AIBT, Summer School

Pesaro, 9th - 11th June 2016

# Le cellule T regolatorie: dal laboratorio all'utilizzo clinico

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OSPEDALE SAN RAFFAELE

FONDAZIONE Telethon

# Tolleranza Immunologica

**Definizione:**

La tolleranza immunologica è la mancata responsività dei linfociti nei confronti di un specifico antigene.

**Significato:**

Un individuo in condizioni normali è tollerante verso i propri antigeni, fenomeno noto come tolleranza verso il self.

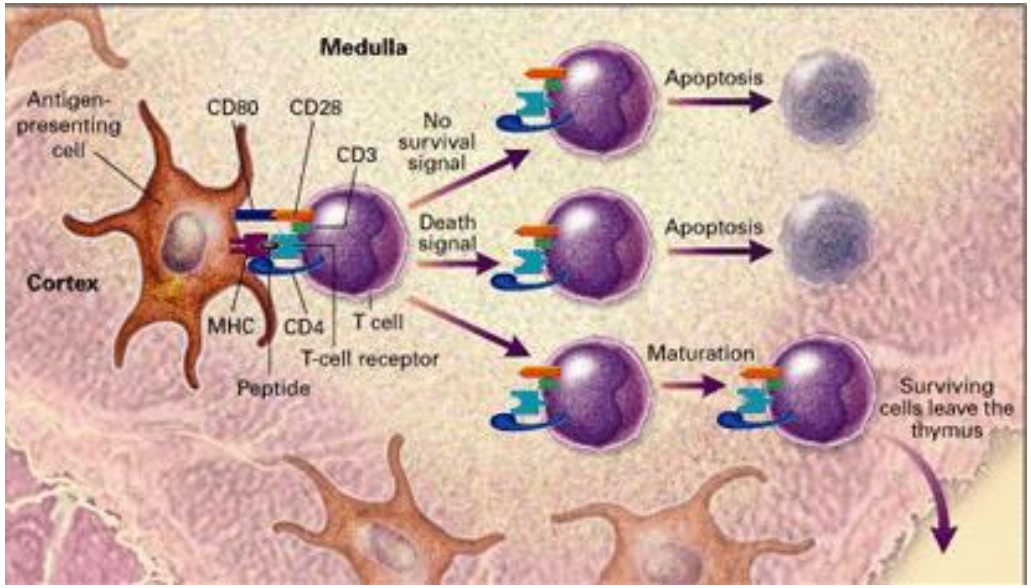
→ la rottura della "self-tolerance" genera le malattie auoimmuni.

**Potenziali terapeutici dell'induzione di tolleranza:**

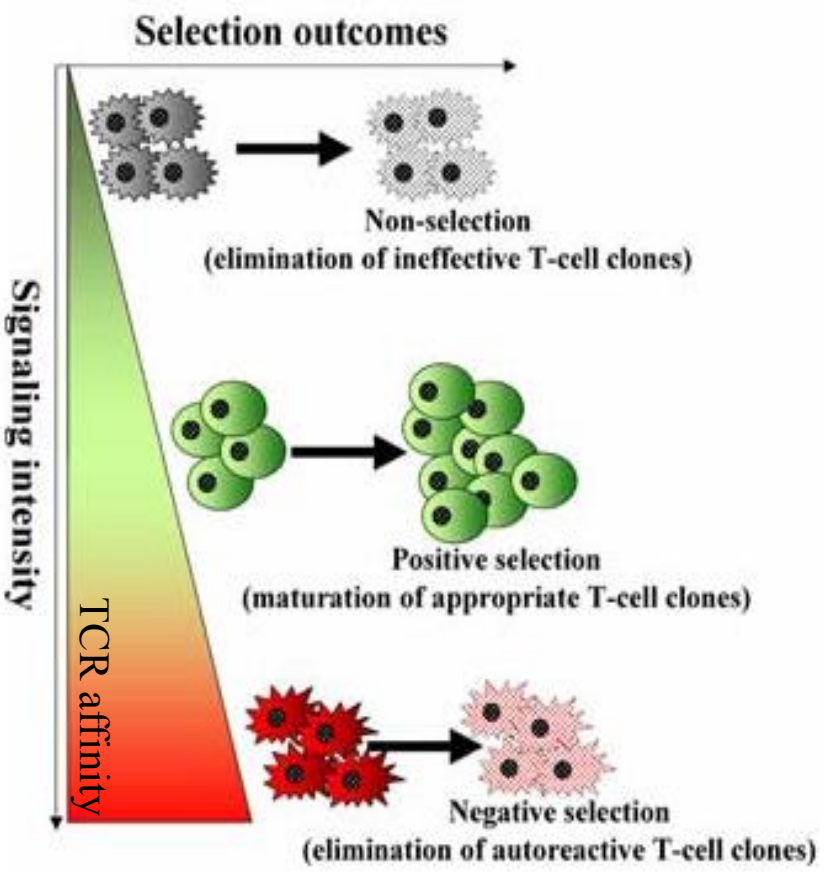
- Prevenzione del rigetto dei trapianti d'organo o le reazioni di GvHD,
- Trattamento di malattie autoimmuni e le allergie,
- Prevenire le risposte immuni dopo tarapia genica.

# Central Tolerance

## CLONAL DELETION (Thymus)

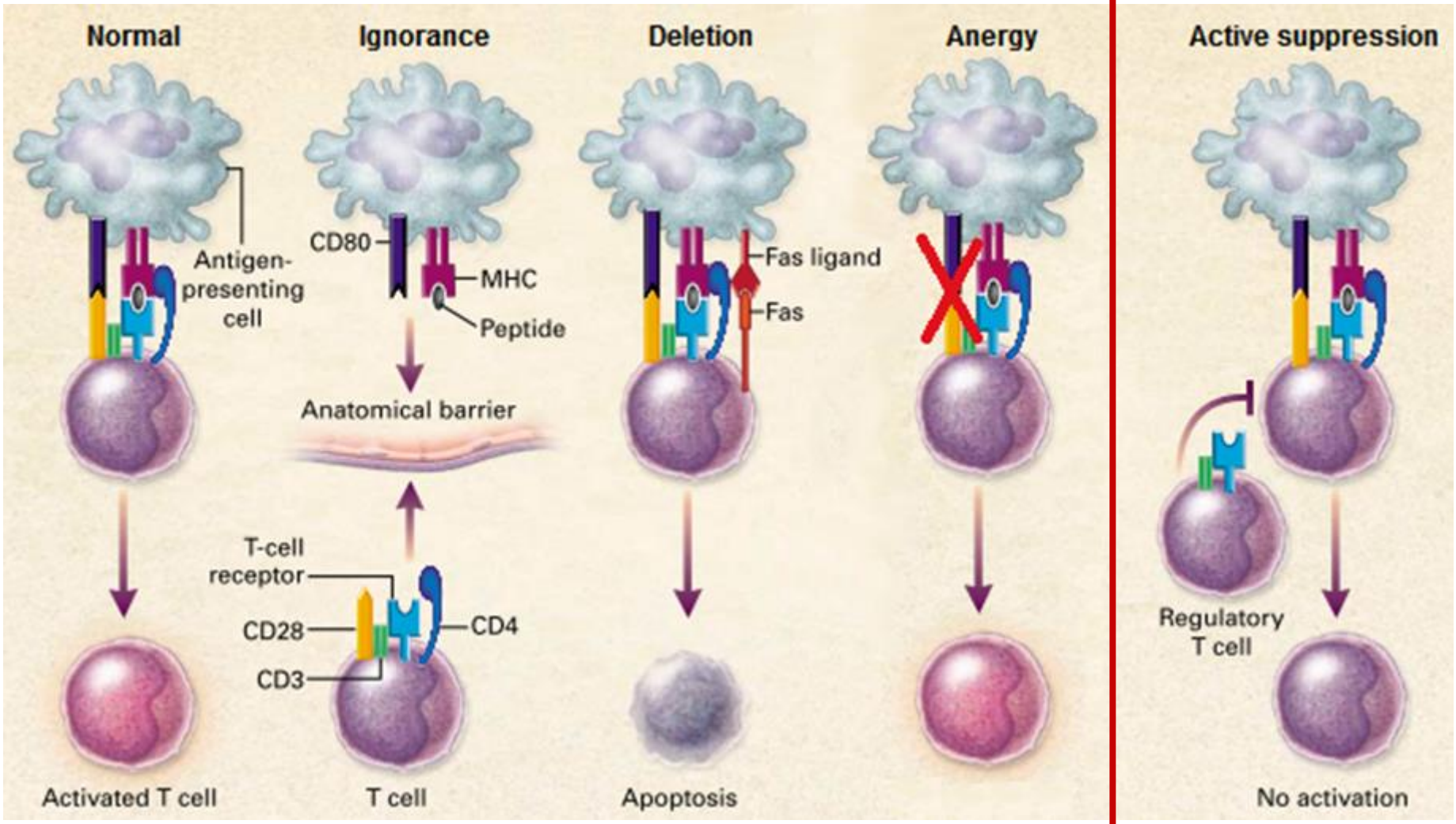



from Kamradt NEJM, 2001



# Peripheral tolerance

*Adapted from Mackay et al. NEJM 2001*



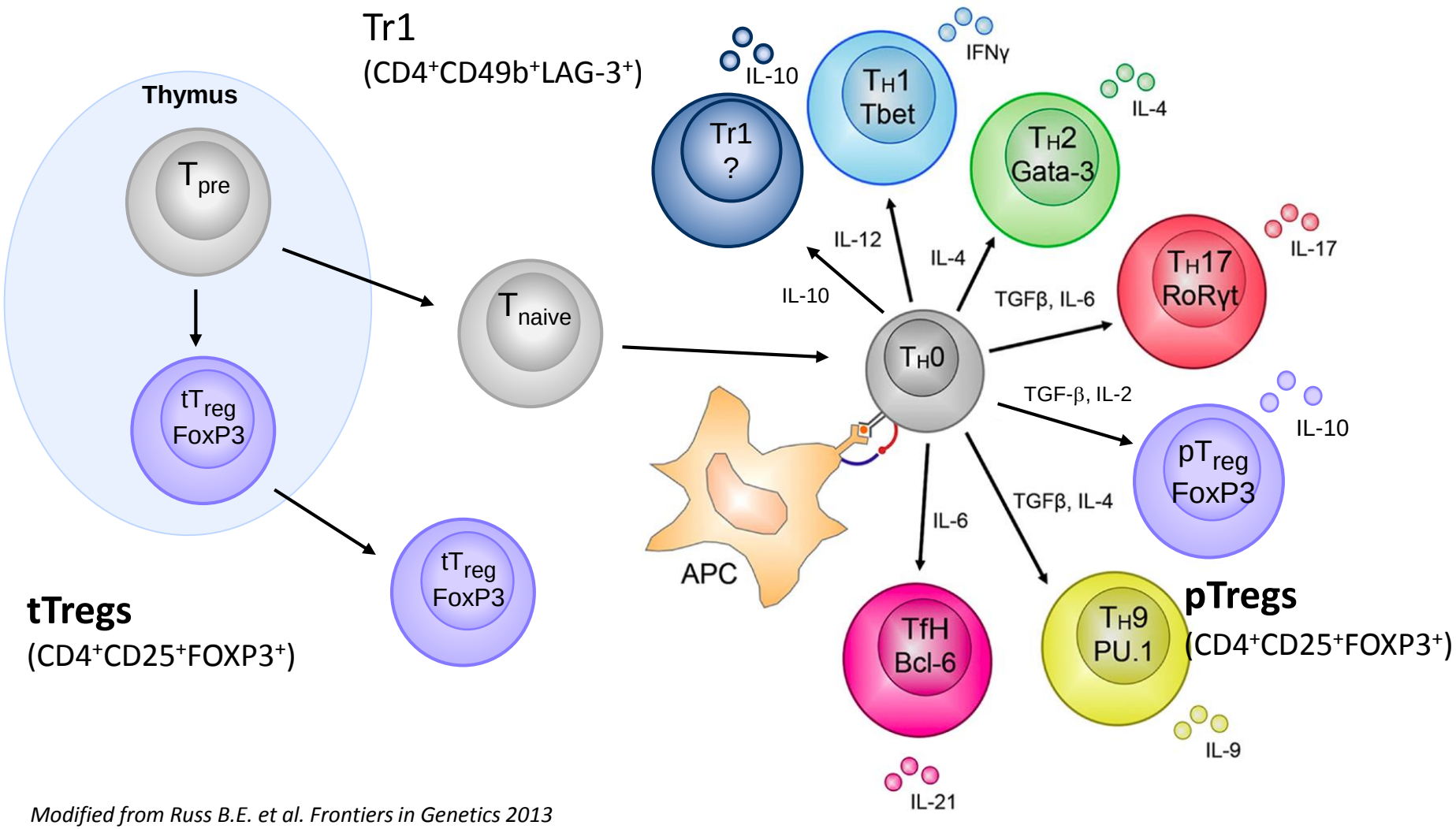

**Anatomic barriers**

**Activation induced apoptosis**

**Absence of co-stimulation (anergy)**

**Active suppression by Regulatory T cells**

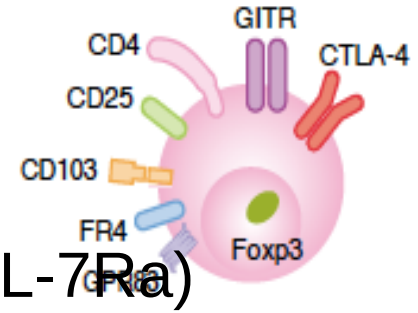
# Treg cells: **n**atural and **i**nducible




