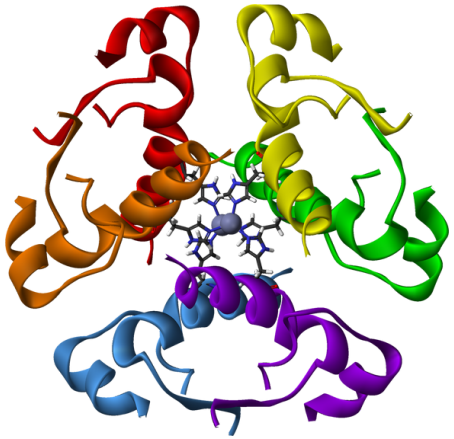
Treatment of type 1 diabetes via α-cell insulin production



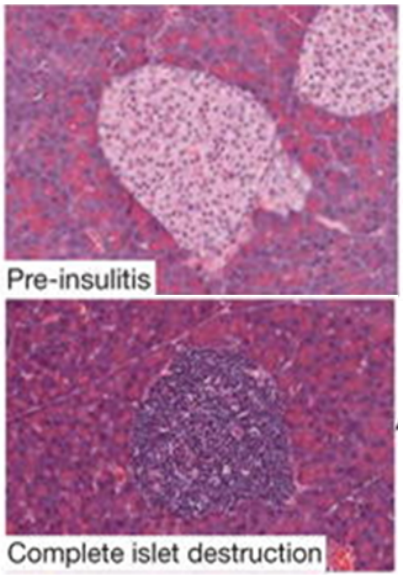
Gene Therapy
Professor Isabella Saggio
A.Y. 2018/2019

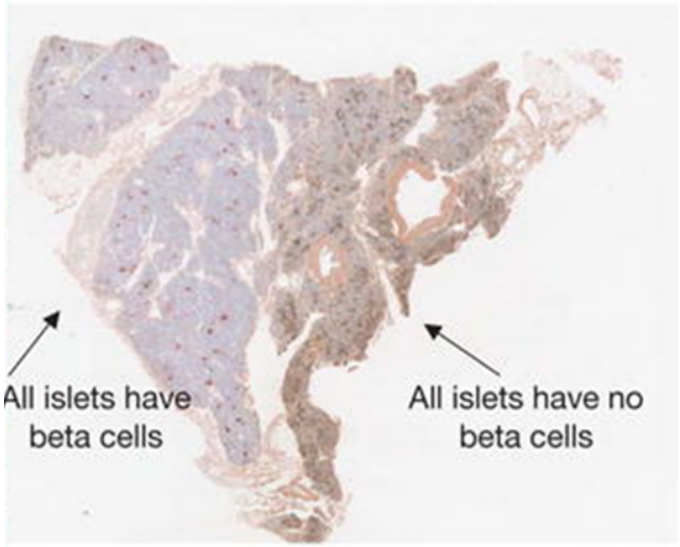
Bertozzi Alessia
Buccioli Lucia
Tosato Federica

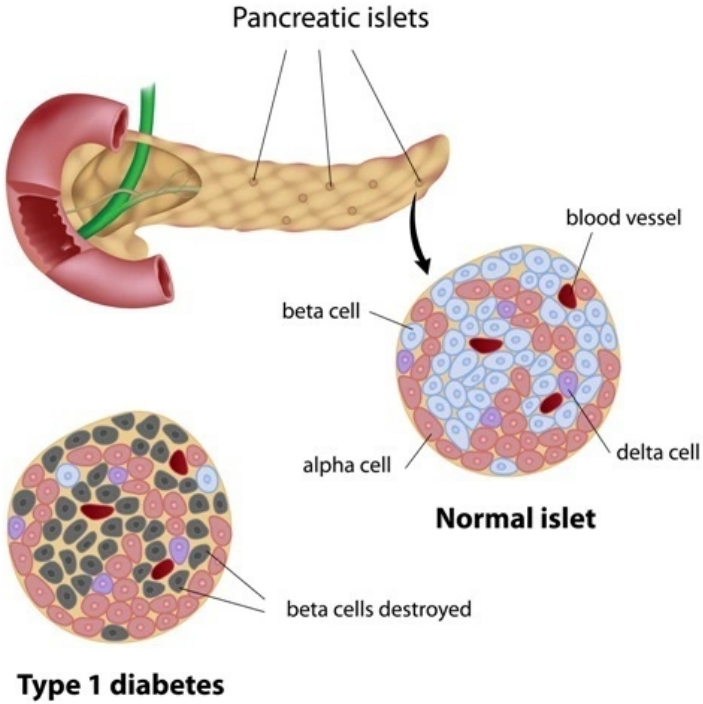
SAPIENZA
UNIVERSITÀ DI ROMA

Background of type 1 diabetes

- Autoimmune disease causing pancreatic β-cell ablation
- Insulin deficiency leading to hyperglycemia







