Proposal of a research project:

Use Mesenchymal Stem Cell derived exosomes targeted to T cells and charged with specific miRNA as a therapeutic tool against Type 1 Diabetes.

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➢ Background information

Type 1 Diabetes (T1D) is an autoimmune disease with increasing incidence that needs improved therapies. New approaches to T1D include:

- Therapies using Mesenchymal Stem Cells (MSC) derived exosomes are already in phase I/II clinical trails.
- Some miRNAs have shown to regulate autoimmunity, so therapies using miRNAs are being developed.

Some of the goals of these therapies are: restore Th1/Th2 balance, enhance functional Treg and switch pro-inflammatory cytokines profiles to protective cytokines profiles.

➢ Objectives

The main objective of these project is to purpose a therapy that will efficiently prevent T1D: **engineered exosomes**.

We are going to use **MSC exosomes**, as they have intrinsic immunosuppressing capacities.

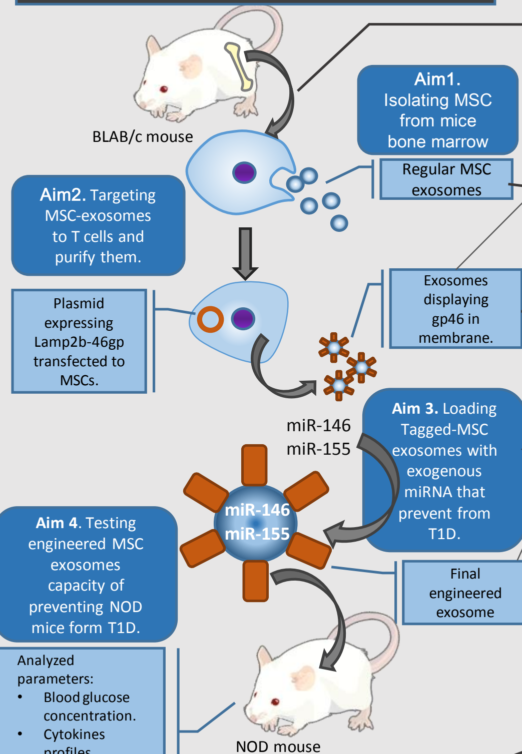
Exosomes will be to target to T cells (the main dysregulated cells in T1D). **gp46** confers tropism to T cells. It will be fused with Lamp2b, a protein found abundantly in exosome membranes

miRNA

Immunosuppressing capacity will be enhanced by charging exosomes with specific immunosuppressing miRNA: **miR-146** and **miR-155** (Treg enhancers).

In order to build engineered exosomes and prove its efficacy, 4 aims described on the right need to be achieved.

➢ Materials and Methods



Flow cytometry. As MSCs will be extracted from bone marrow is important to confirm correct isolation of only MSCs. Flow cytometric will analyze expression of Sca-1, CD90 and CD73 (positive markers for MSC), and expression of CD45 (hematopoietic lineage marker—negative marker).

Ultracen-trifugation Exosomes will be isolated from the cell culture using ultracentrif-gation.

Exosomes characterization will confirm exosomes presence, and thus, successful isolation by ultracentrigufacion. Characterization will be based on size and surface protein analysis.
ELISA. Presence of CD9, CD36 and TSG101 (the main representative protein in exosomes) will be testes using ELISA.
NTA. Nanoparticle Tracking Analysis will determinate diameter of exosomes.

ELISA. Presence of gp46 will be tested using ELISA method.

Confocal microscopy. Gp-46 exosomes and regular MSC exosomes will be label with PKH26 and uptake will be evaluated in in-vitro T cells culture by confocal microscopy.

Electroporation. miRNA will be loaded into exosomes using electroporation. Electroporation increases the permeability of membranes allowing miRNA to be introduced. Electroporation will performed at 400V and 125 µF.

RT-qPCR. In order to confirm miRNA loading, quantification of miR-146 and miR-155 by reverse transcription qPCR will be performed before and after electroporation.

MTT assay. Cytotoxicity of exosomes will be evaluated.

Measure glucose concentration. NOD mice that had two consecutive blood glucose values of more than 250mg/dl be defined as diabetic.
ELISA. Cytokines profiles will be quantified from the blood serum over the time using ELISA. Pro-inflammatory cytokines analyzed will be: IL-2, IFNϒ and TNFα. Protective cytokines analyzed will be: IL-4, IL-10 and TGF-β.
Flow cytometry. Th1/Treg ratio measures will be performed by flow cytometry. Tregs are going to be identify by Foxp3 (a transcription factor unique in Tregs) and Th1 are going to be identify by CXCR3 (chemokine receptor abundantly expressed in Th1).

➢ Expected results

- **Prevention and delay of T1D development.**

The percentage of NOD mice developing T1D is expected to decrease and the onset is expected to be delayed.

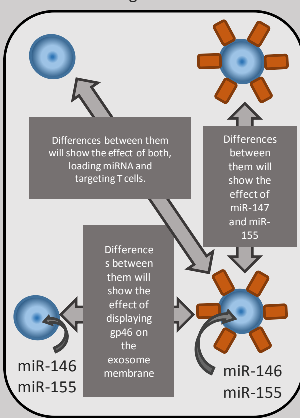
- **Cytokines profiles: switch to protective cytokines**

Assay are expected to show less pro-inflammatory cytokines (IFN- γ, TNF-α, and Il-12) and more protective cytokines (Il-4, Il-10 and TGF-β). Pro-inflammatory profiles are linked with more pancreatic immune infiltration and T cell activation which results in T1D development.

- **Decreasing Th1/Treg ratio**

Tregs proliferation is expected to be increased, as miR-146 and miR-155 are enhancers of Treg and MSC exosomes are also claimed to promote Tregs proliferation. Th1 is expected to be decreased, as enhanced functional Tregs are supposed to restore the Th1/Th2 balance by promoting Th2 and inhibiting Th1.

Other exosomes will be tested to provide information about the single effect of each one of the elements used to form engineered exosomes:



➢ Conclusions

- MSC derived exosomes and miRNA exhibit immunomodulatory capacities.
- Exosomes have a great potential as a drug deliver, since they are well-tolerated in vivo, naturally efficient at delivering functional biomolecules and relatively easy to engineer (they can be targeted and loaded with specific molecules).
- Engineered exosomes will bring new insights into T1D treatment and benefits to T1D patients.

➢ References

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