Progetto Ematologia Romagna
Rimini 8 aprile 2017
Immunologia e Tumori

C’è futuro senza rigetto?

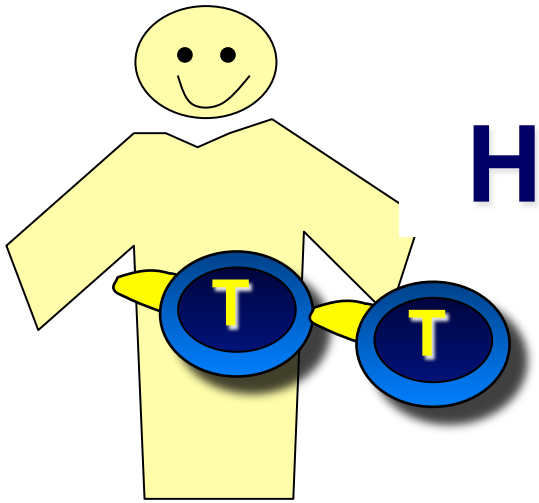
M.Arpinati
Istituto di Ematologia e Oncologia Medica “Seragnoli”

Outline of the talk

- **General mechanisms of alloreactivity**
- **Alloreactivity in HSC transpantation**
- **GVHD as a model to PREVENT alloreactivity**
- **GVHD as a model to TREAT alloreactivity**

The immunological barrier

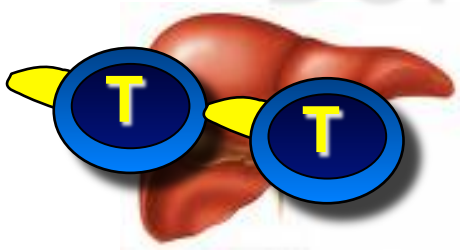
Medawar 1944 described it in skin transplants in mice
Starzl 1967 performs first successful allo liver transplant
Don Thomas 1968 performs first successufl BMT