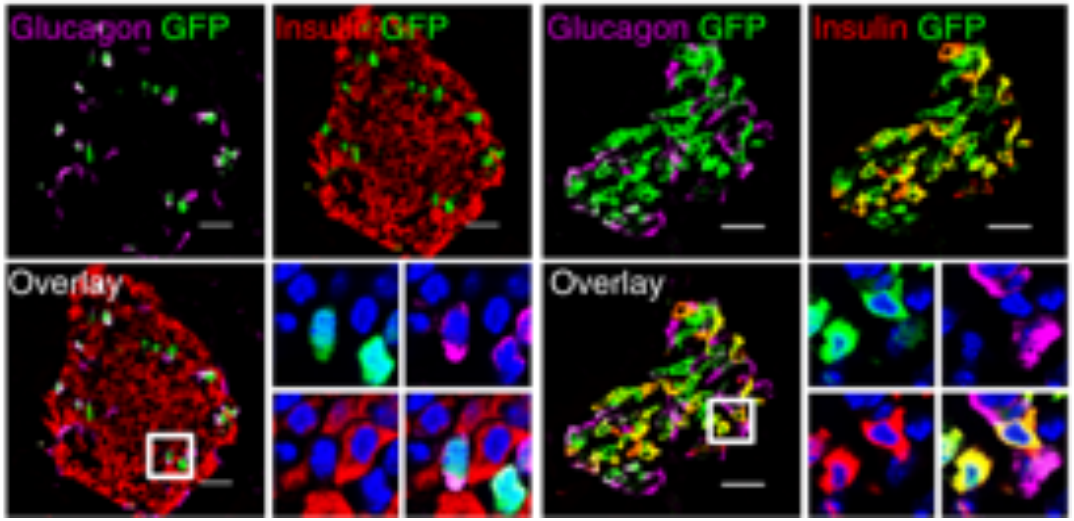
Bluestone J.A. et al. *Nature* (2010)

Our goal

Find a possible therapeutic approach in human

How?

Inducing insulin production and Pdx1 overexpression in pancreatic α-cells

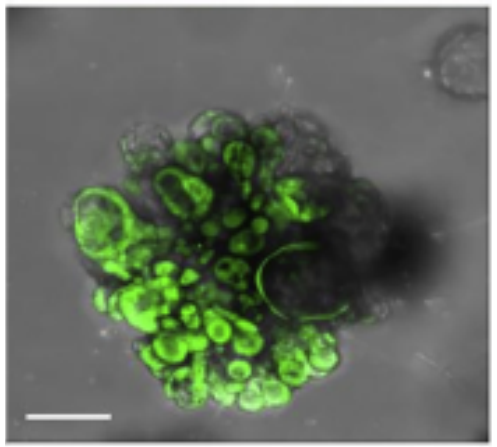


Cigliola V. et al. *Nature* (2018)

