Alorreactividad frente a tolerancia: importancia de la inmunosupresión

Marcos López Hoyos
Inmunología
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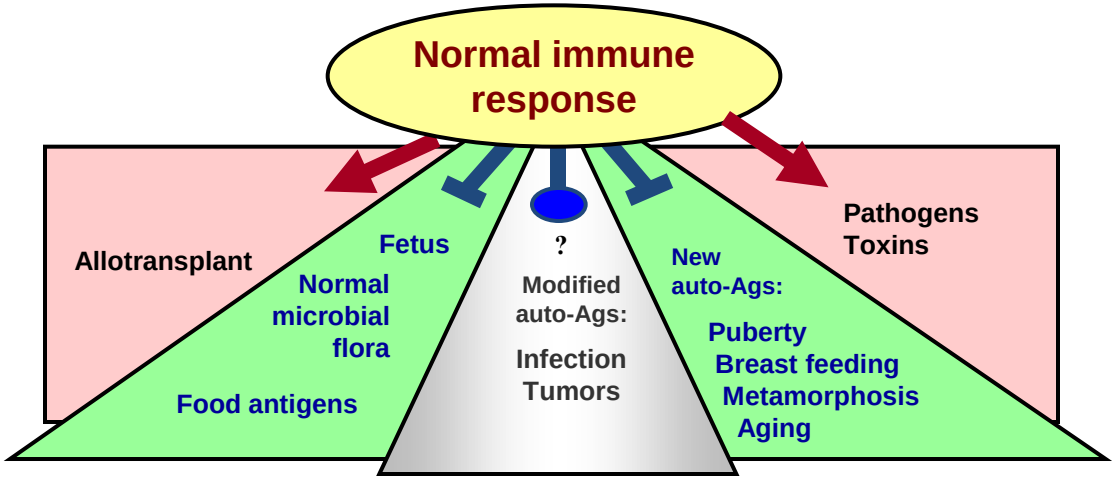
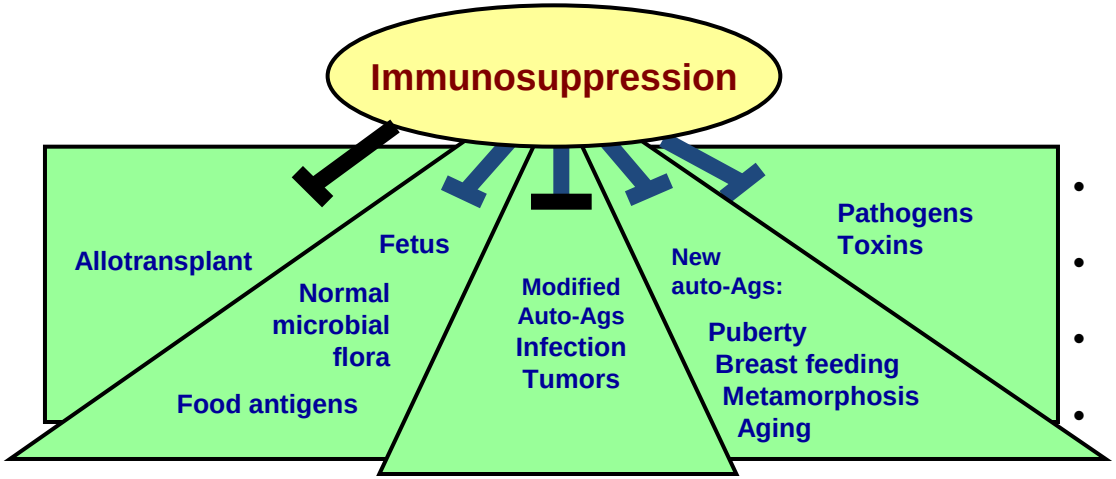
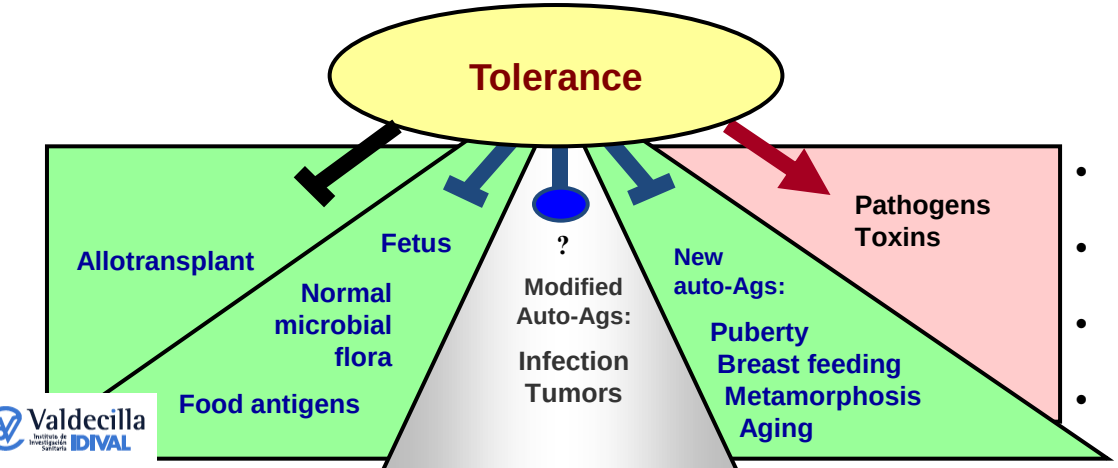
Santander (SPAIN)

The immune system pursued by microorganims





- No immunological specificity
- Risk of immunodeficiency/neoplasia
- Adverse effects
- IFTA



- Immunological specificity
- No adverse effects
- Long-term benefits
- Lack of clinical markers

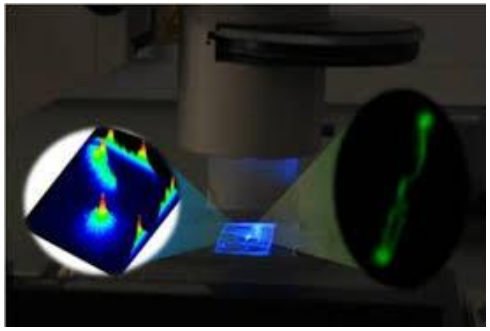
Courtesy: Dr. J Merino (Univ Cantabria)

Immunosuppression measurement

Individualization

OUR NEEDS

Molecular microscopy



Prediction

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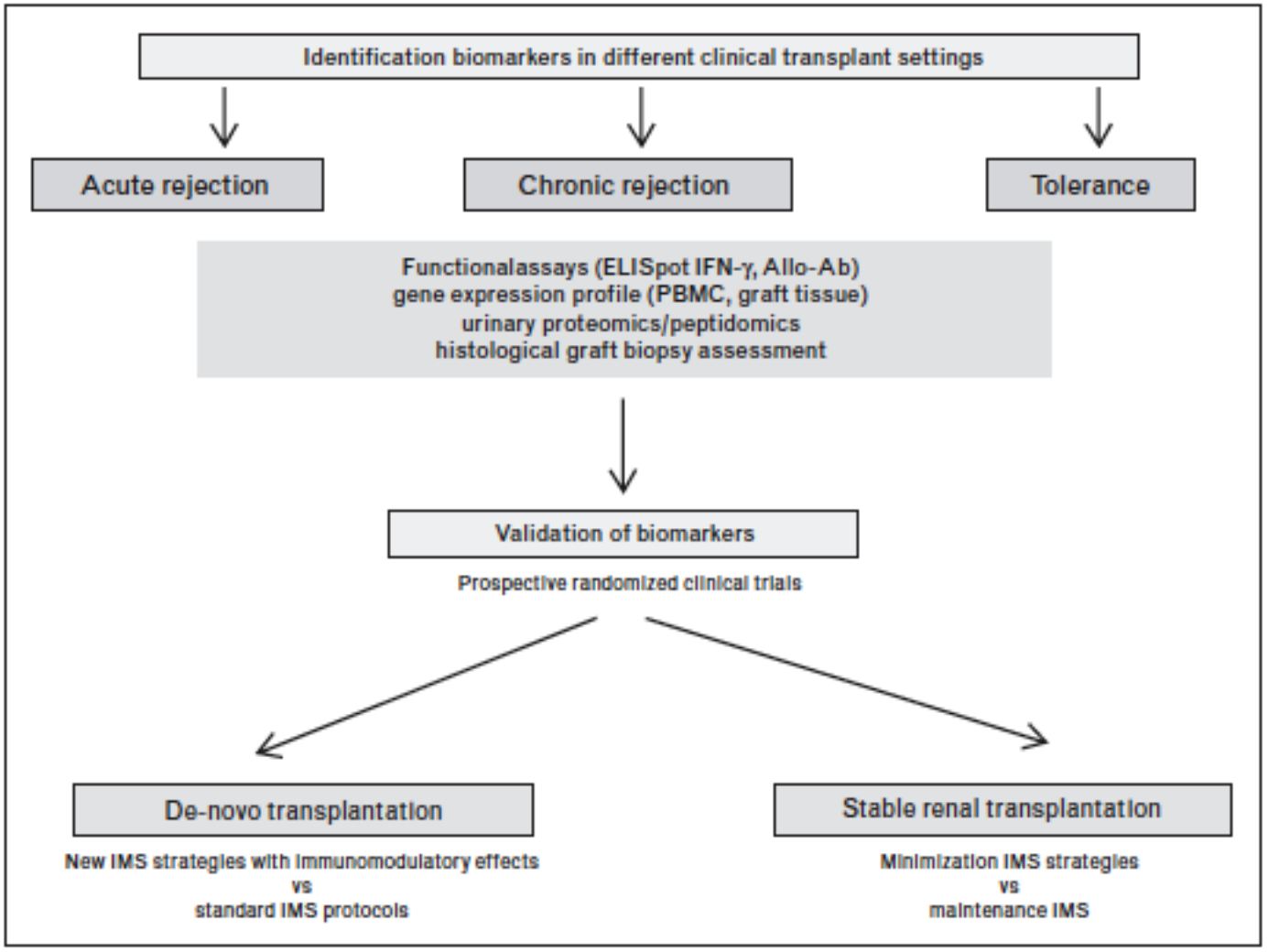
Our approach in renal transplantation



Transplantation Research Immunology Group

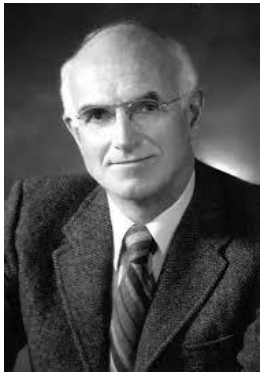
TRIG

Indices of Tolerance

Demonstrating the applicability of novel indices of immunological tolerance clinical practice using immunosuppresion-free transplant recipients

Mechanisms of T cell tolerance in transplantation

Deletion
- Central (thymus)
- Peripheral
  - Active T cell death
  - Passive cell death
  - Anti-T cell treatment

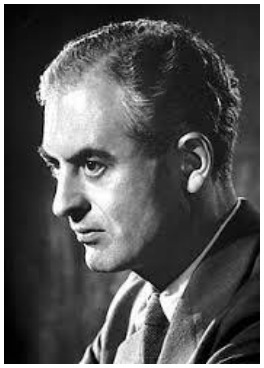


J.E. Murray

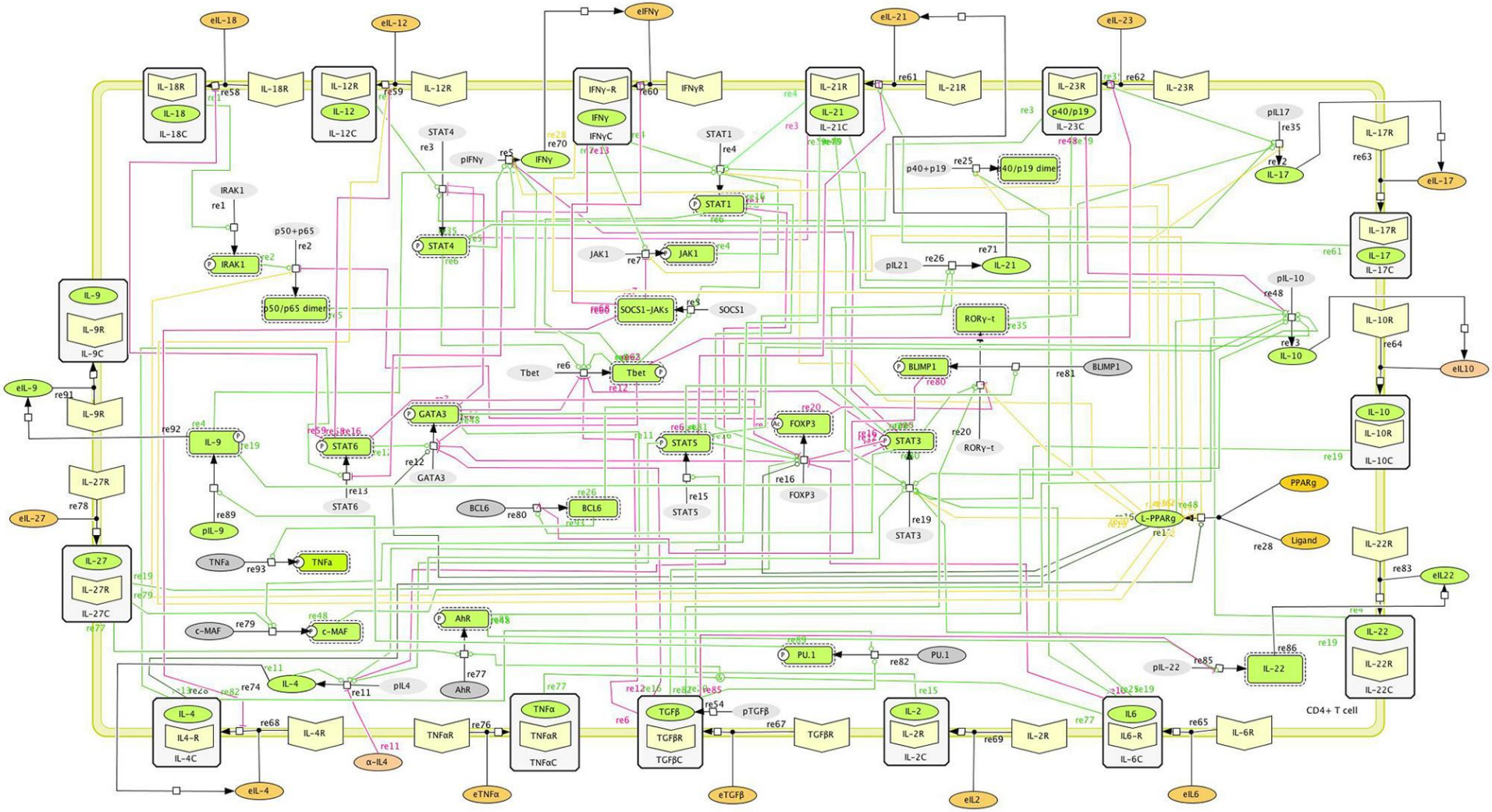
Anergy

Immune deviation
- Th1 (IL-2, IFNγ)
- Th2 (IL-4, IL-10)
- Th17 (IL-17, TGFβ, IL-6)

Suppression
- Tregs
- Other: Tr1, $CD8^+CD28^-$, Tγδ2



P. Medawar



Systems Biology Markup Language (SBML)-compliant network model of CD4+ T cell differentiation, including cytokines, receptors, and intracellular signaling pathways controlling CD4+ T cell fate and function.

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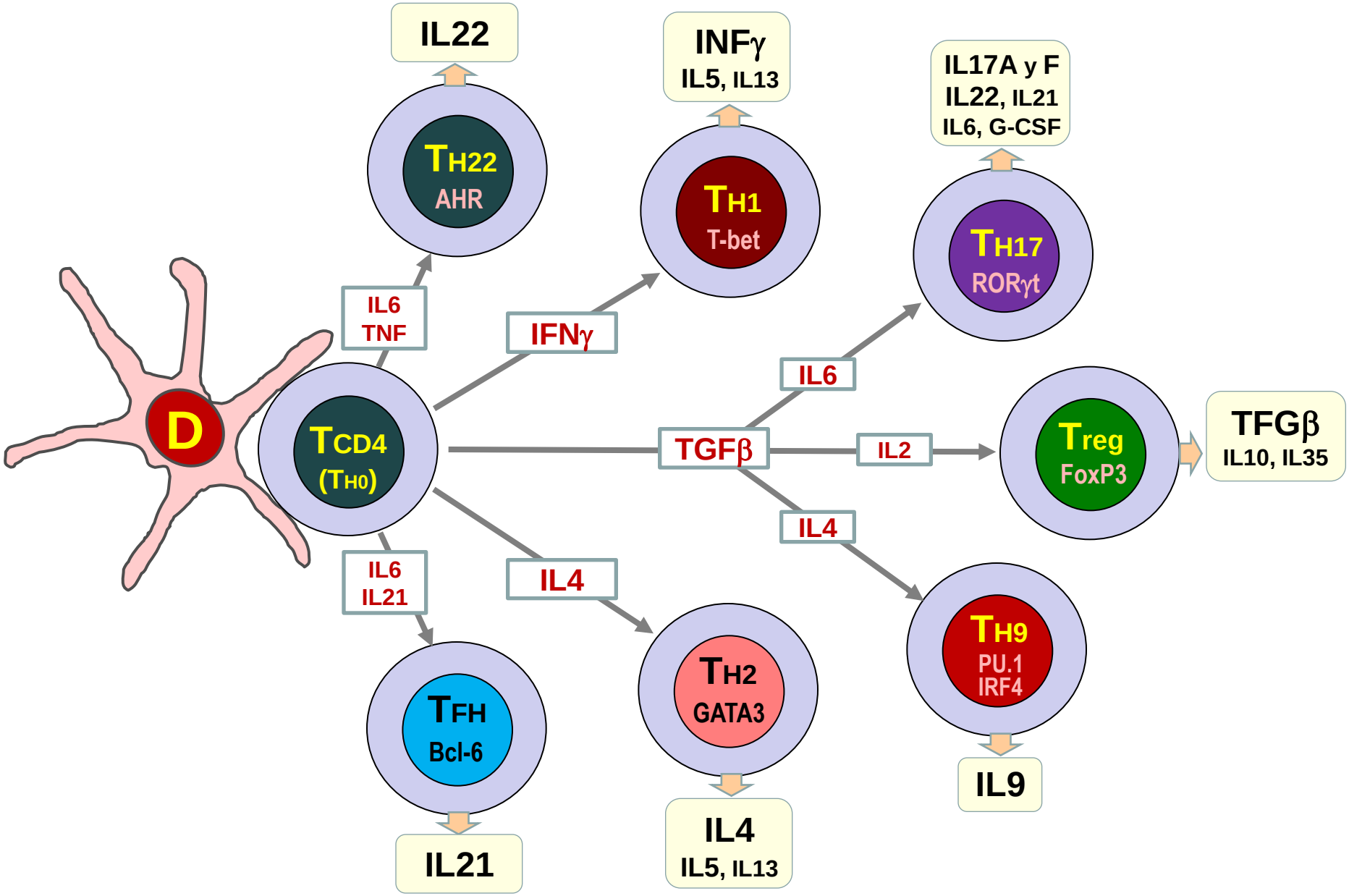
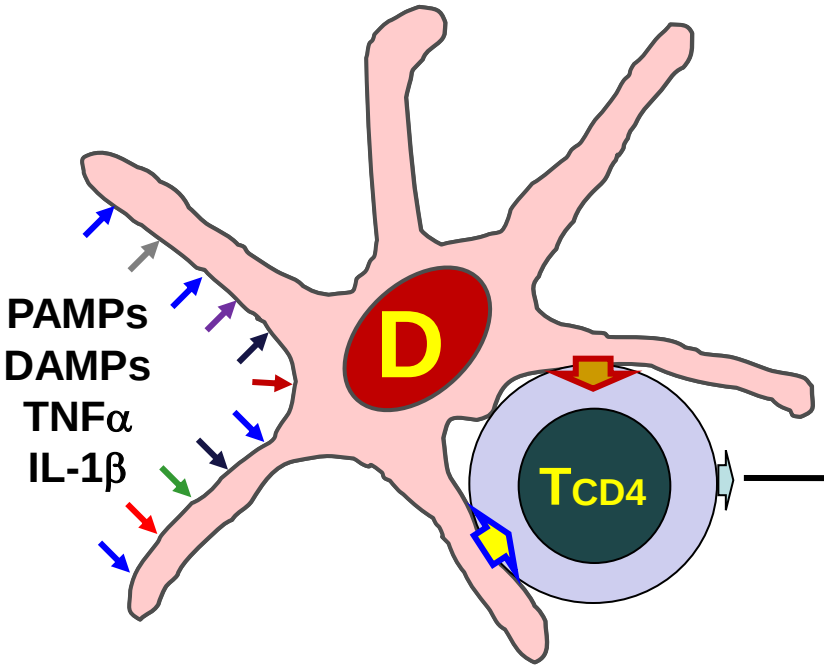
Carbo A. et al, Front Cell Dev Biol, 2014