HOST IMMUNITY

rejection

DONOR IMMUNITY



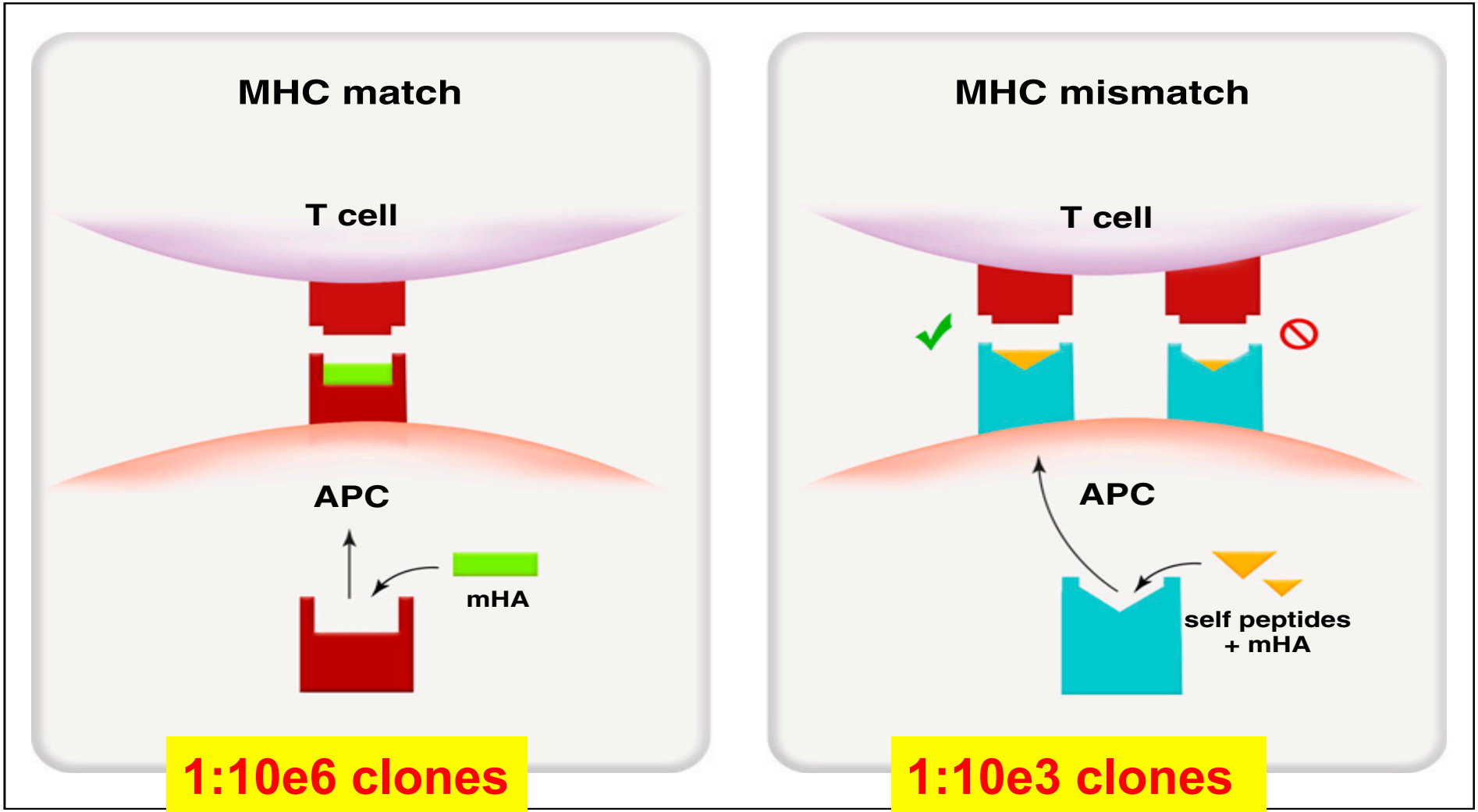
GVHD

Biology of the immunological barrier

Mitchison1964
Billingham 1966
Thomson 1996
Schlomchick 1999

- **Different antigens between host and donor**
- **Functional APC presenting antigens**
- **T lymphocytes.**

