OSR Retreat Abstract

Instructions:

-Title

- Authors: Name Surname (underline the presenting author)

- Affiliations

- Email of the presenting author

- Text divided in Introduction, Methods, Results and Conclusions (max 2500 characters including spaces). Please, do not include references in the abstract.

- ***Title***: Generation of IL-10 engineered VitD3-treated tolerogenic dendritic cell for cell-based approaches to re-establish tolerance in immune-mediated diseases
- ***Authors***: G. Giacomello[1,2], L. Passerini[1], G. Andolfi[1], G. Amodio[1] and S. Gregori[1]
- ***Affiliations***:
[1]Mechanisms of Peripheral Tolerance Unit, San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), San Raffaele Scientific Institute (IRCCS), Milan, Italy.
[2]University of Milano Bicocca, Milan, Italy
- ***Email of the presenting author***: giacomello.gloria@hsr.it

Introduction
Tolerogenic dendritic cells (tolDC) are a promising approach to modulate antigen-specific immune response in autoimmune diseases. Vitamin D3-treated tolerogenic dendritic cells (VitD3DC) has been tested in a Phase I clinical trial for Multiple Sclerosis, the treatment was safe and well-tolerated with limited clinical benefit. IL-10 gene transfer into human monocytes induces differentiation of effective tolDC (DC^{IL-10}) that promote type 1 T regulatory (Tr1) cells. We aim at combining IL-10 gene transfer and VitD3 to generate an improved clinical grade cell product ($VitD3DC^{IL-10}$).

Methods
Peripheral blood CD14+ monocytes were *in vitro* differentiated into DC with or without VitD3 and engineered with lentiviral vector (LV) encoding for IL-10. Viral-like-particles (Vlp) containing virion-associated protein X (VPX) or deoxynucleosides (dNS) were used to overcome monocyte restriction to LV transduction. DC were characterized phenotypically (flow cytometry) and functionally (cytokine profiling, inhibition of IMLR and induction of Tr1 cells, using allo-$CD4^+$ T cells as responder cells).

Results
$VitD3DC^{IL-10}$ acquired the phenotypic features of VitD3DC (CD86) and of DC^{IL-10} (CD14, CD16, CD163, CD141, HLA-G and ILT-4), and the ability to secrete high levels of IL-10. $VitD3DC^{IL-10}$ inhibited CD4+ T cell responses in I MLR and induced Tr1 cell differentiation but at lower levels compared to DC^{IL-10}. Addition of VitD3 during DC^{IL-10} generation affected the overall yield of resulting DC. Thus, to optimize the generation of $VitD3DC^{IL-10}$ we assessed the ability of dNS and Vlp-VPX to improve IL-10 engineered DC cell recovery. Transduction of monocytes with LV in the presence of dNS allowed efficient and reproducible transduction of monocytes (Vector Copy Number-VCN: 5.4±1.5), although at lower levels compared to Vlp-VPX pre-treatment (13.7±8.9). Nonetheless, the resulting DC^{IL-10} acquired the phenotypic features and functions of typical of DC^{IL-10}.

Conclusions
Our preliminary data show that i) IL-10 gene transfer endowed VitD3DC with the ability of promoting Tr1 cells, ii) dNS can be used to induce efficient monocyte

transduction with IL-10-encoding LV, resulting in the acquisition of DC^{IL-10} features. However, addition of dNS did not improve cell recovery. We are currently optimizing the differentiation protocol to improve cell yield and achieve clinical translation of the resulting cell product.

WORD count: 2419 ch.
LIMIT: 2500 ch.