*Modified from Russ B.E. et al. Frontiers in Genetics 2013*

# tTreg cells

Thymic-derived Treg cells

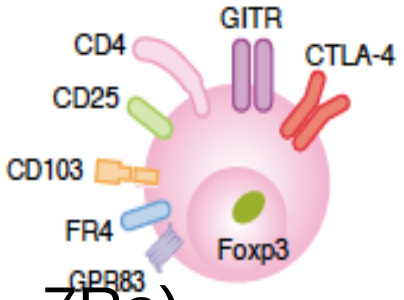



**$CD4^+CD25^+FOXP3^+$**

- CTLA-4, GITR and negative/low for CD127 (IL-7Rα)
- CD39, $CD49d^{low,}$ Helios
- TSDR (Treg-specific-demethylated-region)
- Are anergic *in vitro*
- Are highly dependent on IL-2
- Are mainly involved in the maintenance of tolerance to self-Ags

# pTreg cells

Induced in the periphery

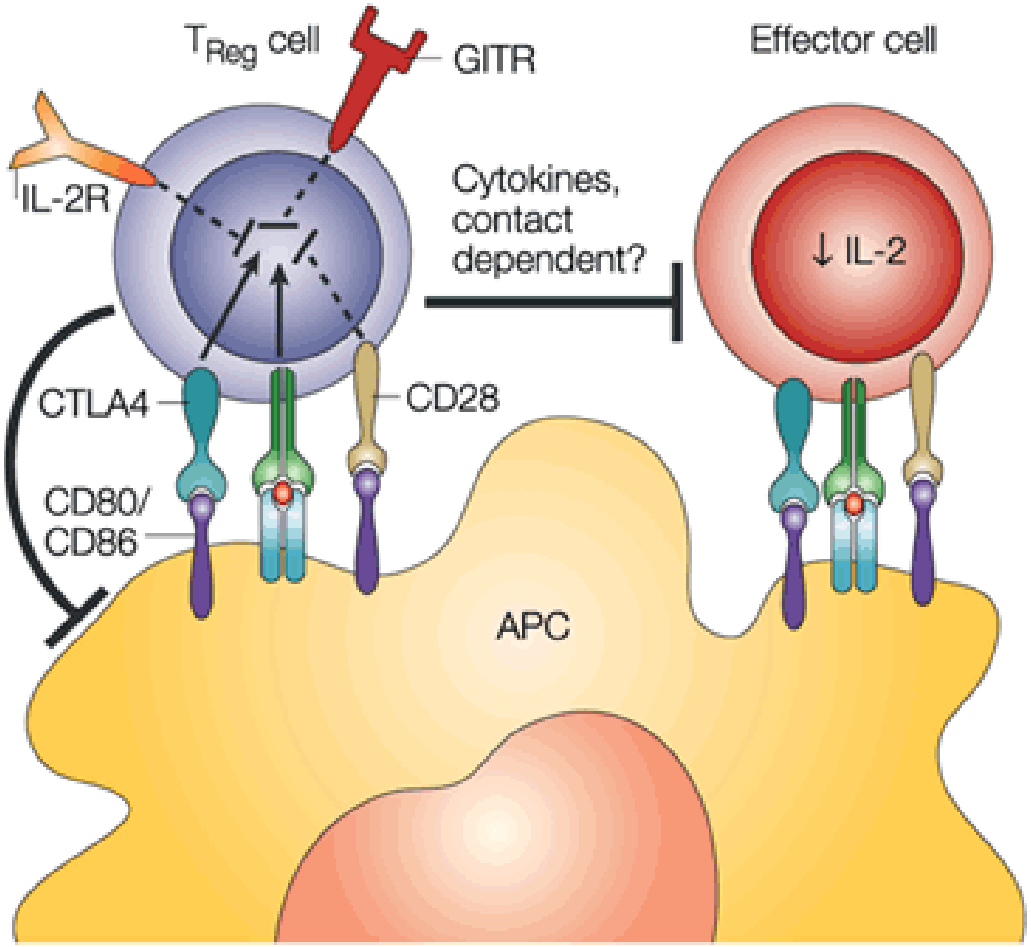



**$CD4^+CD25^+FOXP3^+$**

- CTLA-4, GITR and negative/low for CD127 (IL-7Rα)
- CD39, $CD49d^{low,}$
- Are anergic *in vitro*
- Are highly dependent on IL-2
- Are involved in the maintenance of tolerance to self-Ags and foreing-Ags.

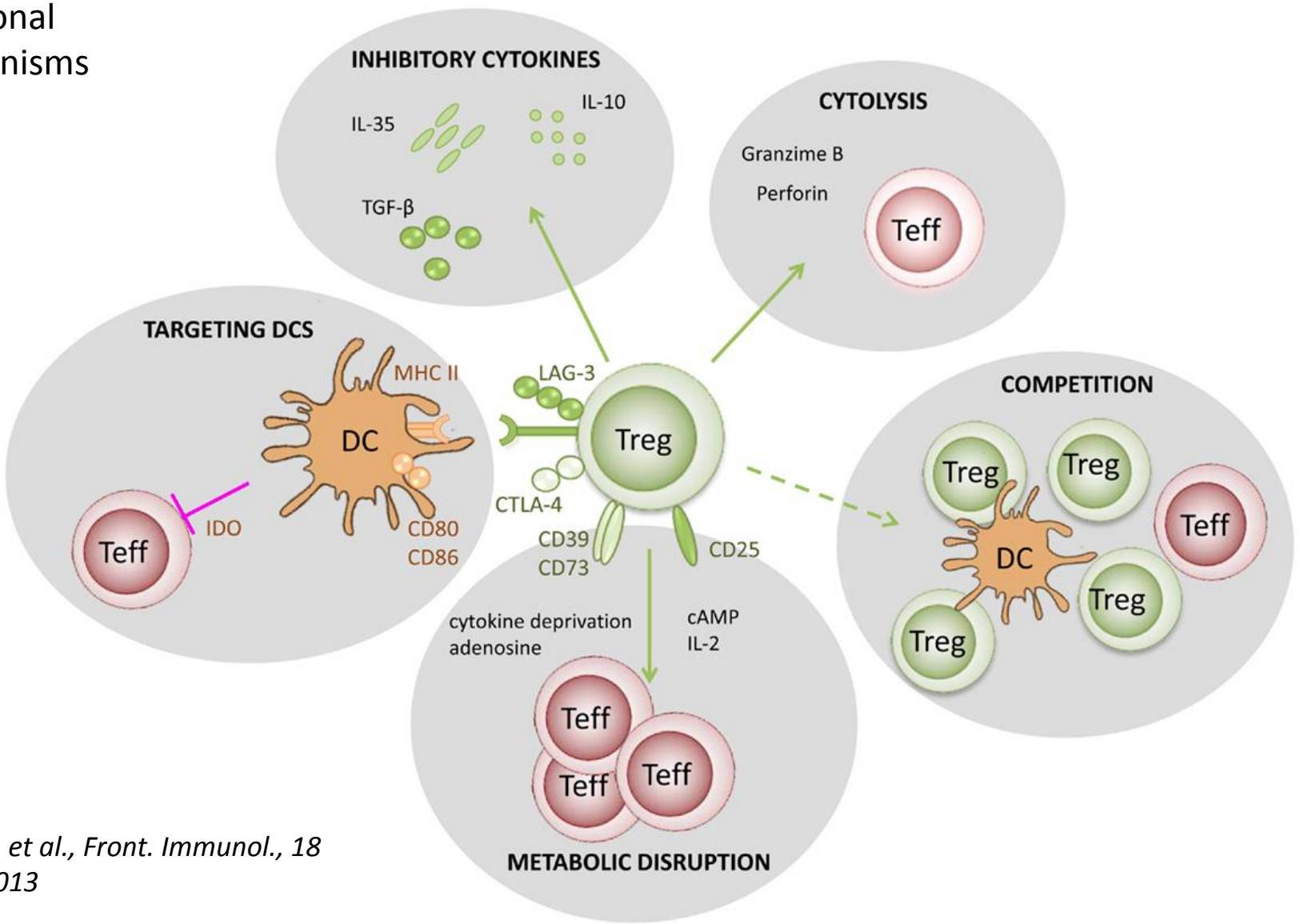
# Mechanisms of suppression by FOXP3$^{+}$ Tregs

The chief mechanisms of suppression mediated by FOXP3$^{+}$ Tregs
Is the cell-to-cell mediated inhibition of effector T cells