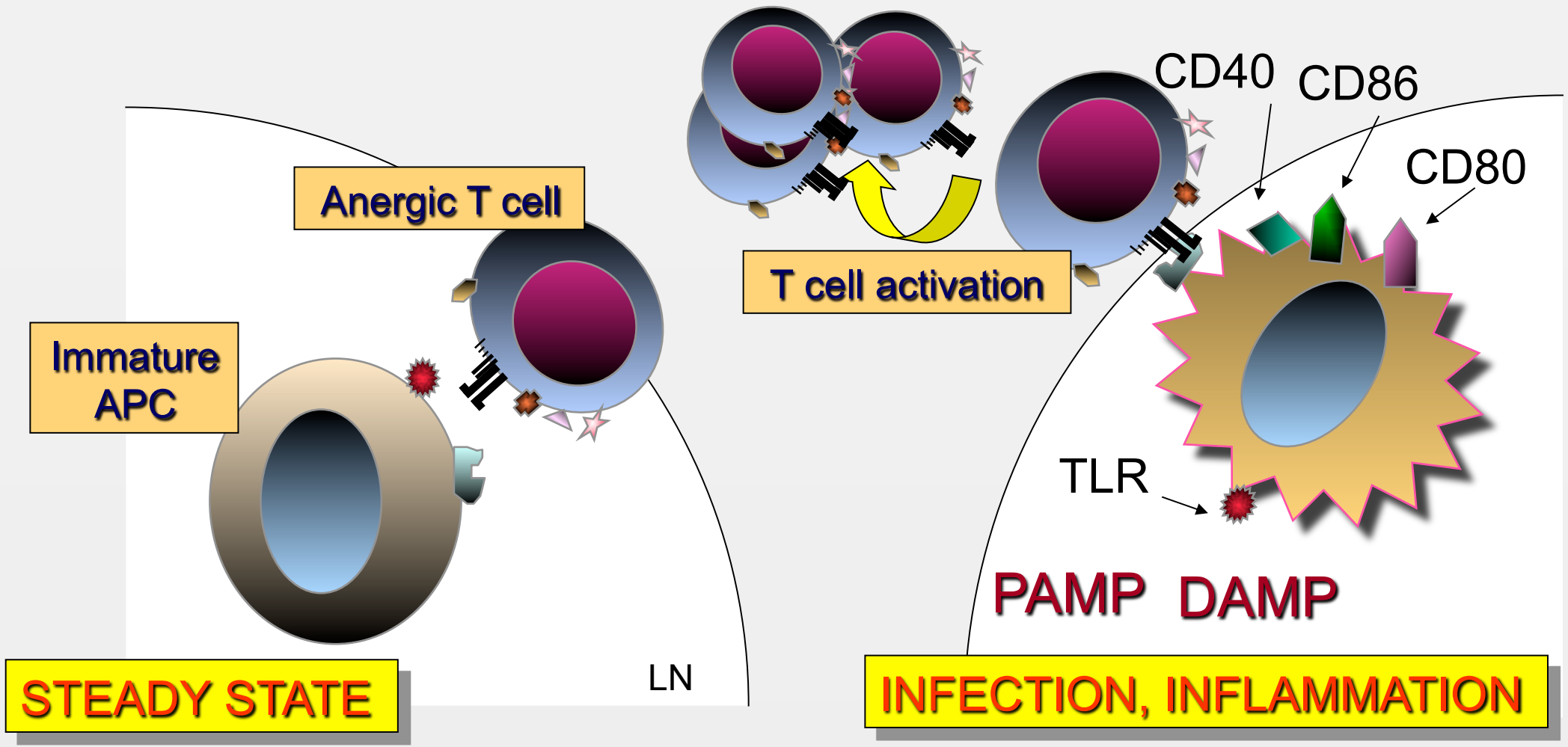
Molecular basis of alloreactivity



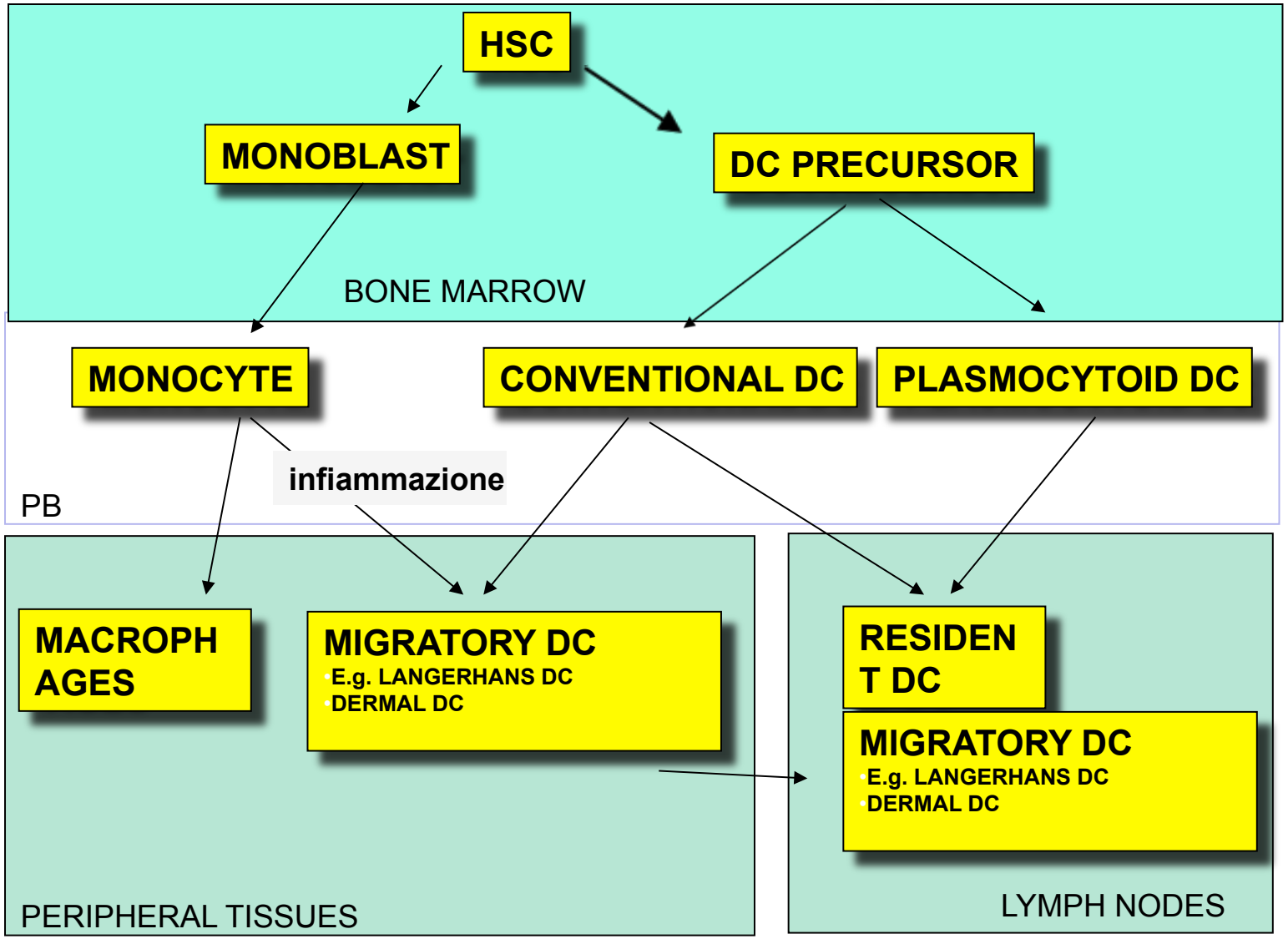
Holtan et al. Blood 2014

APC sense DANGER to activate T cells

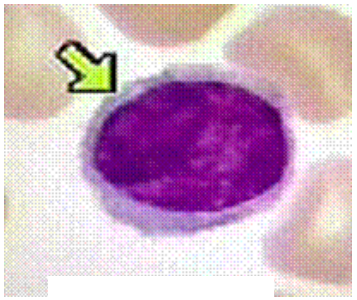
P Matzinger and R Steinman



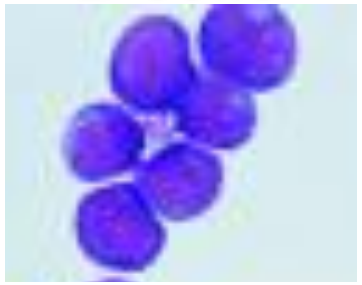