Types of CD4⁺ T cells



Courtesy: Dr. J Merino (Univ Cantabria)

Dendritic cell collect the information and design the type of CD4+ T cell activation



Treg/T_{R1}

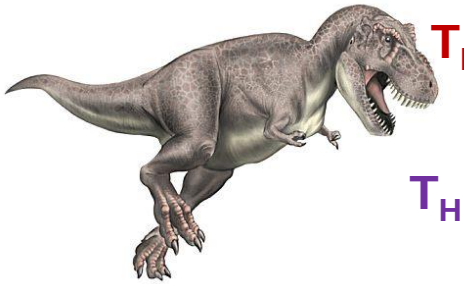
T_H3

T_H22

T_{Fh}

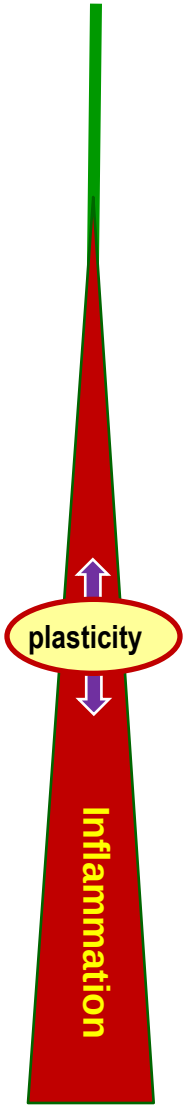
T_H2

T_H9



T_H1

T_H17



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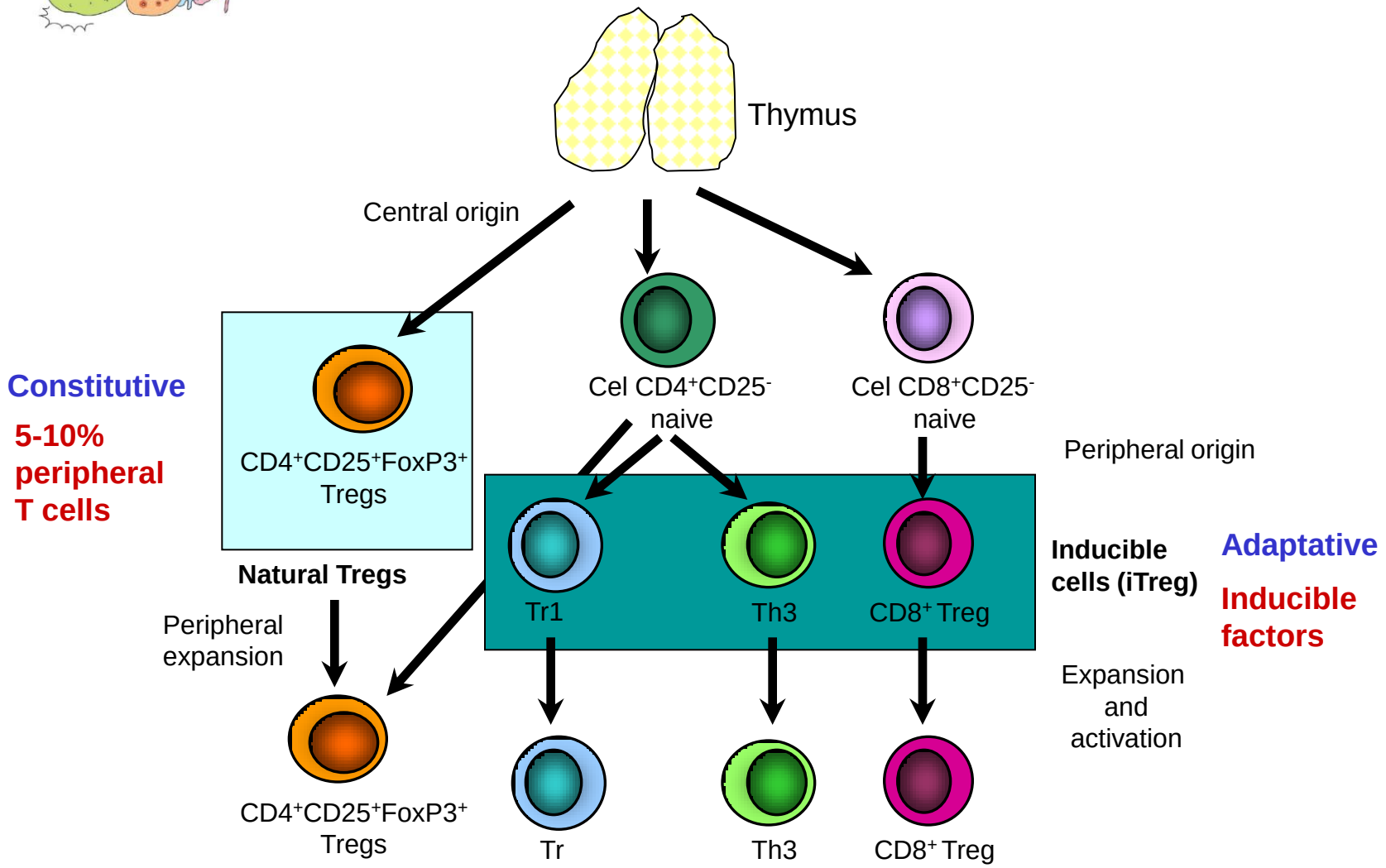
Courtesy: Dr. J Merino (Univ Cantabria)

Regulatory T cell subsets



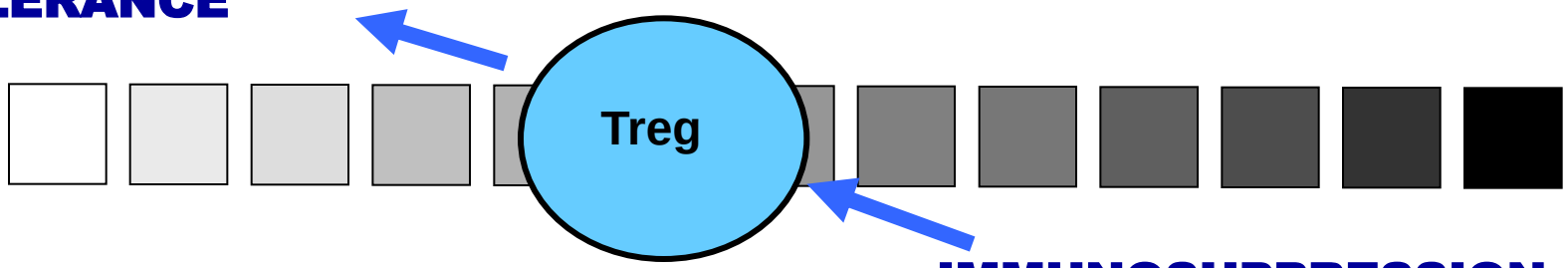
Tregs as tolerance marker in renal transplantation

The aim of tolerance biomarkers is to define those patients in whom immunosuppression can be minimized or withdrawn without risk of kidney allograft function.

It must be kept in mind that any non-tolerant patient defined by a immunological battery of assays may have a normal kidney function.

Immunosuppressant may promote tolerance through the generation and/or the maintenance of regulatory T cells

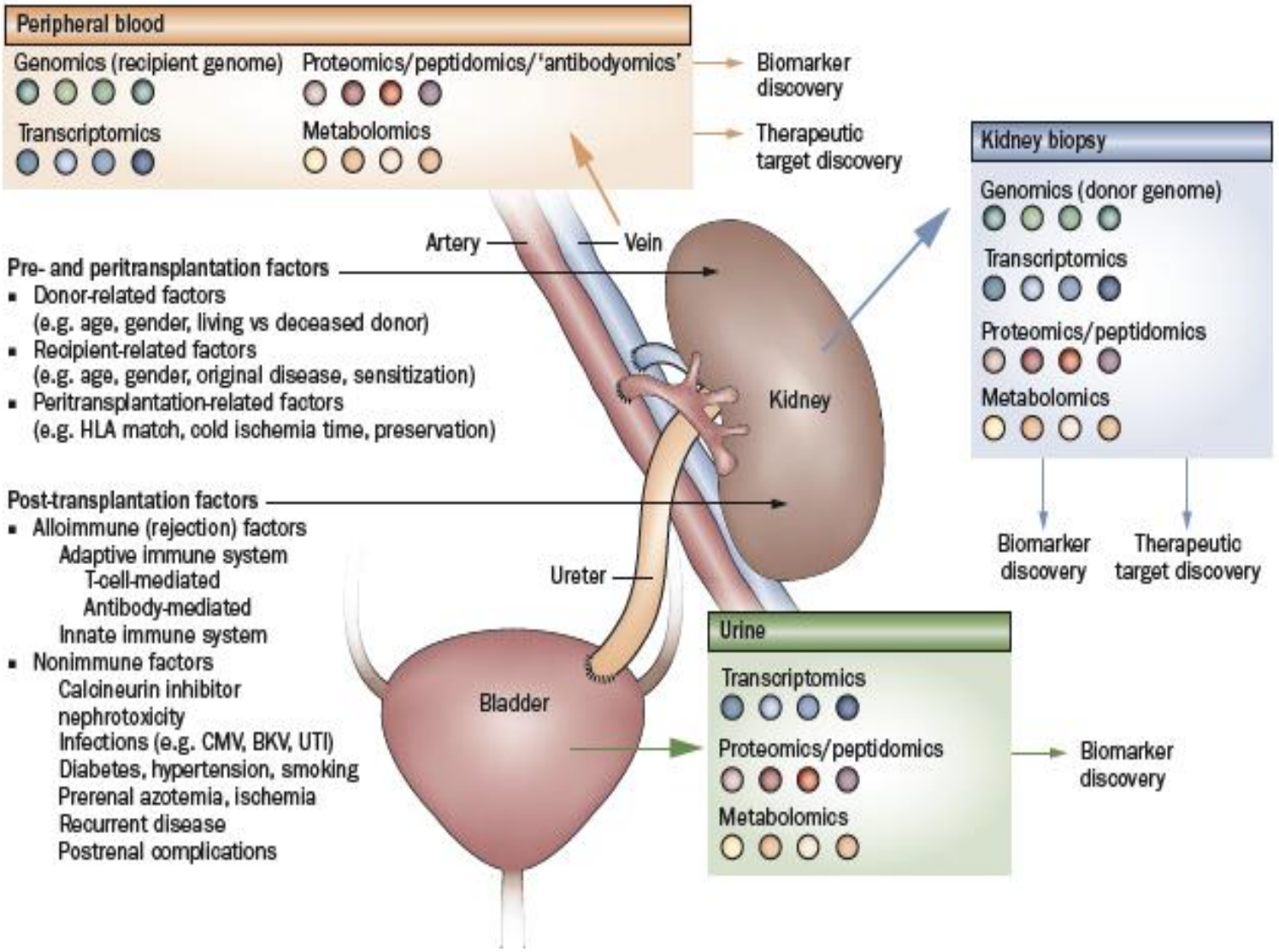
TOLERANCE



IMMUNOSUPPRESSION

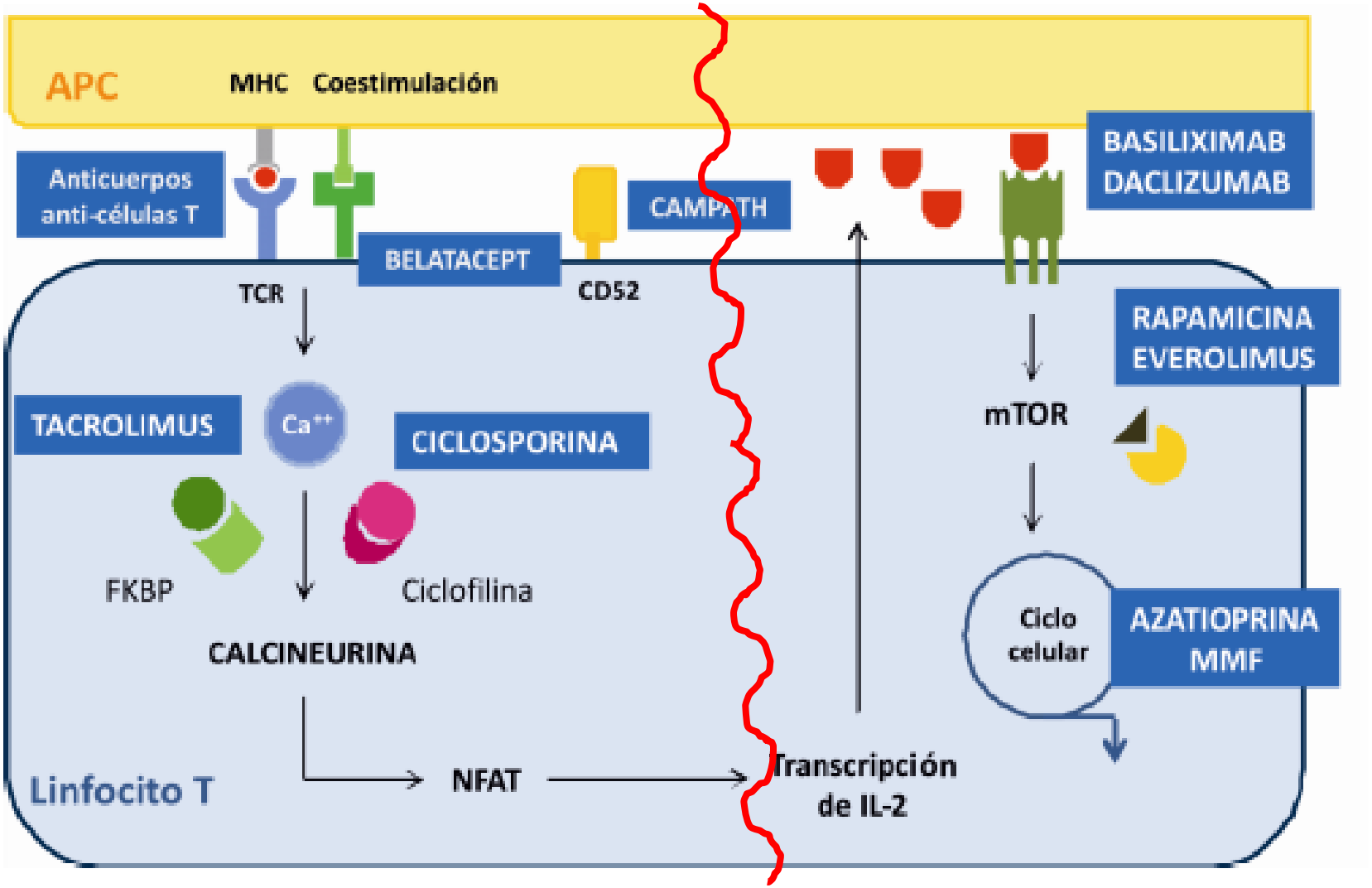
Valmori et al. J Immunol 2006.
San Segundo et al. Transplantation 2006.
Coenen et al. Blood 2006.
Lopez et al. J Am Soc Nephrol 2007.
Lopez-Hoyos et al. Transplantation 2009.

The source of biomarkers in renal transplantation



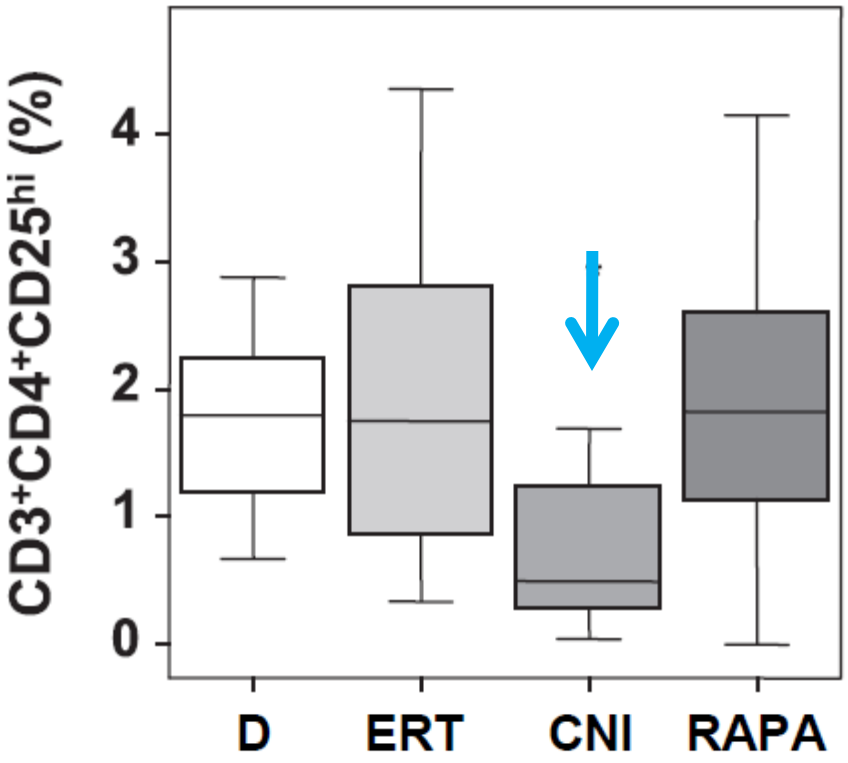
Naesens et al, Nat Rev Nephrol 2010

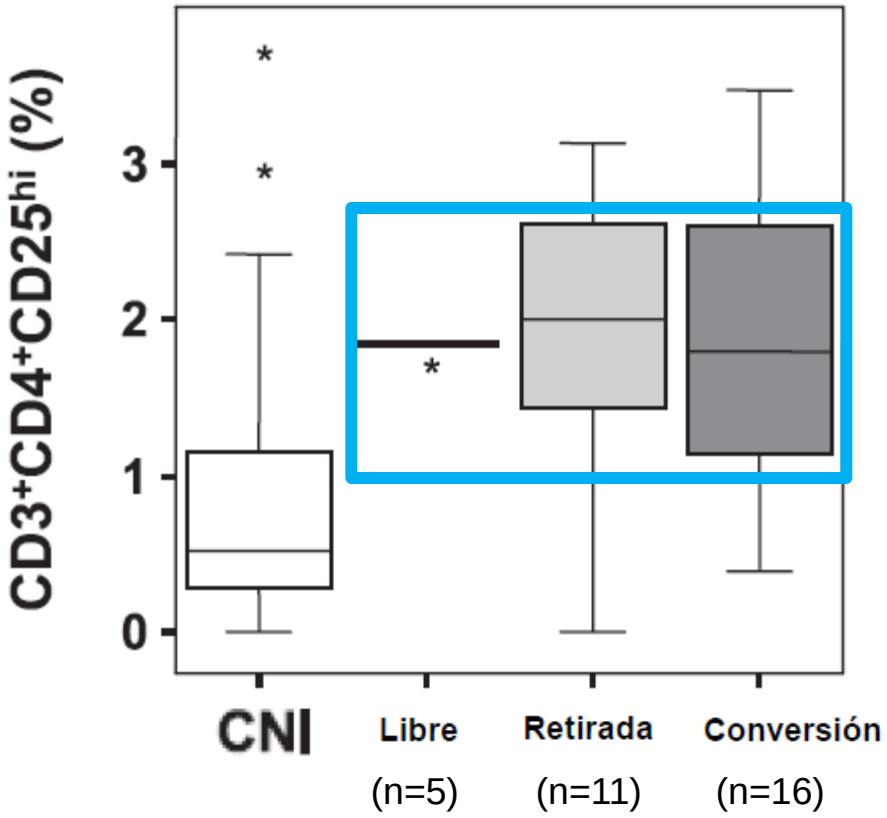
First signal vs second signal blockade



Beatriz Suárez, PhD Thesis

Calcineurin inhibitors, but not imTOR, reduce percentages of $CD4^+CD25^+FOXP3^+$ regulatory T cells in renal transplant recipients





D: Donors
ERT: End stage renal disease
CNI: Calcineurin inhibitors
RAPA: Rapamycin