# Mechanisms of suppression by FOXP3$^{+}$ Tregs

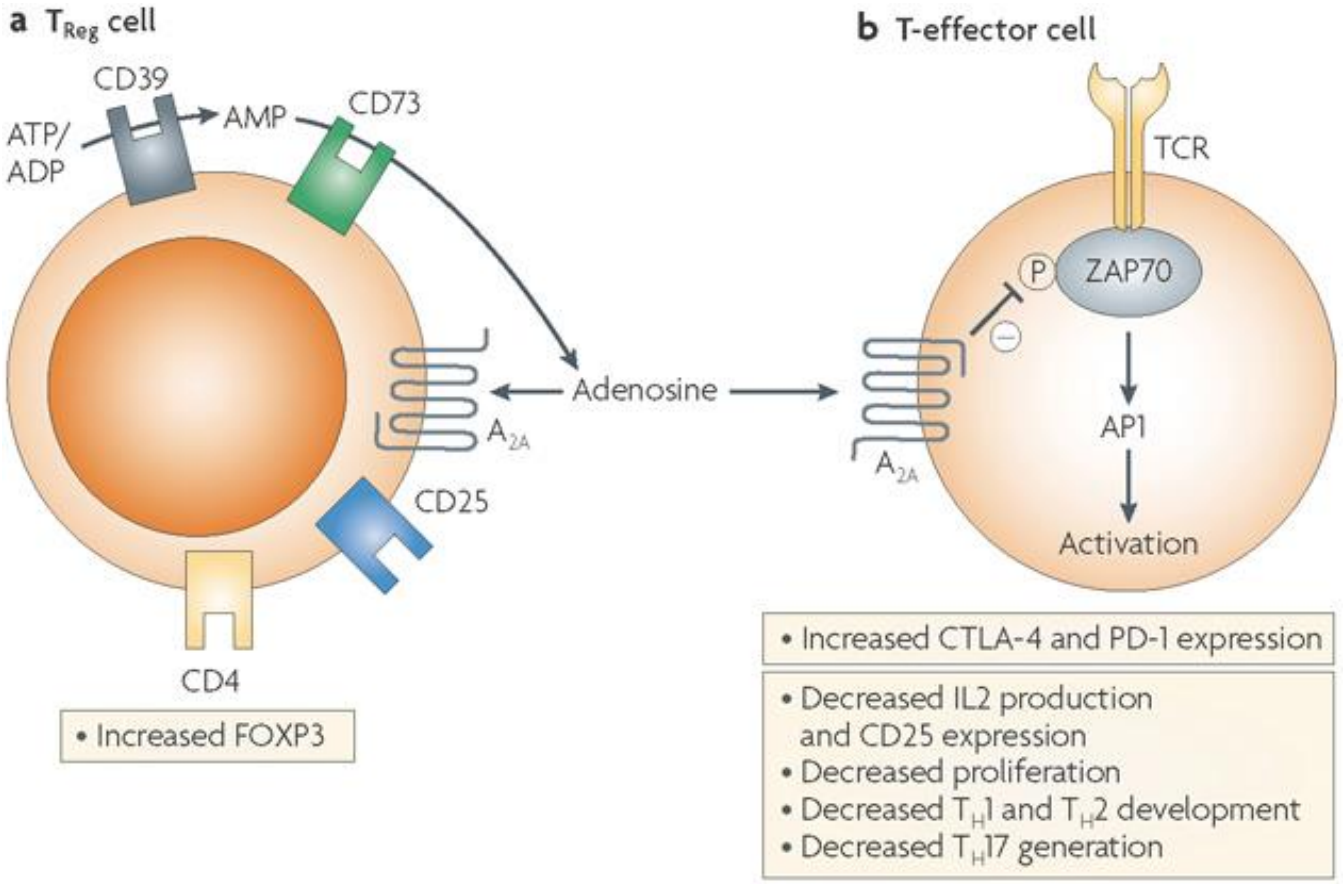
Additional mechanisms




*Caridade M. et al., Front. Immunol., 18 November 2013*

# Mechanisms of suppression by FOXP3$^{+}$ Tregs

**METABOLIC DISRUPTION**

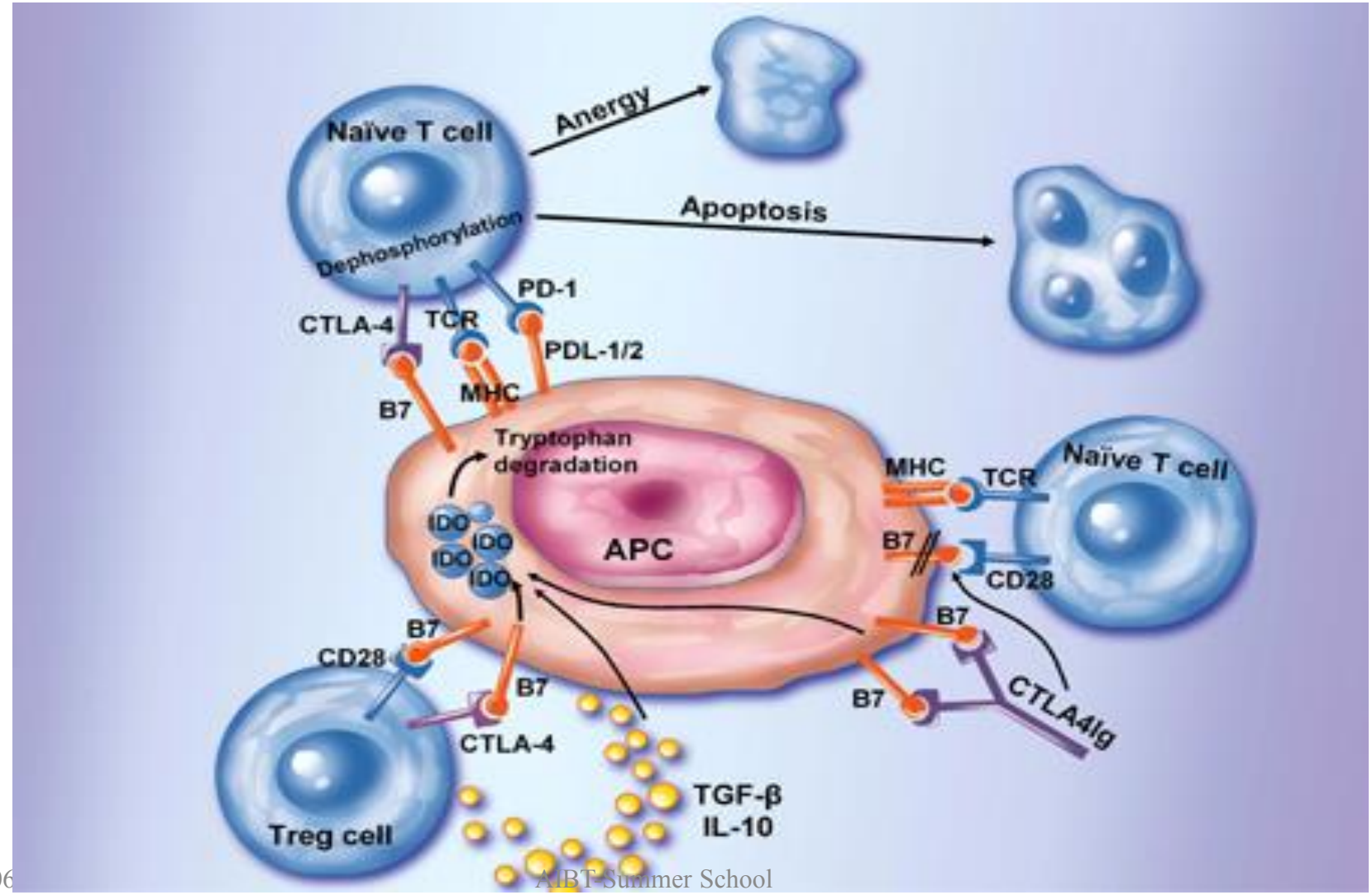



*György H. et al., Nature Reviews Drug Discovery 7, 759-770, 2008*

Nature Reviews | Drug Discovery

# Mechanisms of suppression by FOXP3$^{+}$ T cells

## TARGETING DENDRITIC CELLS



# T regulatory type 1 (Tr1) cells

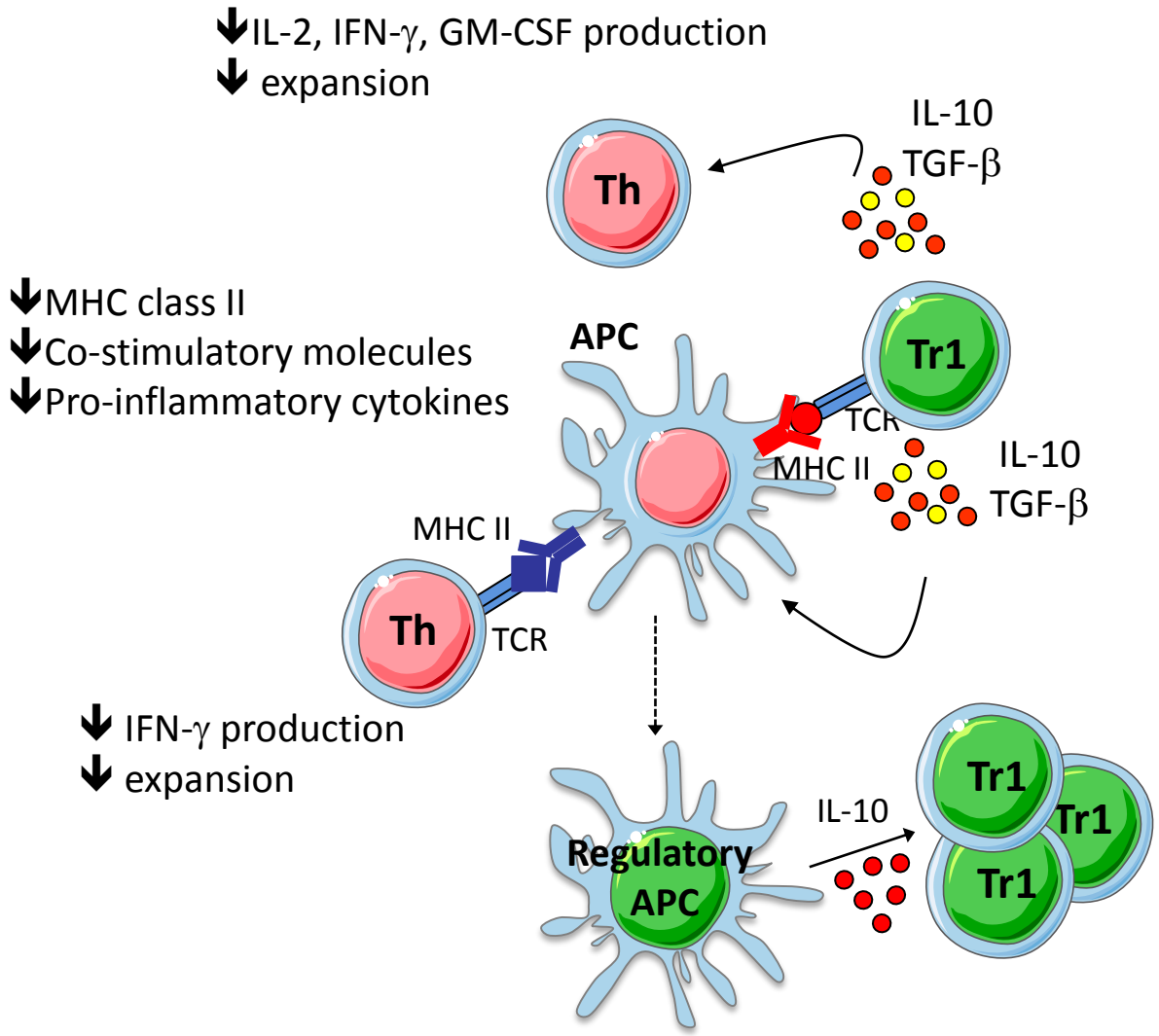
Induced in the periphery

**$CD4^+CD45RA^-CD49b^+LAG\text{-}3^+$**

- Are IL-$10^{++}$ IL-$4^-$ IL-$17^-$ IL-$2^{low/-}$ IFN-$g^+$ TGF-$b^+$ IL-$5^+$,
- FOXP3 not constitutively expressed, but it can be up-regulated upon activation
- Are anergic *in vitro*
- Are mainly involved in the maintenance of tolerance to exogenous-Ags

**Can be generated *in vitro* in an antigen-specific manner**

# Chief mechanisms of Tr1-mediated suppression



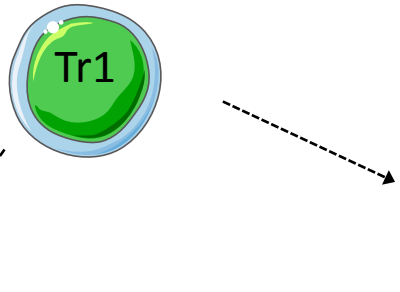

*Magnani CF. and Gregori S., EJI 2011; Gregori and Goudy, Frontiers in T cell Biol 2012*

European Journal of Immunology

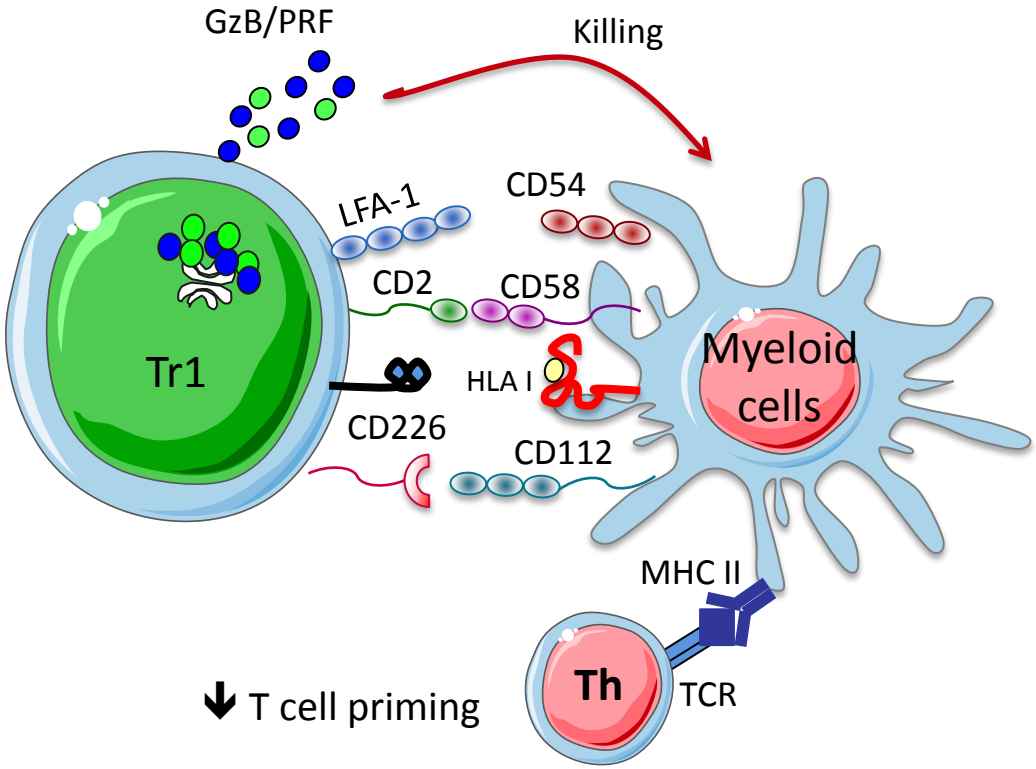
# Killing of myeloid APCs via HLA class I, CD2 and CD226 defines a novel mechanism of suppression by human Tr1 cells

*Chiara F. Magnani[1], Giada Alberigo[1], Rosa Bacchetta[1], Giorgia Serafini[1,2], Marco Andreani[2], Maria Grazia Roncarolo[1,3] and Silvia Gregori[1]*

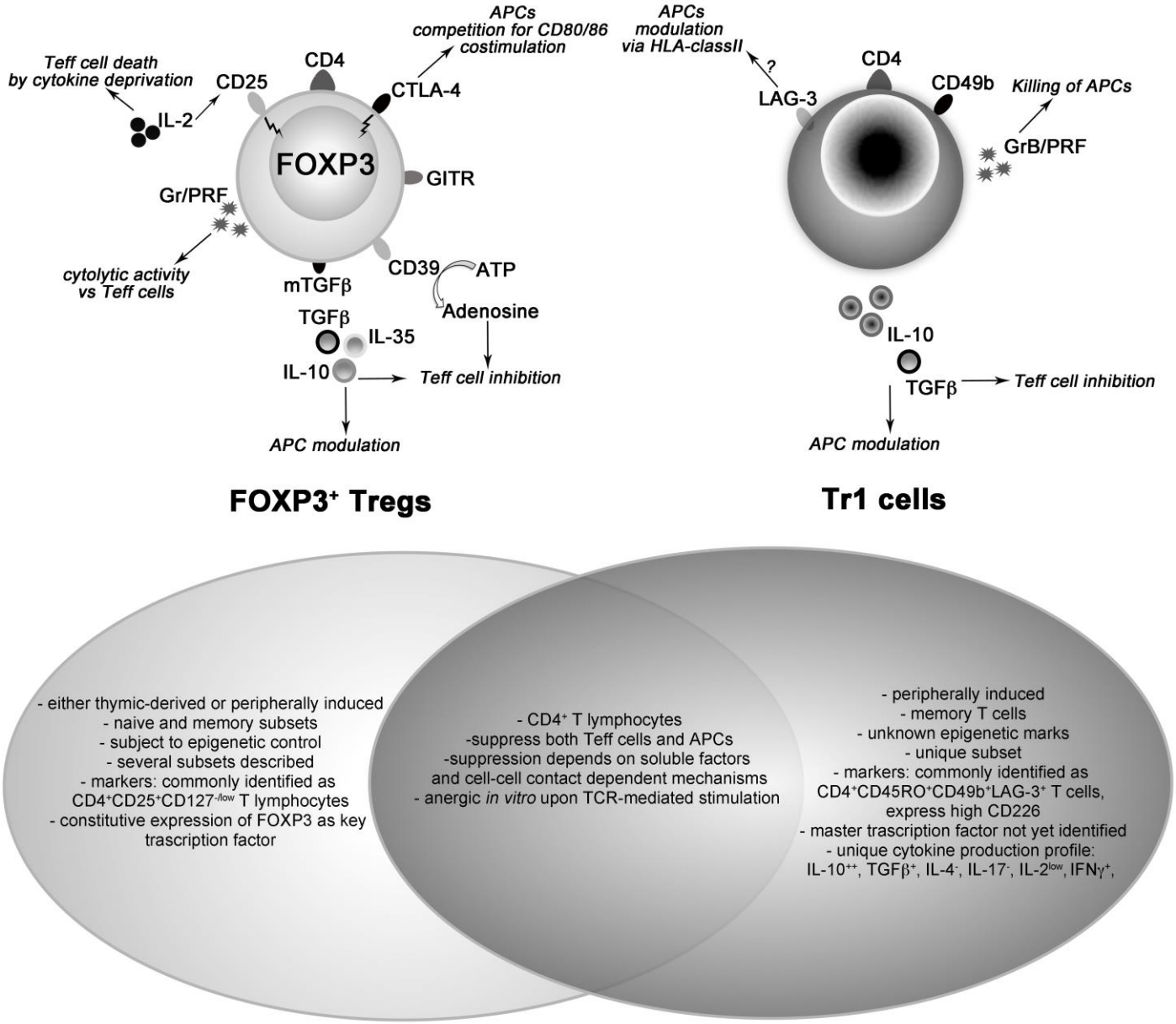
TCR-mediated activation




Direct and indirect suppression of T cell via IL-10 and TGF-β



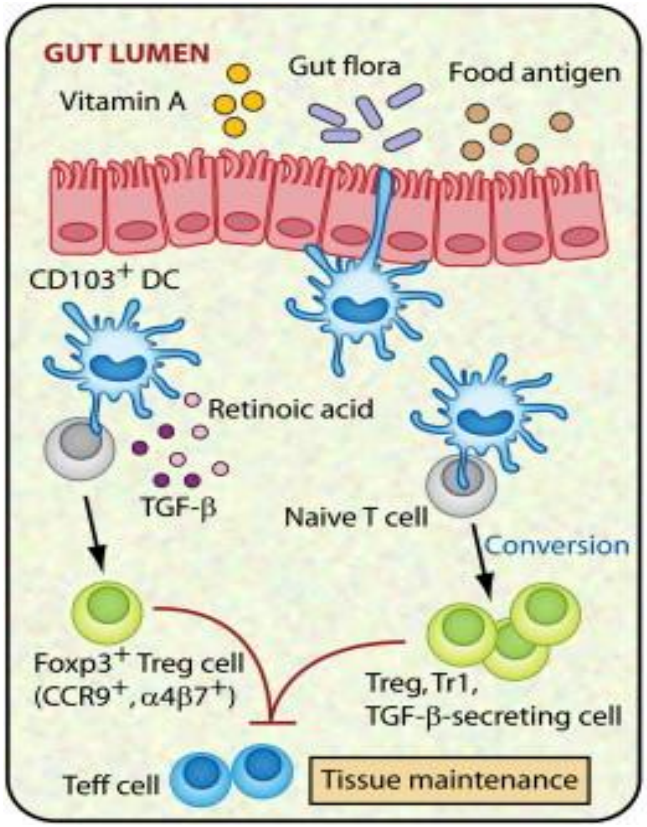
# Similarities and differences between FOXP3$^{+}$ Tregs and Tr1s

