



# Impact of mesenchymal stromal cells and/or everolimus on T-reg lymphocyte expansion in rats

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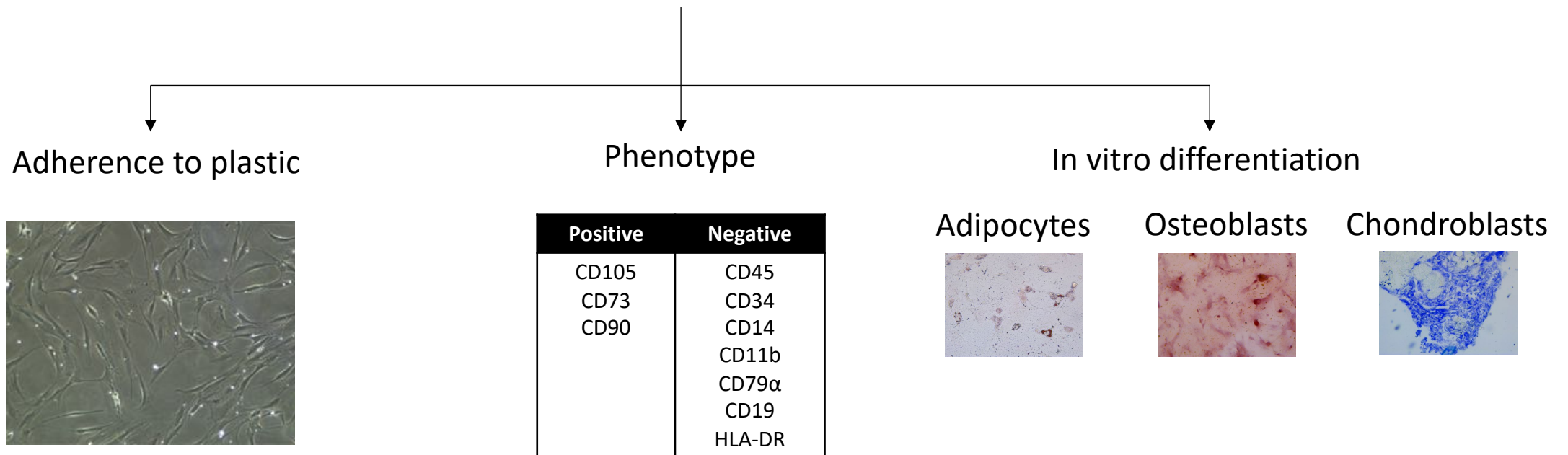
CHU Sart Tilman, Liège, Belgium