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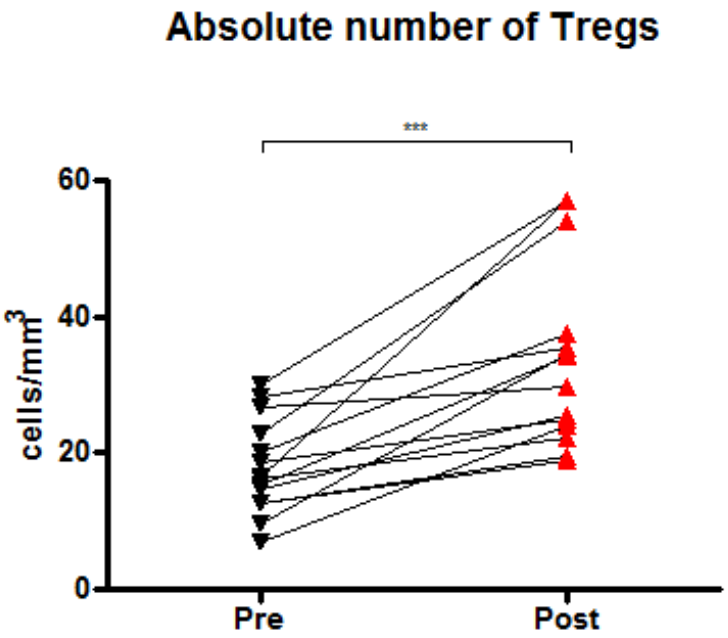
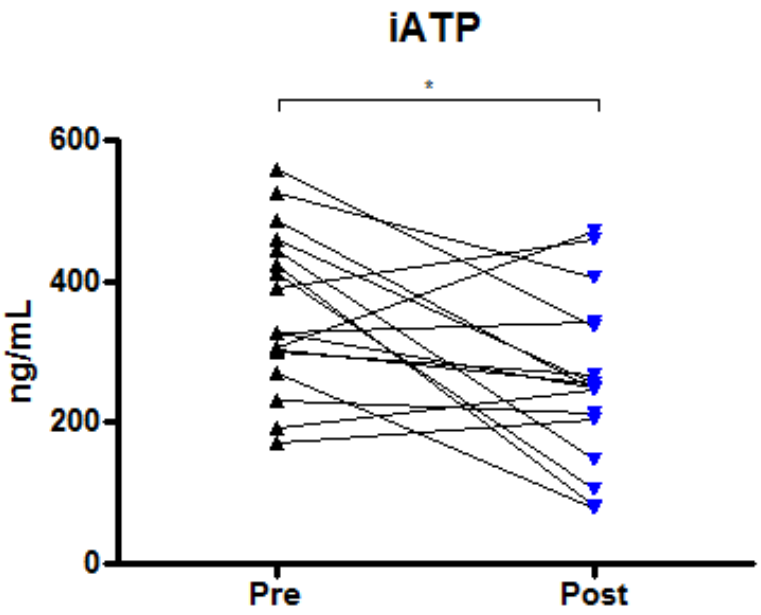
San Segundo et al, Transplantation 2006

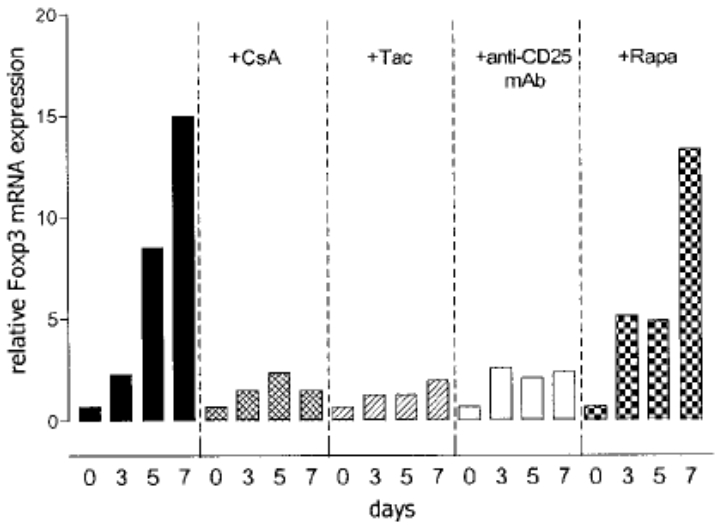
The conversion to imTOR from CNI is accompanied by an increase of circulating Tregs and decrease ATP production



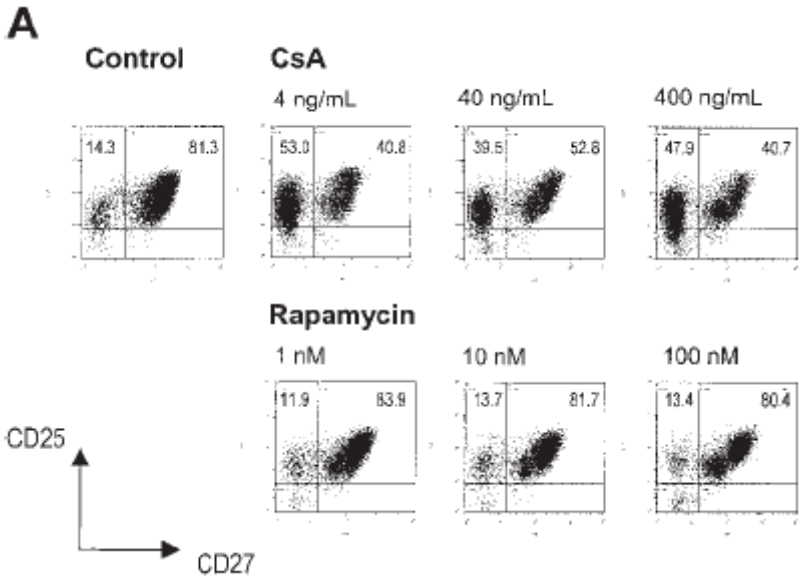


San Segundo et al, Transp Proc 2010

imTOR induce Treg differentiation *in vitro*



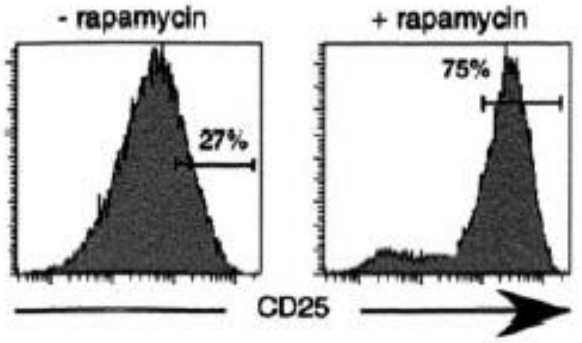
Baan et al; Transplantation 2005; 80: 110-117



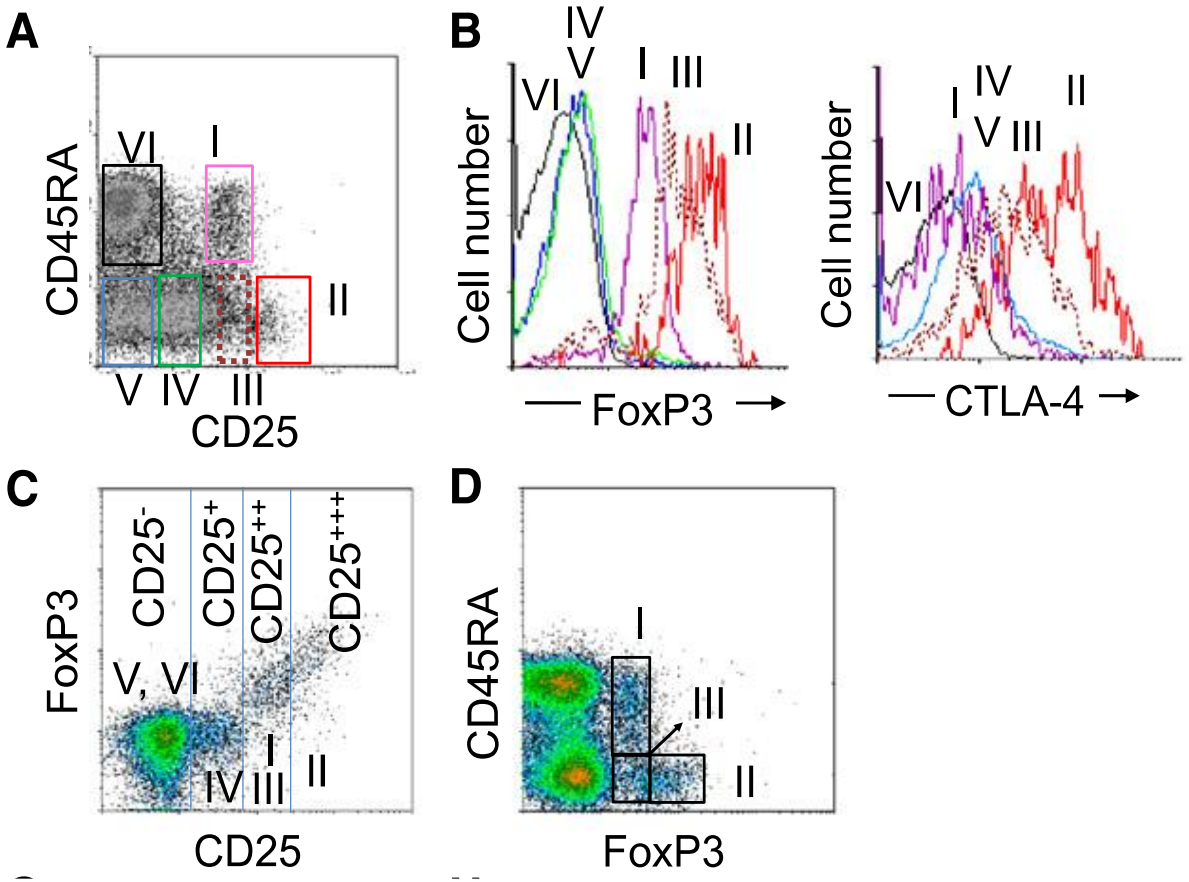
Coenen et al; Blood 2006; 107: 1018-1023

Rapamycin-mediated enrichment of T cells with regulatory activity stimulated CD4+ T cell cultures is not due to the selective expansion of naturally occurring regulatory T cells but to the induction of regulatory functions in conventional CD4+ T cells.

Valmori et al; J Immunol 2006; 177: 944-949.



There is a number of different Treg subsets with different suppressor ability



Fr.I (naive Tregs):	**$CD45RA^{+}$ $CD25^{+}$**
Fr.II (activated Tregs):	**$CD45RA^{-}$ $CD25^{high}$**
Fr.III (silent Tregs):	**$CD45RA^{-}$ $CD25^{+}$**

Miyara et al. Immunity 2009

❑ Multicenter Study

Instituto de Salud Carlos III

Valdecilla
Hospital Universitario Marqués de Valdecilla

CLÍNIC
BARCELONA
Hospital Universitari

Hospital Universitario 12 de Octubre
SaludMadrid
Comunidad de Madrid

Arrixaca
Hospital Universitario "Virgen de la Arrixaca"

❑ 75 patients with end-stage renal disease recruited

Exclusion criteria: pediatric, retransplant, ABO mismatched, HIV-positive

San Segundo D et al. Transplantation 2014

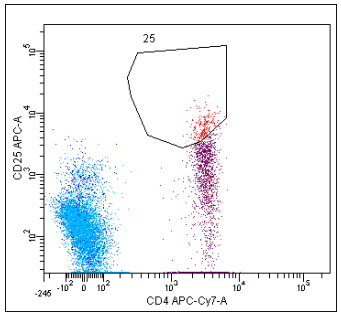
❑ Regulatory T cell subsets

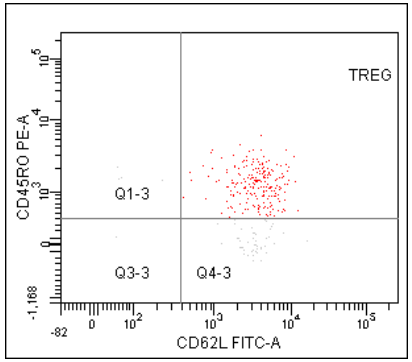
➢ Natural Tregs

✧ $CD4^{+}CD25^{+}CD127^{low}CD27^{+}Foxp3^{+}$

➢ Mature/Activated Tregs

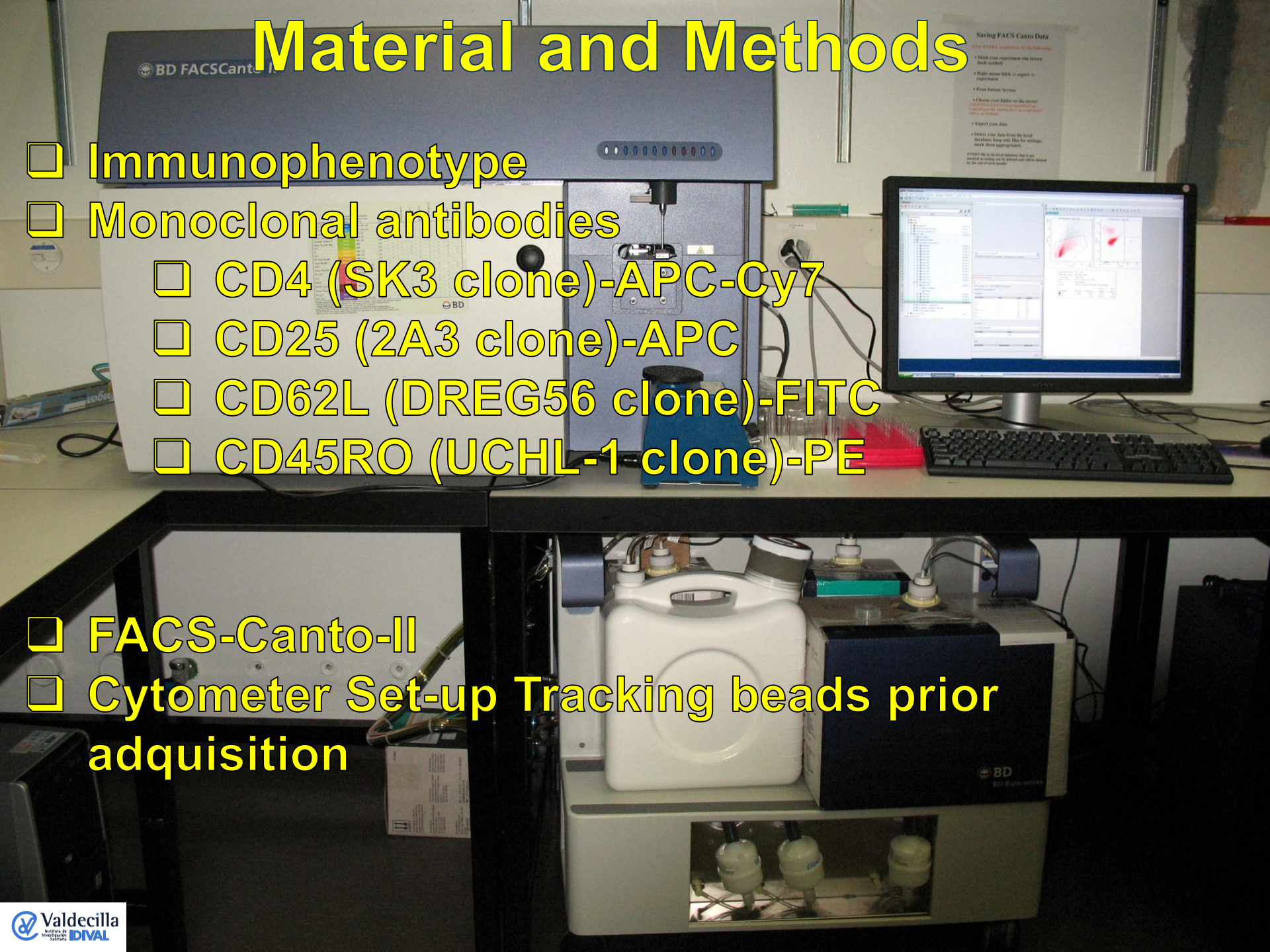
✧ $CD4^{+}CD25^{high}CD62L^{+}CD45RO^{+}$





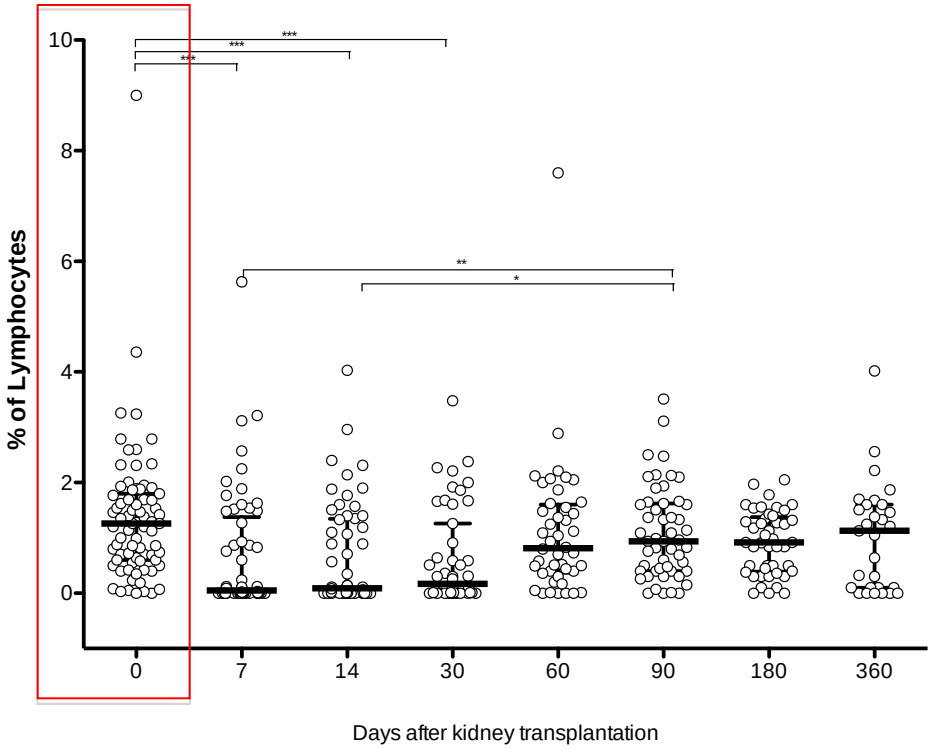
Material and Methods

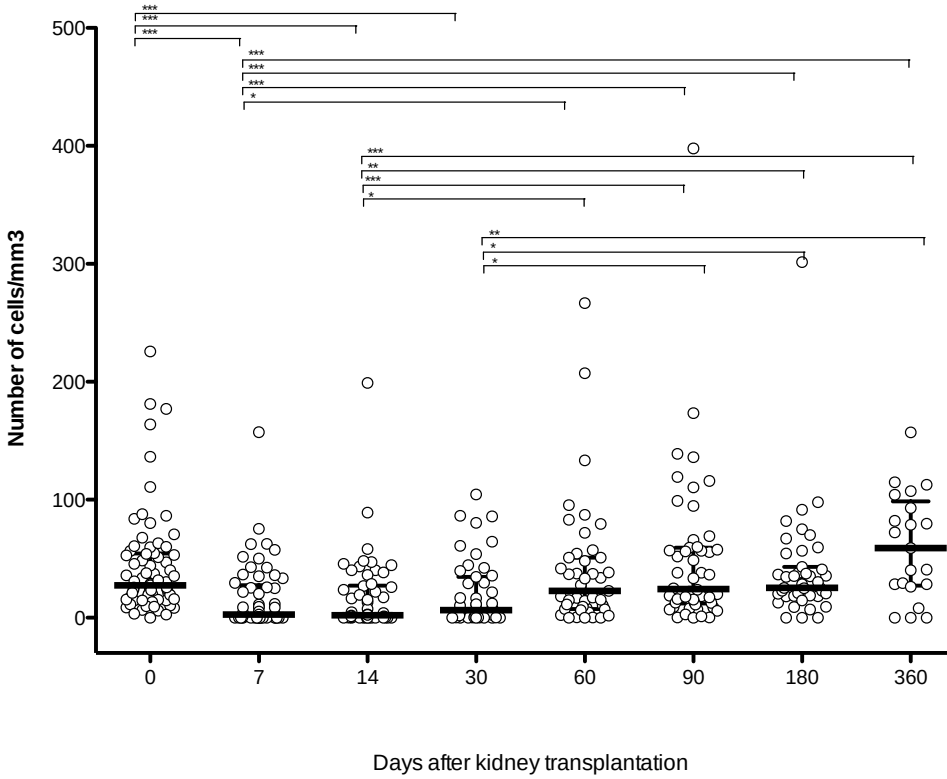
- Immunophenotype
- Monoclonal antibodies
  - CD4 (SK3 clone)-APC-Cy7
  - CD25 (2A3 clone)-APC
  - CD62L (DREG56 clone)-FITC
  - CD45RO (UCHL-1 clone)-PE