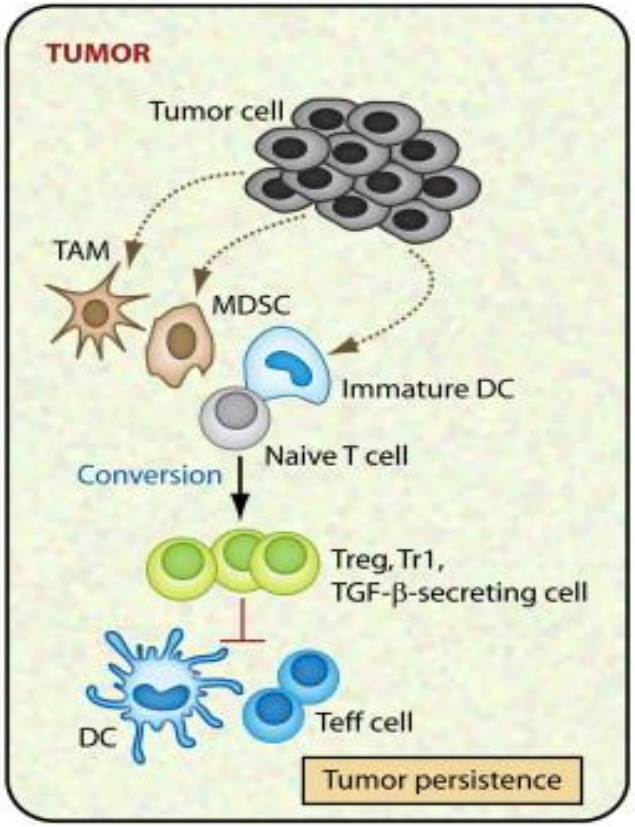


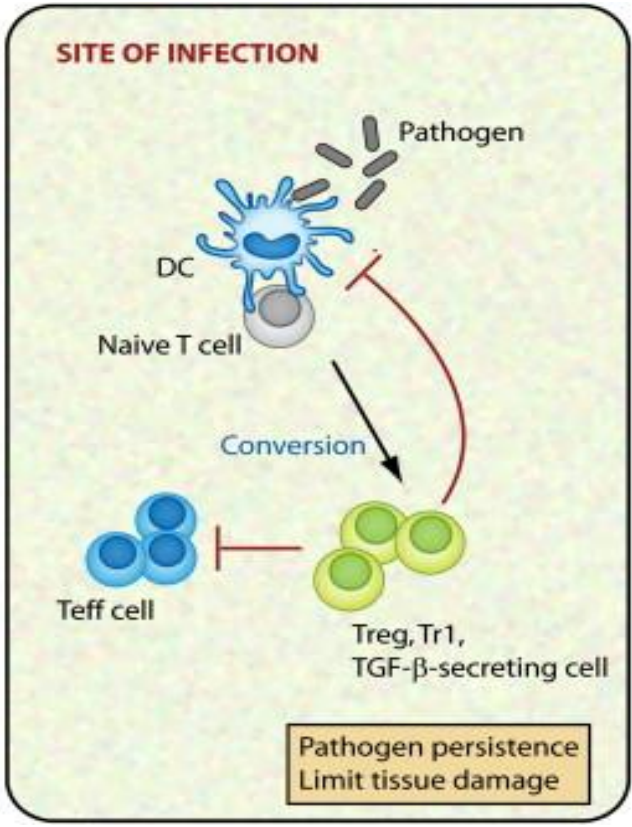
# Dual role of T regulatory cells

**TISSUE HOMEOSTASIS And TOLERANCE**

**IMMUNE ESCAPE**



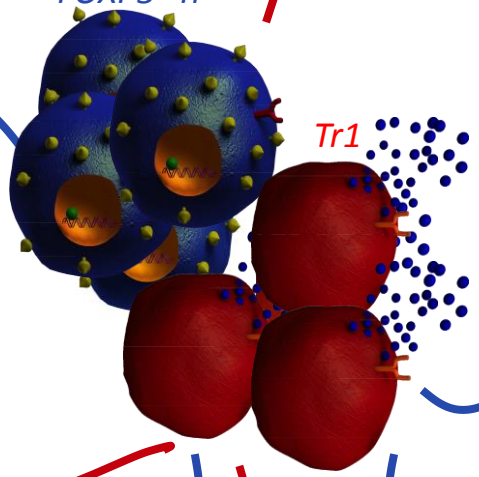
# Treg cell function in health and disease

## Treg defects

## Treg function

*IPEX syndrome*
*FOXP3* mutations
*(Bacchetta, JCI 2006; D'Hennezel, NEJM 2009; Moes, Gastroenterology 2010)*

*HSCT (SCID, β-Thal)*
*↑Allo-Ag specific Treg*
*(Bacchetta, JEM 1994; Serafini, Haematologica 2010)*




*IPEX-like syndrome*
*↓Treg*
*(Barzaghi, J Autoimmunity 2012)*

*Allergy*
↑Allergen-specific Treg in tosils
*(Palomares, JACI 2012)*

*IBD*
IL-10/IL-10R mutations
*(Glocker, NEJM 2009; Glocker Lancet 2010)*

*Liver Tx*
↑Treg in TOL patients
*(Castellaneta, Transplantation 2011)*

*T1D*
*↓ Treg function*
*(Ferraro, Diabetes 2010)*

*Allergy*
*↓Treg ↑Teff*
*(Akdis, JEM 2004)*

*Celiac Disease*
↑Gliadin-specific Treg in the gut
*(Gianfrani, J Immunol 2006)*

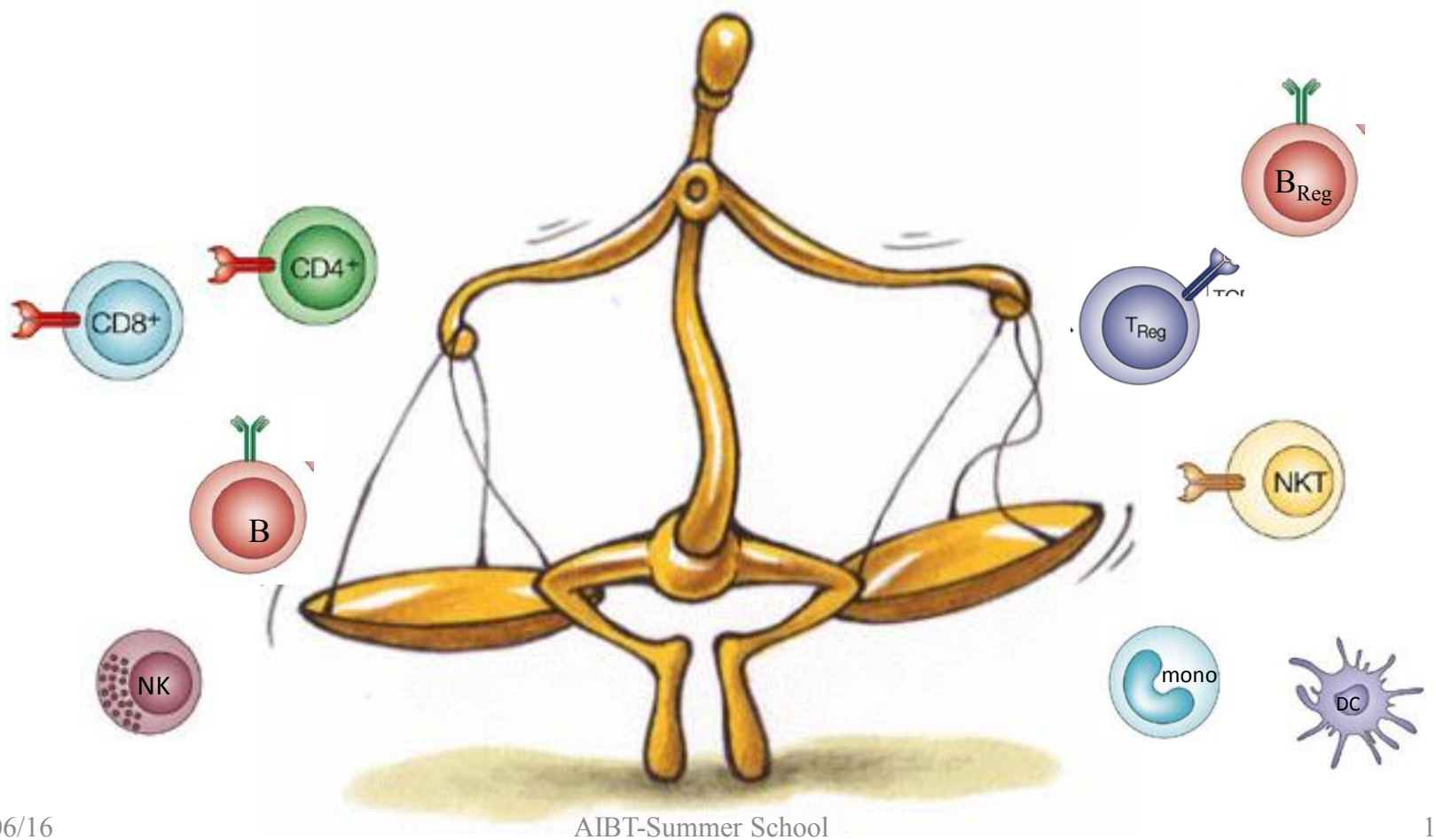
# Rationale for Regulatory cell-based therapy

**THE IMMUNE SYSTEM IN AUTOIMMUNE DISEASES, ALLOGRAFT REJECTION/GVHD, GENE THERAPY**

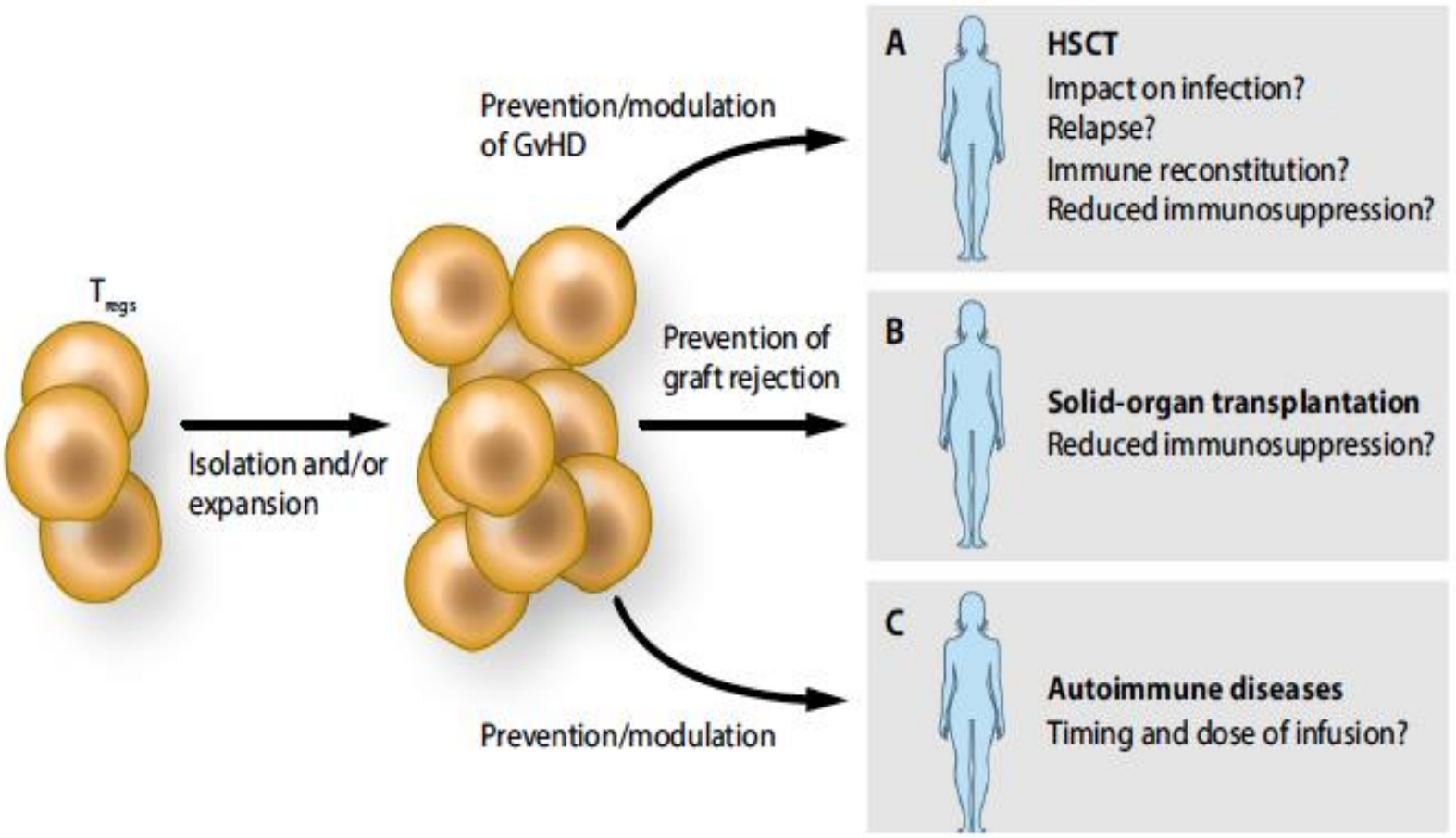
….unbalanced effector/regulatory T cell ratio