COMBINED SIZ AND BTS CONGRESS 2020

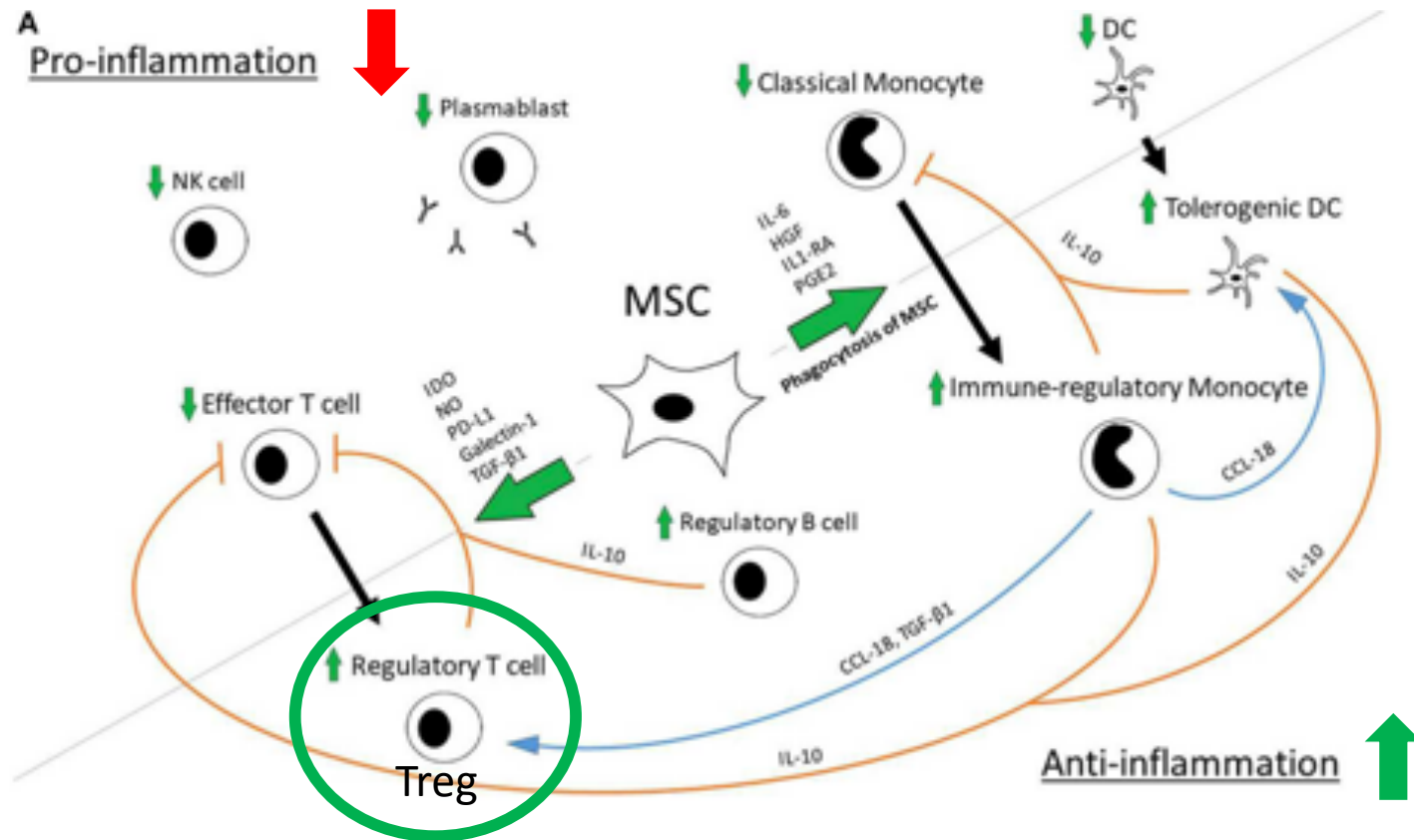
**11th March 2020**

## Mesenchymal Stromal Cells (MSC)

- Adult multipotent and fibroblast-like cells
- Isolated from various origin (bone marrow, adipose tissue, ...)
- **3 minimal criteria**



# MSC immunosuppressive properties



Increased therapeutic interest:

- Degenerative and immune diseases
- Sepsis
- **Solid-organ Transplantation (SOT)**

- ➔ Alternative IS strategy?
- ➔ Ischemia reperfusion injury?

Encouraging pre-clinical data!

