SAPIENZA
UNIVERSITÀ DI ROMA

PD-L1 genetic overexpression: a new strategy for Type 1 Diabetes Mellitus

Andrea Galeone, Antonino La Rocca, Vittoria Peretti, Rebecca Rossetti

Prof.ssa Isabella Saggio

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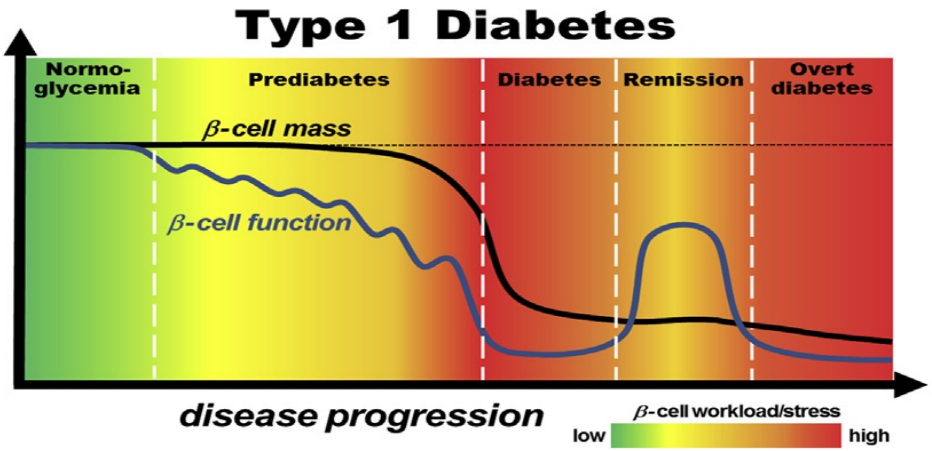
Introduction

Clinical features T1DM

- Autoimmune reaction against pancreatic β-cells
- Lack of insulin production and hyperglicemia
- At the onset of the disease, 30-40% of pancreatic islets are alive



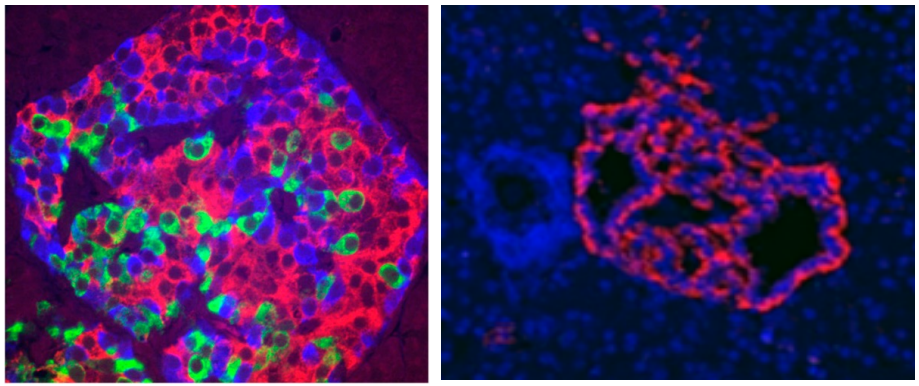
(Chen et al., 2017 Molecular Metabolism)

Current treatments

- Frequent monitoring of blood glucose levels and synthetic insulin administrations
- Clinical pancreatic islet transplantation

T1DM and cognitive dysfunction

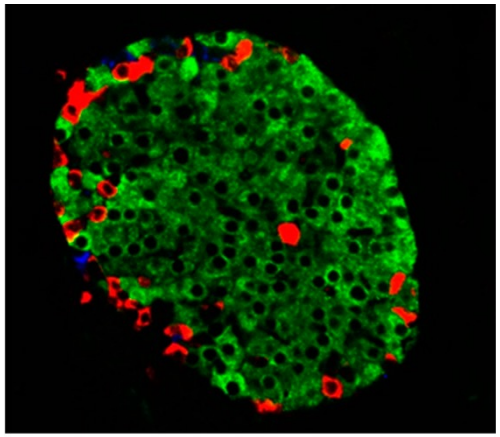
- Slowing of mental speed
- A diminished mental flexibility
- Lerning and memory are spared