Our models

Non Obese Diabetic Mouse

Spontaneous T1D model



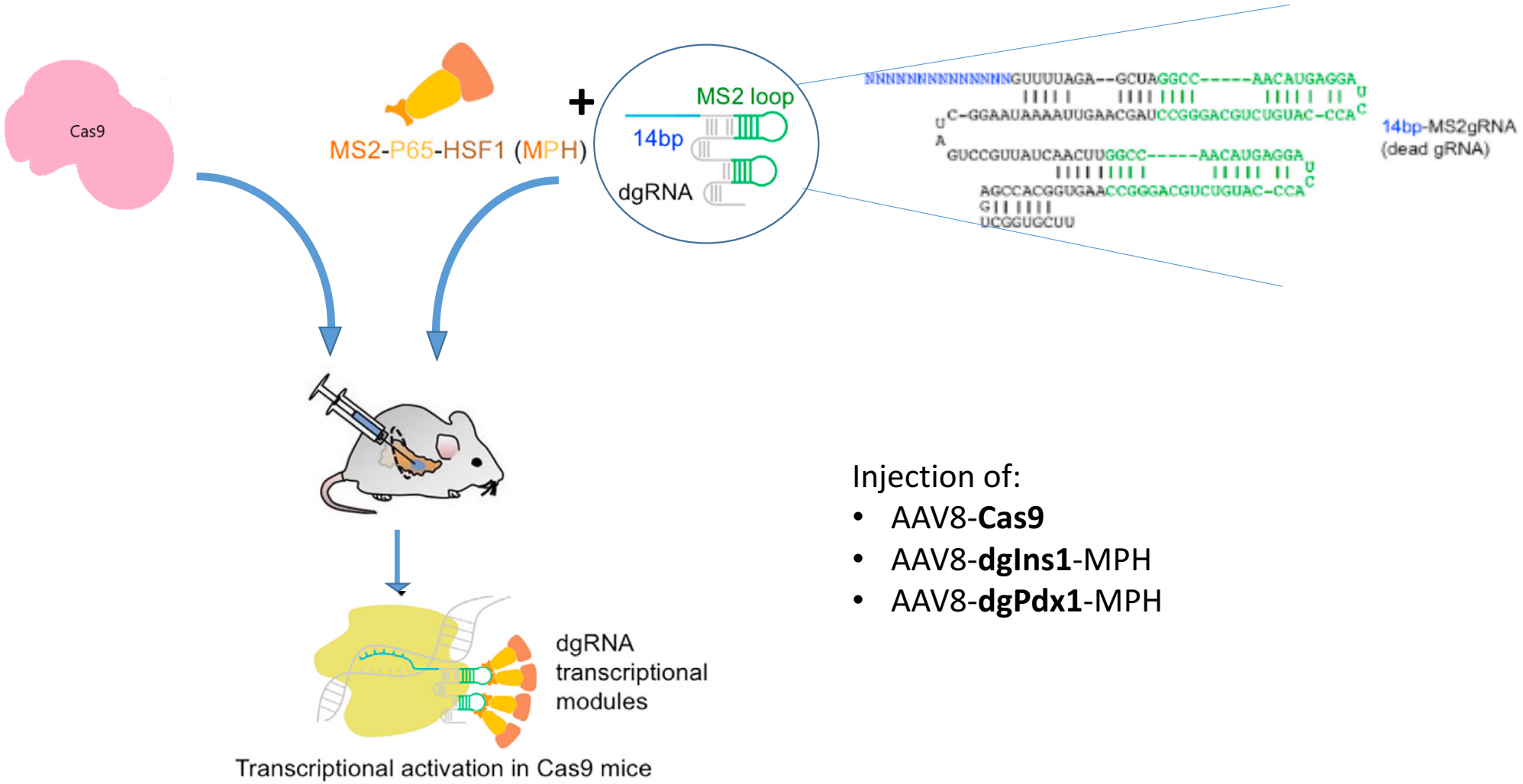
Human pancreatic organoid

Cell-derived *in vitro* 3D organ model

The Jackson Laboratory

Loomans C.J.M. et al. *Stem Cell Reports (2018)*

In vivo therapeutic approach

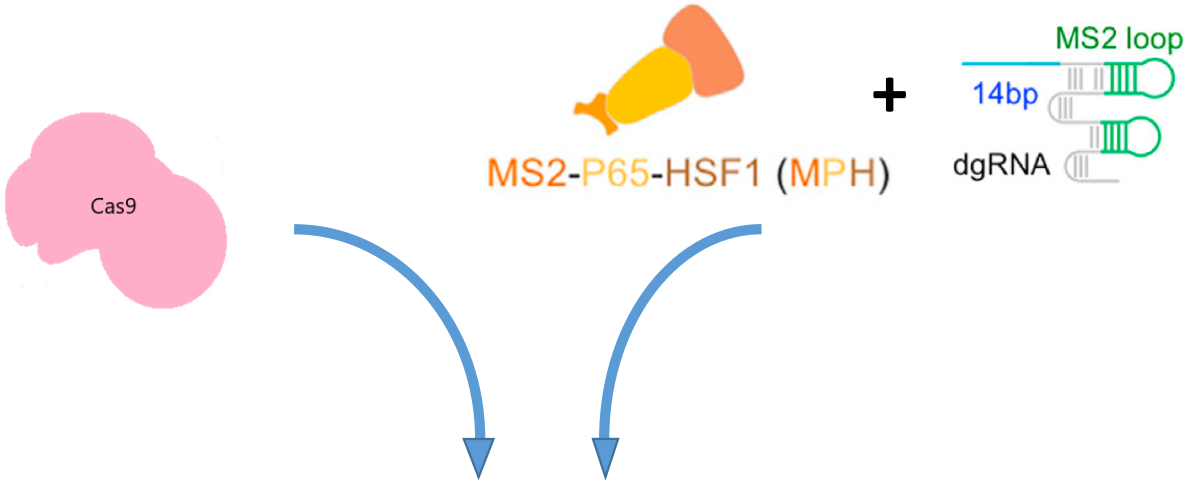


Injection of:

- AAV8-**Cas9**
- AAV8-**dgIns1**-MPH
- AAV8-**dgPdx1**-MPH

Liao H. et al. *Cell* (2017)

In vitro therapeutic approach

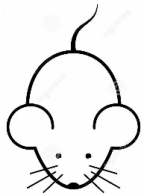


Human pancreatic organoid culture

Transfection of:

