEMdiff™ Pancreatic Progenitor Kit in 3D culture

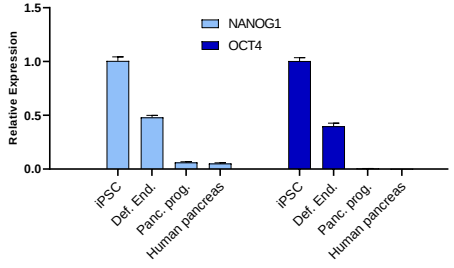




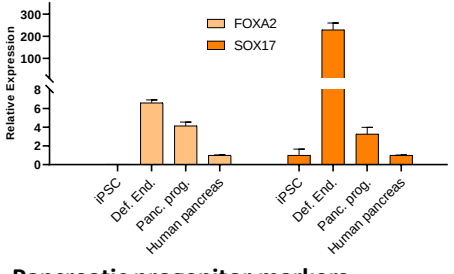
Samples at different stages of differentiation were collected for characterization (RT-qPCR, ICC, insulin)

Pancreatic progenitor differentiation with STEMdiff™ Pancreatic Progenitor Kit in 3D culture

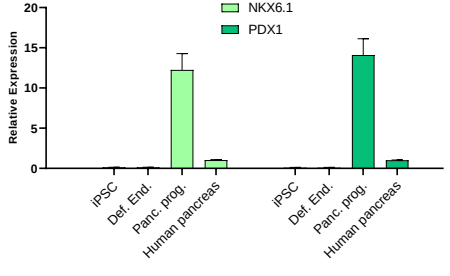
Pluripotency markers

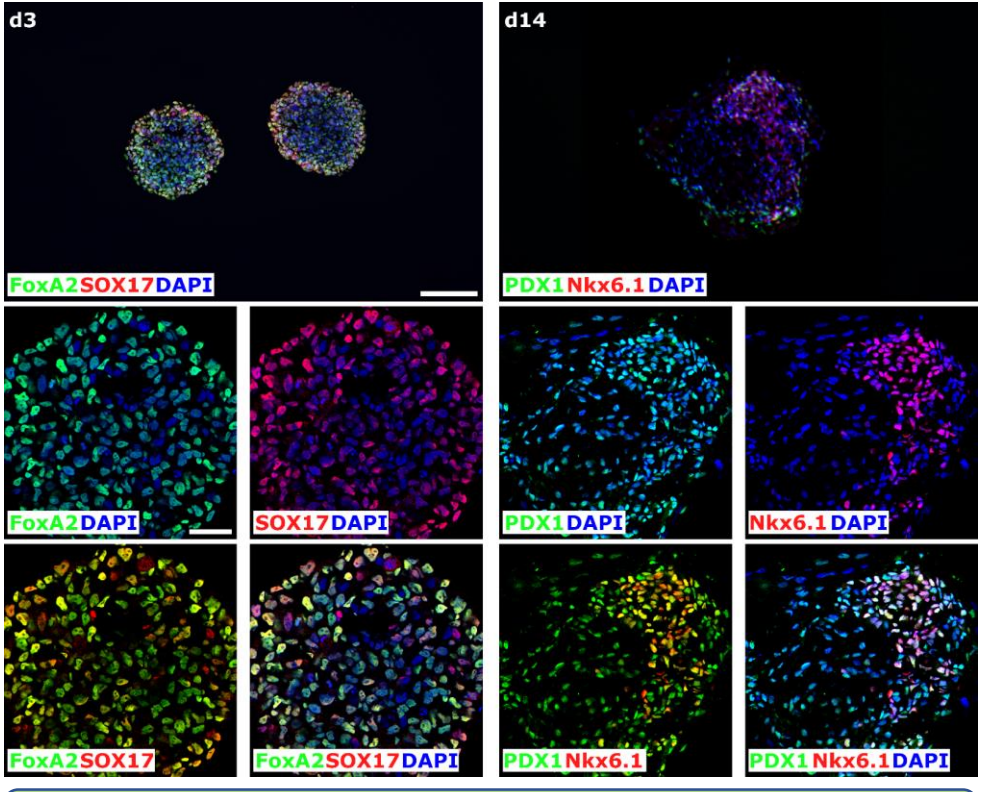


Definitive Endoderm markers



Pancreatic progenitor markers



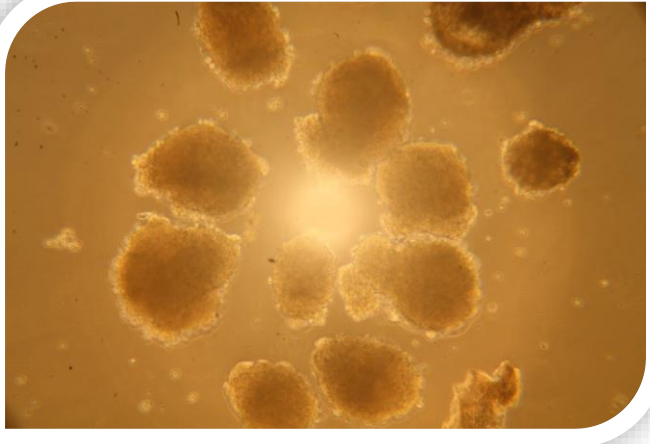


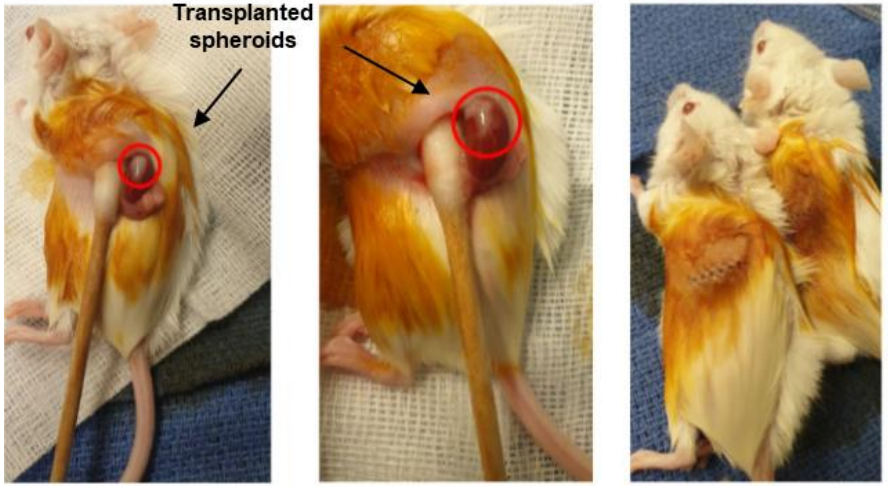
Insulin content: 8.16 µU/spheroid(day14)
Insulin secretion: 1018 ± 141 µU/600 spheroids(day14)/hour in G15

hiPSC derived pancreatic progenitor transplantation and *in vivo* maturation in mice