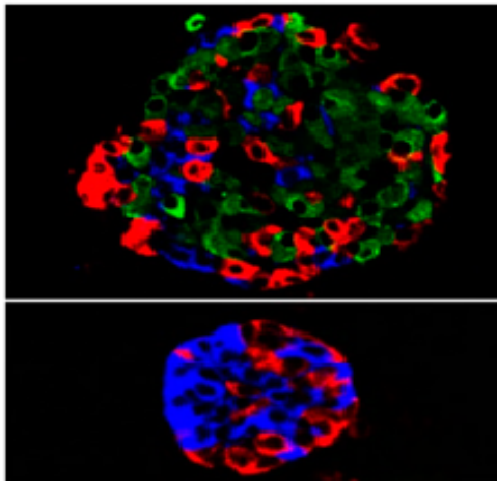
(Allison L.O'Kell et al., 2017 Diabetes)

Non-obese diabetic mice (NOD)

WT



NOD



(Allison L. O'Kell et al., 2017 Diabetes)

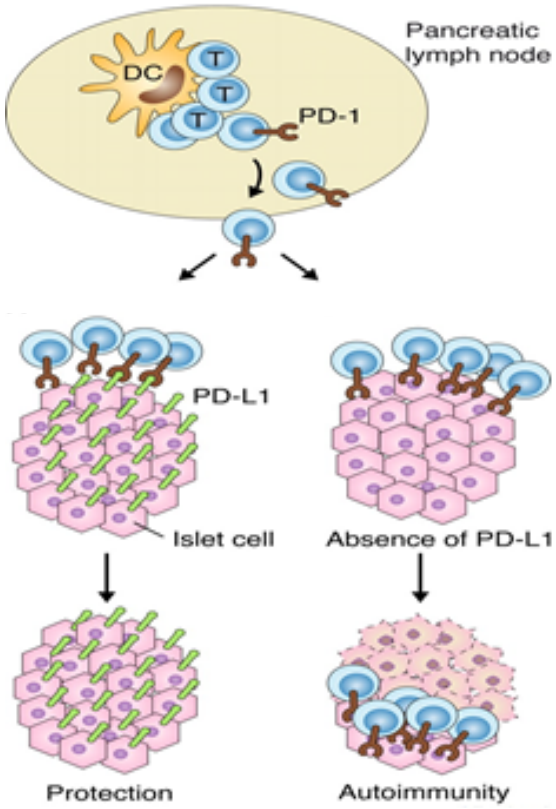
Why?

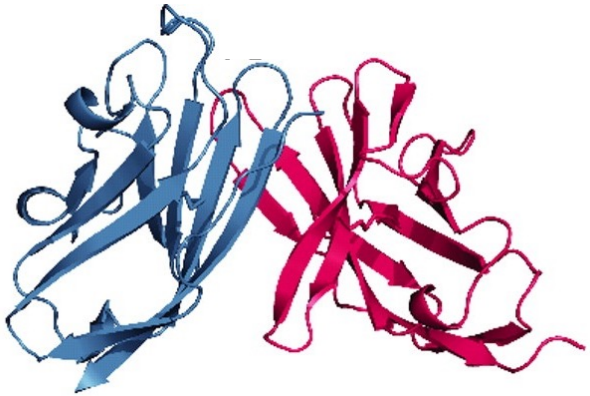
Striking resemblance to human T1DM:

- Pathophysiology
- Disease development
- **Autoimmune rejection of islet transplants**
- MHC class 2 share structural similarities