- AAV8-**Cas9**
- AAV8-**dgIns**-MPH
- AAV8-**dgPdx1**-MPH

Delivery system of gRNAs

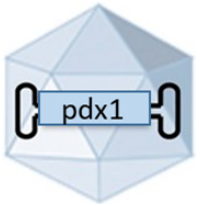


sgRNA	Ins1 (5'->3')
1	GGGACATCAATATT
2	TCTACTTAGTCTTA
3	TATAGTCTTAATAA
4	ACCTTCTTCATCTT

AAV8

sgRNA	Ins (5'->3')
1	GCTGAGGCTGCAAT
2	GCACCAGGGAAATG
3	ATGACCCGCTGGTC
4	TCTGGCCACCGGGC

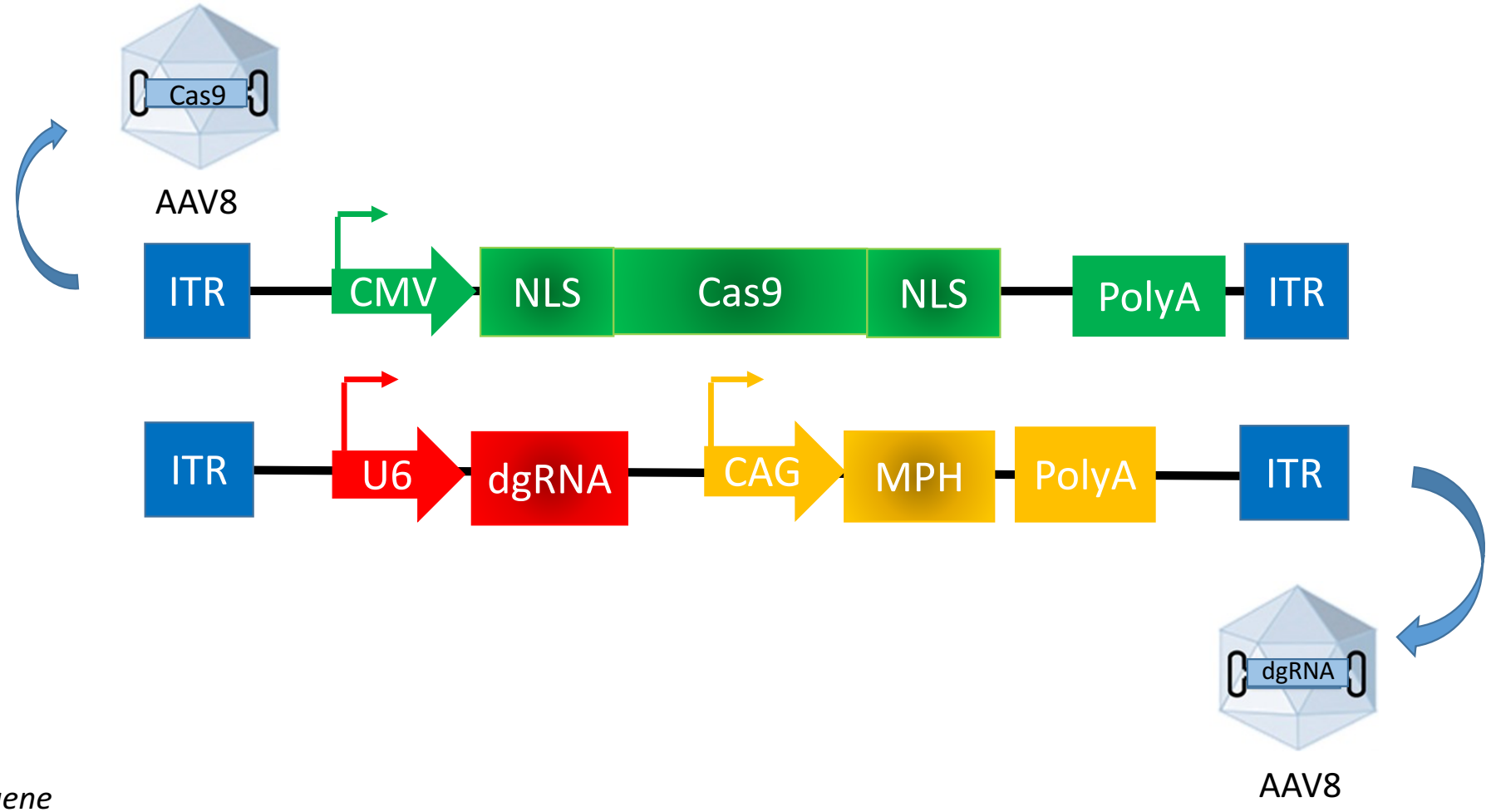
sgRNA	Pdx1 (5'->3')
1	TGTGCGCCCCGTTT
2	AAGCCTCCTTCTTA
3	GCCGTGCCATAGGC
4	GTGCAGGTGTTCGC