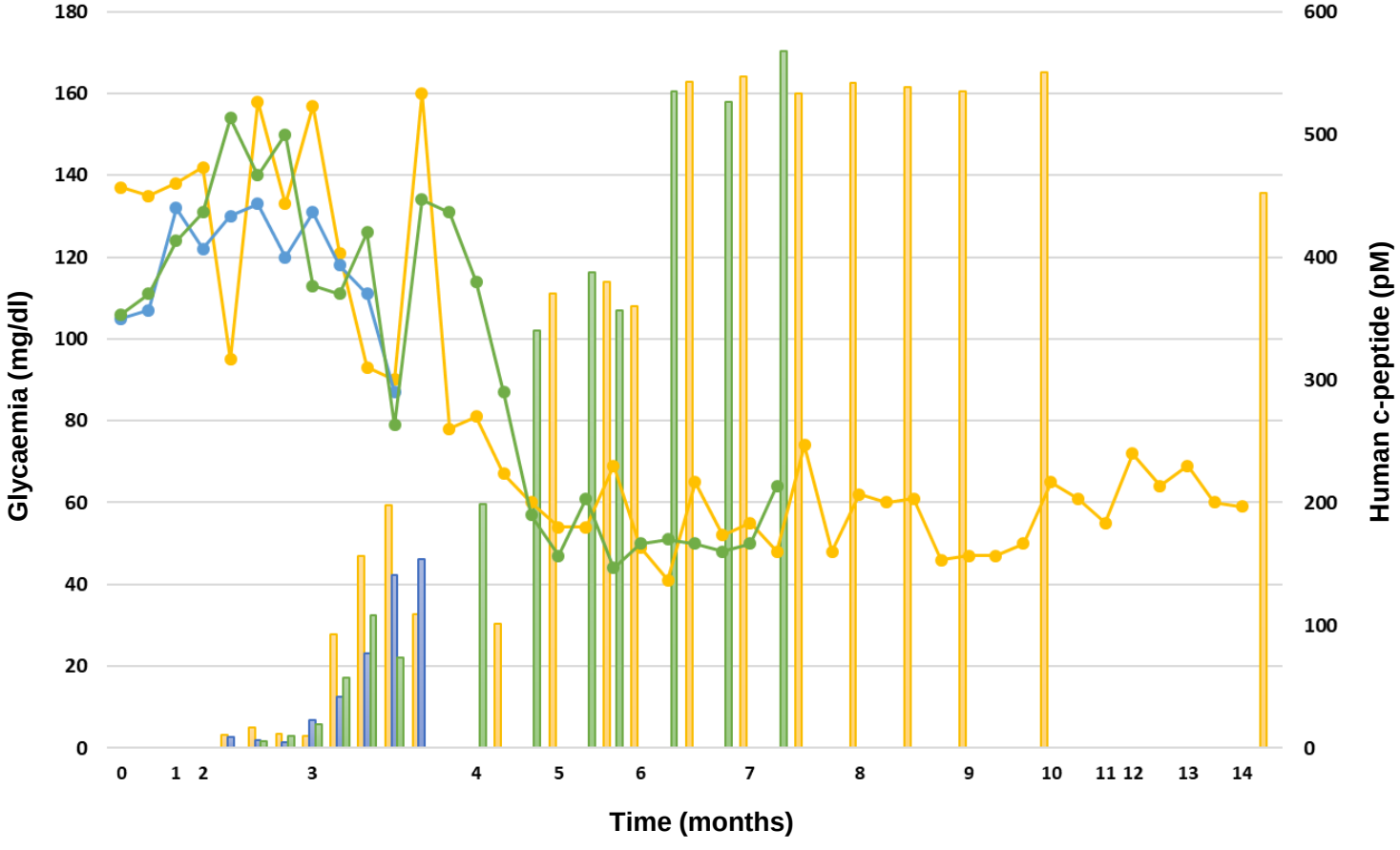
- **Immature human beta-cell (pancreatic progenitor spheroid) implantation into non-diabetic SCID mice**
- **Implantation under the left kidney capsule (600 spheroids/mouse; n=3)**