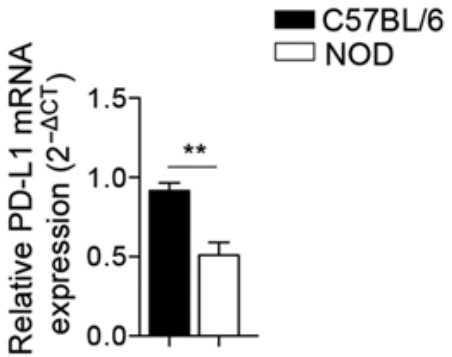
(King et al., 2012 British Journal of Pharmacology)

PD-L1/PD-1 pathway





PD-L1 **PD-1**



Islet cells protect themselves by expressing PD-L1. Binding of PD-L1 to PD-1 receptor downregulates T-cell effector function.

(Natalia Martin-Orozco et al., 2006 JEM)

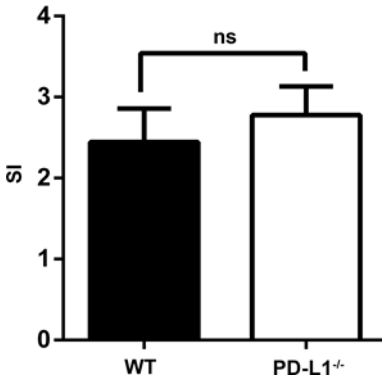
(Ben Nasr et al., 2017 Science Translational Medicine)

RESEARCH ARTICLE

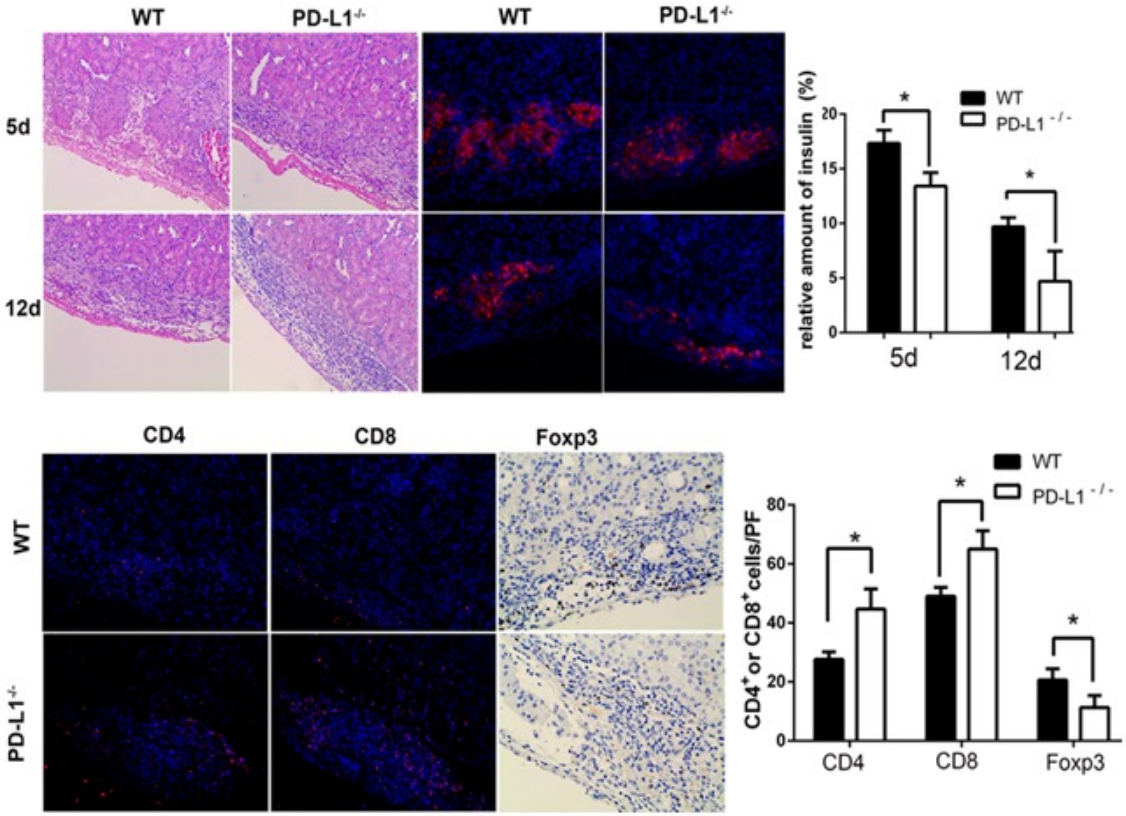
PD-L1 Deficiency within Islets Reduces Allograft Survival in Mice