sgRNA	Pdx1 (5'->3')
1	GTGCTCCCCAAAAT
2	AAGAGGCTAGGCCC
3	GGCCGCCGCACCAT
4	CGCACTAAGAGGCT

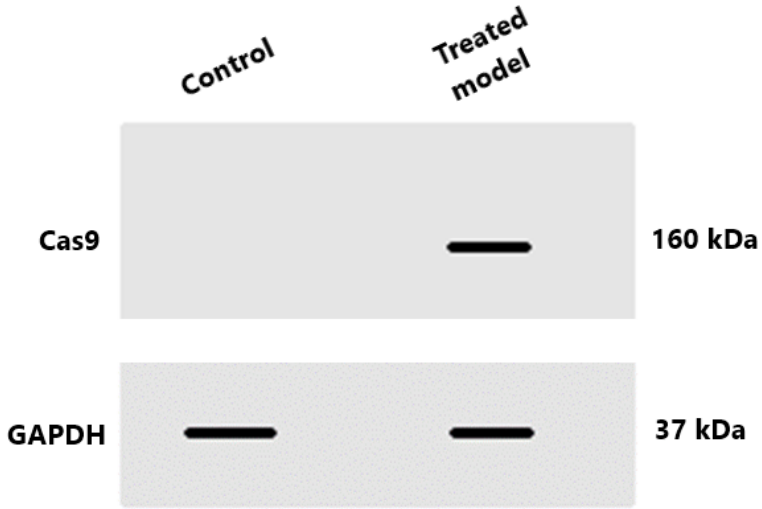
CHOPCHOP

Delivery system

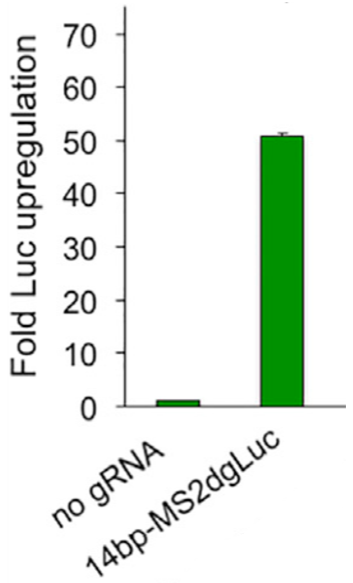


Addgene

Predictive results



Expression of Cas9 in both injected NOD mouse and transfected human organoid.

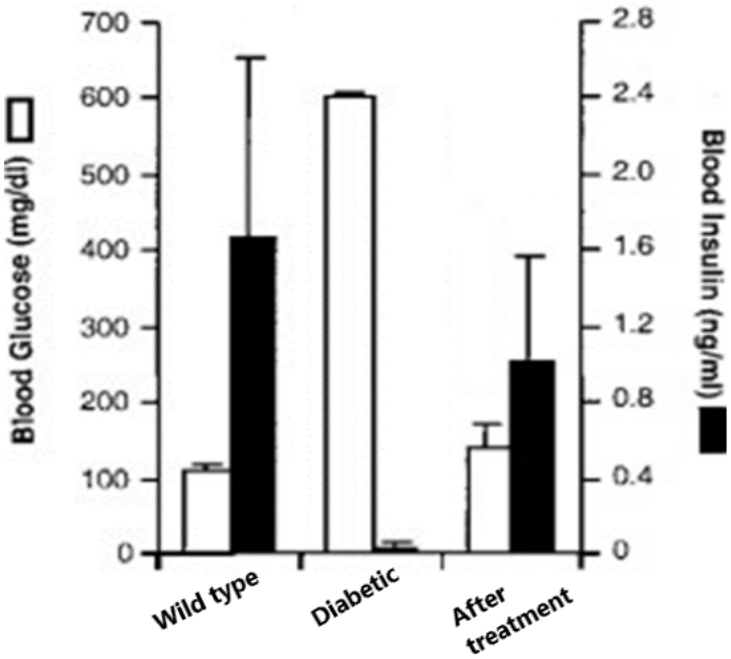


14bp-modified dgRNA improves Luciferase gene activation in α cells.

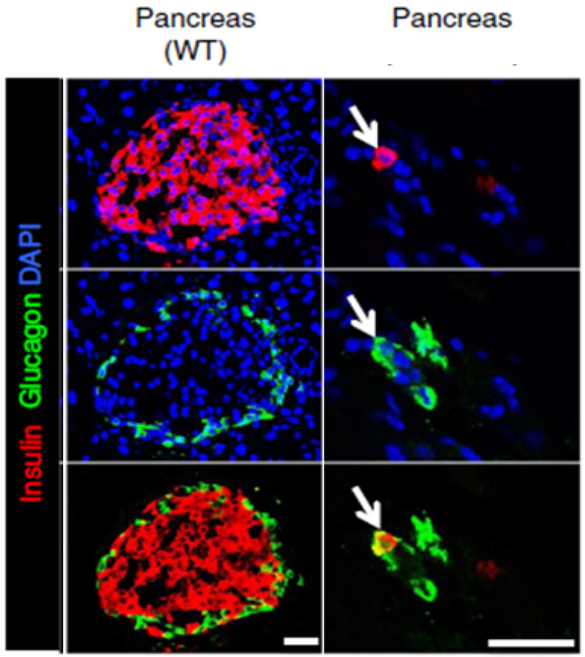
Adapted from Liao H. et al. *Cell* (2017)

Predictive results

In vivo



Glucose-responsive insulin production.