# MSC in solid-organ transplantation – Clinical Trials

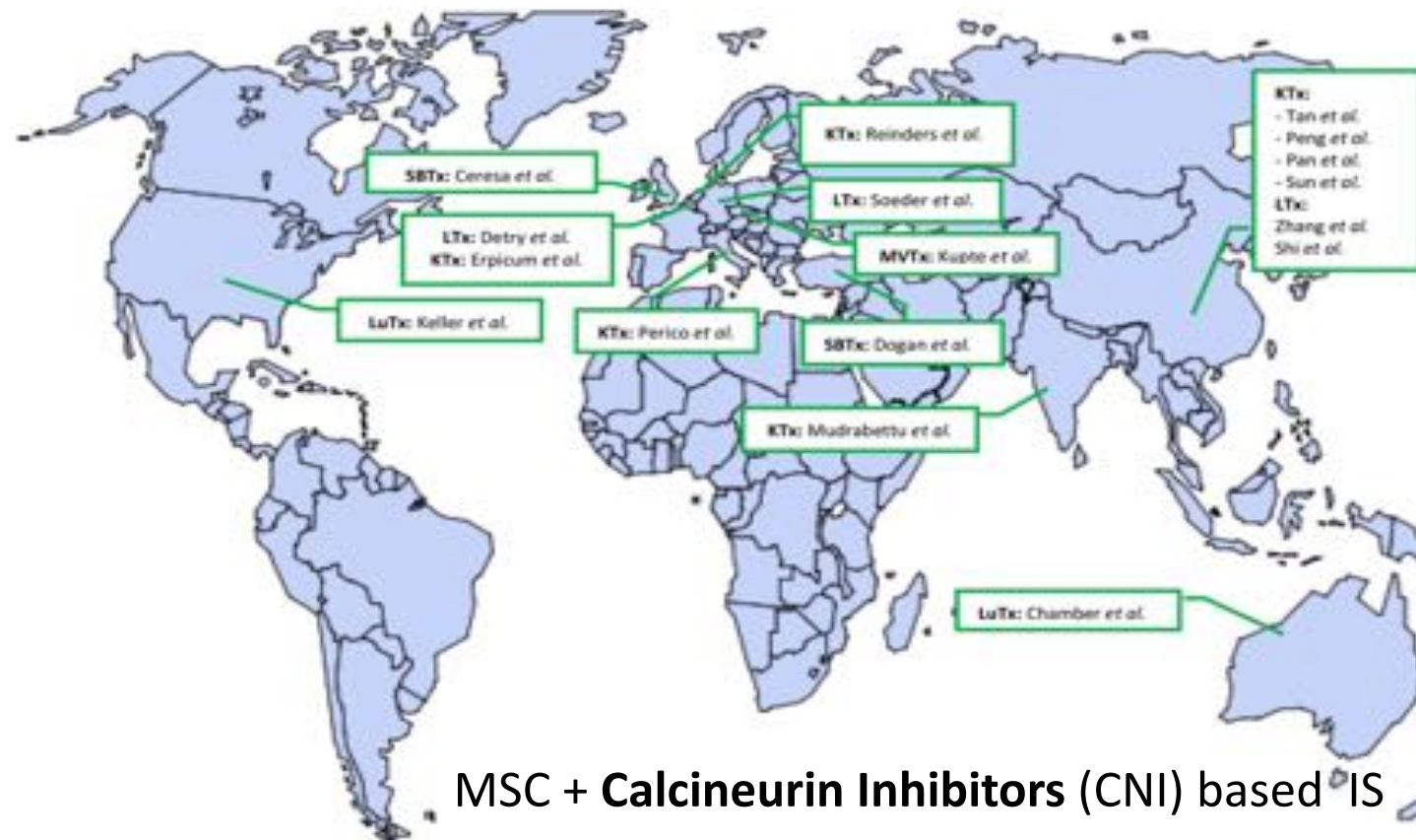
- 1) Safe
- 2) Effective?  
Inconstant  
Not-convincing clinical impact

Many variables!

Questions to resolve:

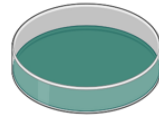
- Precise mechanism of action
- Best timing? Single vs Repeated?
- Best origin/source?
- **Associated immunosuppressive drugs?**

- Effect on MSC?
- Effect on Treg?



# Impact of ISD on MSC and on Treg ? - Preclinical data

In vitro



Effect on MSC



**CNI**  
(CsA, TAC) → **Toxic**  
**Inhibitory effect**

Effect on Treg



**Inhibitory**  
(proliferation/function)

In vivo



Effect on MSC



**Variable**

Effect on Treg



**Inhibitory**

CNI + MSC → contradictory association?

**mTor inhibitors**  
(rapamycin, everolimus) →

**Not toxic but**  
**Inhibitory effect**

**Promote proliferation**

**Synergistic**

**Function and**  
**proliferation**  
**promotion**

mTor inhibitors + MSC → better association?

# mTor inhibitors and MSC in SOT - Preclinical data

- **No liver / kidney transplantation models** associating mTor inhibitors with MSC
- Pre-clinical data from RAPAMYCIN ... = EVEROLIMUS ??
  - ≠ bio-pharmacological properties
  - ≠ action on vascular inflammation
  - ≠ effect on mitochondrial oxidation
  - ➔ **Not equivalent**