APC include DC and monocytes



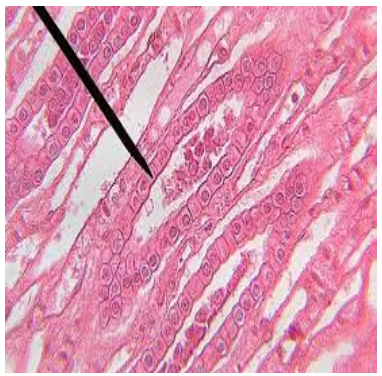
But also…



B cells



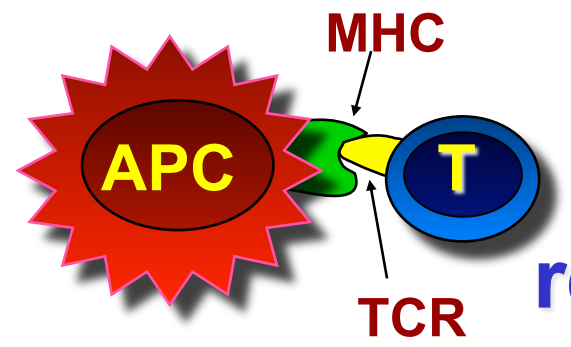
CD34+ cells



Stenger et al. Blood 2012

Non hematopoietic cells

DIRECT ANTIGEN PRESENTATION



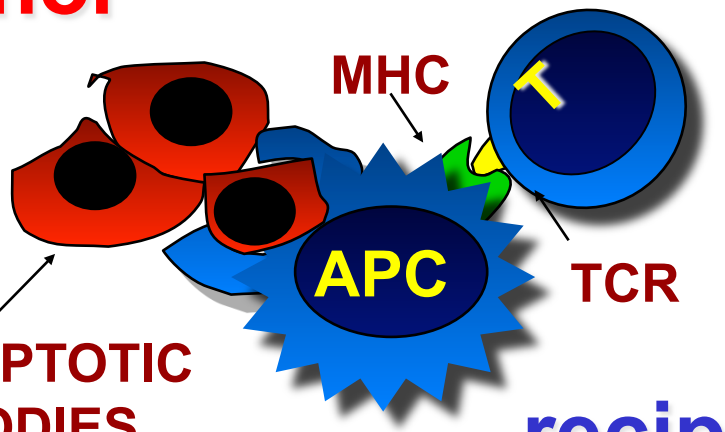