Published: March 18, 2016

Dongxia Ma[1], Wu Duan[2], Yakun Li[1], Zhimin Wang[1], Shanglin Li[1], Nianqiao Gong[1], Gang Chen[1], Zhishui Chen[1], Chidan Wan[3]*, Jun Yang[1]*



PD-L1 KO doesn't show impairment of insuline release.

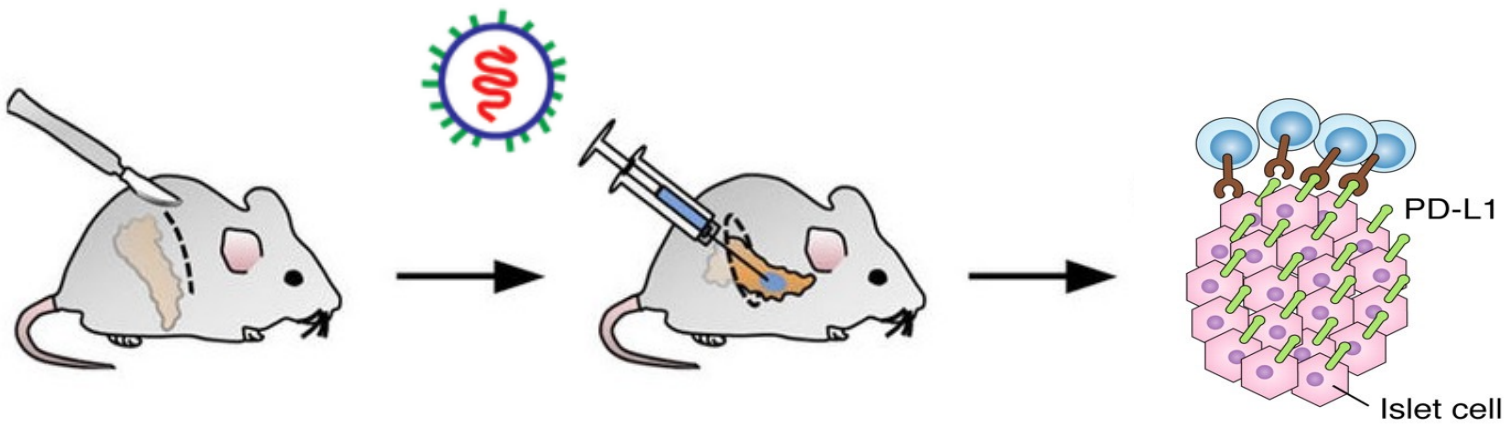


PD-L1 KO in grafted islets favored immune cell infiltration and decreased the islet function.

Objective

Restore the expression of PD-L1 in pancreatic islets to block the autoimmune reaction, using a modified 3^{rd} generation Lentiviral vector.

