Proposal of a research project:

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

**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 trials.
- 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 this project is to purpose a therapy that will efficiently prevent T1D: engineered exosomes.

1. We are going to use MSC exosomes, as they have intrinsic immunosuppressing capacities.
2. Exosomes will be targeted to T cells (the main dysregulated cells in T1D), go through tropism to T cells. It will be fused with Lamp2b, a protein found abundantly in exosome membranes, enhancing capacity to be internalized.
3. 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.

**Expected results**

- Prevention and delay of T1D development.
- Cytokines profiles: switch to protective cytokines.
- Exosomes will be targeted to T cells using Lamp2b.
- Immunosuppressing capacity will be enhanced by charging exosomes with specific immunosuppressing miRNA: miR-146 and miR-155 (Treg enhancers).

**Materials and Methods**

**Flow cytometry.** MiRs will be extracted from bone marrow to confirm correct isolation of only MSCs. Flow cytometry will analyse expression of CD3, CD4 and CD8 (positive markers for MSC), and expression of CD45 (hematopoietic lineage marker - negative marker).

**Ultracentrifugation.** Exosomes will be isolated from the cell culture using ultracentrifugation.

**ELISA.** Presence of CD9, CD36 and TSG101 (the main representative proteins in exosomes) will be tested using ELISA.

**NTA.** Nanoparticle Tracking Analysis will determine diameter of exosomes.

**Confocal microscopy.** Qp-46 exosomes and whole MSC exosomes will be labeled with PNA-206 and evaluated in vitro T cells culture by confocal microscopy.

**Electroporation.** miR will be loaded into exosomes using electroporation. Electroporation increases the permeability of membranes allowing miRNA to be introduced. Electroporation will performed at 1000 and 125 μF.

**MTT assay.** Cytotoxicity of exosomes will be evaluated.

**Differences**

- miR-146 and miR-155 will be loaded into exosomes.
- Flow cytometry: Tregs are going to be identified by FoxP3 (a transcription factor unique in Tregs) and Th1 are going to be identified by CD4 (hematopoietic lineage marker - negative marker).

**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**