Stage-4(Day14) pancreatic progenitor spheroids

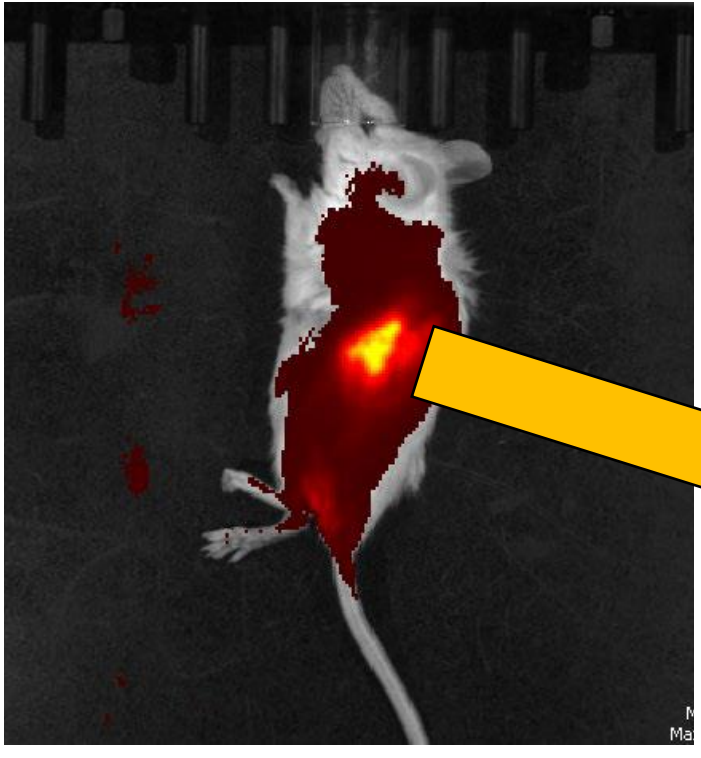




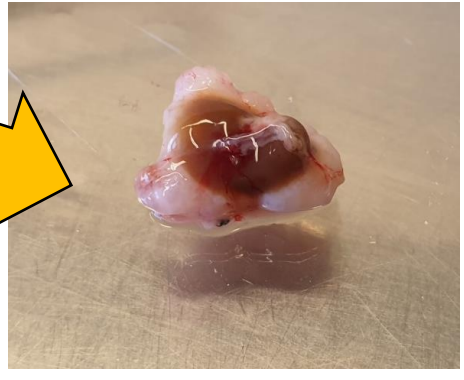
Follow-up of glycaemia and human c-peptide levels in sera