# Rationale for Treg-based therapy

….restore correct effector/regulatory T cell ratio



# Treg-based cell therapy

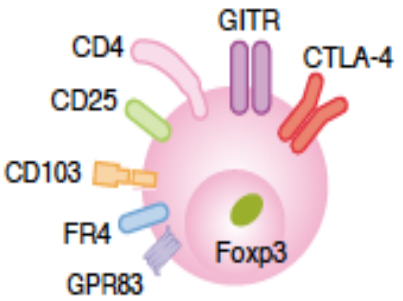



*P. Trzonkowki, et al, Science Trans Med 2015*

# $CD25^+$ Treg-based therapy

Peripheral blood

Freshly isolated
$CD8^-CD25^+$
$CD4^+CD25^+CD127^-$

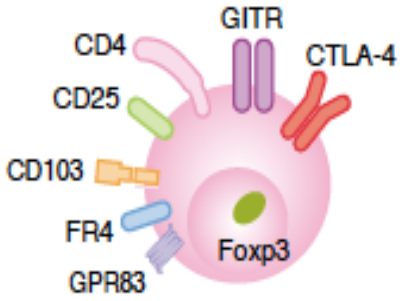



*In vitro* expanded
with Rapamycin

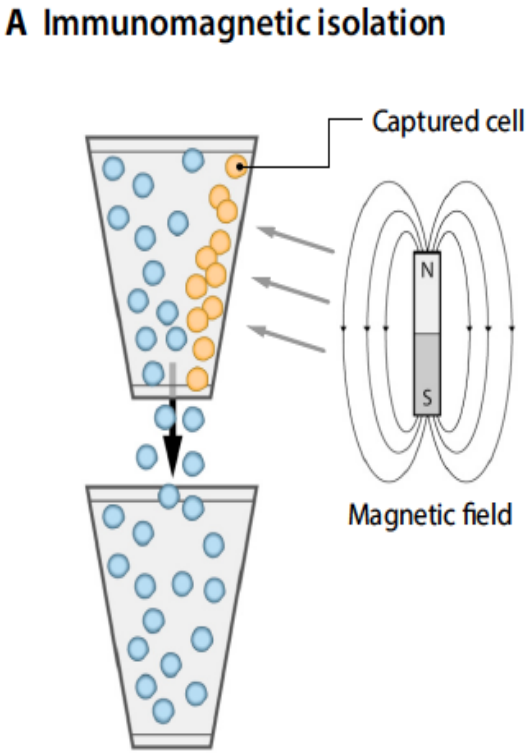
*In vitro* expanded
with antiCD3/CD28 and IL-2

# $CD25^+$ Treg-based therapy

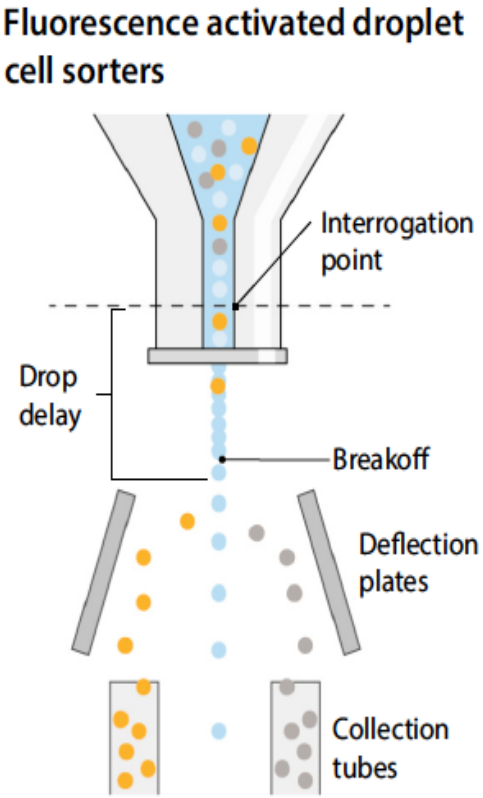
Freshly isolated from peripheral blood




**$CD8^-CD25^+$**



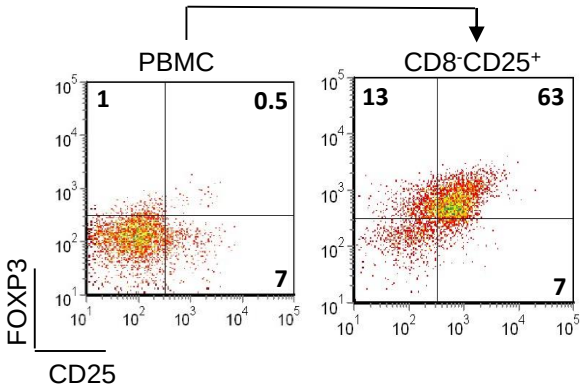

**$CD4^+CD25^+CD127^-$**

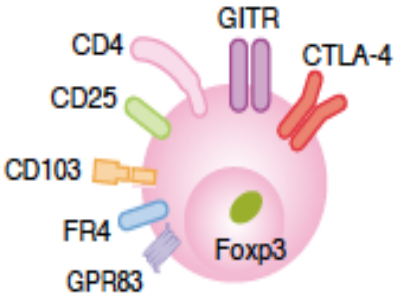


# Expansion of CD4$^+$CD25$^+$ Tregs with RAPAMYCIN

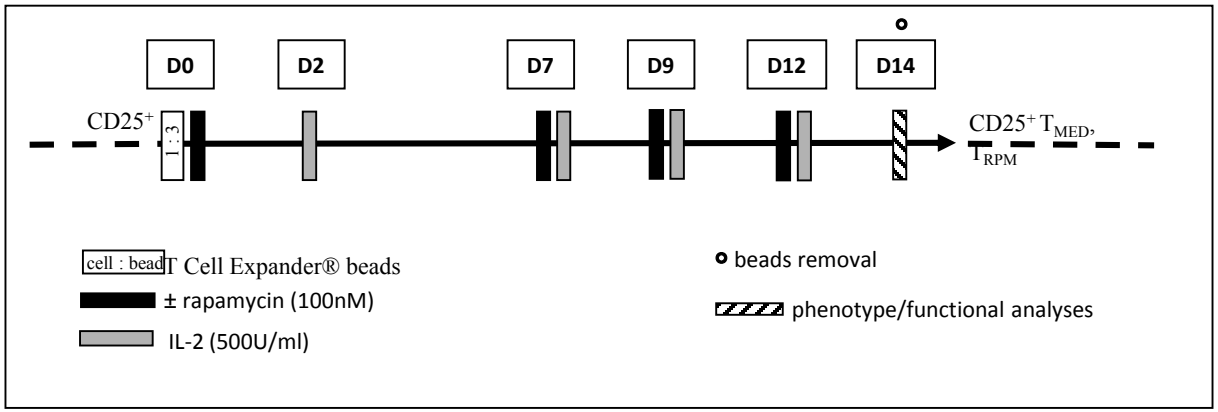
- **GMP purification of human nTregs:**

magnetically purified CD8$^-$CD25$^+$ are enriched in nTregs




- **GMP expansion of human nTregs**



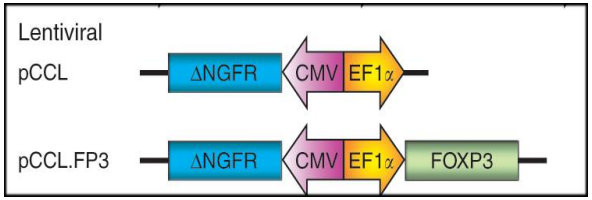

*E. Tresoldi, Haematologica 2011*

# FOXP3-Engineered Human CD4$^+$ T Cells (CD4$^{FOXP3}$)

**Generation of Potent and Stable Human CD4$^+$ T Regulatory Cells by Activation-independent Expression of FOXP3**

Sarah E Allan[1,2], Alicia N Alstad[1,2], Natacha Merindol[3–5], Natasha K Crellin[1,2], Mario Amendola[6], Rosa Bacchetta[6], Luigi Naldini[6,7], Maria Grazia Roncarolo[6,7], Hugo Soudeyns[3–5] and Megan K Levings[1,2]

www.moleculartherapy.org vol. 16 no. 1, 194–202 jan. 2008




Transduction of CD4$^+$ T cells with Bd.LV.FOXP3 generates potent and stable human Tregs

**Transduction protocol**

Total PBMC

CD4$^+$ T cells

alloAPCs+aCD3
IL-7 & IL-2

16hrs

LV-FOXP3/ΔNGFR

MOI20
1 day

9 days

IL-7 (10 ng/ml)
IL-2 (100 U/ml)

**Day 10** beads sorting
CD4$^+$NGFR$^+$ T cells

4 days

IL-15 (10 ng/ml)
IL-2 (100 U/ml)

**Day 14** restimulation and expansion

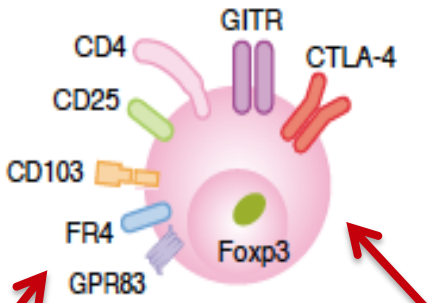
**Day 28 Read outs**
Phenotype
Proliferative response
In vitro/vivo suppressive activity

# Induction of Ag-specific CD25$^{+}$ Tregs

Induction of donor-alloantigen specific Tregs

TCR engineered Tregs or classical CD4$^{+}$ T cells

**Ag-specific FOXP3$^{+}$ Tregs**



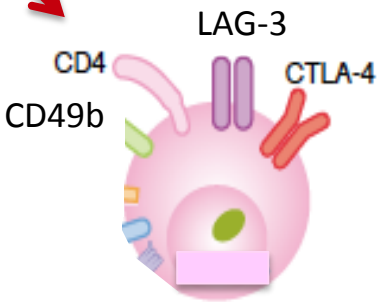

CAR engineered Tregs

Conversion of Ag-specific CD4$^{+}$ T cells into Tregs by FOXP3 gene transfer

# Tr1 cell-based therapy

In vitro induction of Tr1 cells by IL-10+APCs

In vitro induction of Tr1 cells by DC-10



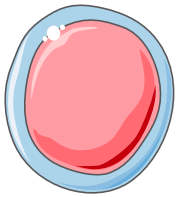

In vitro expansion of Ag-specific Tr1 cell clones

# *Ex vivo* induction of Tr1 cells

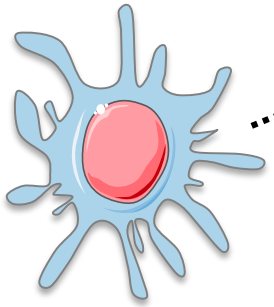
**IL-10 + alloAPC**

*(Groux H. 1997 Nature, Bacchetta R. 2002 EJI, Bacchetta and Gregori Hematologica 2010)*