Experimental plan

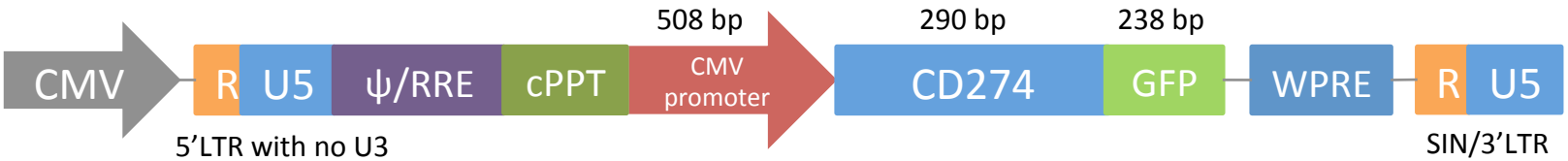


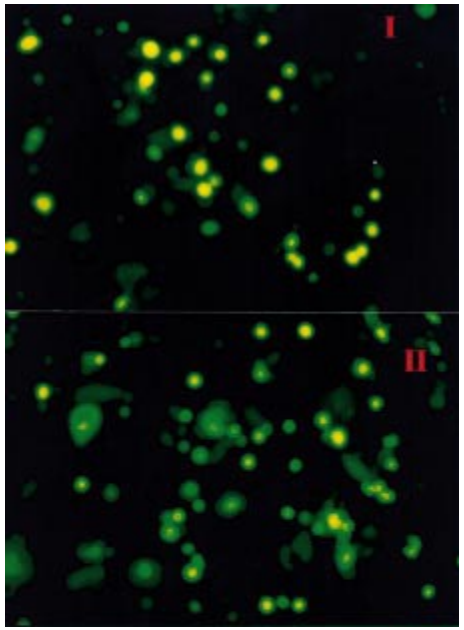
Laparotomy and pancreas mobilization

Lentiviral injection

PD-L1 overexpression

3rd generation Lentivirus





Ad-GFP

LV-GFP

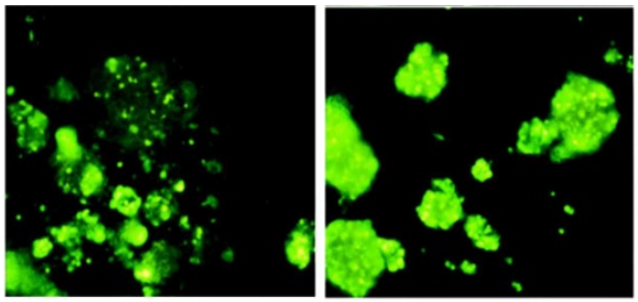
(Giannoukakis N. et al., 1996 Gene Ther)

Advantage:

- Long term gene expression via stable vector integration into host genome;
- Capacity of infecting both dividing and non-dividing cells;
- Lack of immunogenic viral proteins after vector transduction;
- Relatively easy system for vector manipulation and production.

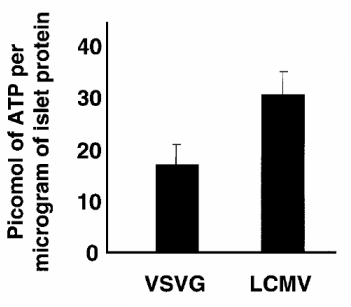
Vector building – Lentivirus

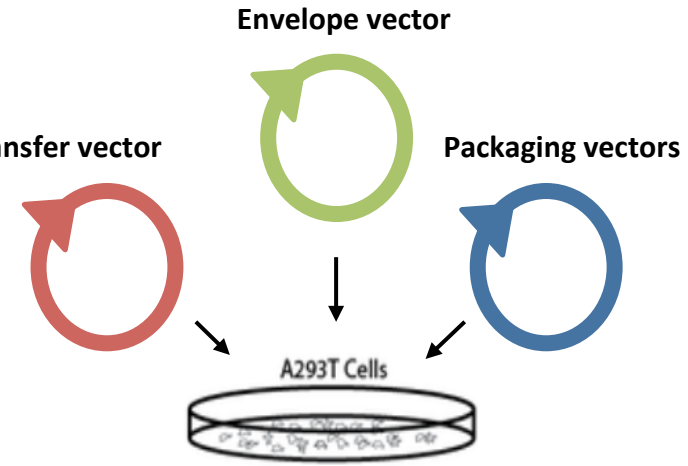
Envelope vector



VSV-G **LCMV**

(Kobinger, 2004 Hum Gene Ther.)