donor

recipient

donor DC → rejection

INDIRECT ANTIGEN PRESENTATION

donor

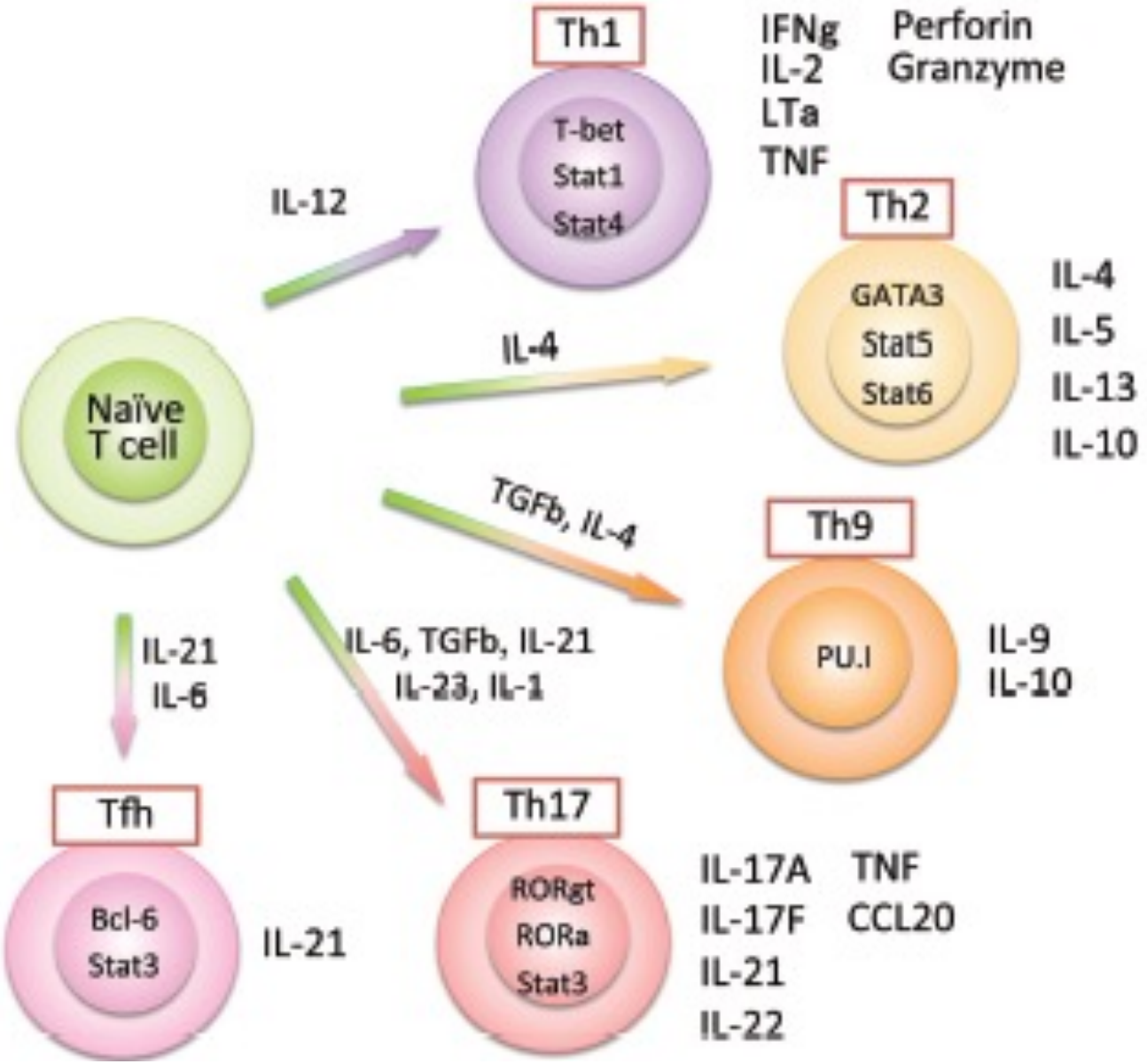


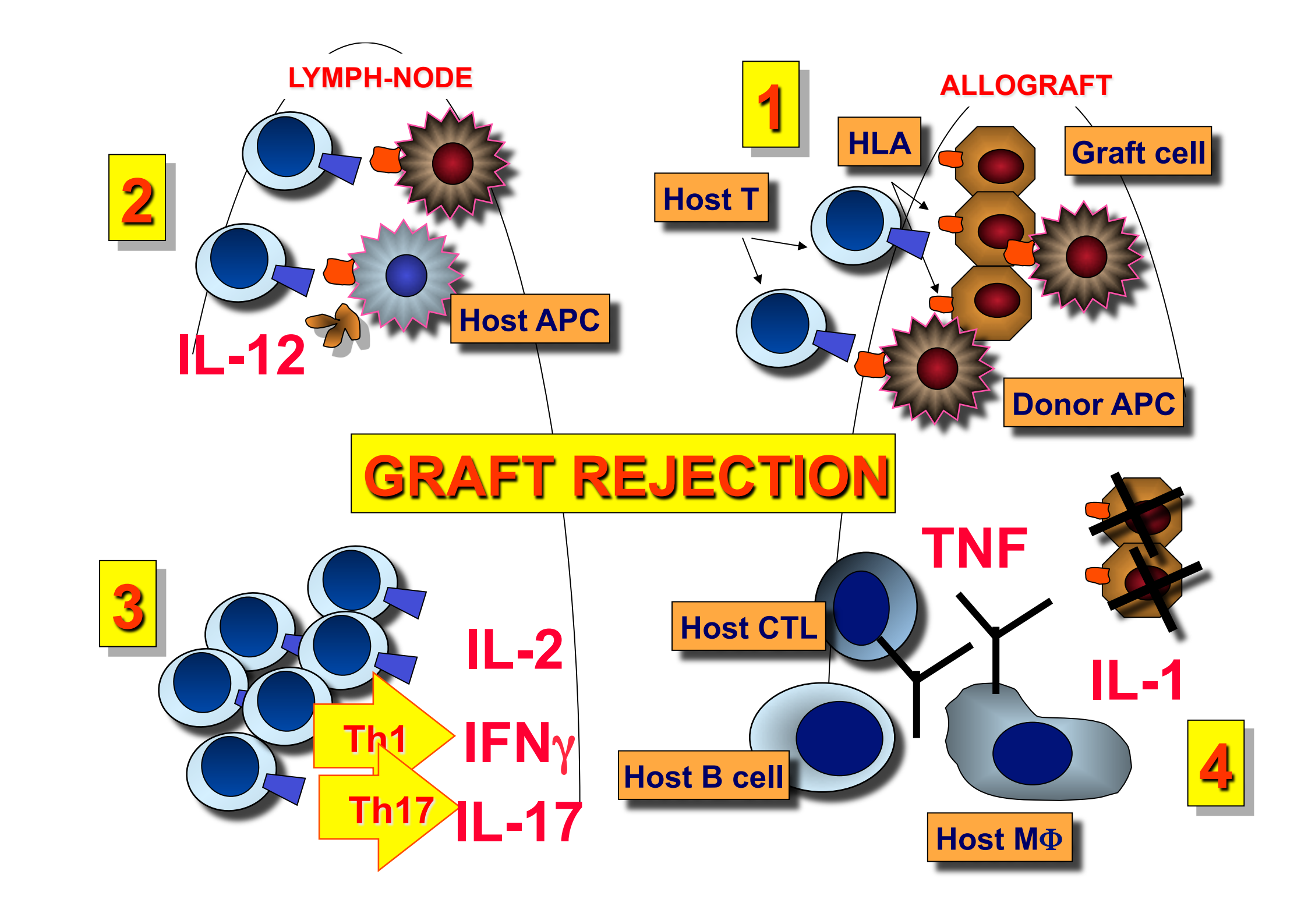
recipient

recipient DC → rejection

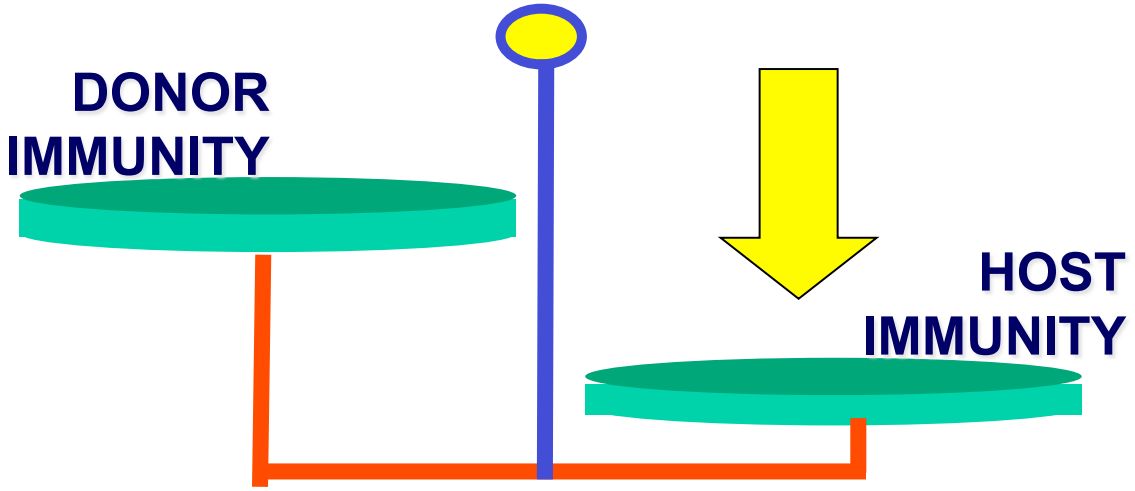
Adapted from Wood et al. Transplantation 2012