**APC (monocytes)**



**Tr1 cells**



**Immature DC**

*(Levings and Gregori, Blood 2006)*

**IL-27**

**MOUSE**

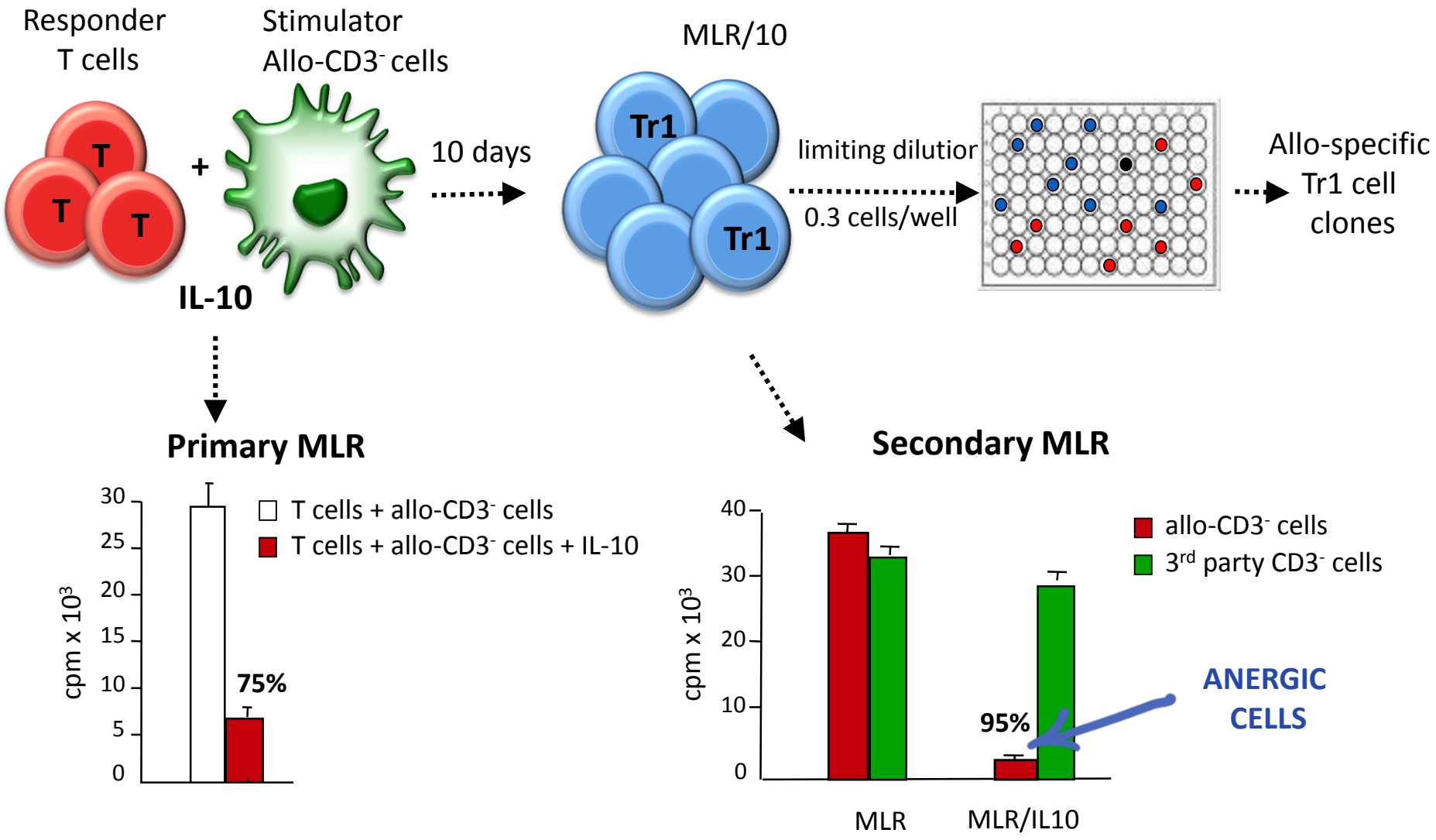
*(Pot. C, JI 2009, Apetoh L. Nat Imm. 2010)*

**HUMAN ?**

**Vit D3 + dexa**

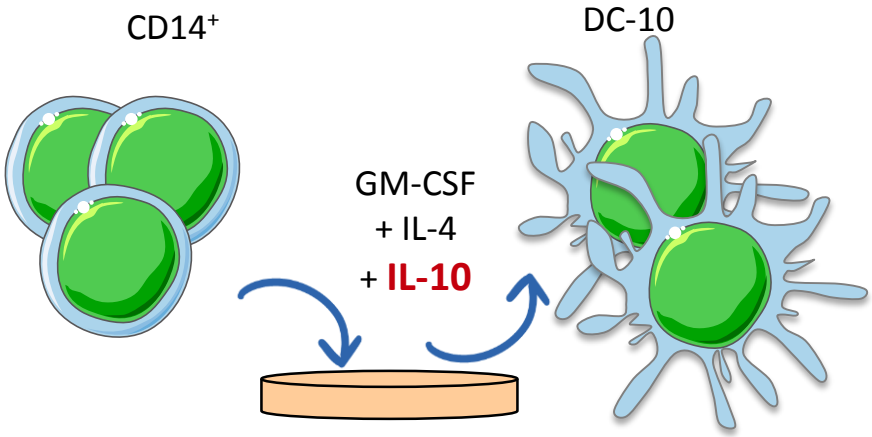
*(Barrat et al., J Exp Med 2002)*

# *Ex vivo* induction of host-specific human Tr1 cells

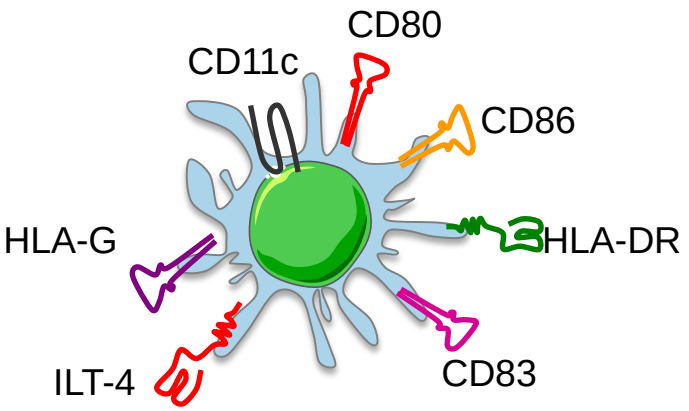



*Groux H. 1997 Nature, Bacchetta R. 2002 EJI, Bacchetta and Gregori Hematologica 2010*

# Tolerogenic DC-10




DC-10 are generated *in vitro* from **monocytes in the presence of IL-10**

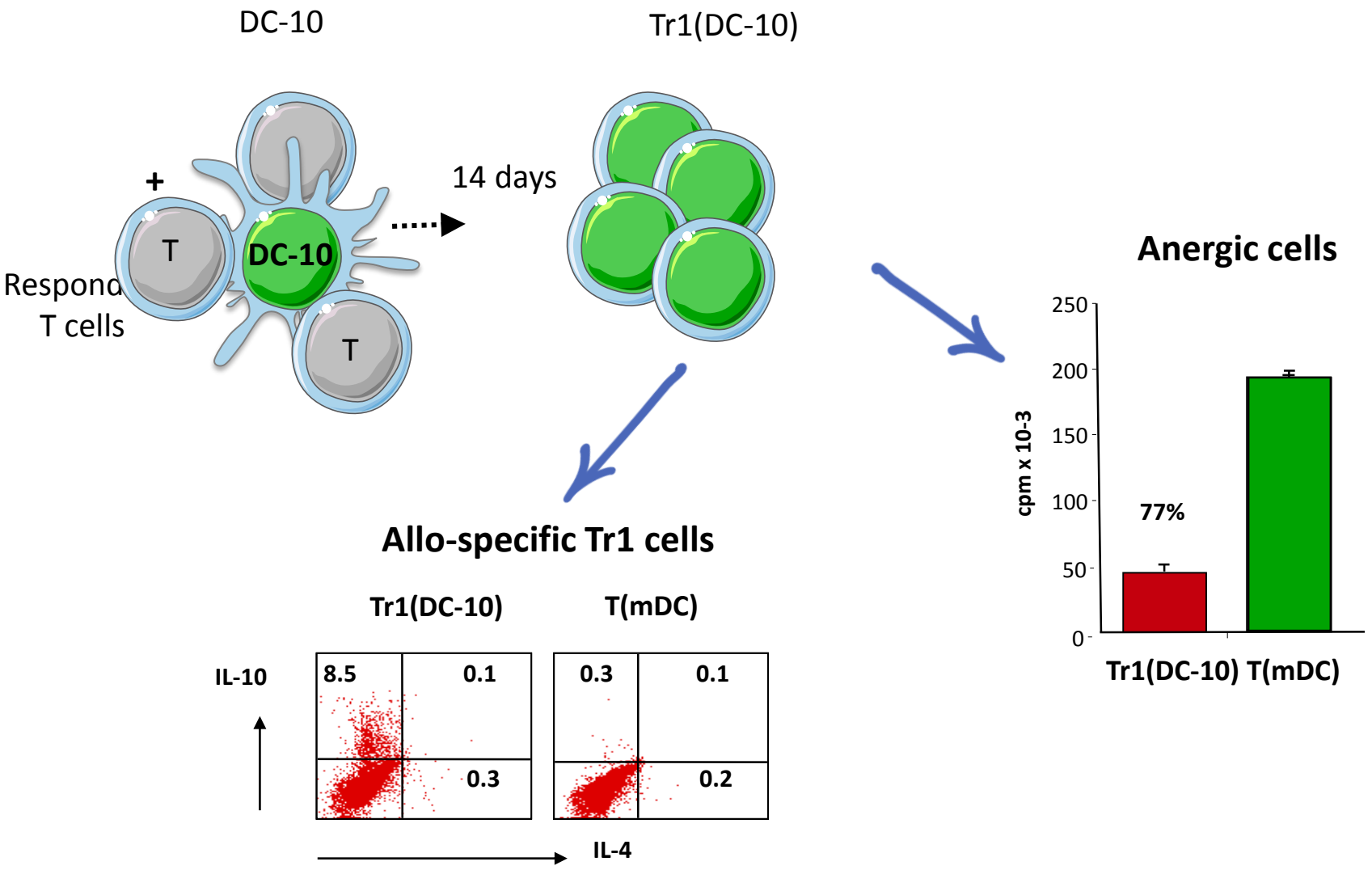



DC-10 are a subset of **mature myeloid cells**

**DC-10** promote the induction of **Type 1 Regulatory (Tr1) T cells**.
Their tolerogenic potential relies on **IL-10 secretion** and on the expression of **HLA-G** and **ILT4**

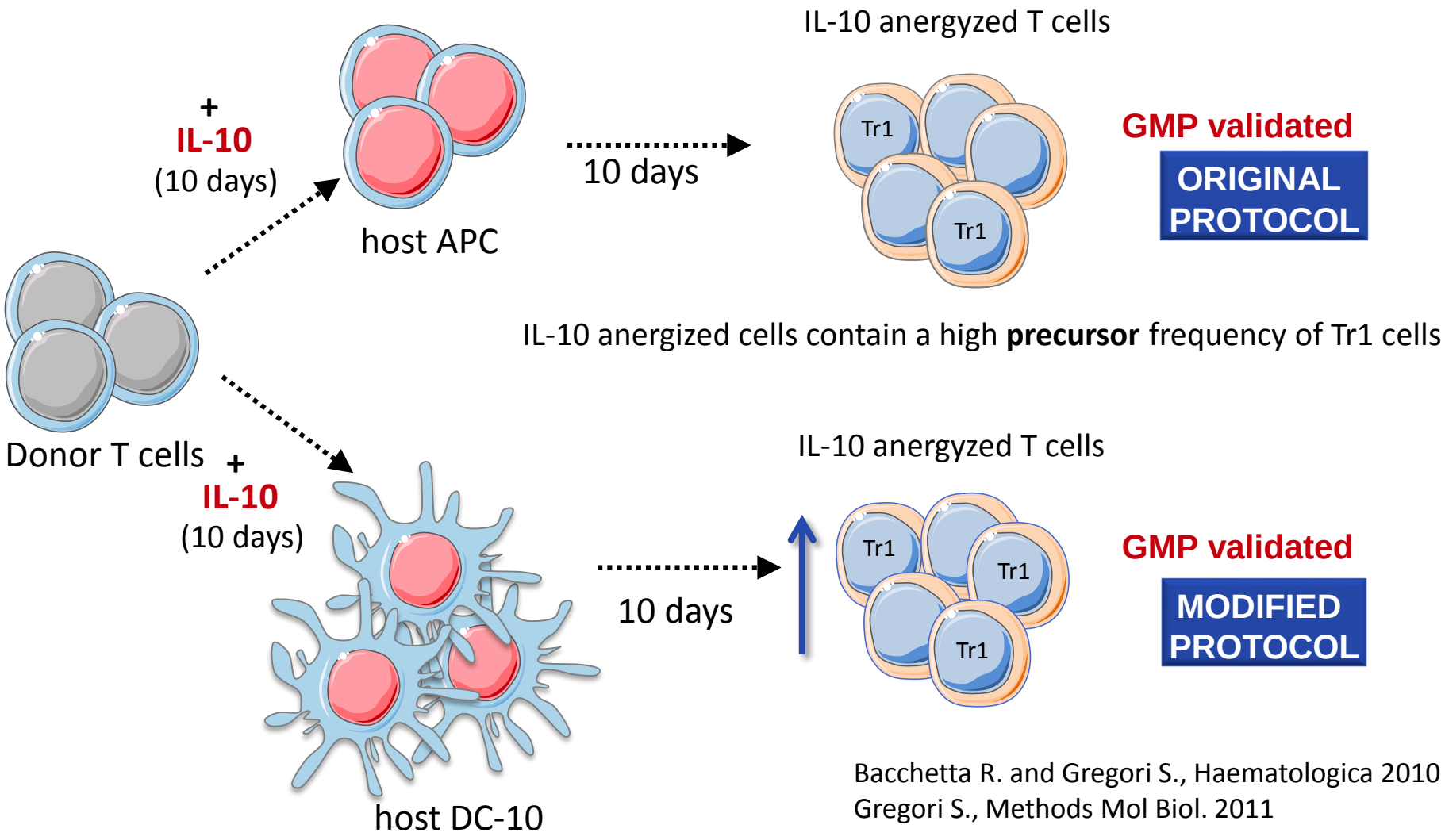
*Gregori, Blood 2010; Gregori , Tissue Antigens 2011; Amodio and Gregori, Transplant Res 2012.*