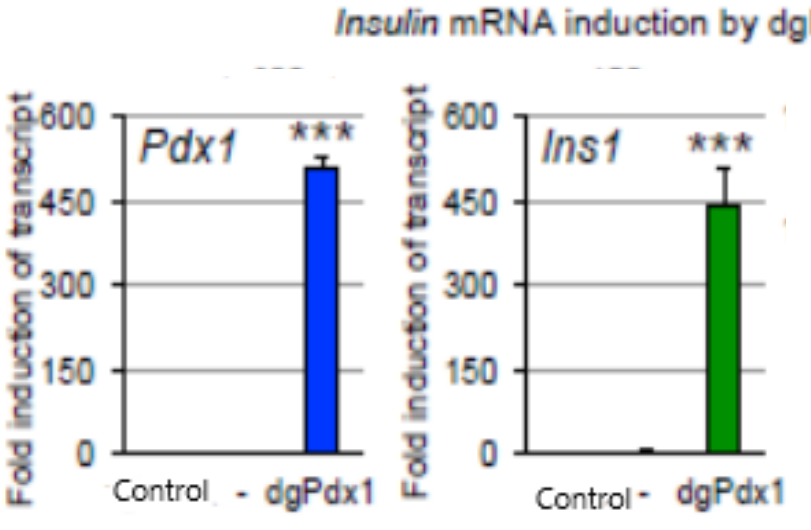


Immunofluorescence of bihormonal glucagon-insulin α-cells.

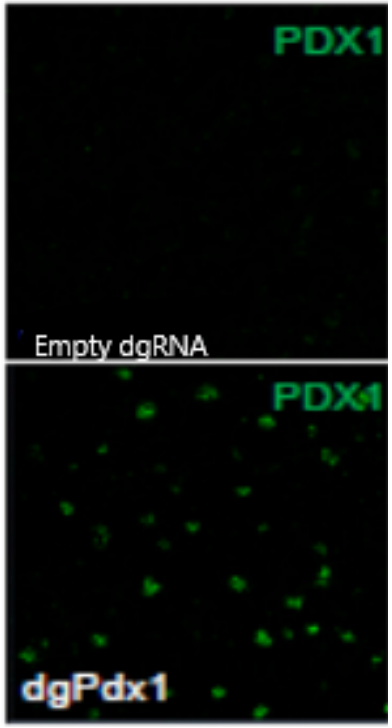
Adapted from Cigliola V. et al. *Nature* (2018)

Predictive results

In vivo



Pdx1 gene induction analysis (qRT-PCR).

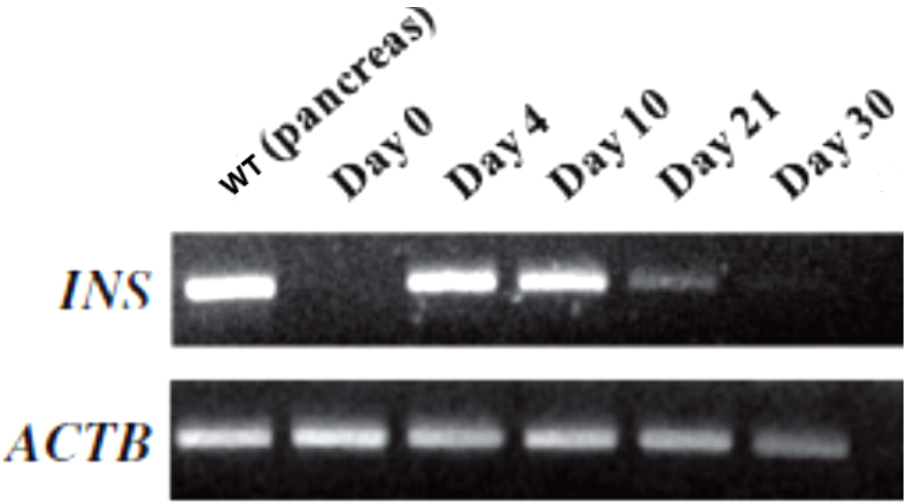


Immunofluorescent analysis of PDX1 protein level in pancreas tissue.