T cell differentiation Wood et al Transplantation 2012

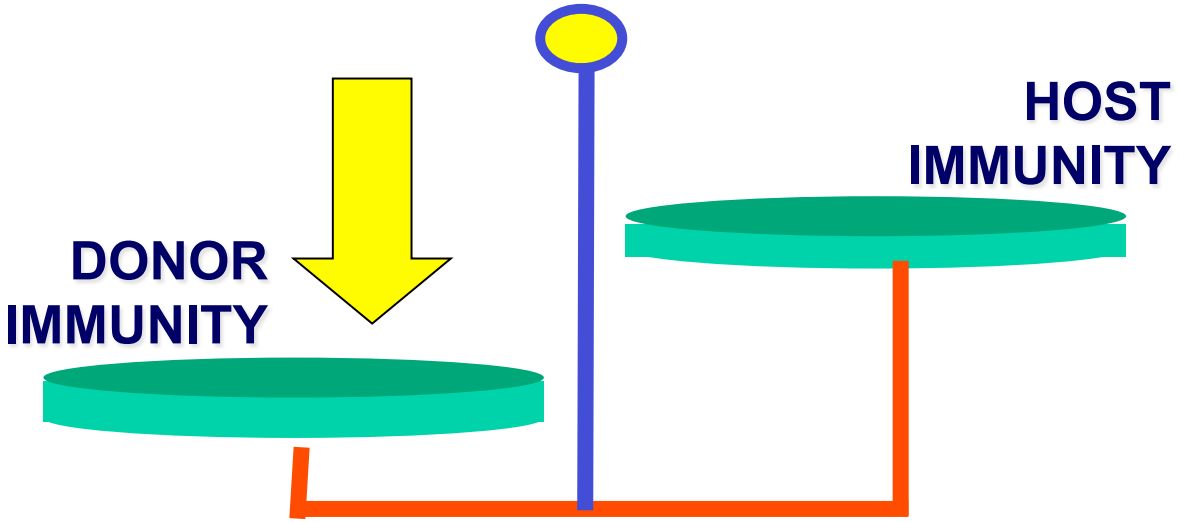



LYMPH-NODE
1
ALLOGRAFT
HLA
Graft cell
Host T
2
Host APC
IL-12
Donor APC
GRAFT REJECTION
TNF
3
IL-2
Host CTL
IL-1
Th1
IFNγ
Th17
IL-17
Host B cell
4
Host MΦ