# *Ex vivo* induction of host-specific human Tr1 cells with DC-10



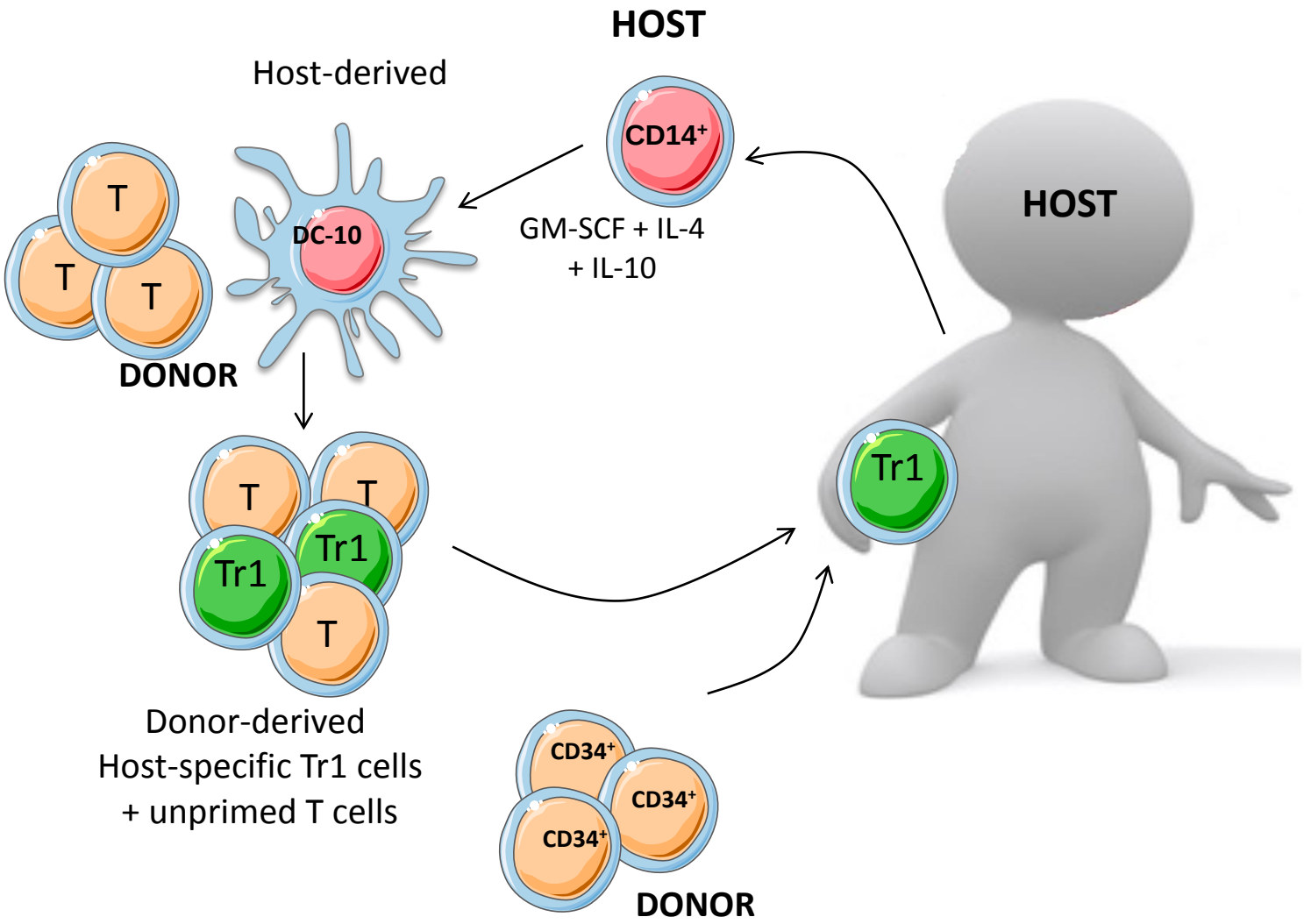

*Gregori et al., Blood 2010; Amodio G, Transplant Research 2012*

# *Ex vivo* induction of Tr1 cells




Bacchetta R. and Gregori S., Haematologica 2010
Gregori S., Methods Mol Biol. 2011

# Tr1-based cell therapy in HSCT to prevent GvHD



# Clinical trials with Tr1 cells

## 1. Induction with exogenous IL-10 and allogeneic APC.

**Completed a phase I clinical trial with IL-10-anergized T cells (IL-10 DLI) in haplo-HSCT**
**(ALT-TEN)** (***Bacchetta et al., Frontiers in Immunol 2014***)**:**

- mild GvHD (grade II or III responsive to therapy),
- tolerance signature,
- faster immune reconstitution,
- long-lasting disease remission.

# The ONE Study in kidney transplanted patients

PATIENT

Leukapheresis from the PATIENT prior kidney Tx

Patient CD4⁺ T cells

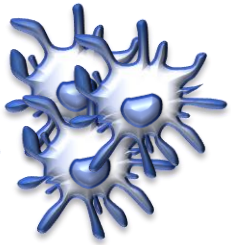

Patient donor-specific Tr1 cells

10 days

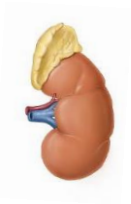
LIVING DONOR

Leukapheresis from the DONOR prior kidney Tx

7 days



Donor DC-10



KYDNEY TRANSPLANTATION

PATIENT

IS treatment

# Tr1 cell-based therapy to prevent graft rejection

Original Basic Science

## Generation of Donor-Specific T Regulatory Type 1 Cells From Patients on Dialysis for Cell Therapy After Kidney Transplantation

Alessandra Petrelli, MD,[1,2] Eleonora Tresoldi, BSc,[3] Bechara G. Mfarrej, MSc,[3] Alessia Paganelli, MD,[1] Donatella Spotti, MD,[4] Rossana Caldara, MD,[1] Antonio Secchi, MD,[1,5] and Manuela Battaglia, PhD[3]

**Induction with tolerogenic DC-10.**

**Clinical Protocol to induce tolerance with T10 cells in kydney transplantation (ONE-T10):**

(Battaglia et al.)

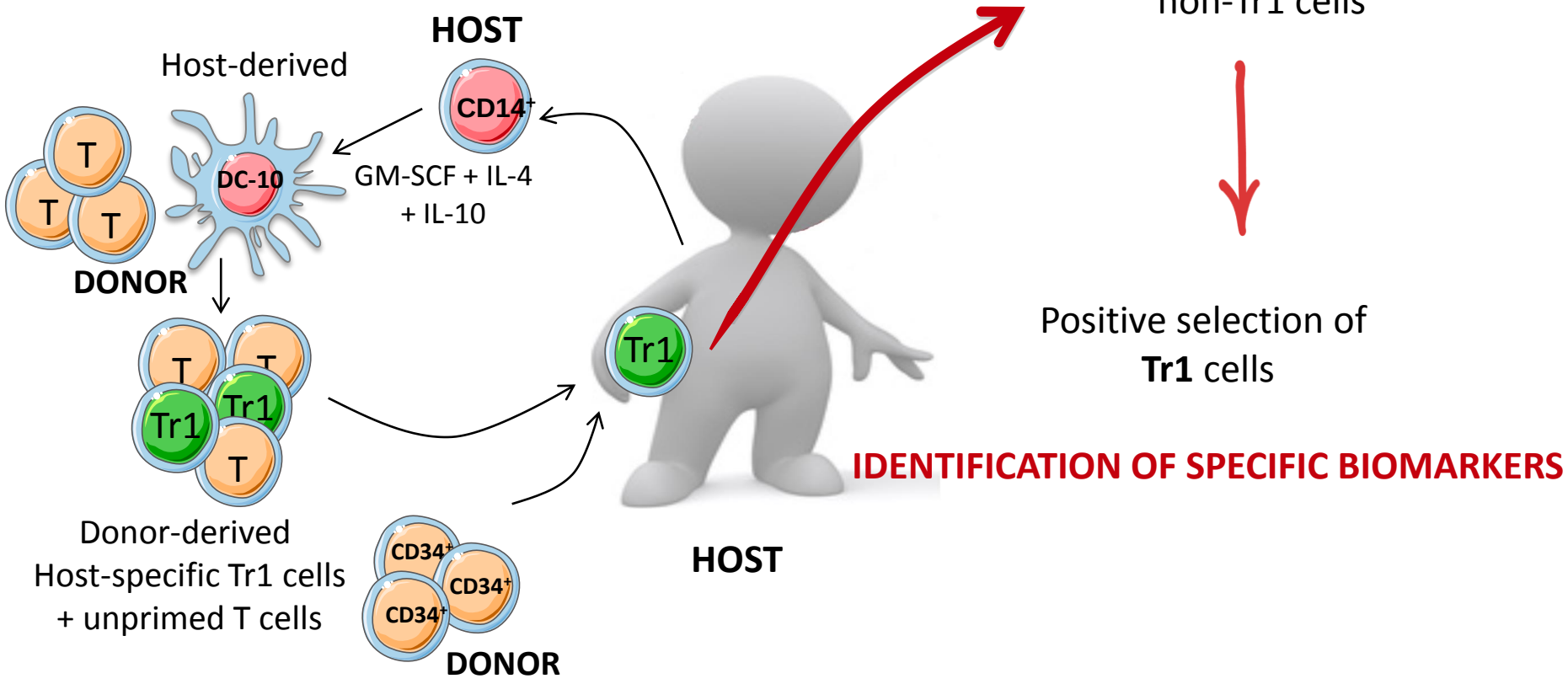
- allo-specific Tr1 of host origin,
- GMP protocol scale up done,
- GMP validation runs ongoing

# Adoptive immunotherapy with Tr1 cells

An efficient GMP protocol for the *ex vivo* induction of functional Allo-specific Tr1 cells has been developed