Adapted from Liao H. et al. *Cell* (2017)

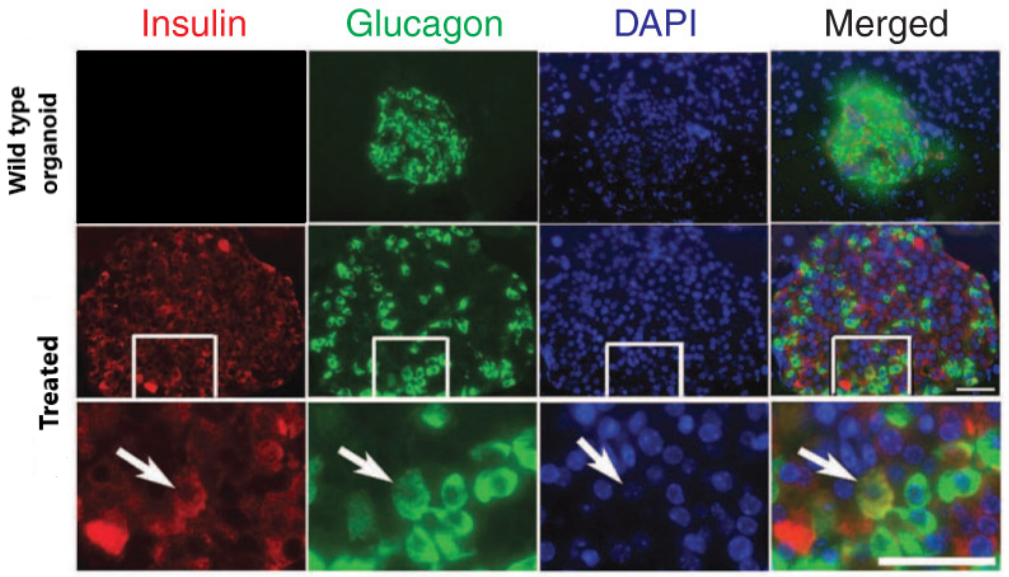
Predictive results

In vitro



Ins gene expression in human pancreatic organoid by RT-PCR.

Adapted from Gimènez C.A. et al. *Gene Therapy* (2016)

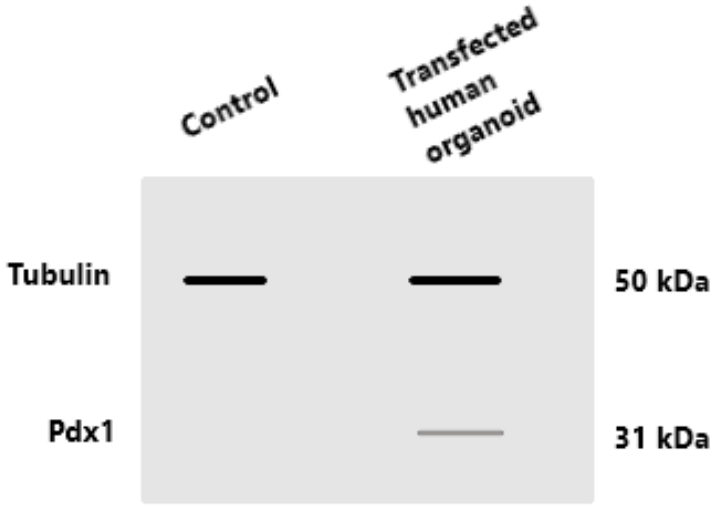


Immunofluorescence of insulin and glucagon in human pancreatic organoid.

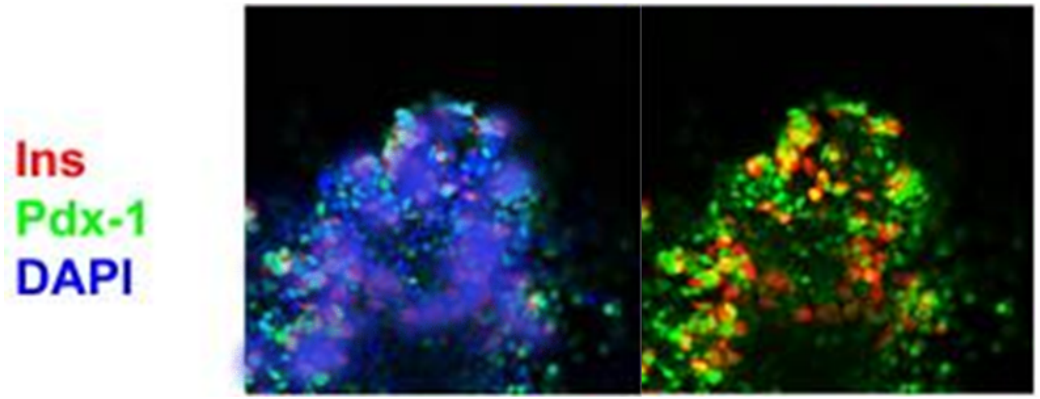
Adapted from Zhao C. et al. *Molecular therapy* (2015)

Predictive results

In vitro



Detection of PDX1 level.