- FACS-Canto-II
- Cytometer Set-up Tracking beads prior adquisition

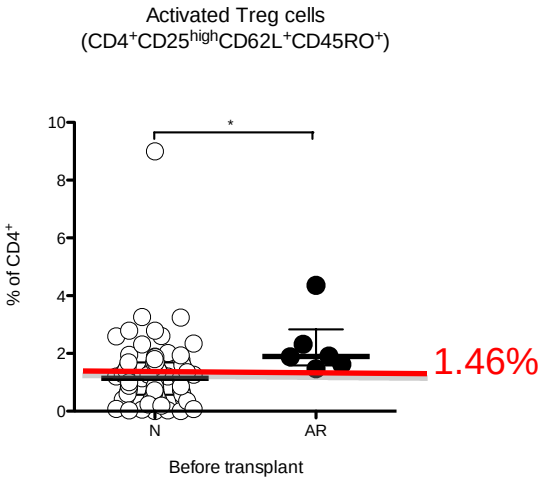
❑ Activated Tregs ($CD4^{+}CD25^{high}CD62L^{+}CD45RO^{+}$)



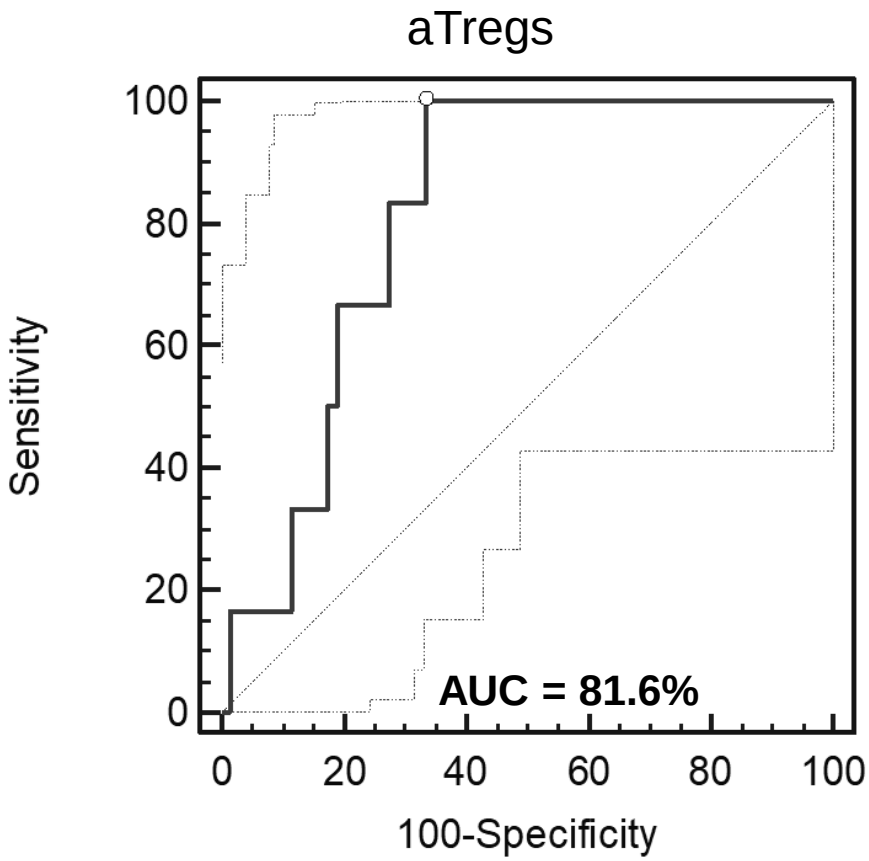


San Segundo D et al. Transplantation 2014

Recipients suffering from acute rejection showed higher frequencies of activated Tregs before kidney transplantation



Pretransplant activated Tregs >1.46 % are a risk factor for acute rejection



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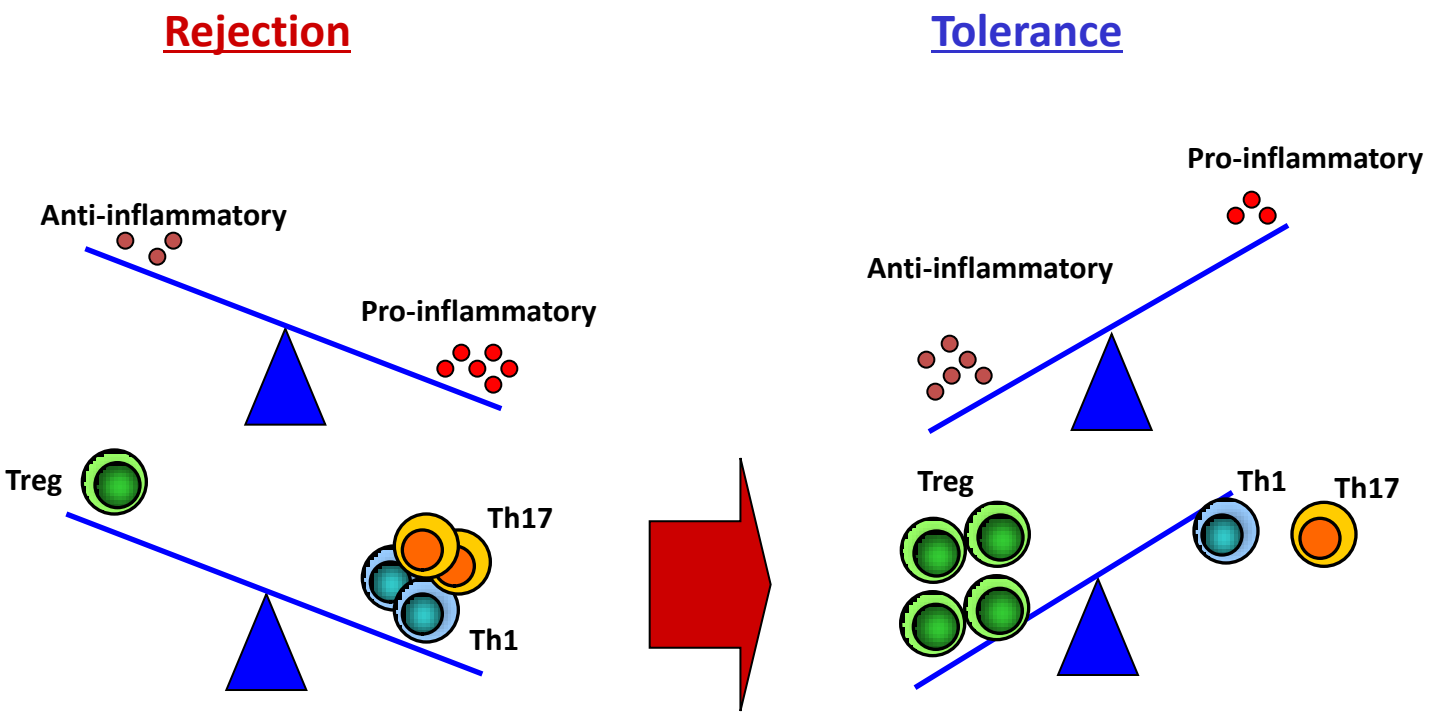
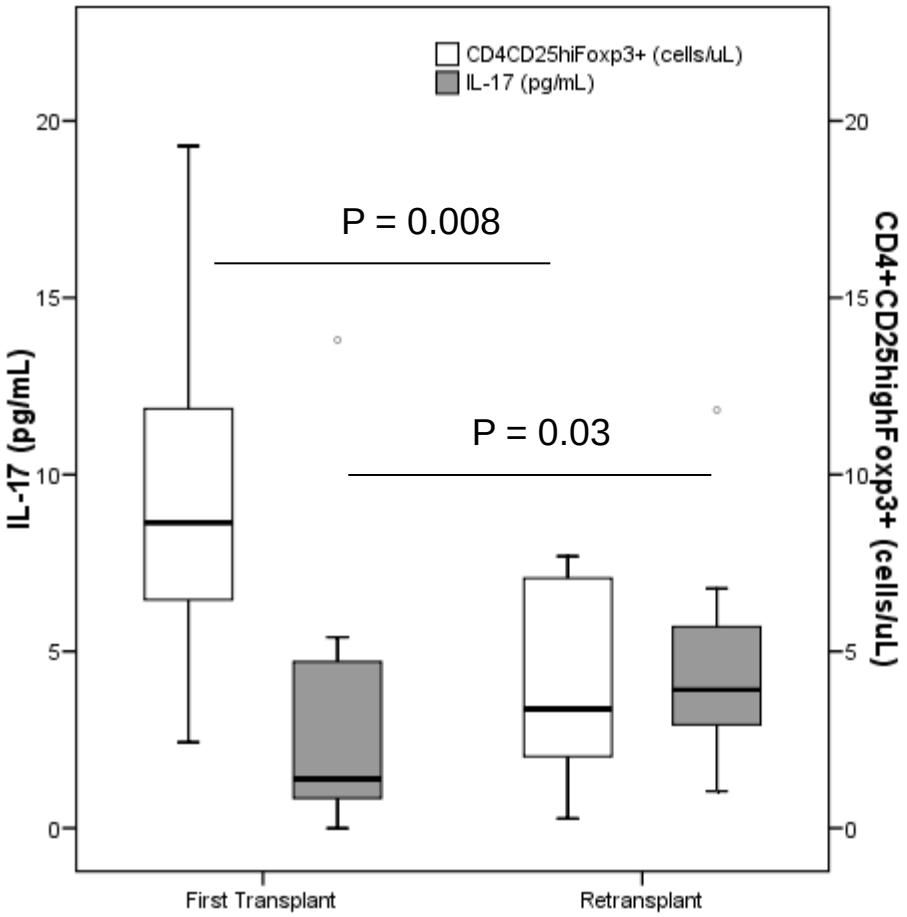
San Segundo D et al. Transplantation 2014

Th17-Th1/Treg balance Tolerance/Rejection

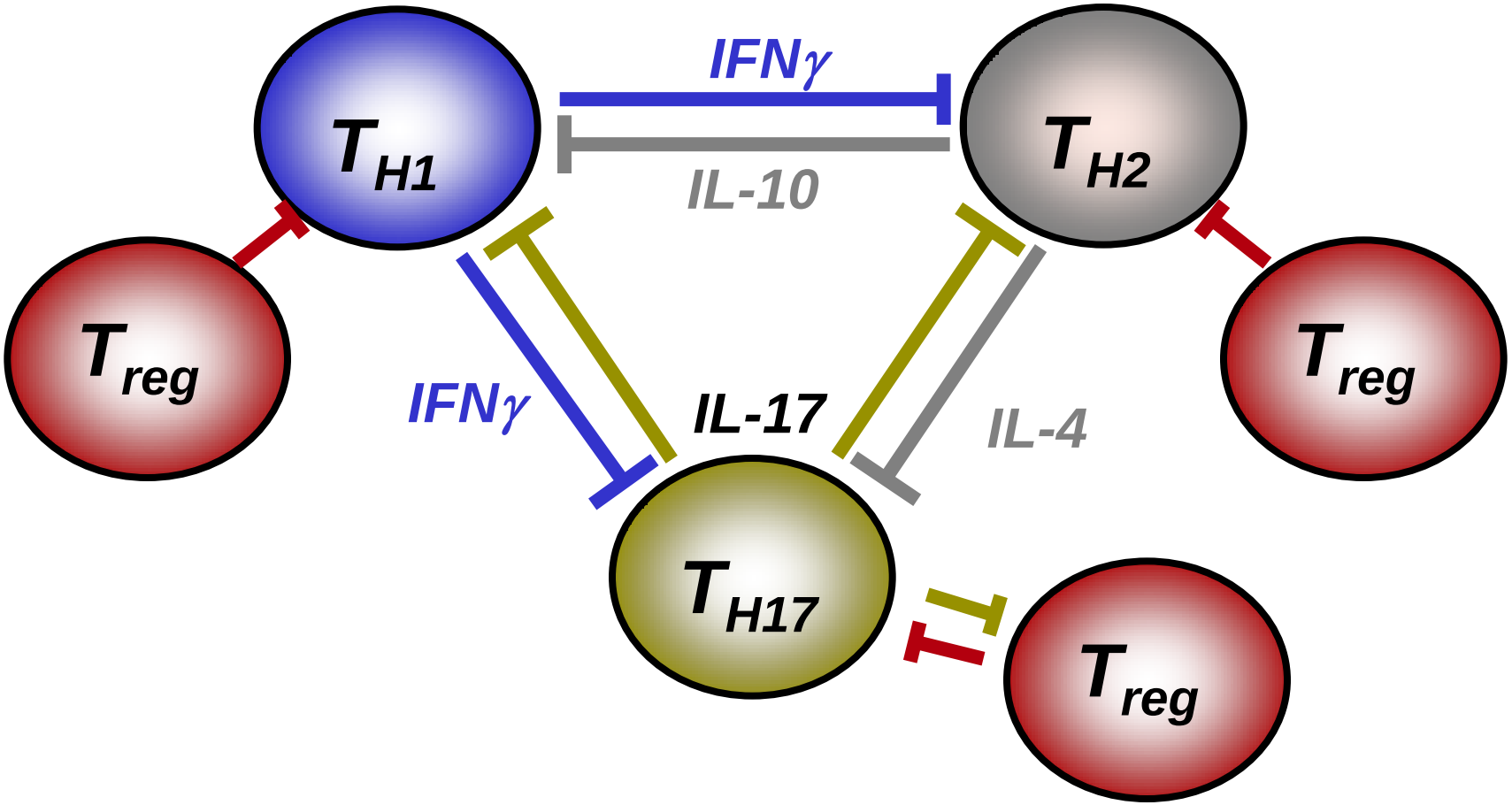


The balance between Th17 and Tregs changes in renal transplant recipients if they have received a previous renal allograft



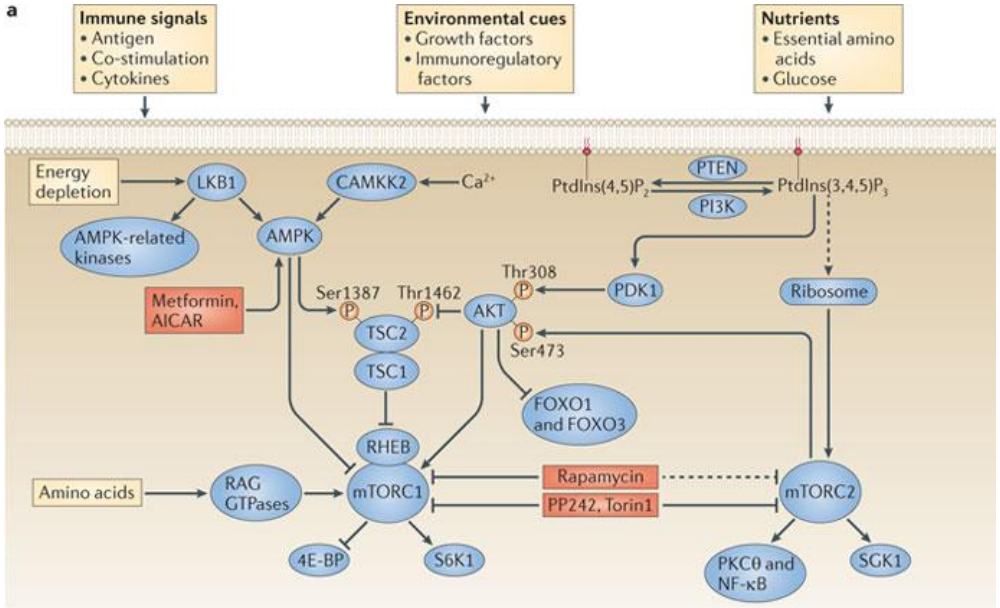
San Segundo D et al. Transplant Proc 2008

Types of CD4$^+$ T cells: functional connection



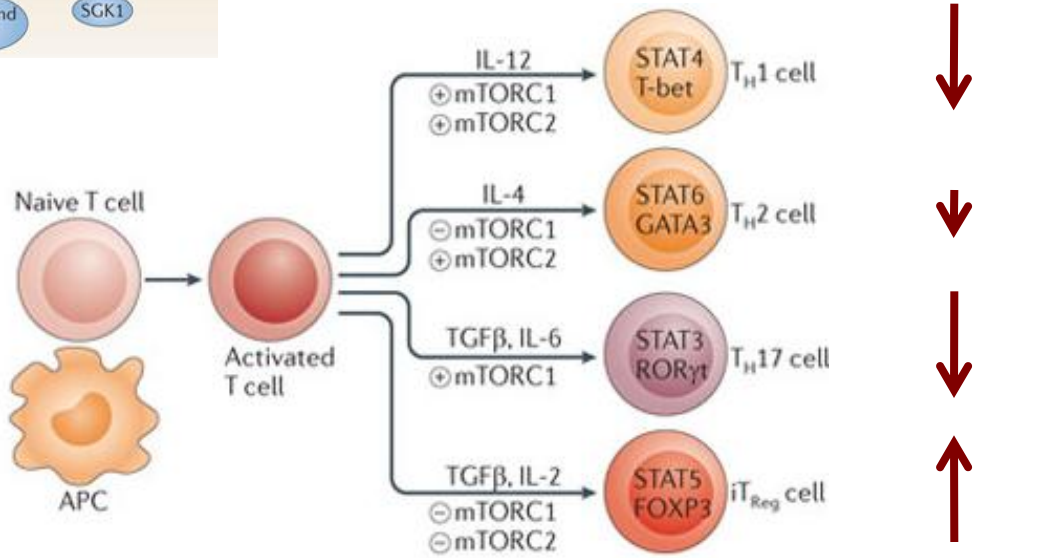
Courtesy: Dr. J Merino (Univ Cantabria)