Klawitter et al., *Expert Opin Drug Saf*, 2015

➔ **Preclinical data are needed for everolimus**

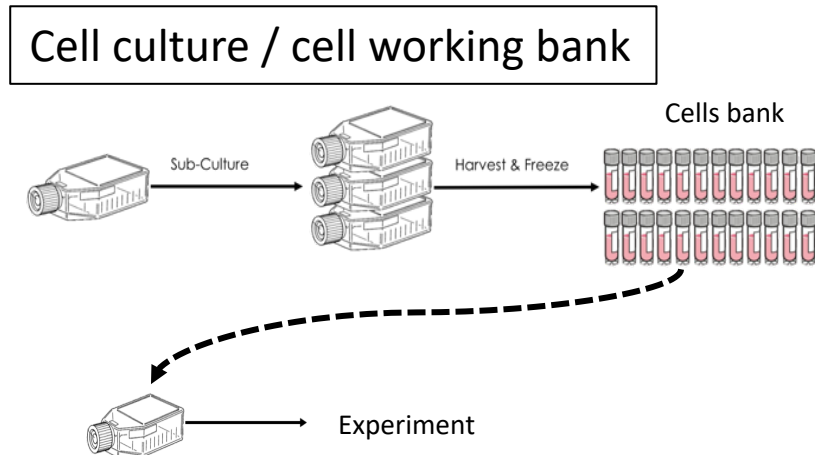
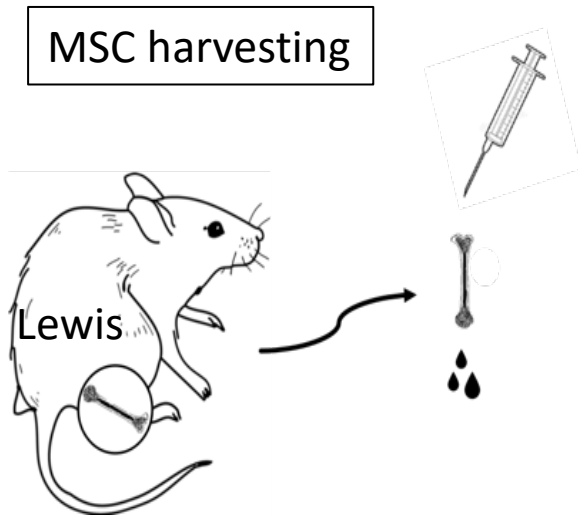
Impact of **MSC + Everolimus** association in **Liver Transplantation model?**



Preliminary investigations:

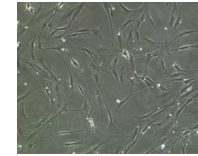
- Continuous administration of everolimus with osmotic pump in rats
- Effect of MSC and/or Everolimus on **Treg proliferation** in rats

## Isolation, culture and characterization of MSC

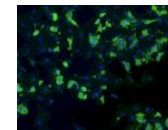
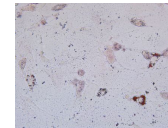


**Characterization**

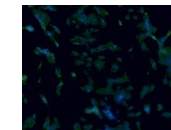
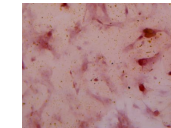
- ✓ Adherence to plastic
- ✓ In vitro differentiation



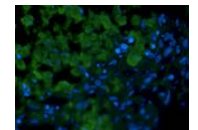
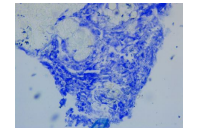
Adipocytes



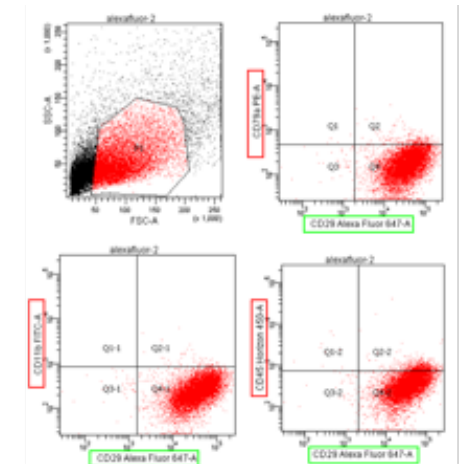
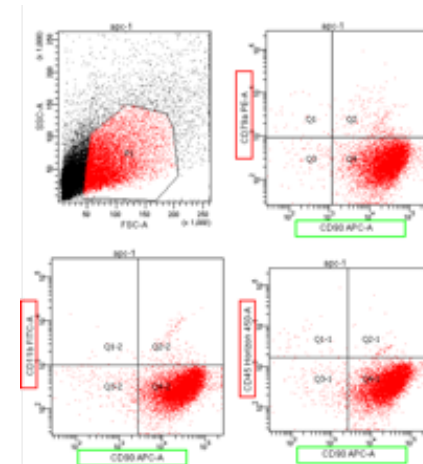
Osteoblasts



