Preclinical assessment of Self-replicating DNA/RNA vaccine encoding HIV gp140

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Background and Aim

- Non-amplifying and Self-Amplifying mRNA(replicon) based DC Vaccines
  
  - **pDNA:** Conventional mRNA
  
  - **RREP:** *alphavirus RNA* launched replicon
  
  - **DREP:** *alphavirus DNA* launched replicon

- **Objectives:** RNA-based DC vaccines to be compared (all expressing the ZM96gp140 insert)
  
  - To study the capture and transmission of different immunogens by DCs.
  
  - To characterize the phenotypic and morphologic, changes induced by immunogens in DCs.
  
  - To analyse the antigen processing and presentation by DCs of different immunogens.

Piotr S. Kowalski et al. Molecular Therapy, 2019
Schematic outline of the study

**DAY 0**
- PBMCs isolation and culture → monocytes adherence

**DAY 2**
- Collecting PBMCs depleted monocytes (lymphocytes) after 2h incubation
- Cytokine supplement (IL-4, GM-CSF) → obtaining immature MoDCs

**DAY 5**
- Collecting immature MoDCs
- Transfection iMoDCs with replicons

**DAY 6**
- Capture efficiency evaluation (24h after the pulsing)

**DAY 7**
- MoDCs and T cell co-culture to determine the proliferative capacity of T cells (CFSE staining)
- Immunophenotype of DCs:
  - Viability (Cytometry)
  - Maturation (Cytometry)
  - gp140 expression (Cytometry)
- Fluorescence microscopy

**DAY 13**
- T lymphocytes proliferation measurement (Cytometry)
- Evaluation of cytokine and chemokine secretion (Luminex)
- Evaluation of IFN-γ-producing specific T lymphocytes (Elispot)
Effects of RREP/DREP on cell viability and DC maturation

Viability
- RREP n=4
- DREP n=12

Maturation
- RREP
- DREP

Insert expression in CD83+ / HLADR+

Capture efficiency evaluation (24h after the pulsing)

Immunophenotype of DCs:
- Viability (Cytometry)
- Maturation (Cytometry)
- gp140 expression (Cytometry)
Effects of RREP/DREP on cell viability and DC maturation

Capture efficiency evaluation (24h after the pulsing)

Immunophenotype of DCs:
- Viability (Cytometry)
- Maturation (Cytometry)
- gp140 expression (Cytometry)

Viability

Man RREP n=4
Man DREP n=12

Maturation

Insert expression in CD83+ / HLADR+
Effects of RREP/DREP on T cell proliferation, cytokine production and HIV-specific T cell responses.

- **Elispot Day 1**
- **Elispot Day 6**
- **DREP gp140**

RREP n=3  DREP n=11

- IL-1β
- TNF-α
- IFN-α
- IL-6

Evaluation of IFN-γ-producing specific T lymphocytes (Elispot)
Conclusions

• Our findings suggest that these RREP and DREP replicons were able to activate DC and efficiently deliver HIV gp140.

• Moreover, by inducing env-specific immune responses.

• DREP vector seems to be a more promising candidate to be used in the preventive vaccine approaches against HIV.
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