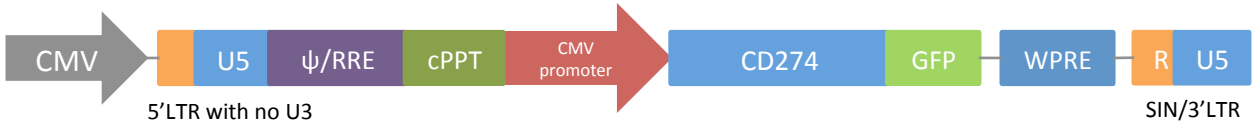




Collect supernatant after 24-48 h

Packaging vectors

Transfer vector



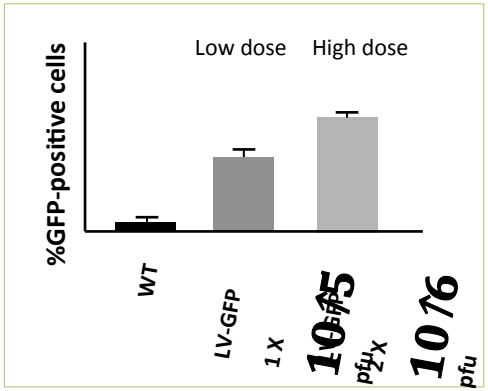
5'LTR with no U3

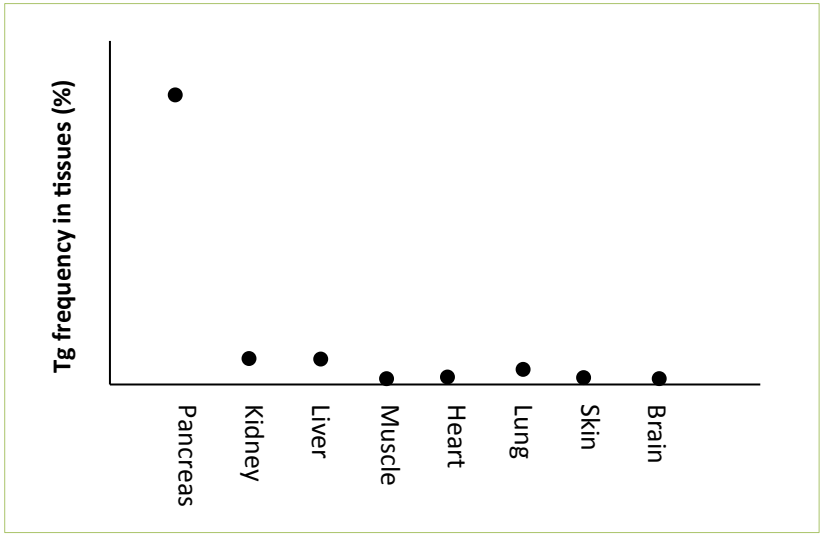
SIN/3'LTR

Transduction *in vitro*

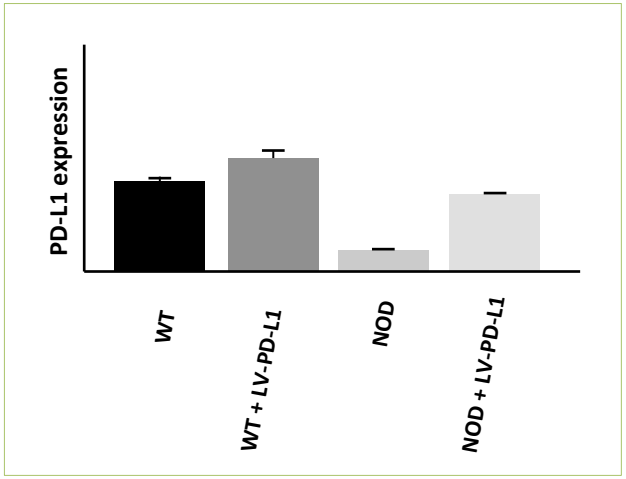
Islets infected with LV-PD-L1 at high dose show PD-L1 overexpression and no toxicity.