imTOR effects in Th cells



Differential activation of mTORC isoforms in the induction of Th subsets

mTORC1 and mTORC2 in the induction of Th subsets



Chi H. Nat Rev Immunol 2012 Am J Transplant 2011
Hurez V, et al Ageing Cell 2015

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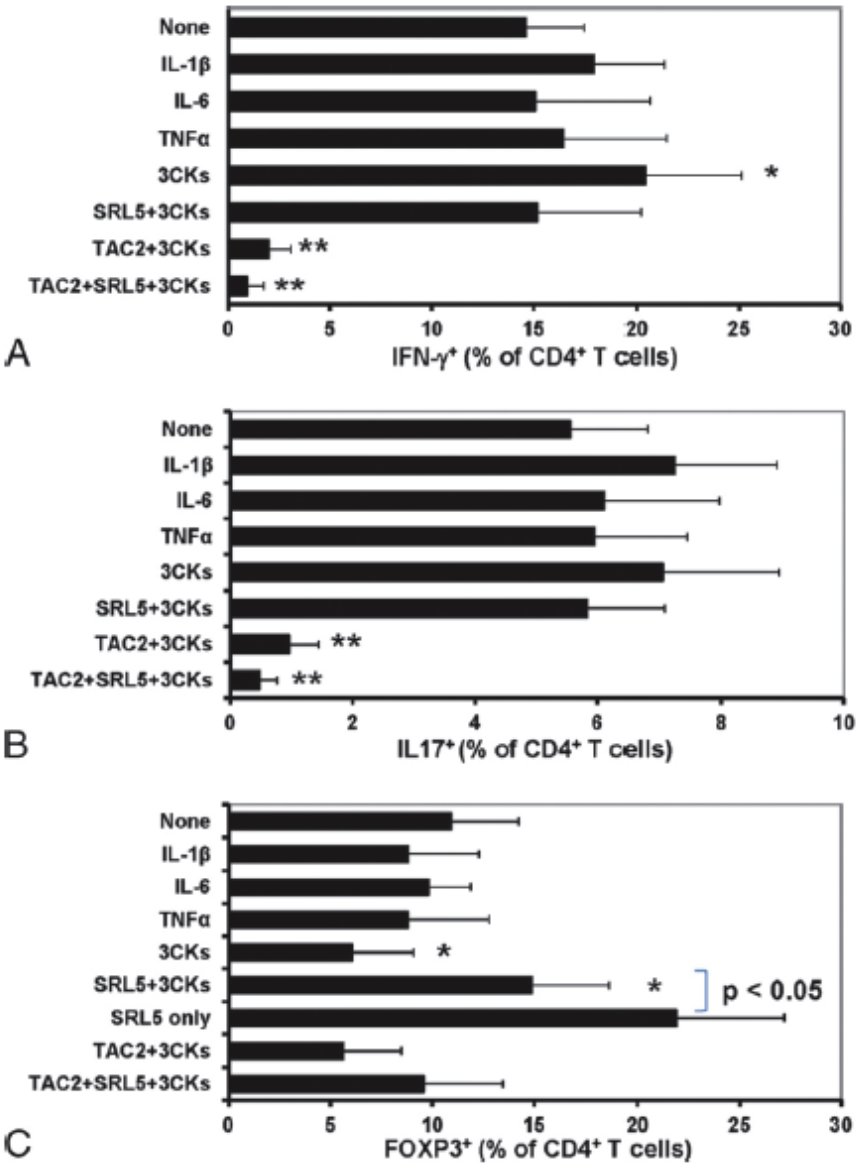
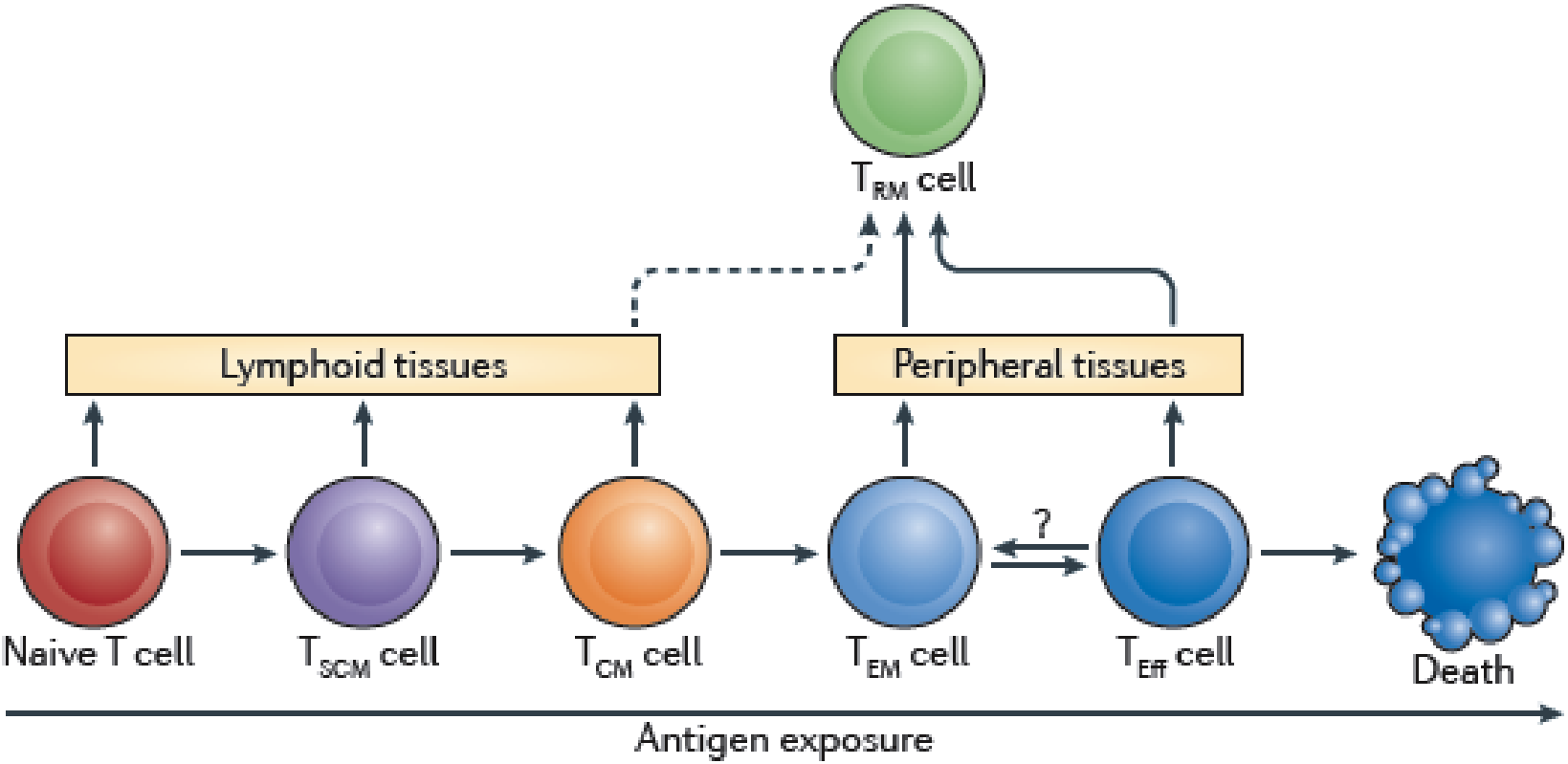
The combination of CNI and imTOR synergizes in the inhibition of Th1 and TH17 cells and recovers the decrease of Tregs induced by inflammatory cytokines or CNI



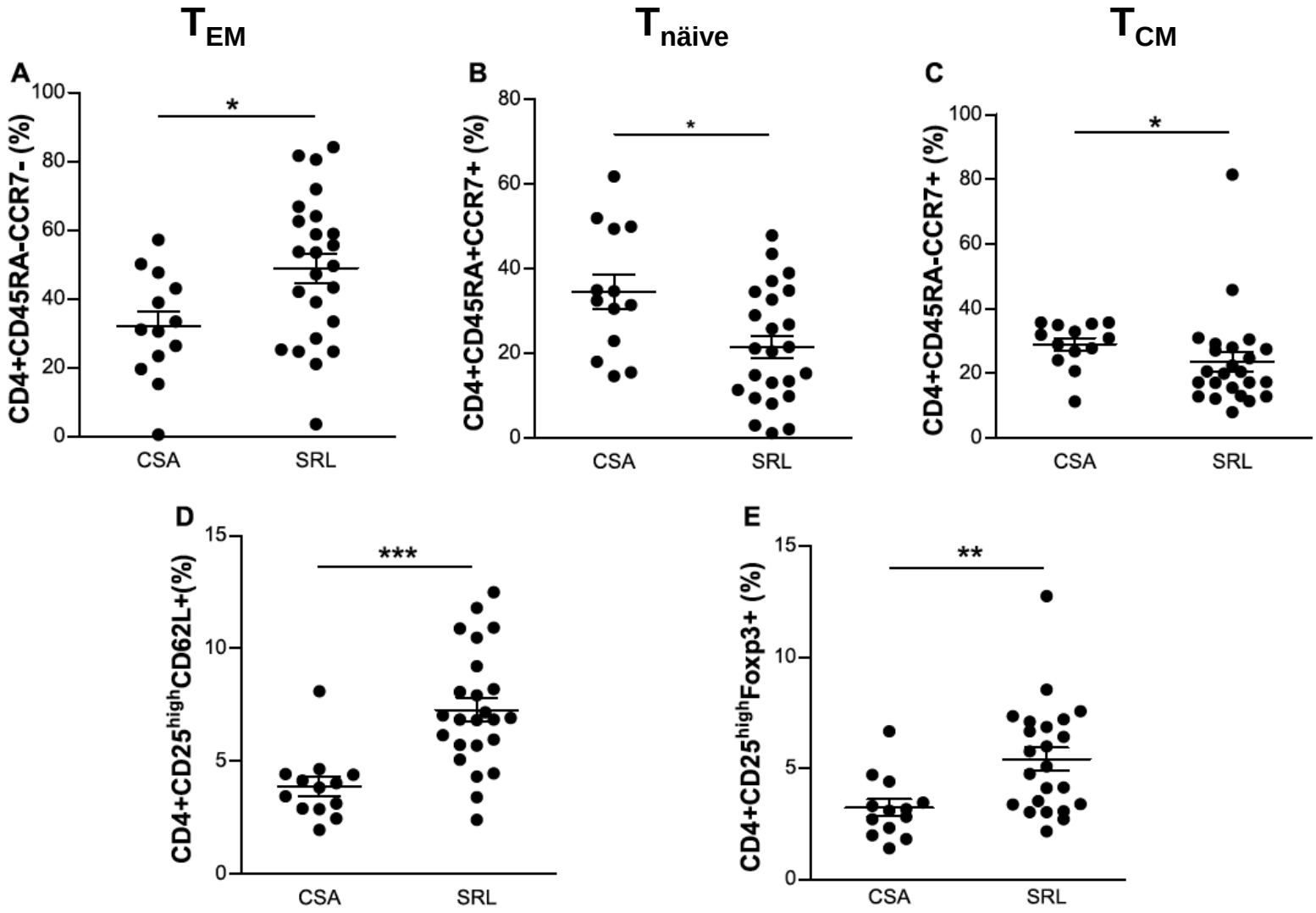
Gallon L et al, Transplantation 2015

Memory T cell generation



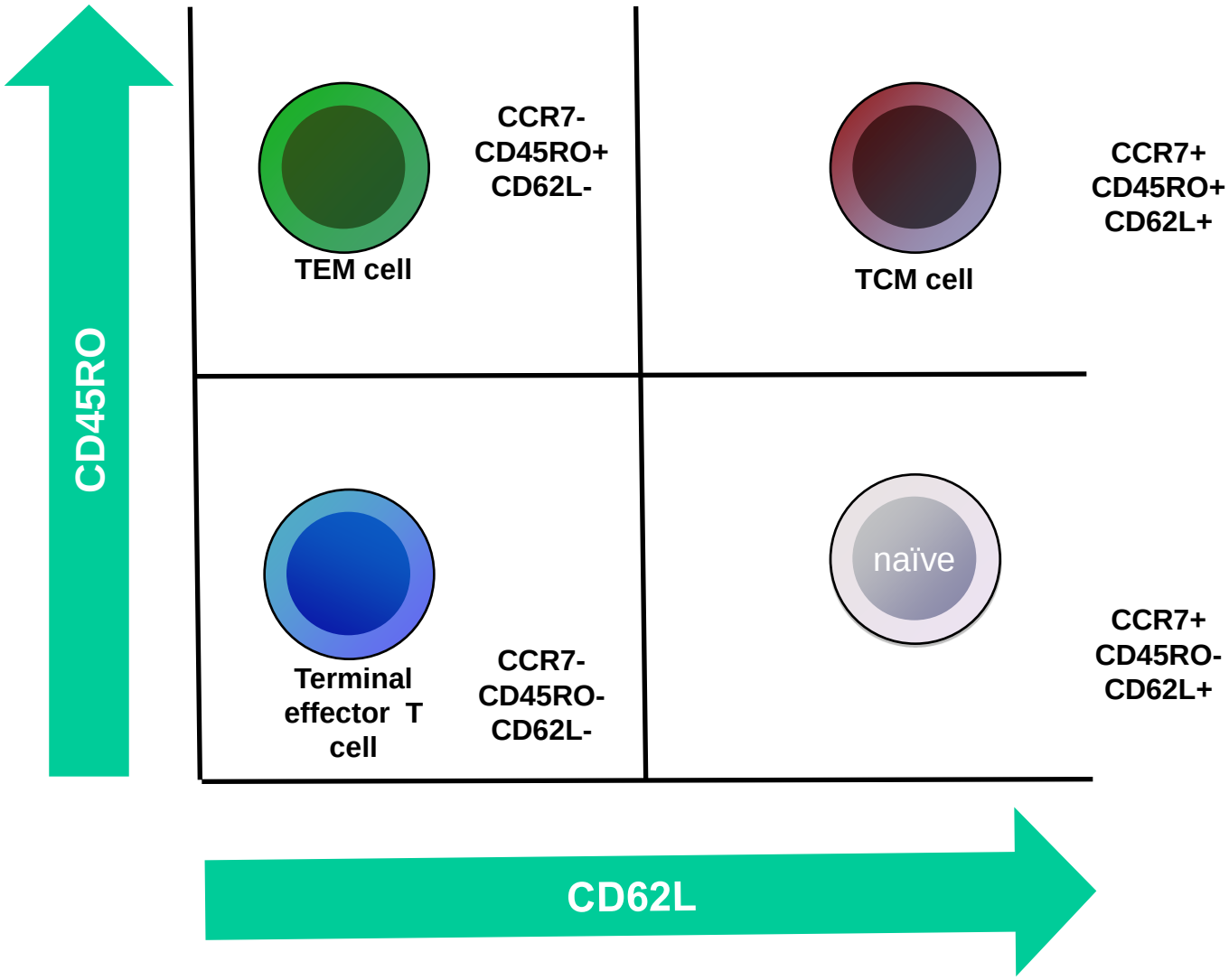
Farber DL, et al; Nat Rev Immunol 2014

Monotherapy with sirolimus induced an in vivo increase of T_{EM} cell and Tregs in renal transplant recipients as compared with CsA

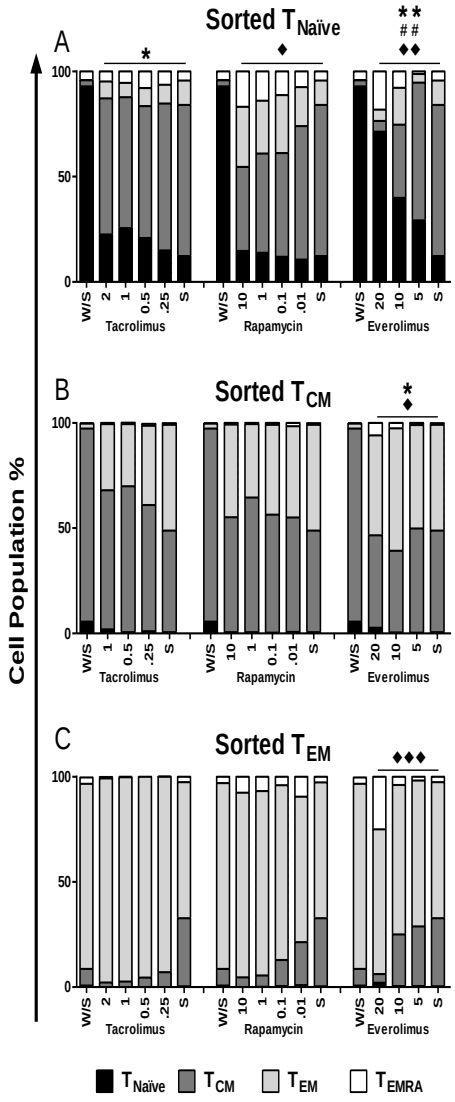


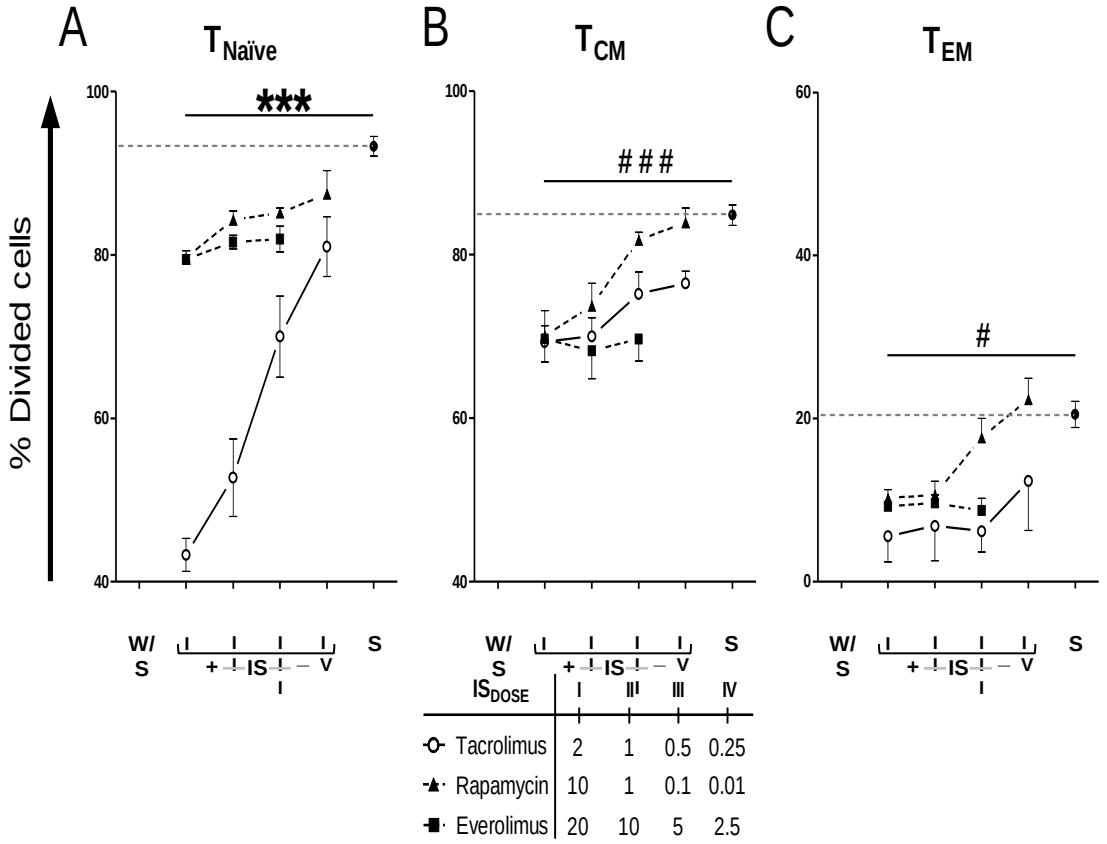
Brouard et al, Am J Transplant 2010

Immunophenotyping Memory T cells



Everolimus, but not Sirolimus, blocks maturation of naïve Th cells after *in vitro* stimulation



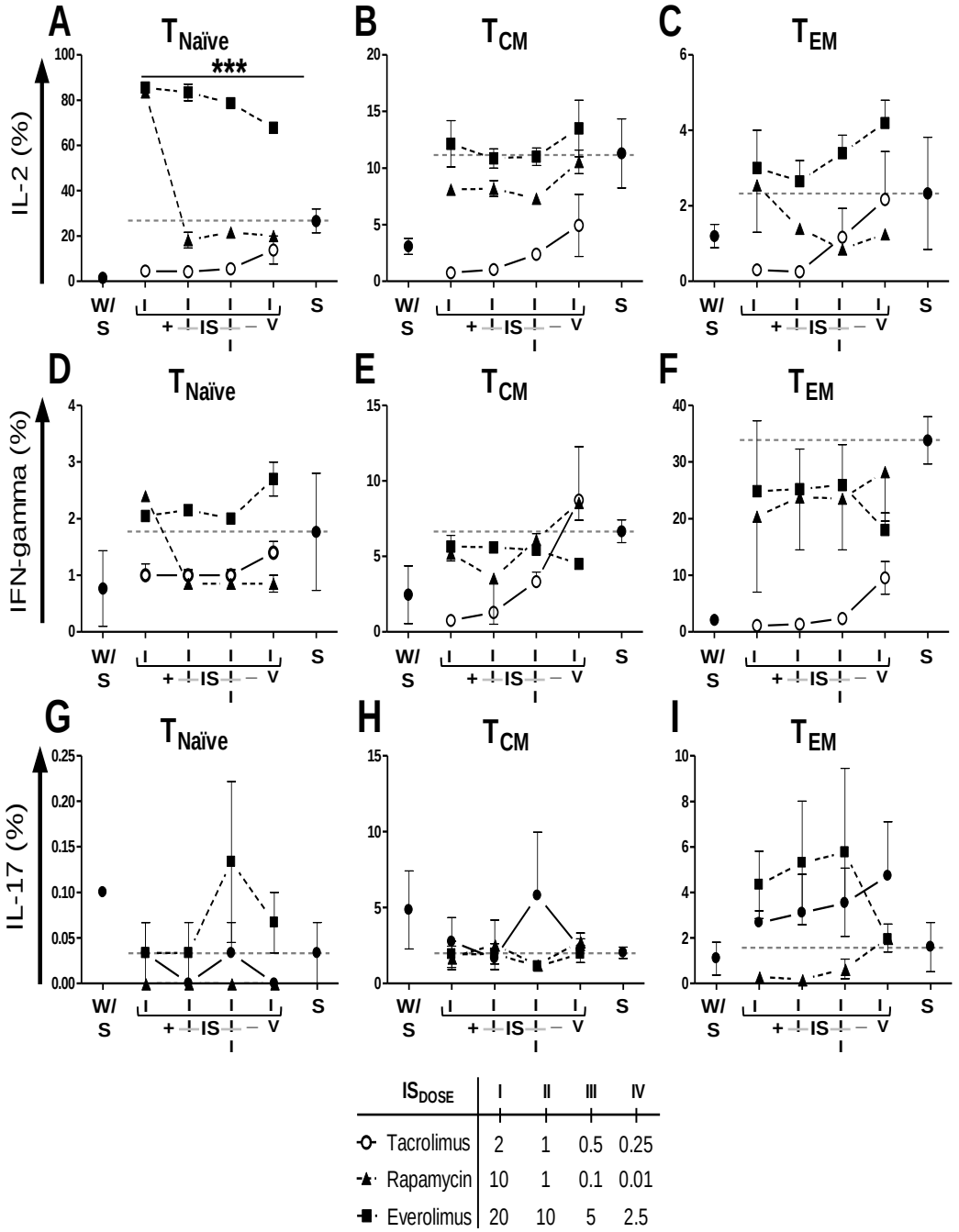


Tacrolimus inhibits the proliferation of naïve Th cells but it does not affect their maturation
Everolimus inhibits TCM and TEM proliferation better than Sirolimus