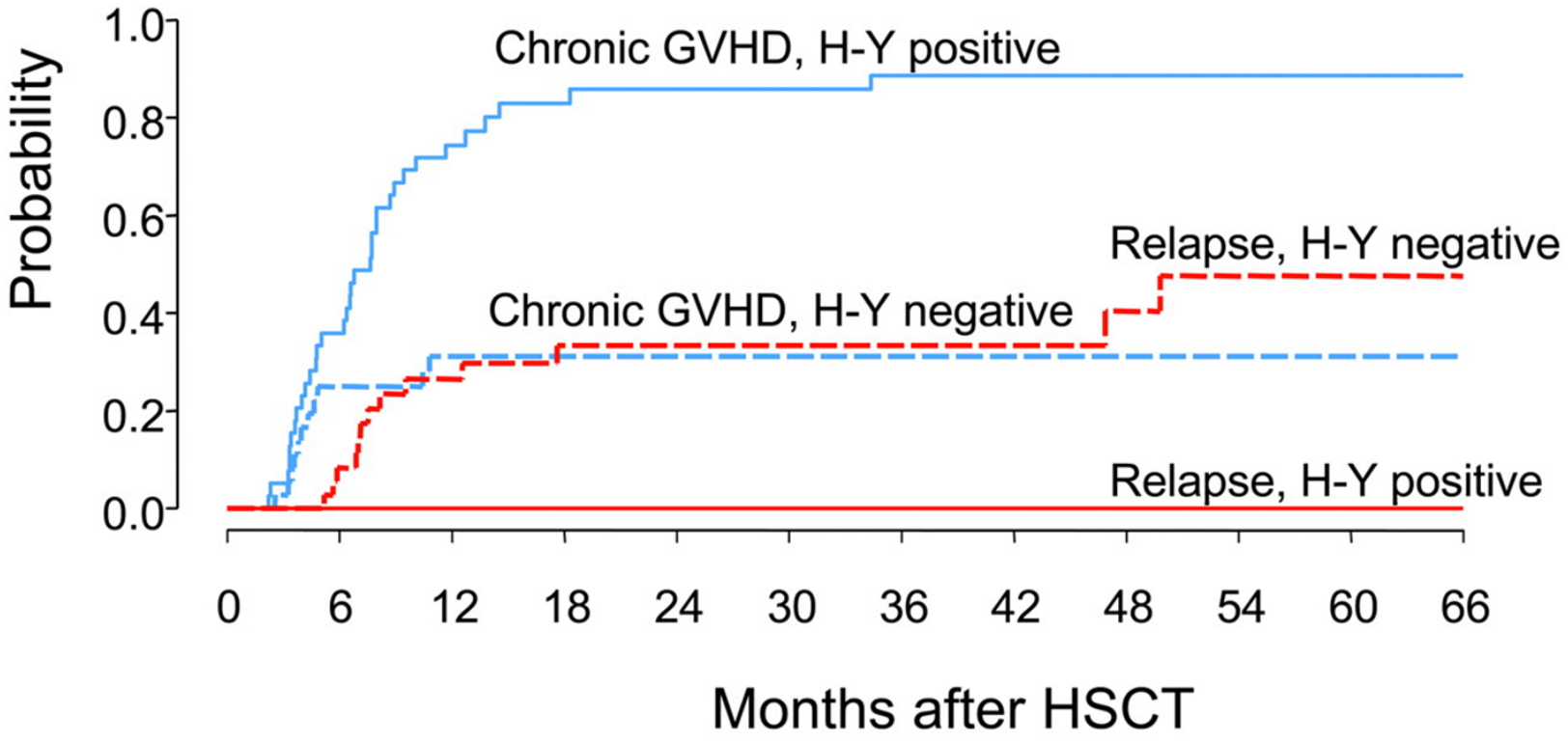
In SOLID ORGAN TRANSPLANTATION



In HSC TRANSPLANTATION



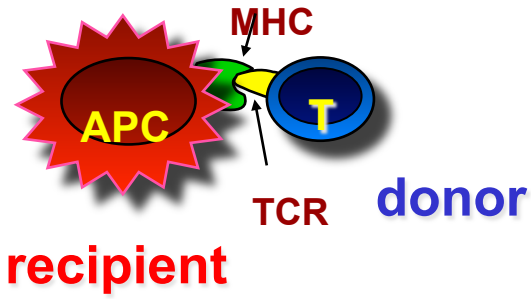
Specificity of BMT I:
Minor Histocompatibility Antigens (mHA)



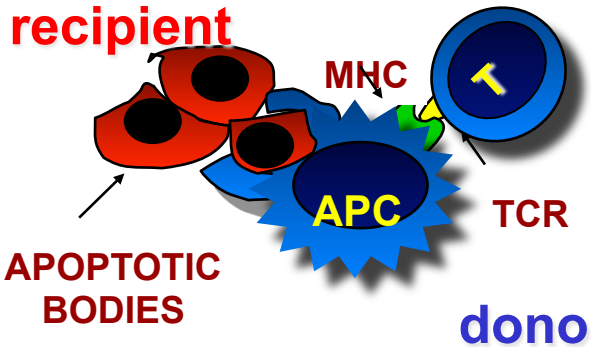
Miklos Blood 2005

Specificity of BMT II: DONOR vs RECIPIENT APC?