FACS

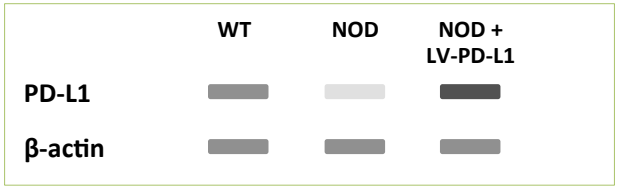




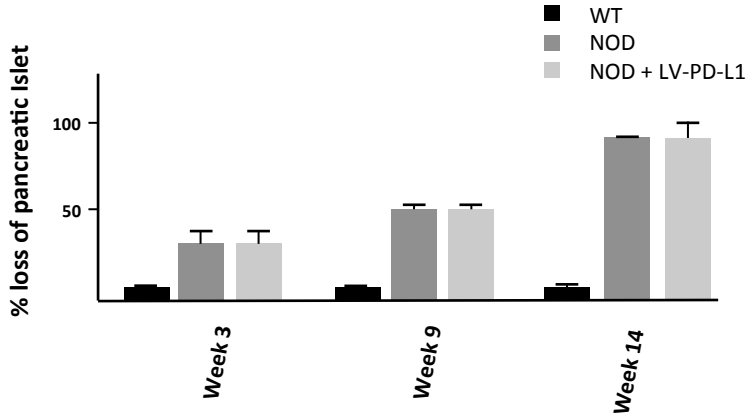
Real-time quantitative polymerase chain reaction (PCR)



WB

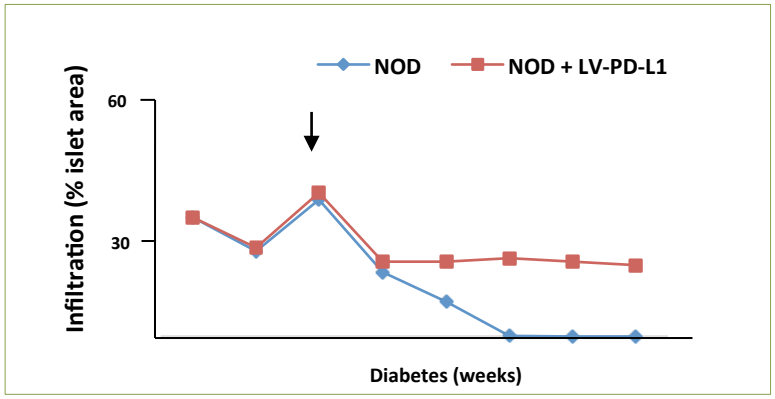


Transduction *in vivo*



NOD mice were transducted 5 days before the analysis.