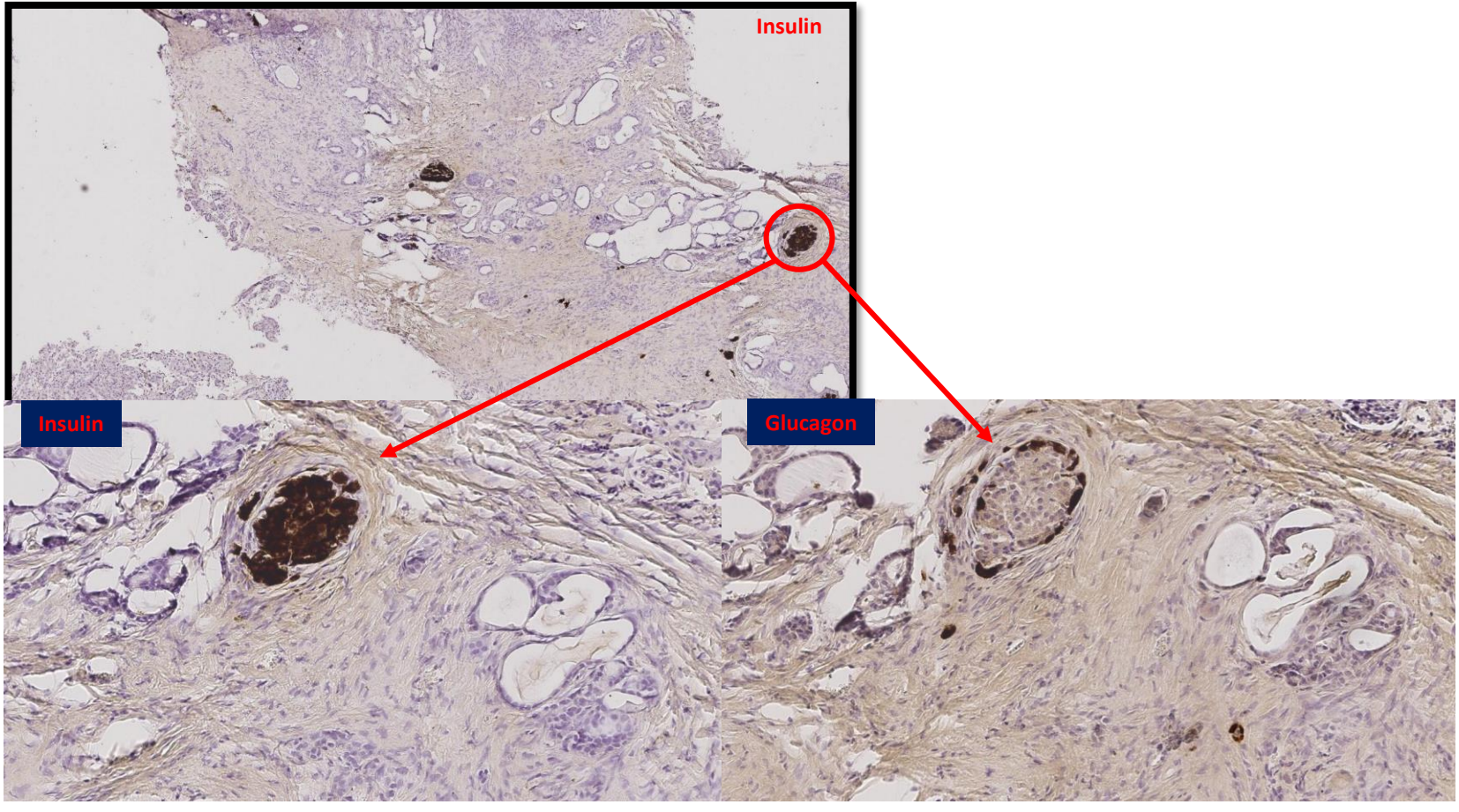
In vivo detection of iRFP720 expression



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



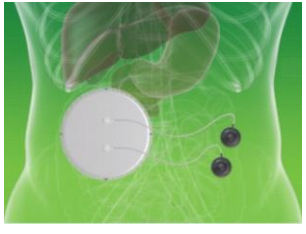
Defymed.

Mailpan®

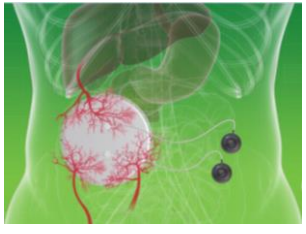
Three functions are absolute prerequisites :

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

1) Implantation in abdominal site T=0

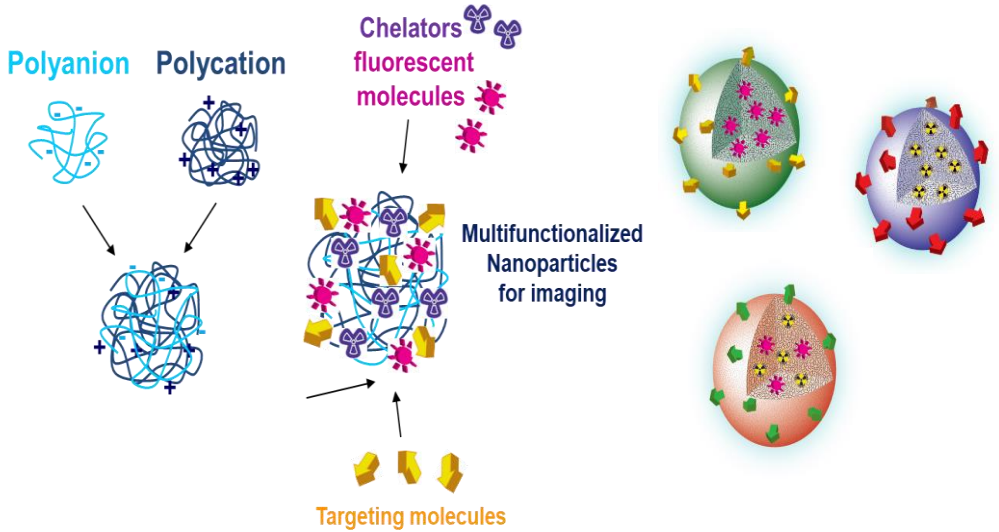