Merino D, et al. Immunology (submitted)

Valdecilla IDIVAL

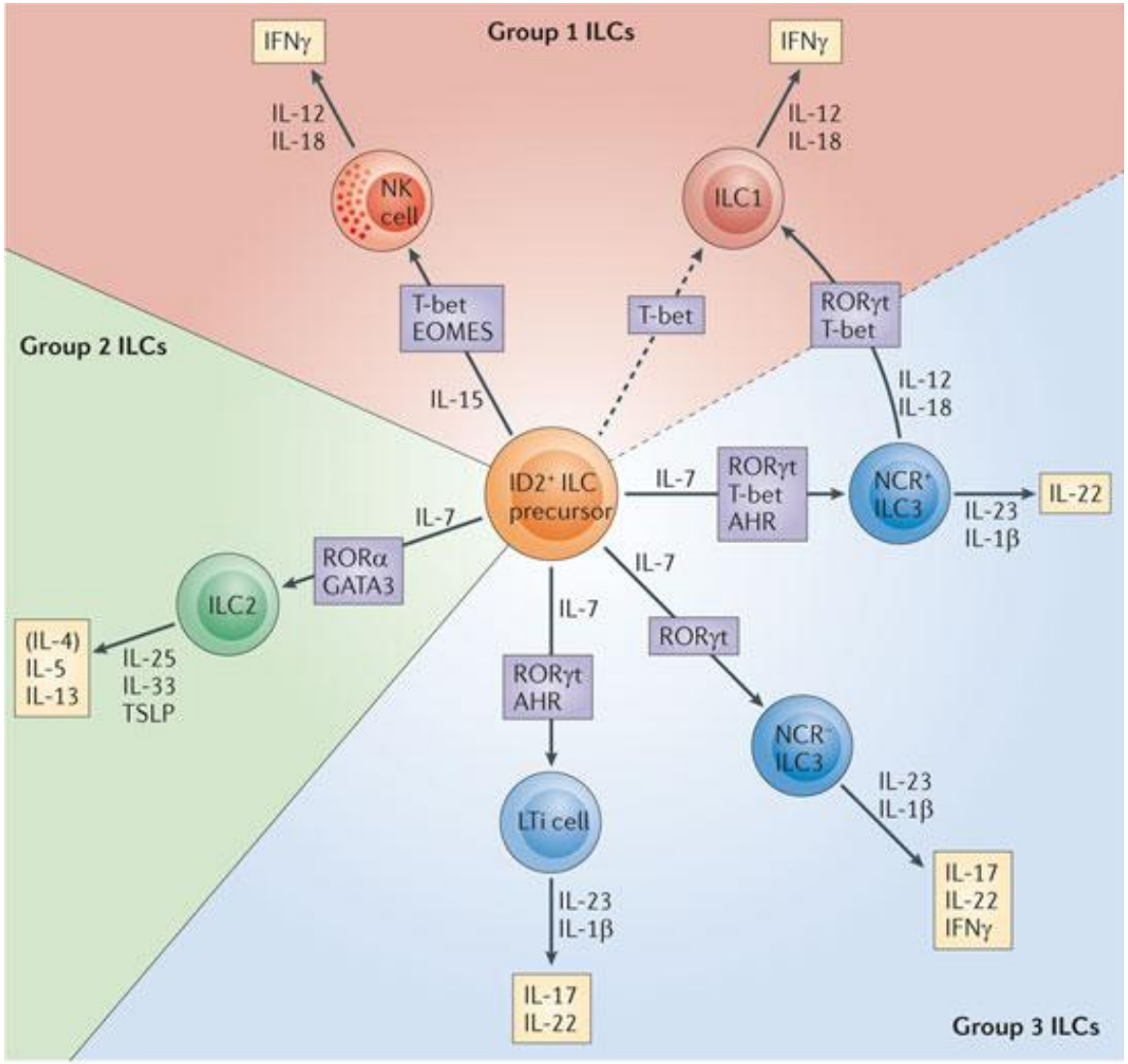
The production of cytokines by effector or naïve Th cells in vitro was not significantly affected by Tac or imTOR in *in vitro* culture



Merino D, et al. Immunology (submitted)

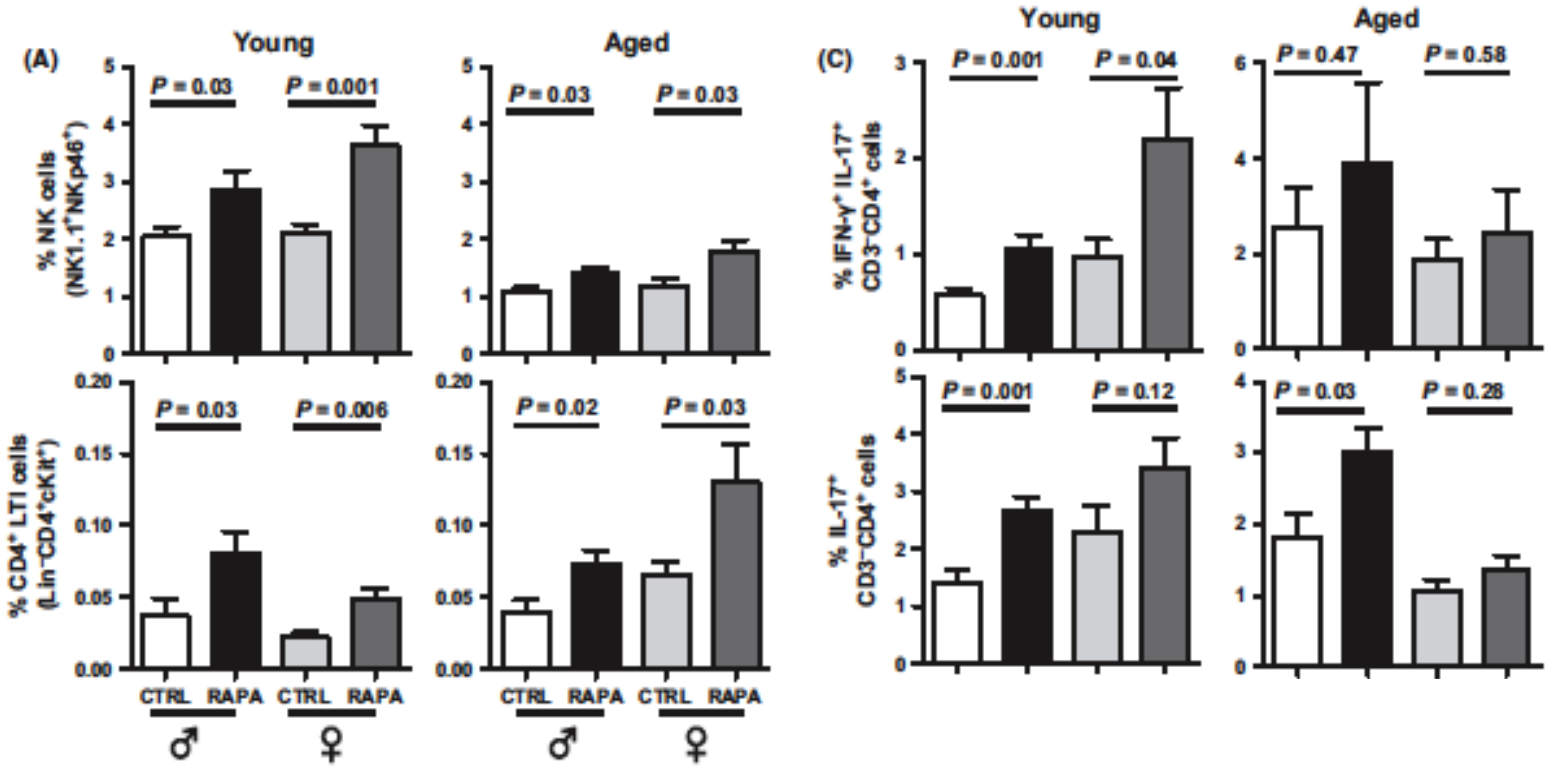
Innate lymphoid cells (ILCs)

A family of developmentally related cells that are involved in immunity and in tissue development and remodelling



Spits H, et al. Nat Rev Immunol 2013

imTOR may also affect ILC



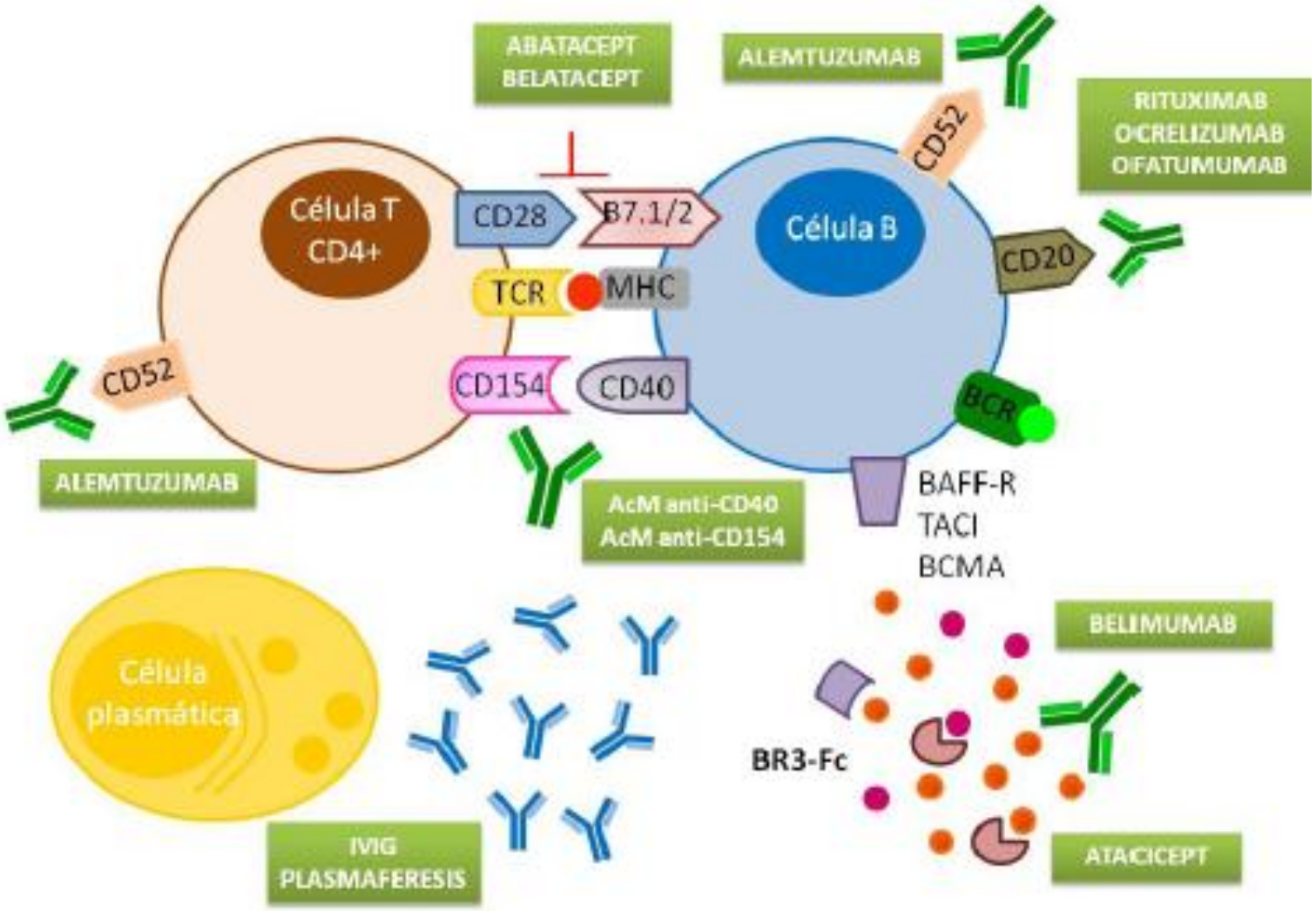
ILC-1

ILC-3

Hurez V, et al Ageing Cell 2015

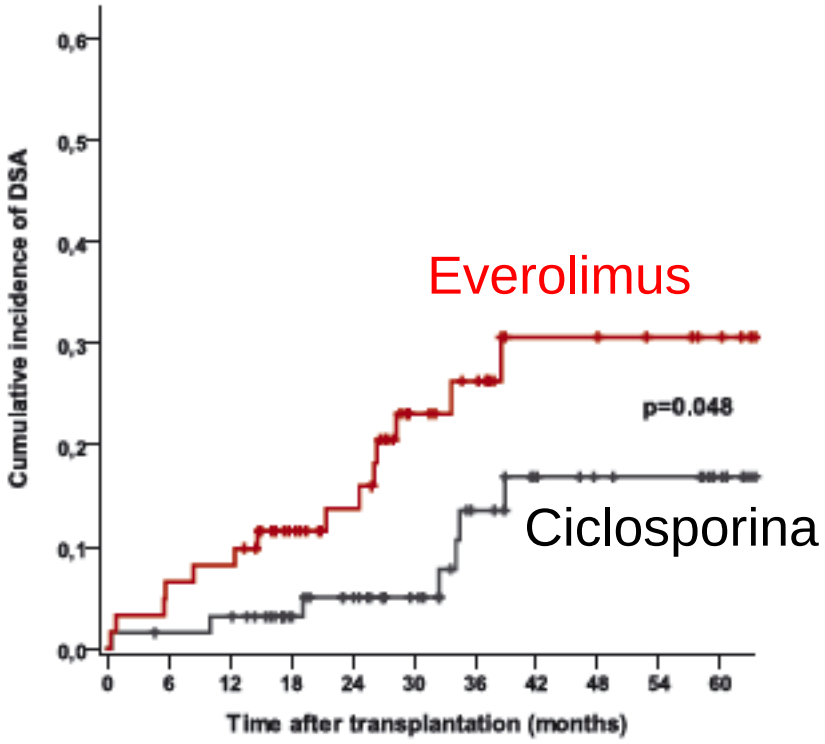
imTOR and B cells:

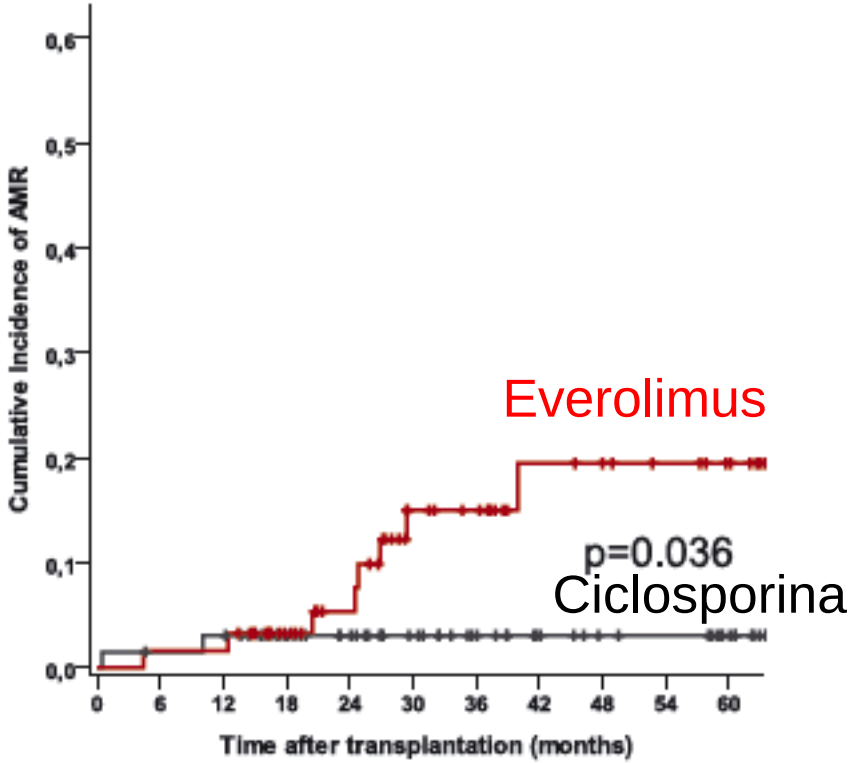
Not only humoral specific therapies



Beatriz Suárez, PhD Thesis

CNI inhibits more effectively the production of DSA (donor specific antibodies) and humoral rejection incidence than imTOR





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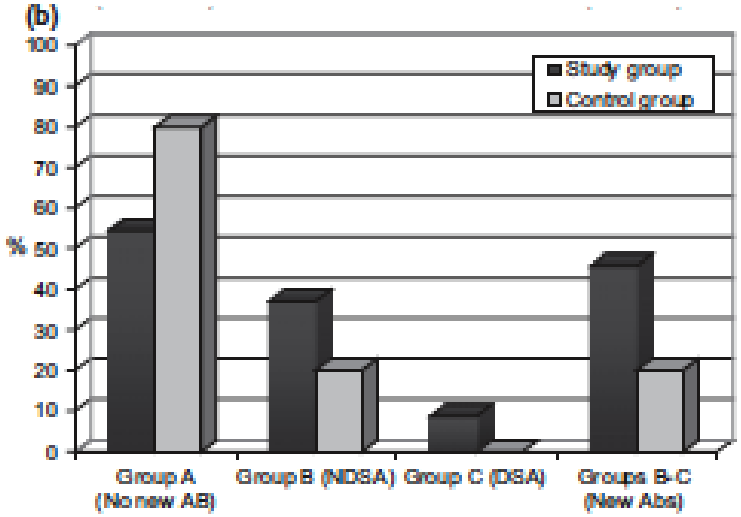
Liefeldt L et al. AJT 2012

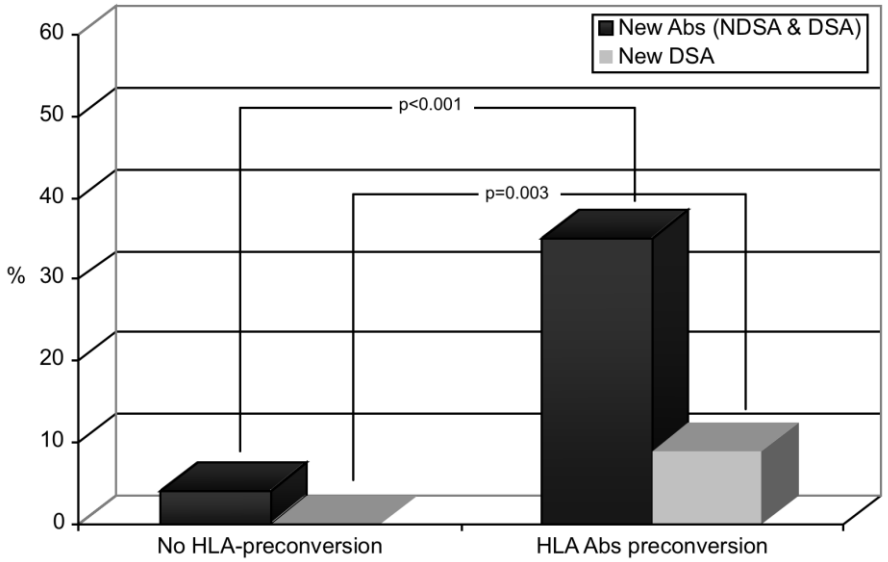
The group of patients converted produces higher HLA Abs after conversion, although most of them are NDSA

	mTORi conversion (n = 184)	CNI therapy (n = 63)	P value
Period of study	Oct-1999/ Feb-2010	Oct-1999/ Feb-2010	
Gender (male/female)	121/63	45/18	NS
Ethnicity (Caucasian/other)	183/1	63/0	NS
Donor type (Living/Deceased)	2/182	0/63	NS
Time after Tx (months): median (range)	65 (3–375)	38 (3–236)	NS
Mean age at conversion (years ± SD)	53.4 ± 13.4	53.2 ± 11.8	NS
Indication for conversion			
IFTA	88		
Neoplasia	65		
Pre-emptive	17		
Other	14		
Mean sCr preconversion (mg/dl ± SD)	1.86 ± 0.72	1.74 ± 0.70	NS
Number of Tx (1st/2nd/3rd) (%)	79.3/19.0/1.6	78.9/17.3/3.8	NS
PRA CDC Pre-Tx (<10%/≤10%)	90%/10%	88%/12%	NS
Preconversion immunosuppression			
TCR/CsA	86/93	29/34	NS
MMF/AZA	97/25	41/8	NS
Esteroids/No esteroids	96/88	39/24	NS

PRA CDC, panel reactive antibodies by complement-dependent cytotoxicity; sCr, serum creatinine; Tx, transplantation.