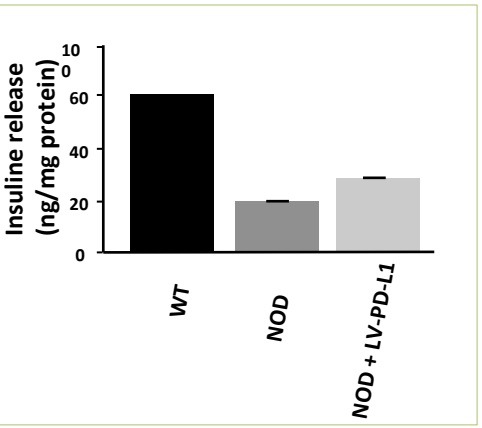
n = 45



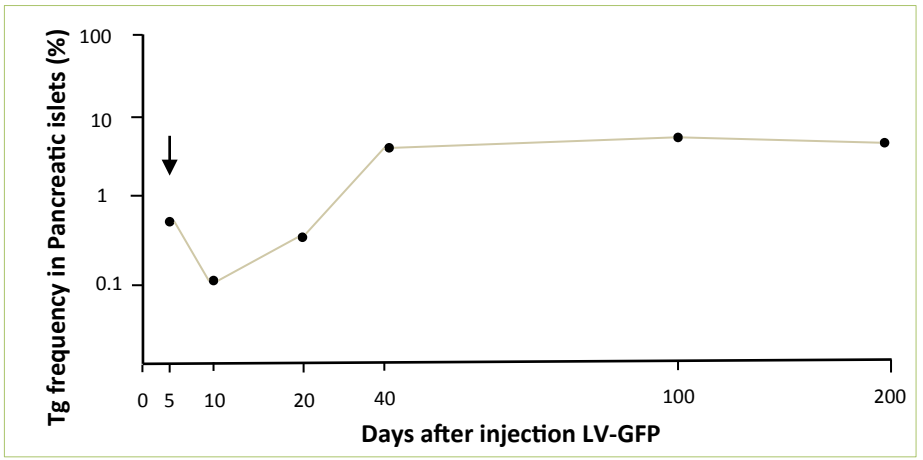
n = 10

PD-L1 expression block the autoimmune reaction, avoiding the progressive distruction of pancreatic islets.

ELISA



We expect stable LV-GFP expression for more than 200 days.



n = 25

Pitfalls and solutions

In the human T1DM is impossible to know the accurate rate of loss of pancreatic islets. Unfortunately, there's no diagnostic way to detect diabetes in the early stage of the disease and this is the main obstacle to our project.

Hopefully, in future we could treat diabetes before the onset of the hyperglicemia and so save more islets direct in the patients' pancreas, avoiding transplants.

Moreover, islets are only a small fraction of the entire pancreas and the surgery shows a high percentual of allograft rejection.

In the future, it might be possible to work directly on human pancreas, in order to restore the PD-L1 expression and avoid autoimmune reaction.

Cost and time

30 WT mice C57BL/6J ≈ € 530

85 NOD mice ≈ € 2.787

Lentivector production ≈ € 1500

Western blot kit ≈ € 2000

RT-PCR kit ≈ € 500

A293 Cells 100 µg: € 690

Ultra sensitive mouse Insulin ELISA kit: € 320

PD-L1 antibody 3000 µl: € 300

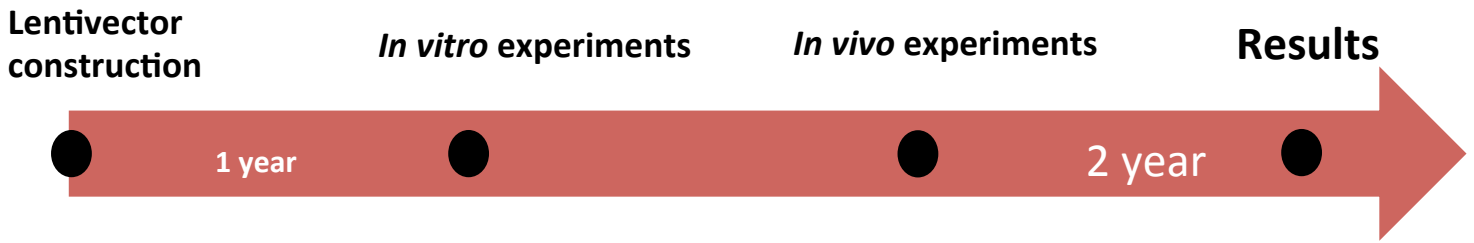
Stabulation: € 25.000/years

The Jackson Laboratory

LSBio
LifeSpan BioSciences, Inc.

addgene
The nonprofit plasmid repository

Crystal Chem
High Performance Assays



In total ≈ € 35.000 (without salary of researchers)

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