2) Pre-vascularization of the device T= 6-8 weeks



3) Injection of cells through chamber T= 2 months

Strategy to generate multimodal nanoparticles (Milano Bicocca University/IT and BBS Nanotechnology/HU).



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.

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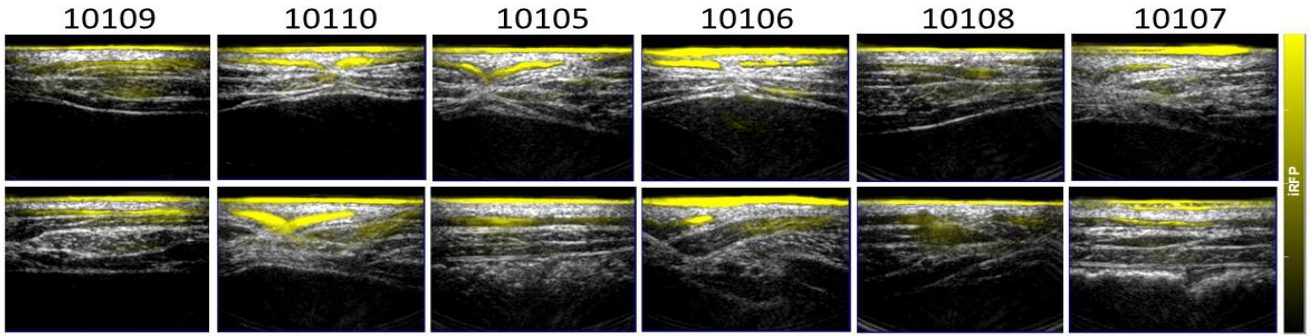
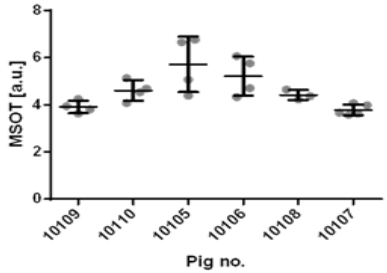
Project No.: 760986