The risk is higher when there is presensitization before conversion



Ruiz-San Millán JC et al. Transplant Int 2014

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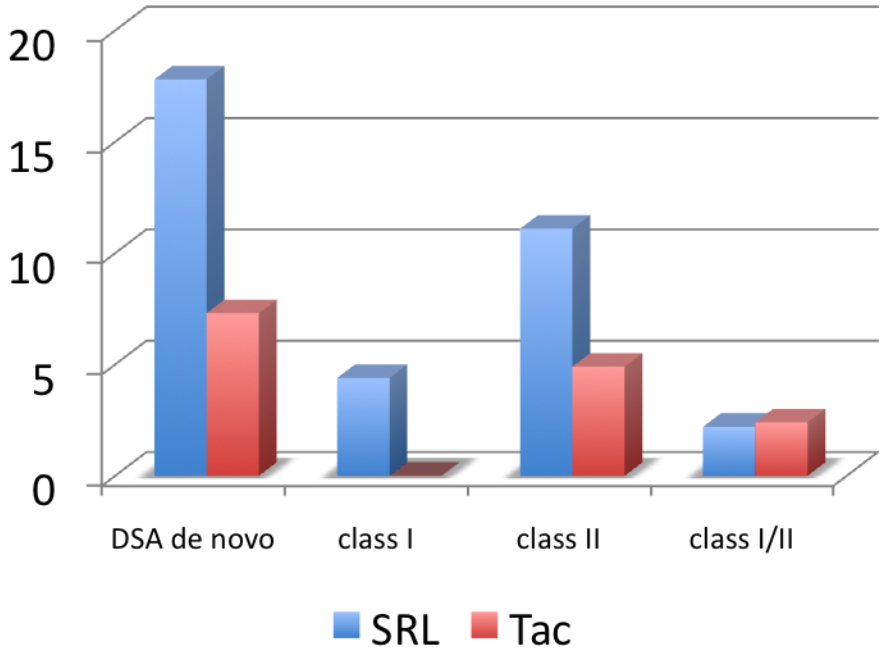
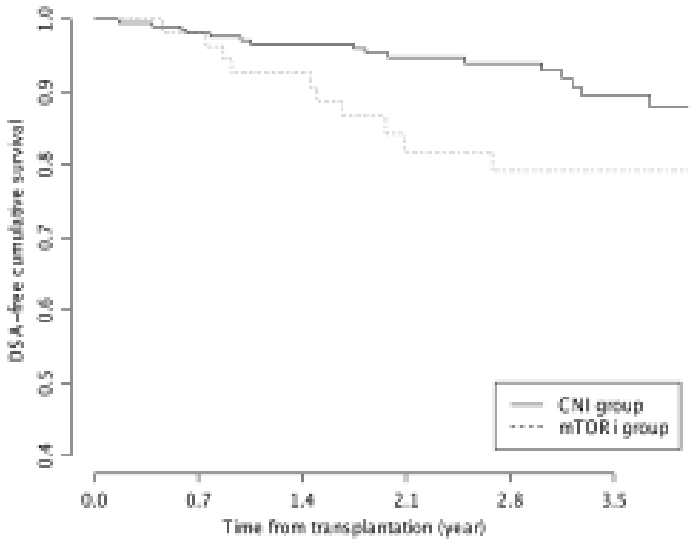
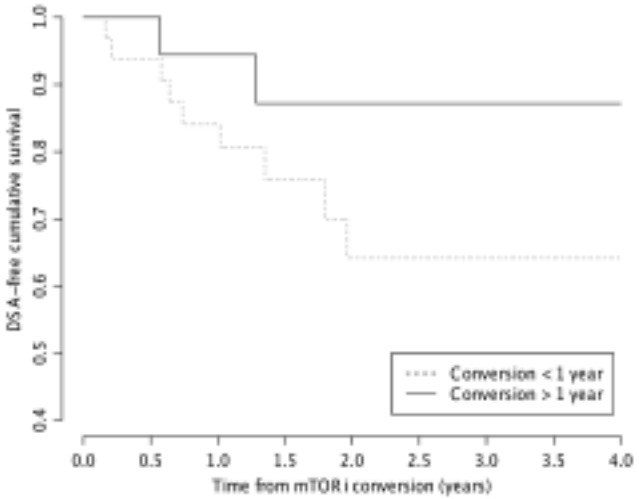
Patients converted from Tac to SRL at 3mo post-Tx showed a tendency to higher incidence of DSA



Veras de Sandes-Freitas TV, et al. Transplantation 2015



But…, the risk seems to be reduced when conversion is not done early



Croze L-E, et al. Transplant Int 2014

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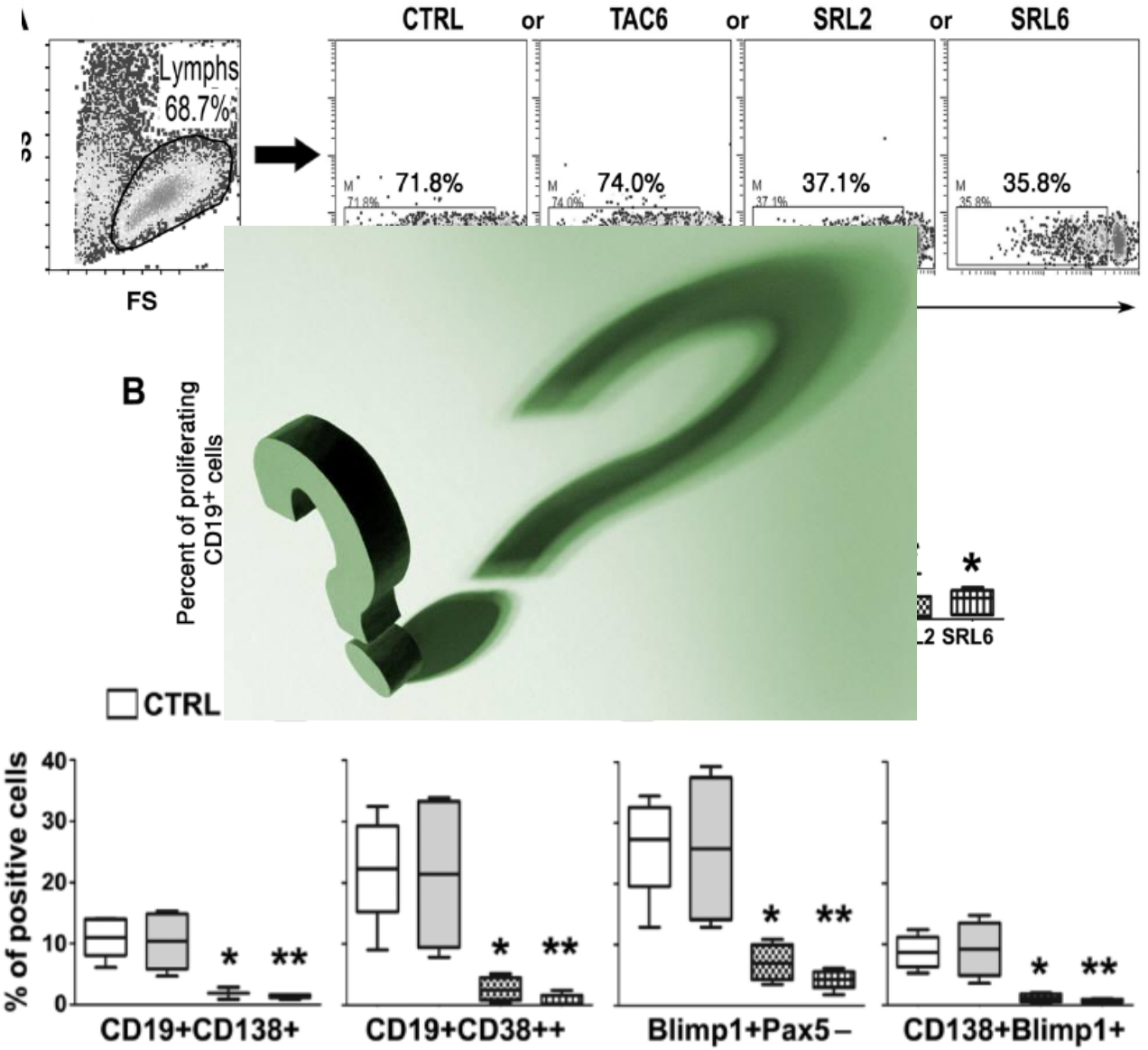
However, CNI does not affect *in vitro* human B cell proliferation or plasma cell differentiation but imTOR does



Traitanon O et al, PLoS ONE 2015

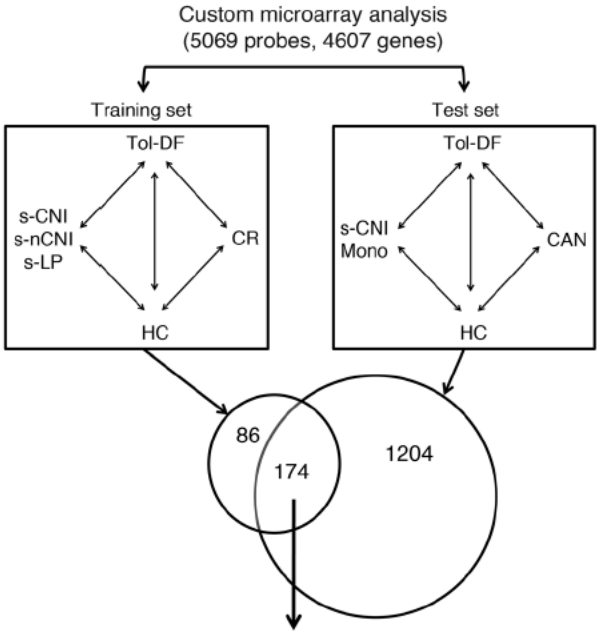
ALLOREACTIVE B CELLS SUBSETS BASED ON FUNCTION

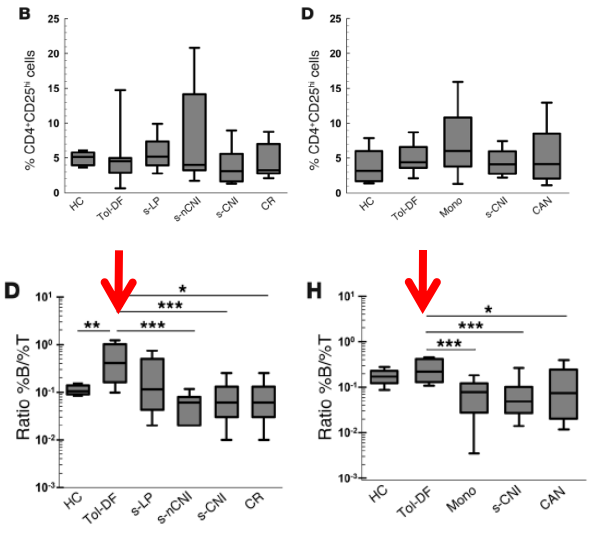
PATHOGENIC B CELLS

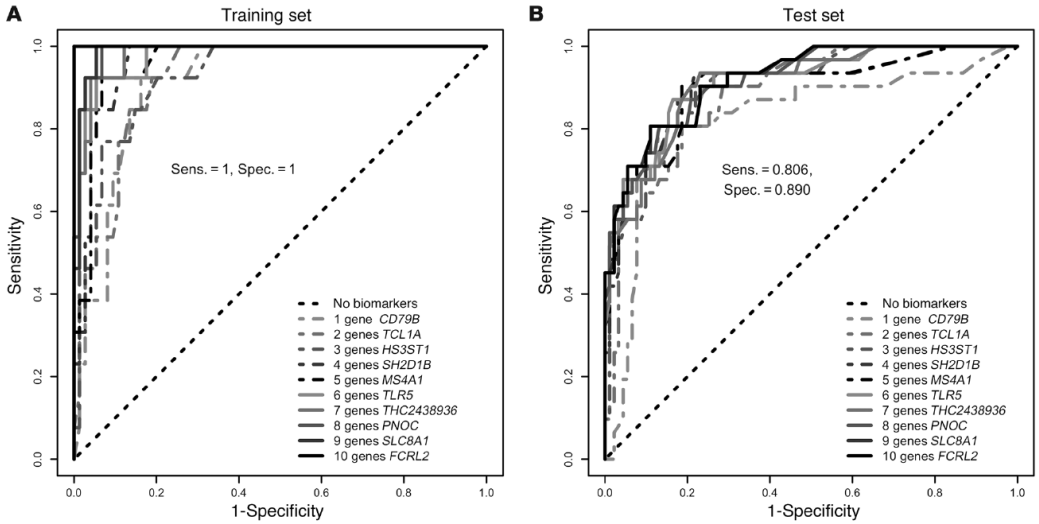
- Produce alloantibodies that mediate rejection
- Produce proinflammatory cytokines
- Function as antigen presenting cells
- Maintain memory T cells

REGULATORY B CELLS

ENRICHED B CELL SIGNATURE IN OPERATIONAL TOLERANT RENAL TRANSPLANT PATIENTS







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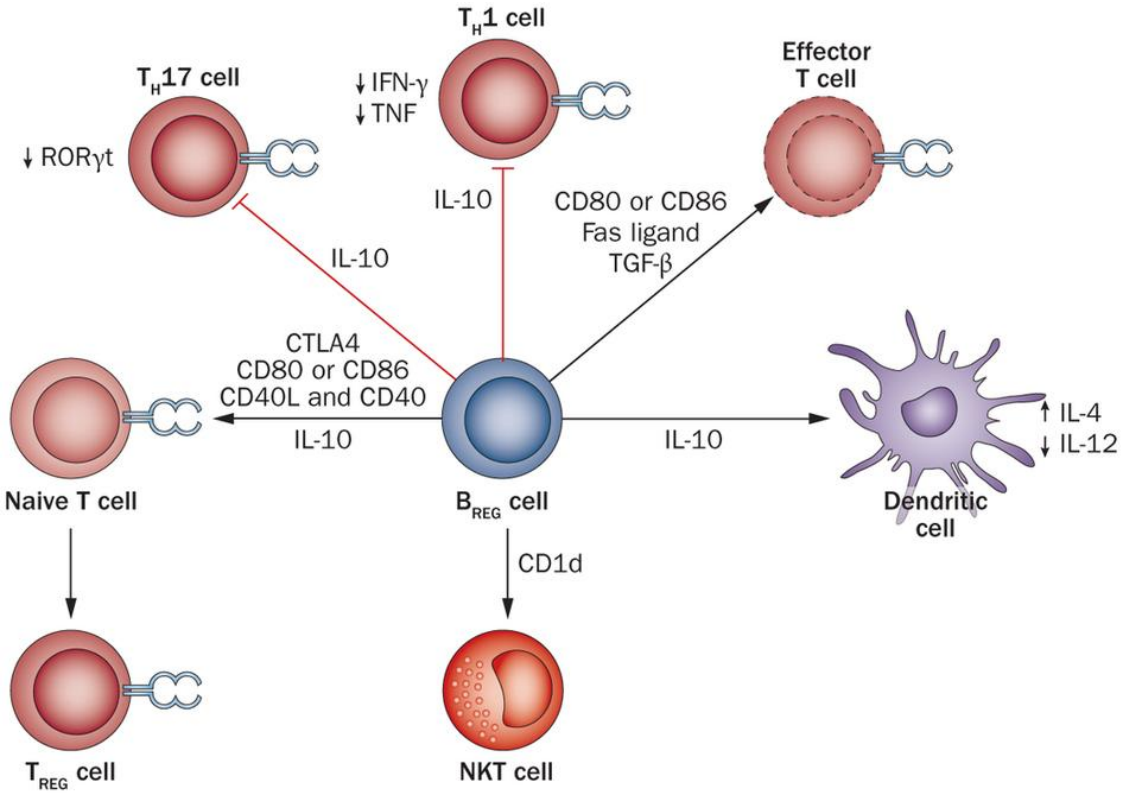
Sagoo P et al, JCI 2010

REGULATORY B CELLS AND TRANSPLANTATION

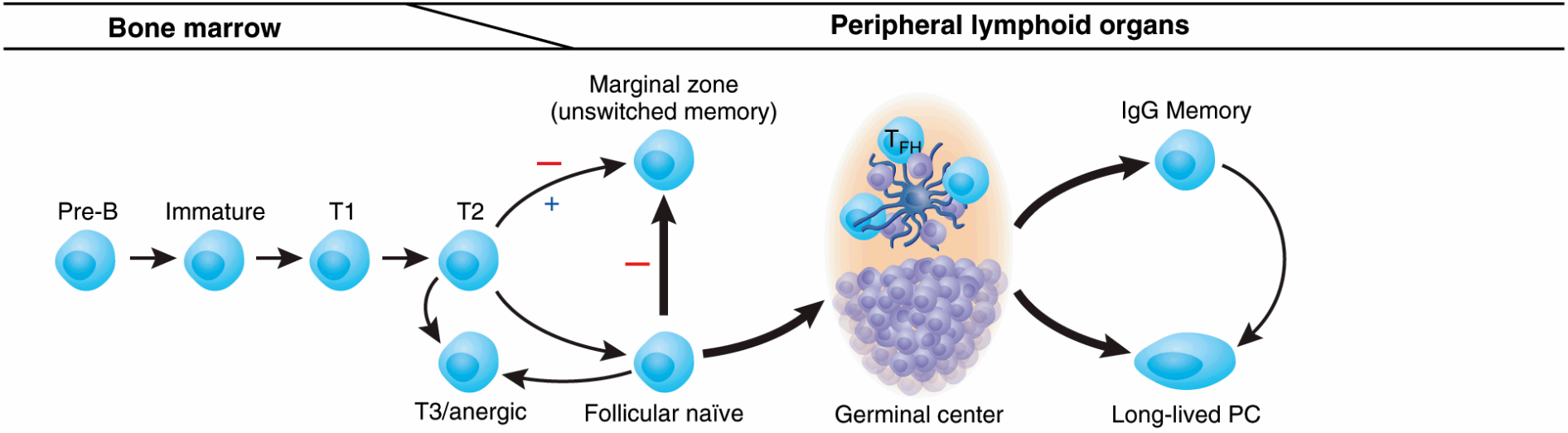
DISTINCT PHENOTYPES BUT MOST REQUIRE IL-10

- Regulatory B cells: $CD19^{+}CD20^{+}CD24^{hi}CD27^{-}CD38^{hi}IgD^{hi}IgM^{hi}$
- Transitional B cells: $CD19^{+}CD24^{+}CD38^{+}IgD^{+}$



Stolp J et al; Nat Rev Nephrol 2014

Marginal Zone B cells ($CD19^+CD27^+IgD^+CD38^+$)



Mechanisms of tolerance

Central	Germinal center	Late	
Editing Deletion	Revision Deletion (Ag or Fas mediated)	**Memory B cells:** -Anergy? -Block of differentiation into effector B cells -Block of differentiation into plasma cells (IL-6and CD40L?	**Plasma cells:** -Inability to compete for long-term survival niches -Inhibition of antibody production?
Anergy Ignorance	Anergy Ignorance Lack of T cell help Competition for BAFF Somatic hypermutation		

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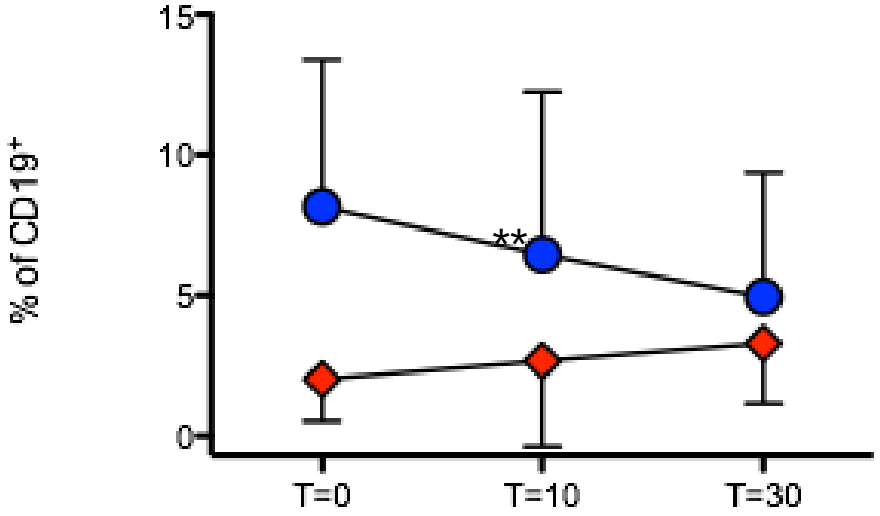
Orduño NM et al; J Invest Dermatol 2009