**BUT**

**IL10 DLI** still contains contaminating non-Tr1 cells



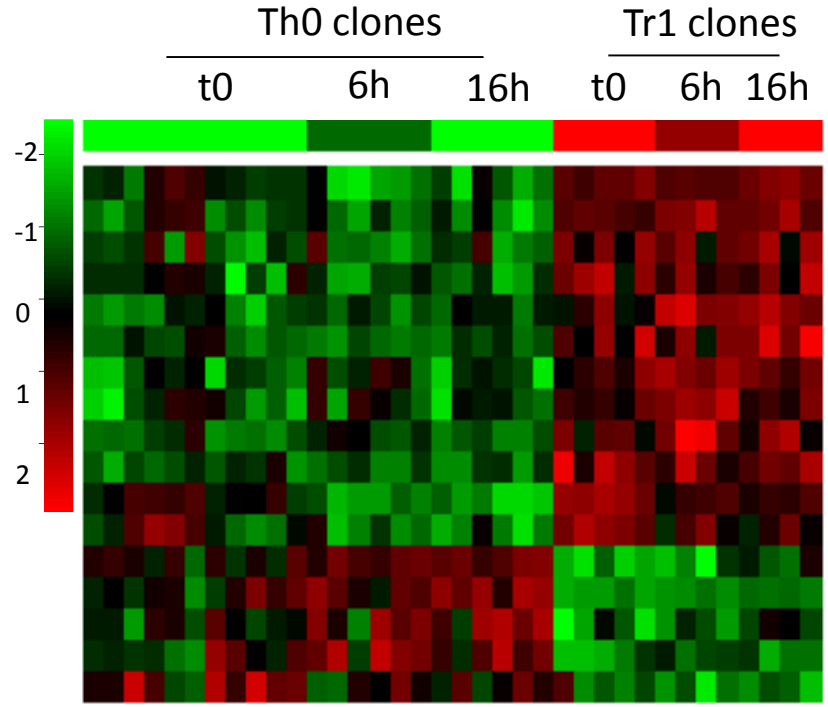

Positive selection of **Tr1** cells

**IDENTIFICATION OF SPECIFIC BIOMARKERS**

# Identification of CD49b and LAG-3 by gene expression profiling of Tr1 cells

## SURFACE MOLECULES

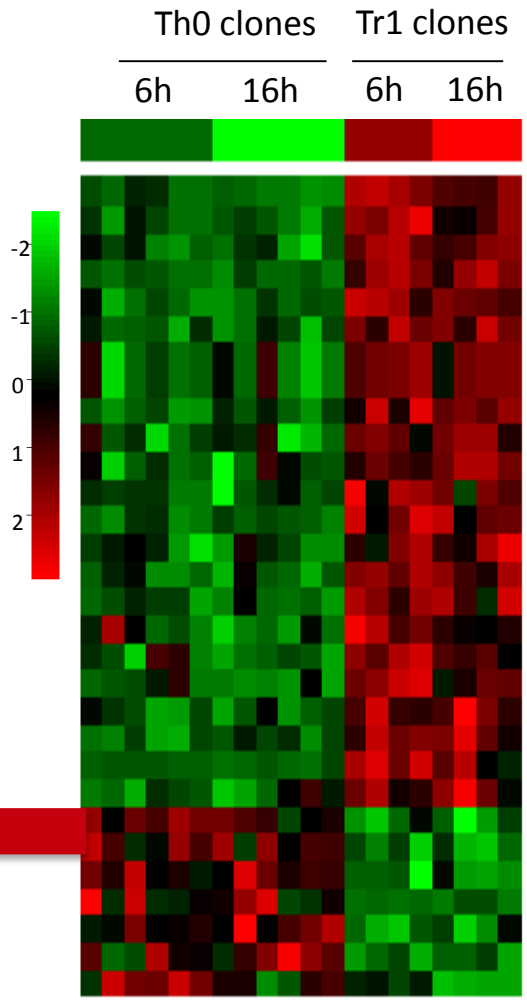
up-regulated at all time points




**CD49b**

integrin alpha 2 subunit of VLA-2 receptor

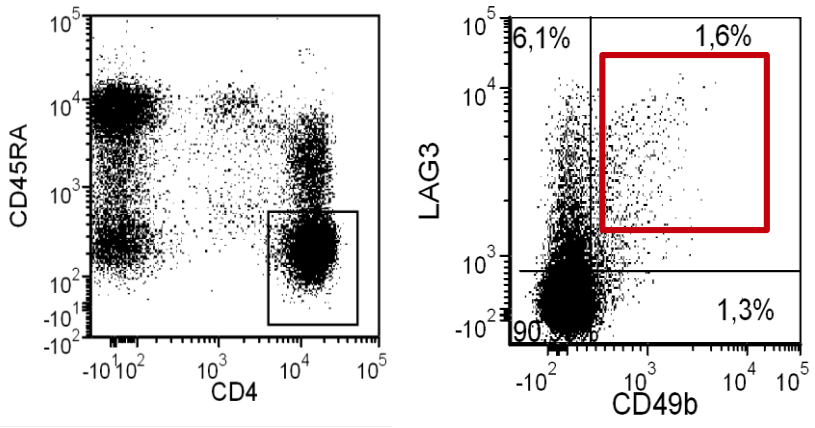
up-regulated upon activation

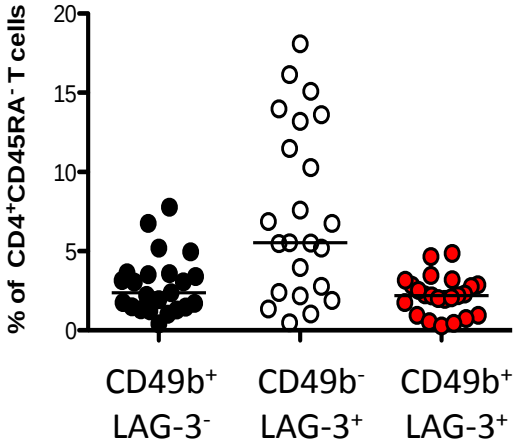


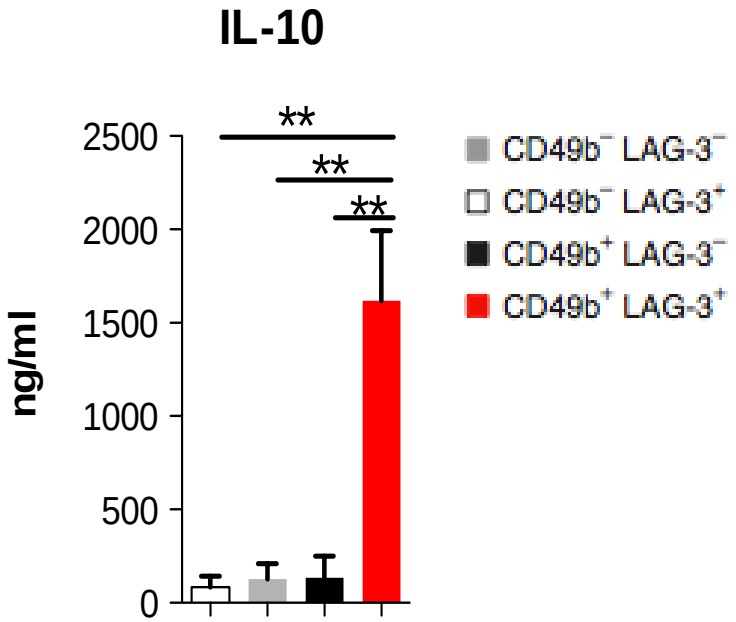

**LAG-3**

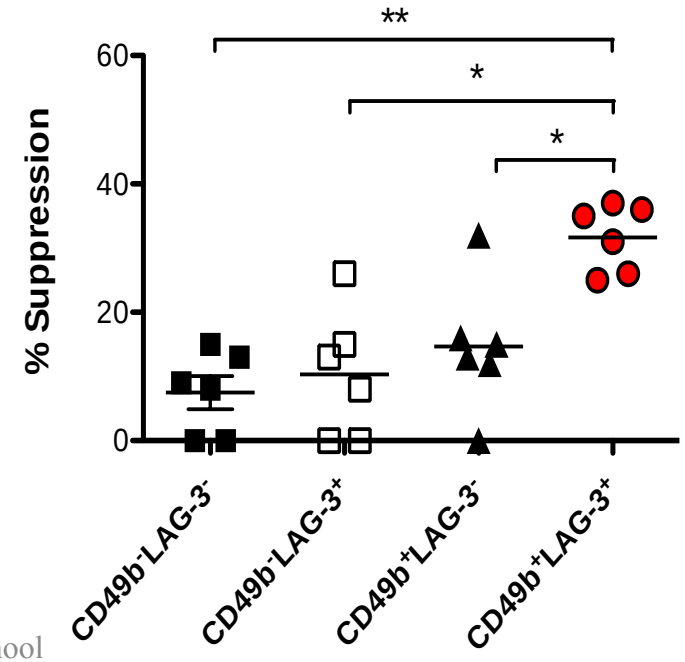
Lymphocyte Activation Gene 3

# Human Tr1 cells are CD45RA$^{-}$CD49b$^{+}$LAG-3$^{+}$



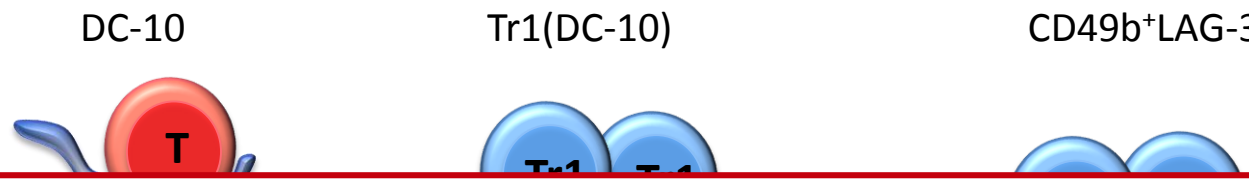



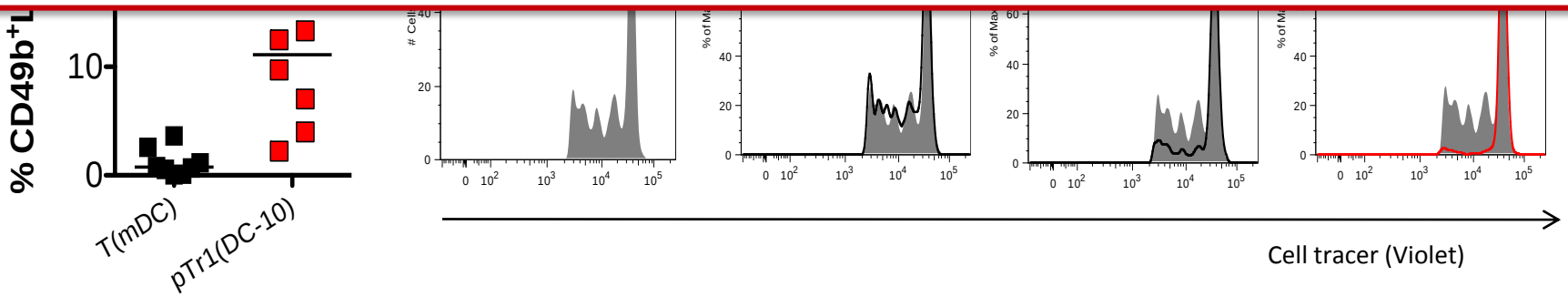




*Gagliani N., Magnani C., and Huber S:, Nat Med 2013*

# In vitro induced Tr1 cells can be selected with CD49b and LAG-3



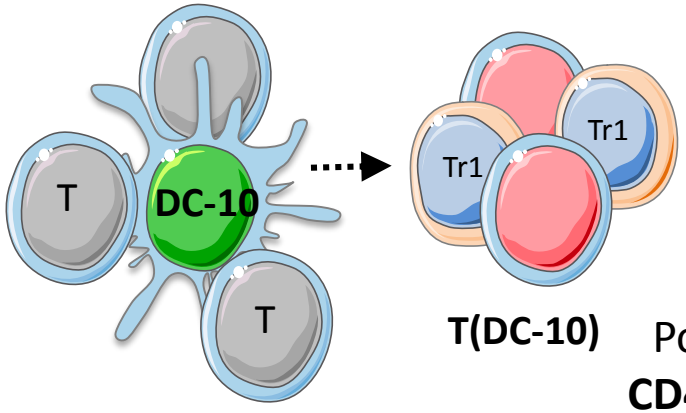

**CD49b and LAG-3 are biomarkers of Tr1 cells that allow their selection from *in vitro* cultures**






*Gagliani N., Magnani C., and Huber S:, Nat Med 2013*

# Generation of Tr1 cells by IL-10 gene transfer




CD4⁺ T cells

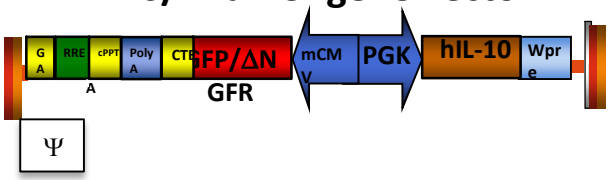
T(DC-10)

**T(DC-10) cells** contained contaminating effector T cells

Positive selection of **CD49b⁺LAG-3⁺ Tr1** cells

*(Gagliani et al., Nat Med 2013)*

**LV-hIL10/ marker gene vector**




**Protocol**

Total PBMC

CD4⁺ T cells

aCD3 (30ng/ml)
aCD28 (1μg/ml)
IL-2 (50U/ml)

2 days

LVs

1 day

14 days

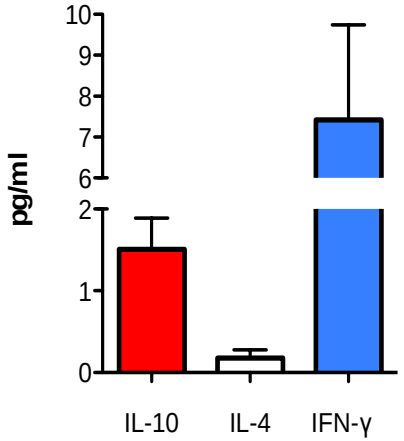
FACS sorting
CD4⁺GFP⁺ T cells
or
CD4⁺ΔNGFR⁺

Feeder mixed expansion

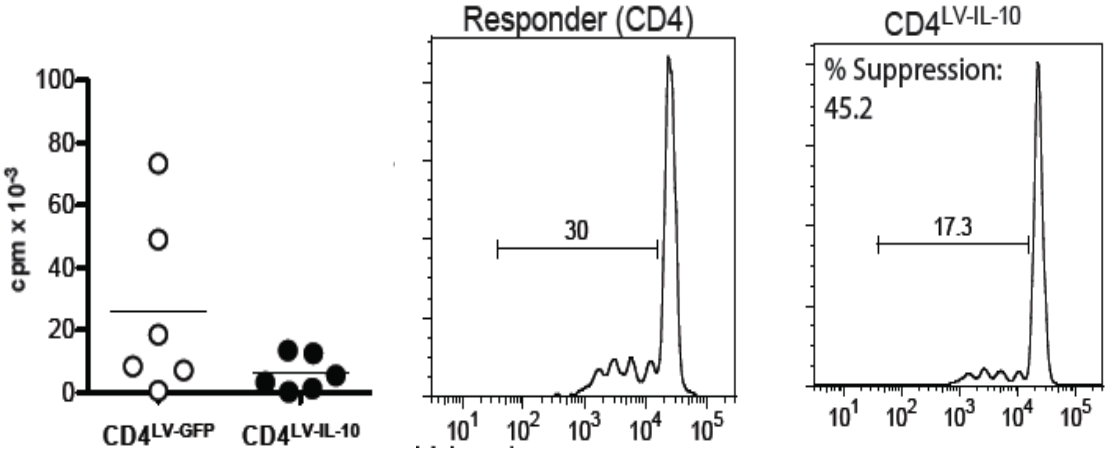
**Read outs:**
Cytokine production
Proliferative responses
Suppressive activity
Lytic activity

# Generation of Tr1 cells by IL-10 gene transfer

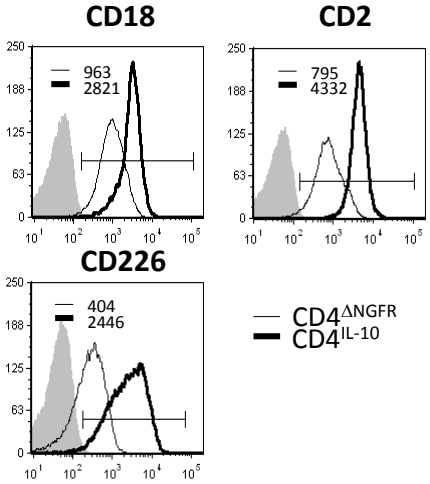
## Tr1 cytokine profile



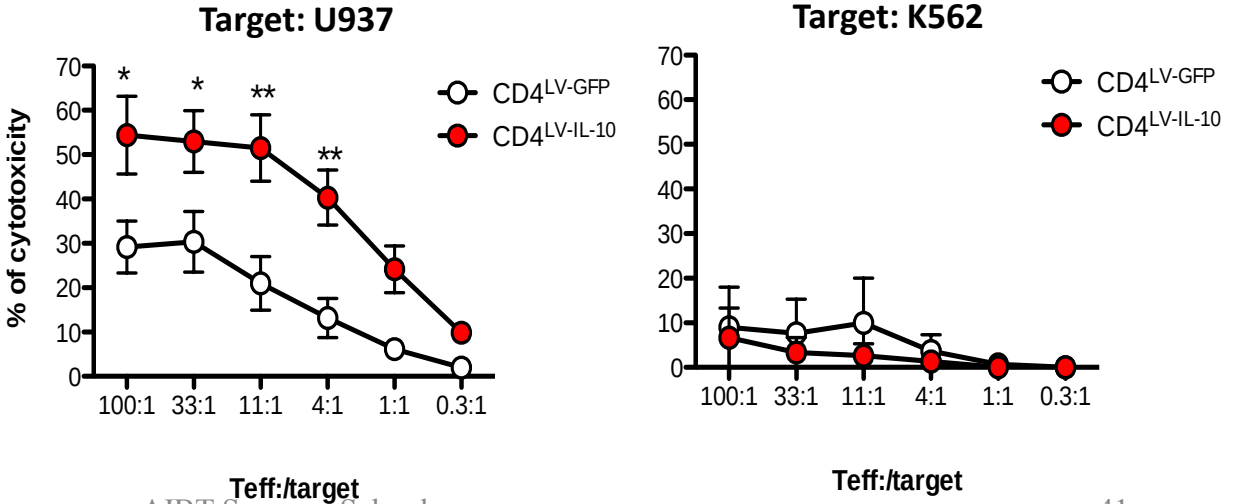

## anergy and suppressive activity




## Tr1 phenotype




## Lytic activity




*Andolfi and Fousteri, Molecular Therapy 2012*

# Protocols to generate regulatory T cells for cellular therapy