In vivo MSOT analysis of iRFP expression in CAG-iRFP720 transgenic founder pigs (LMU and iThera, Munich/Germany).

Optoacustic imaging machine





- 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

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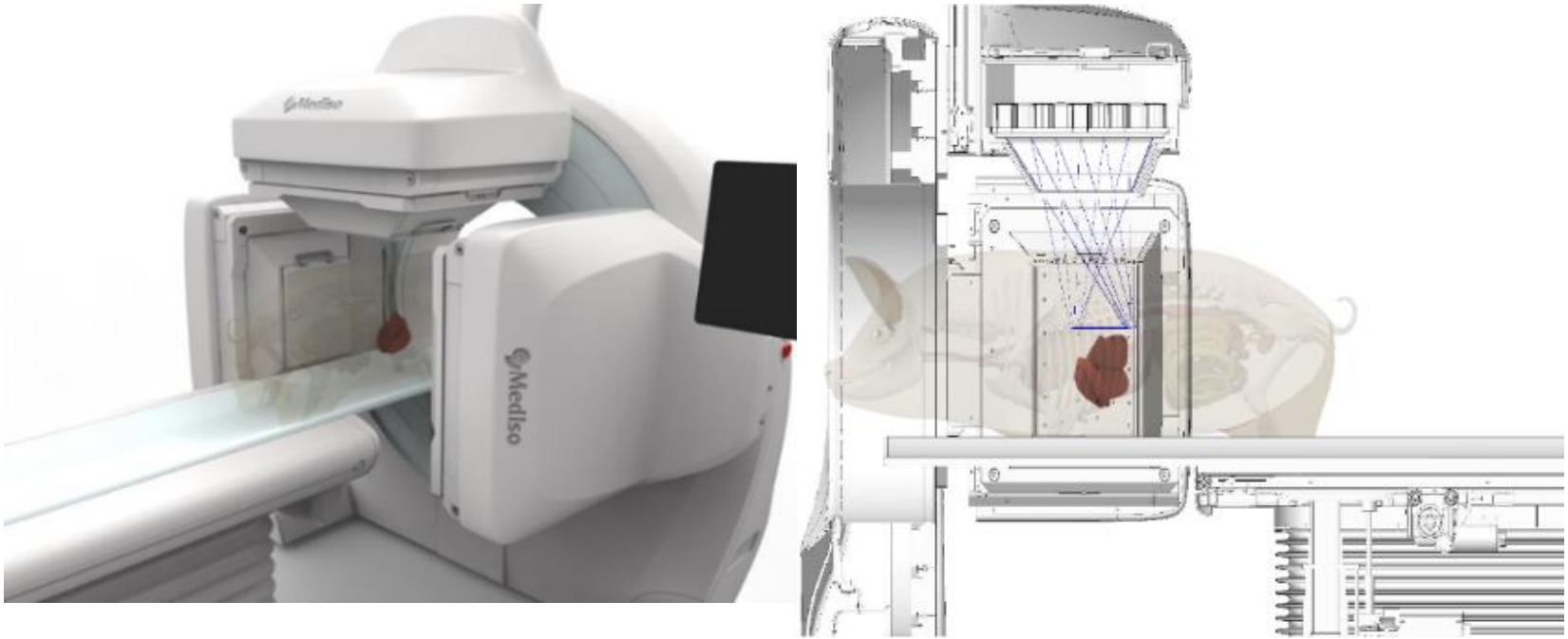
The newly developed AnyScan TRIO system with the new multi-pinhole collimator design focusing on the pig's liver (Mediso/Hungary)



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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

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