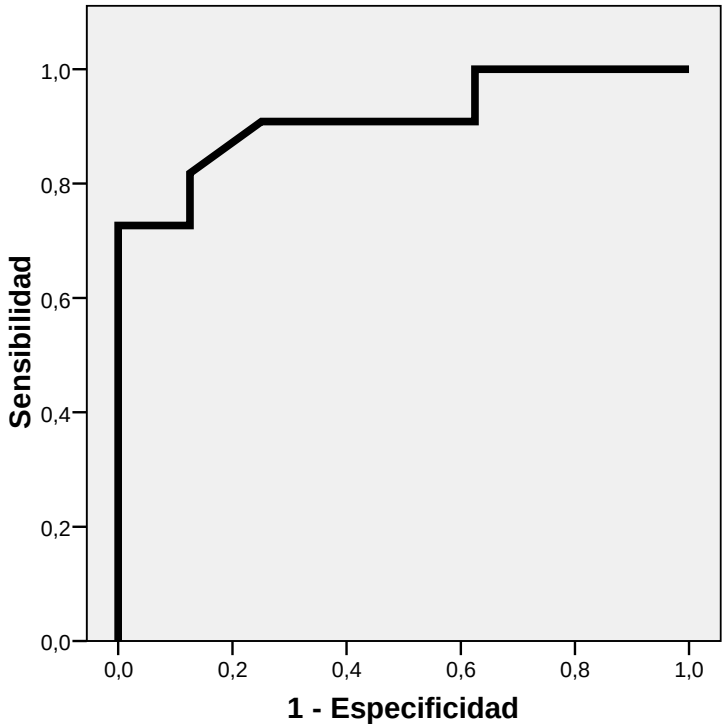


MZB cells are decreased in renal graft rejection

Coordenadas de la curva

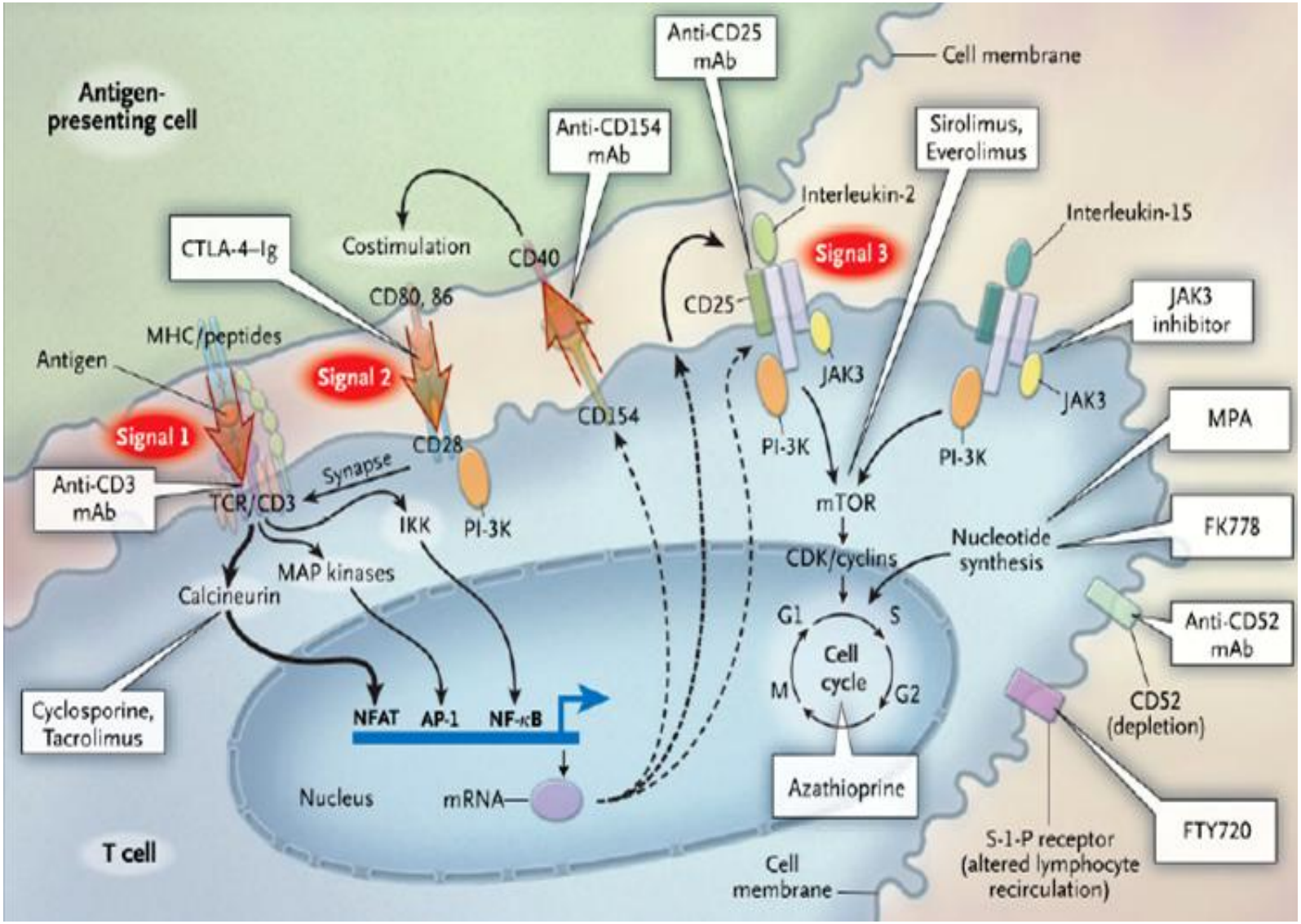
Variables resultado de contraste: USCD38
Positivo si es mayor o igual que(a)

	Sensibilidad	1 - Especificidad
,3000	1,000	,875
,7000	1,000	,750
1,2500	1,000	,625
1,7500	,909	,625
2,2500	,909	,500
2,7500	,909	,375
2,8500	,909	,250
3,5500	***,818***	***,125***
4,3500	,727	,125
5,1000	,727	,000
6,1500	,636	,000
6,8000	,545	,000
7,5500	,455	,000
8,4500	,364	,000
9,1500	,273	,000
13,1000	,182	,000
17,5500	,091	,000
19,4000	,000	,000



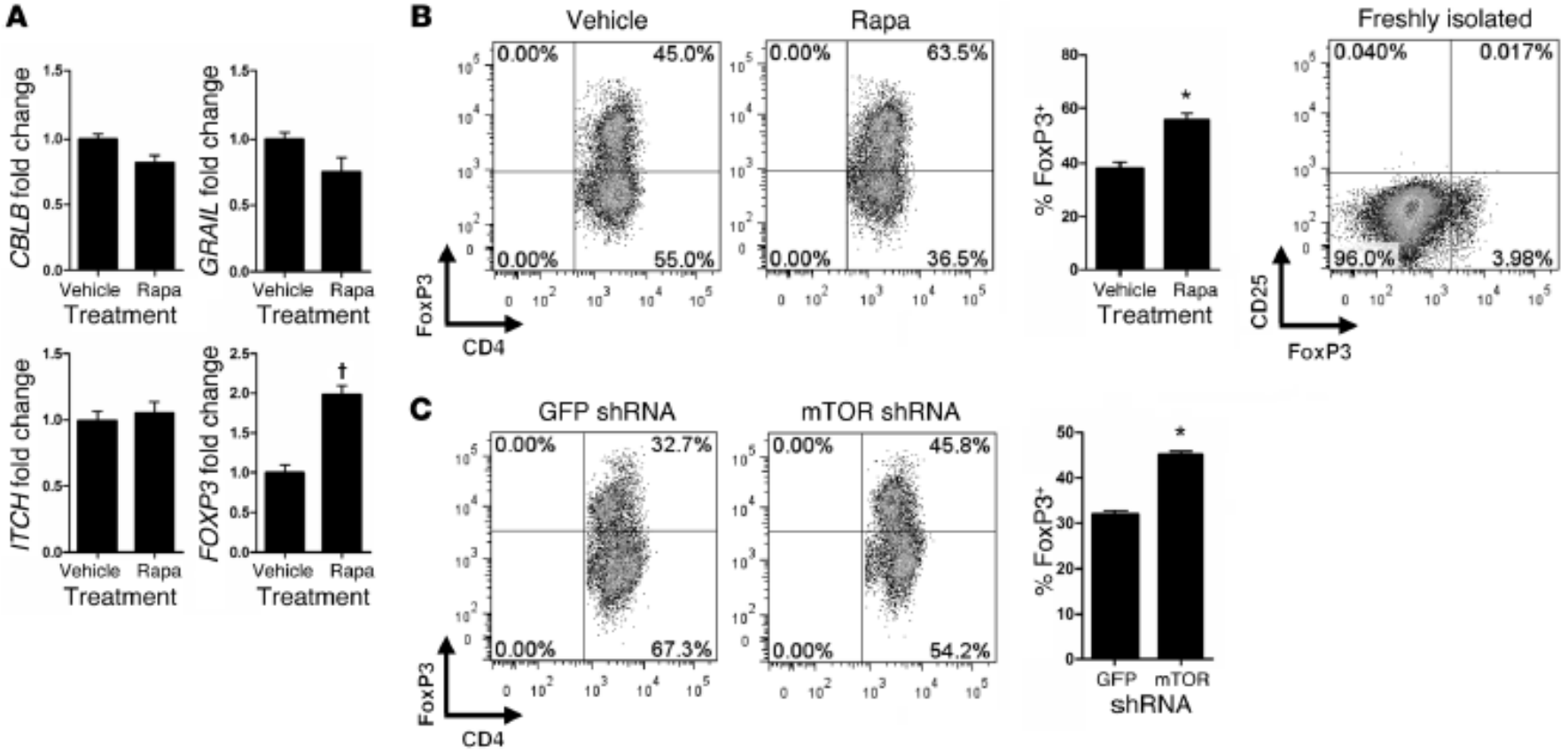
San Segundo et al; Trends in Transplant 2013

… and Antigen-Presenting Cell: the missing player



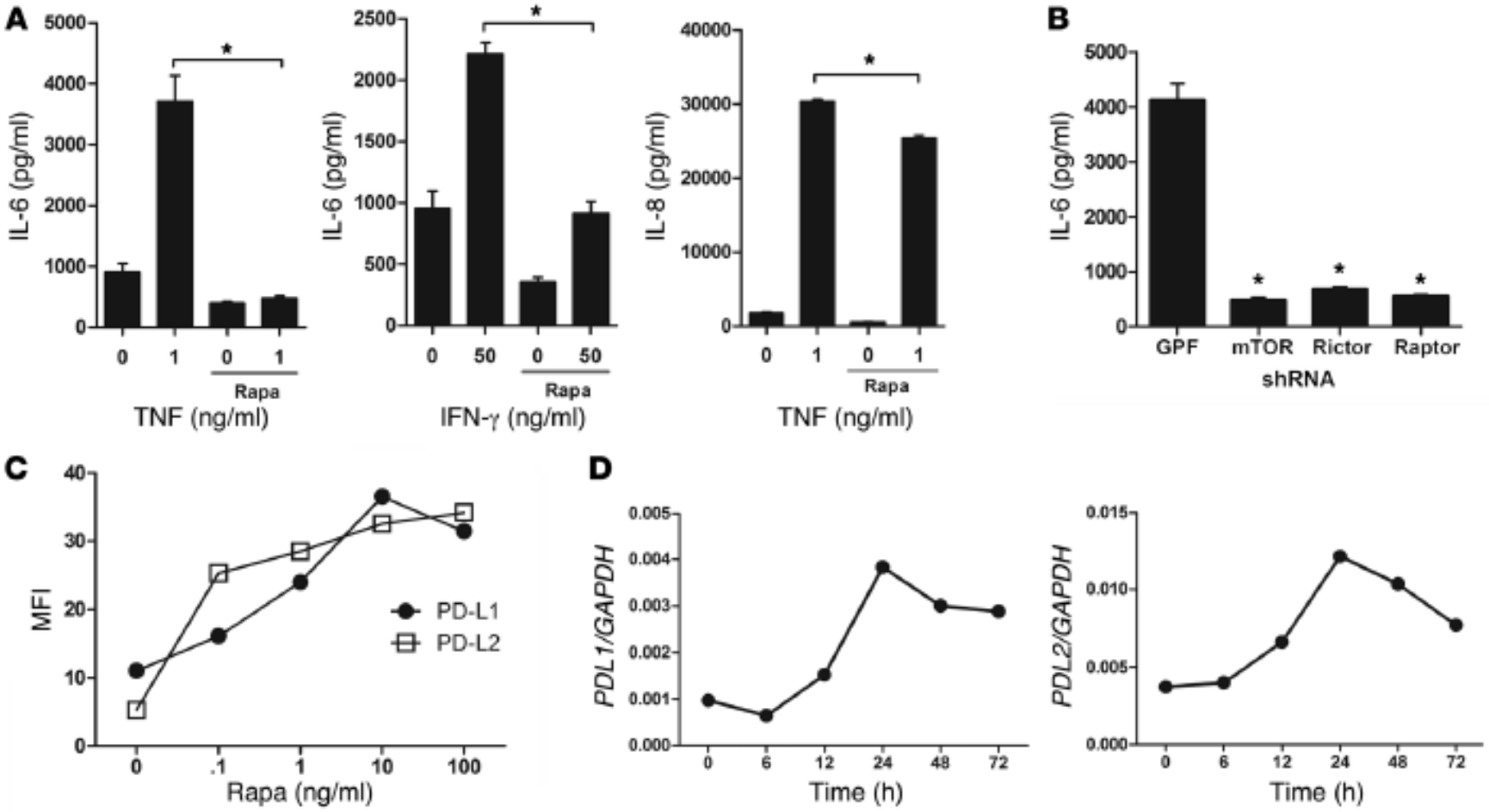
Halloran PF. N Engl J Med 2004; 351: 2715

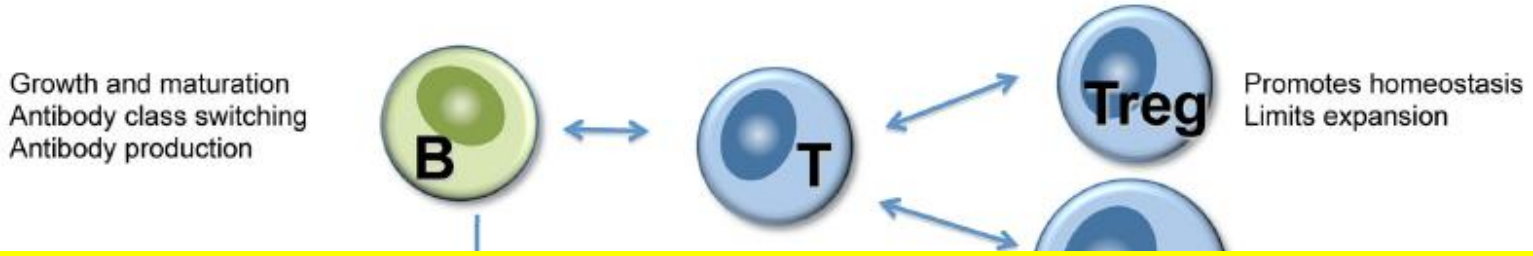
Pretreatment of endothelial cells with imTOR induces the expansion of Tregs



Wang C, et al. JCI 2013

Pretreatment of EC with imTOR inhibits secretion of IL-6 and augments the expression of PD-L1 and PD-L2

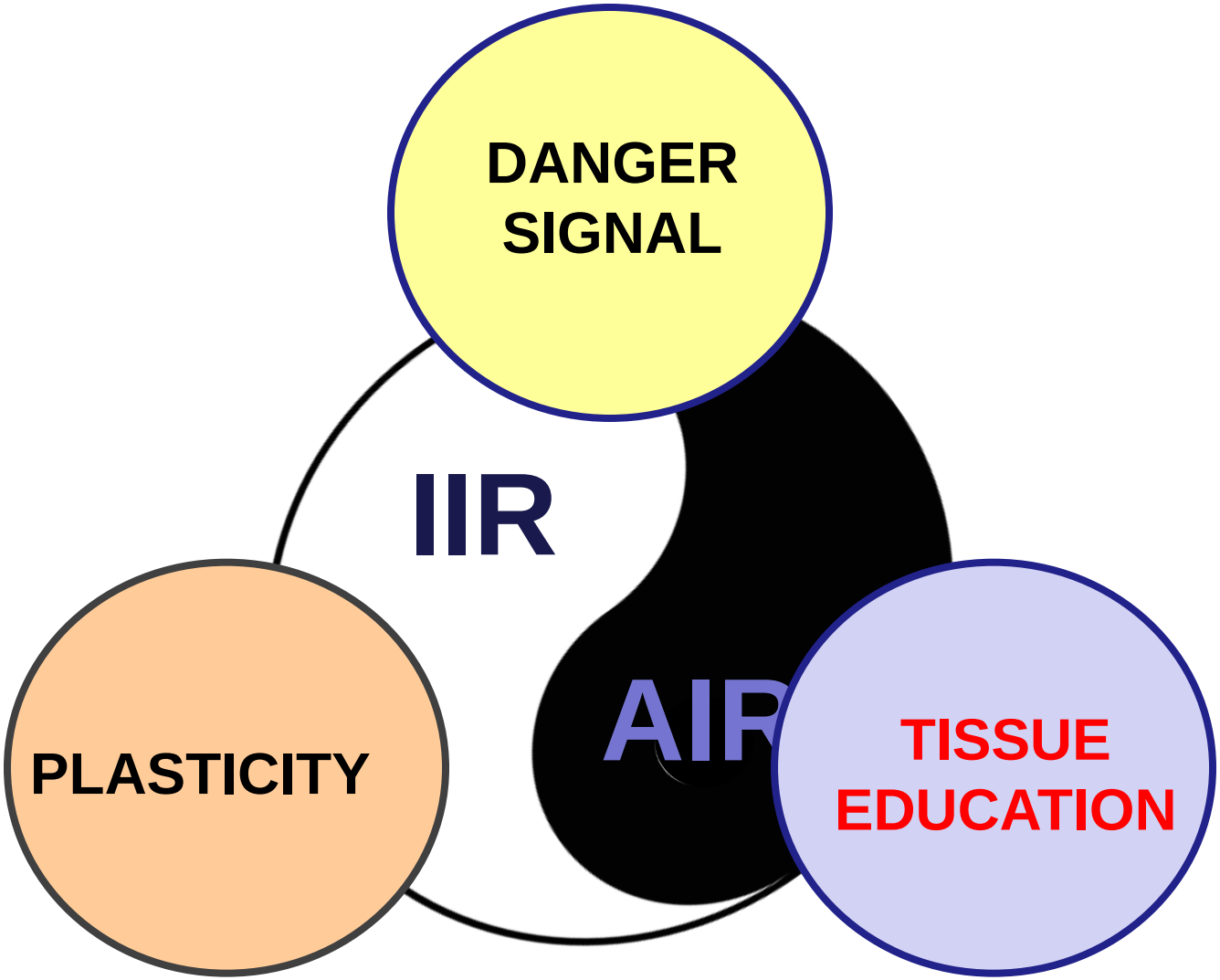




Last two words...

Valdecilla IDIVAL

Fantus D & Thomson AW; AJT 2015



Courtesy: Dr. J Merino (Univ Cantabria)

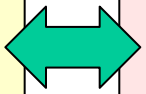
Tissue-based class control: the other side of tolerance.

Polly Matzinger, Tirumalai Kamala (Nat Rev Immunol 2011, 11: 221)

The Ghost Lab, Laboratory of Cellular and Molecular Immunology, T-Cell Tolerance and Memory, National Institutes of Health, Bethesda, Maryland, USA.

Even in the absence of any autorreactivity, an uncontrolled immune response may totally destroy a tissue

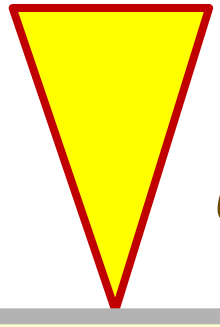
The immune system adapts the response to the exposed danger



The immune system adapts the response to the tissue where it acts

Tissue control

Time-dependent class switch



Damage- or stimulus-dependent class switch

Intensity of the immune response

TAKE HOME MESSAGES

- **Real immune tolerance is the holy grail in transplantation but impossible to reach to date.**

- **Operational tolerance is the main approach to understand mechanisms of allotolerance and to develop markers of tolerance.**

- **The use of biomarkers related to immune tolerance, especially active suppression, are of utility to define the best immunosuppression.**

- **The effects of clinically used immunosuppressants are not only directed to the main Th cells.**

- **New effects of both CNI and imTOR are being described in parallel with new immune cell subsets and their role in transplant outcome.**

- **It is possible that the best option from an immunological point of view is to combine and sinergize effects of CNI and imTOR**

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