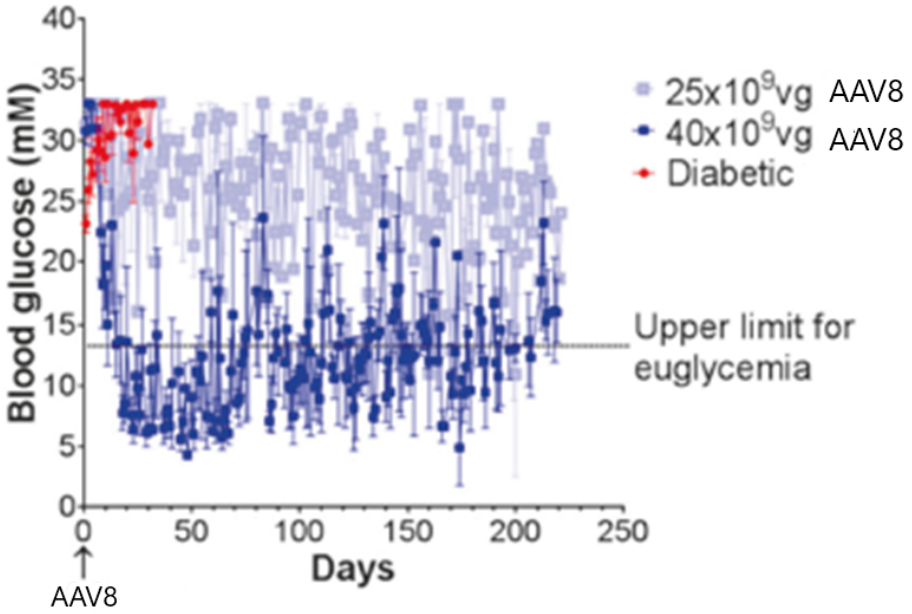


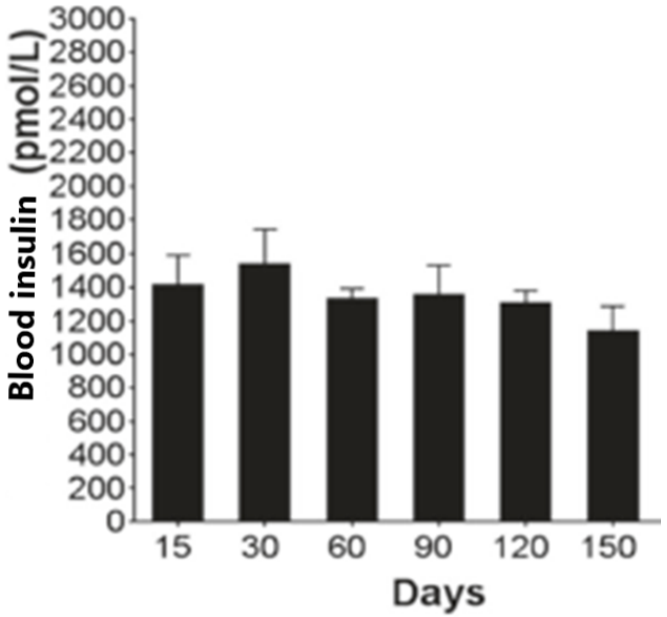
Immunofluorescence of PDX1 in human pancreatic organoid.

Adapted from Kubo A. et al. *PLoS* One (2011)

Long term predictions

In vivo





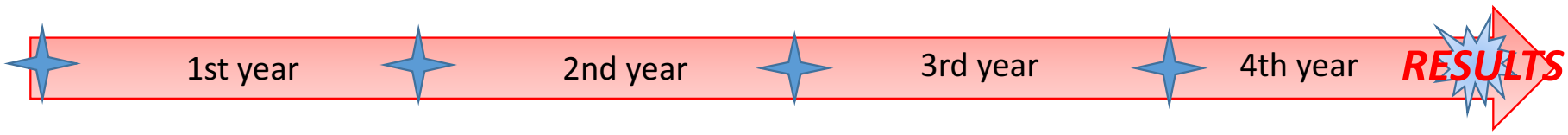
Adapted from Recino A. et al. *Nature* (2018)

Pitfalls and possible solutions

- Cas9 off-targets → new Cas9 types with enhanced specificity (eSpCas9)
- AAV specificity → Y447F +Y733F mutants of AAV8
- Restoration of autoimmune response against insulin-producing α cells →

Costs and time

Nod mice (x40)	1.550€ (Jackson laboratory)
Stabulation for mice	about 440€/month
Pancreatic organoids	1.400€
Cas9 vector	57€ each (Addgene)
dgRNA vector	57€ each (Addgene)
AAV8 (10^12 GC/mL x 20mL)	10.000€ (Addgene)
Immunofluorescence kit	516€ (ThermoFisher)
Western blot kit	about 1500€
Immunohistochemistry antibodies	about 300€ x Ab + secondary Abs
qRT-PCR kit	690€ (Sigma-Aldrich)
Luciferase	300€
Molecular Biology Lab Apparatus	500€



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