Chondroblasts



- ✓ Flow cytometry



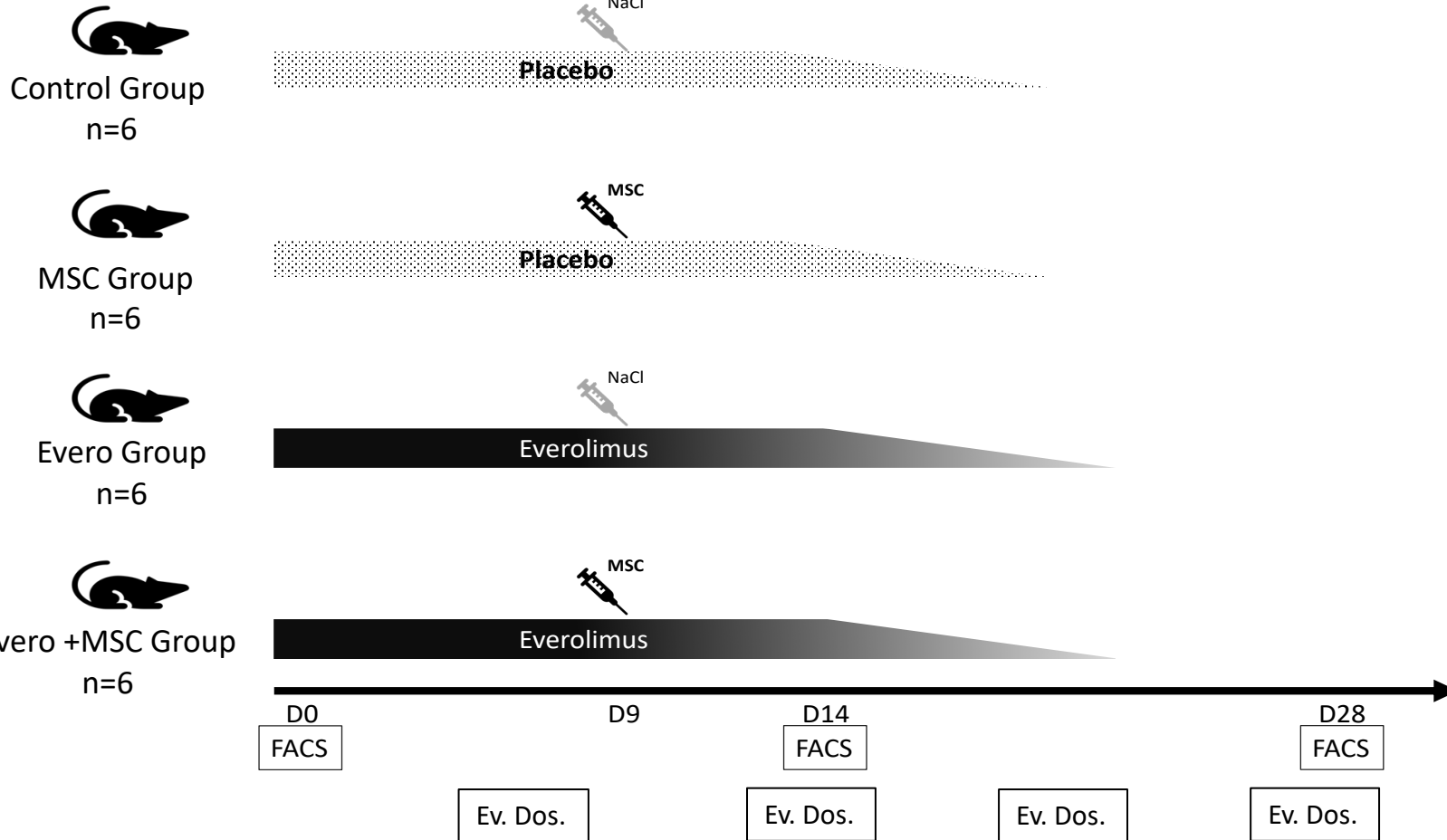
Positive for:

- CD90-APC
- CD29-Alexafluor

Negative for:

- CD45-V450
- CD11b-FITC
- CD79a-PE





- 4 groups of 6 Lewis rats
- Evero/placebo in osmotic pump (14 days)



- **MSC** (i.v, 1ml,  $10^6$ cells/kg)
- Everolimus dosage: D7-14-21-28
- **%T-reg** flow cytometry analysis D0-14-28

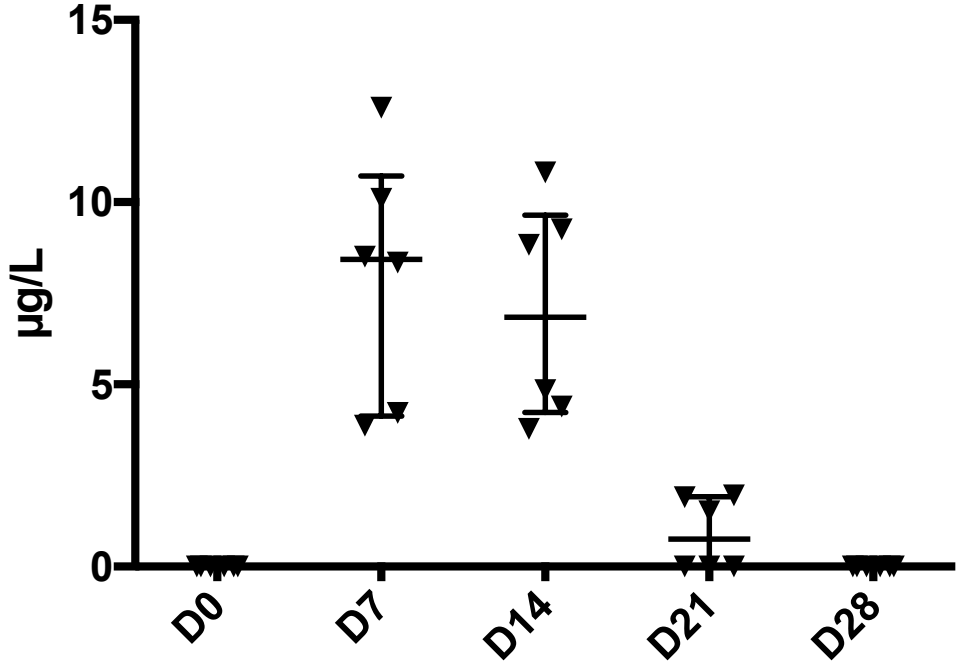


# Results

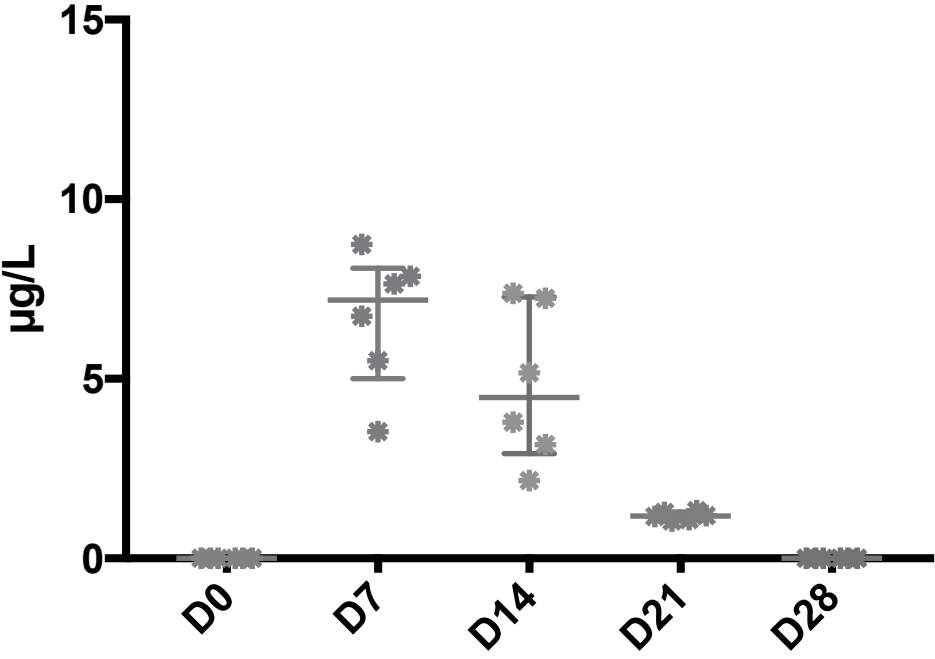
## Everolimus blood concentration

0.25mg/kg/day

### Evero Group



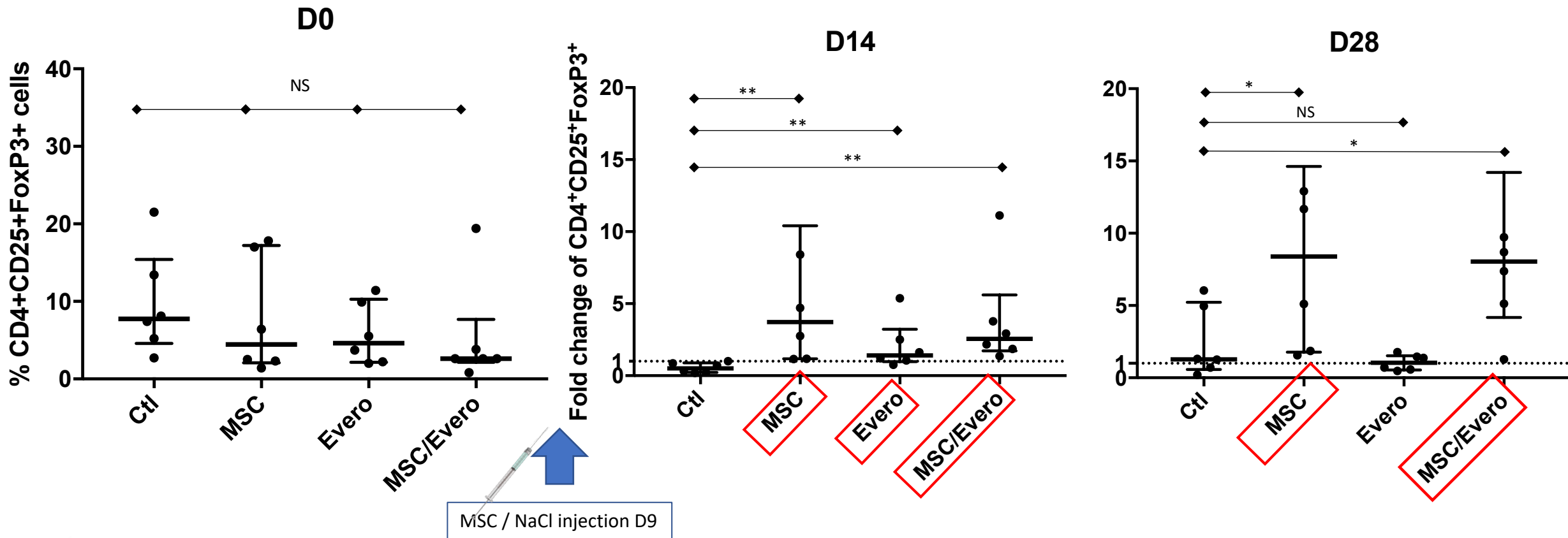
### Evero +MSC Group



→ No significant difference

# Results

Treg cells basal percentage in blood and fold changes on D14 and D28



MSC / NaCl injection D9



Everolimus/placebo : 14 days

# Conclusion

## MSC

- ✓ Promote Treg expansion among CD4+ T cells in rats

## Everolimus

- ✓ Can be delivered by osmotic pumps in rats
- ✓ Have no observed toxic effect on Treg
- ✓ **Do not impair MSC-effect on Treg expansion**
  - ➔ May be an interesting drug to associate with MSC in SOT?

## PERSPECTIVES

- Development of liver transplantation model in rat ✓
- Evaluation of **MSC + Everolimus** association for acute rejection in that model
- New Clinical trials design ?

THANK YOU FOR YOUR ATTENTION