DIRECT PRESENTATION

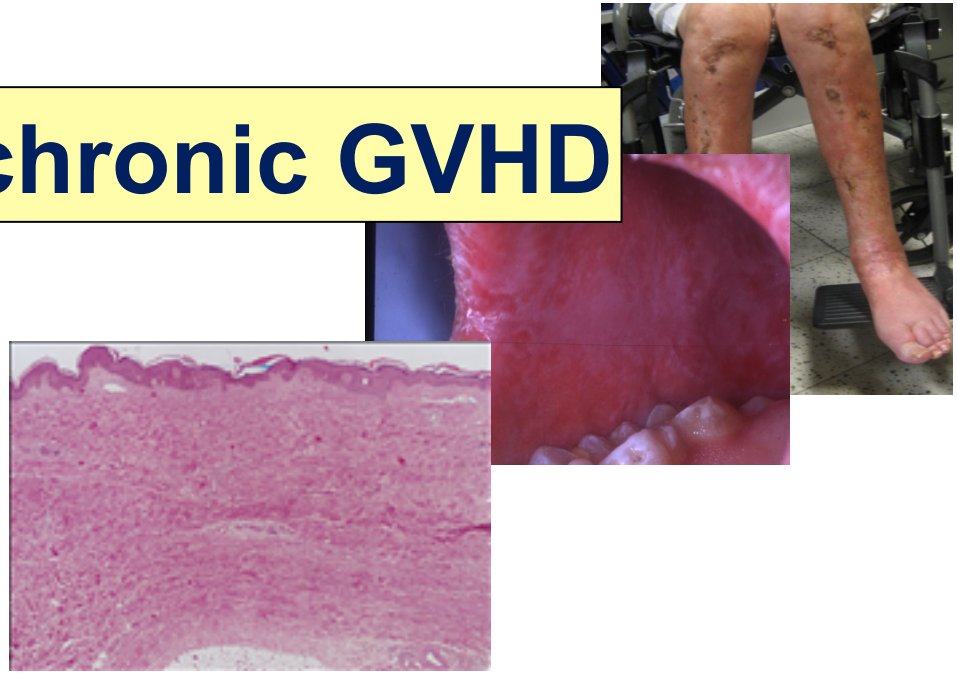


INDIRECT PRESENTATION



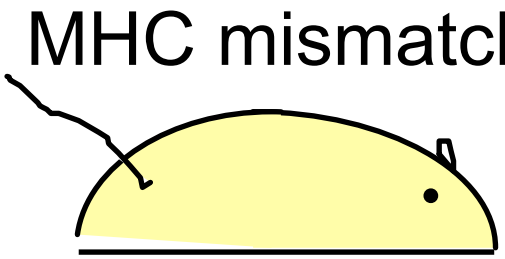
acute GVHD

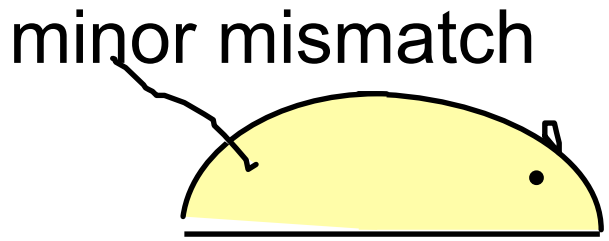
chronic GVHD



Direct presentation drives ACUTE GVHD

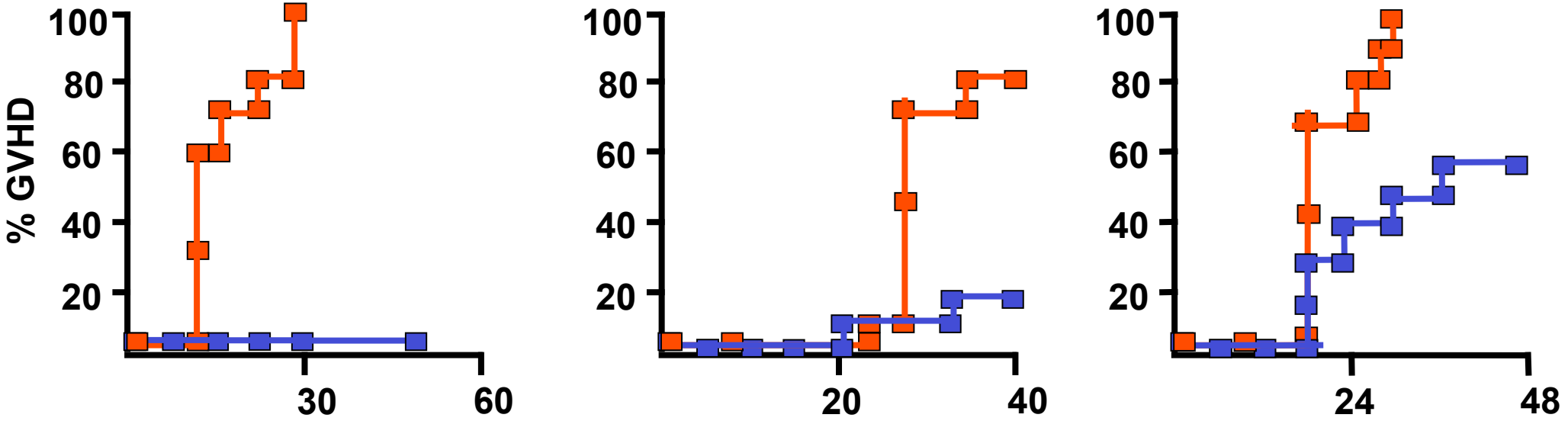
Ruggeri 2002
Schlomchick 1999
Matte 2004
Duffner 2004
Zhang 2002





CD8+

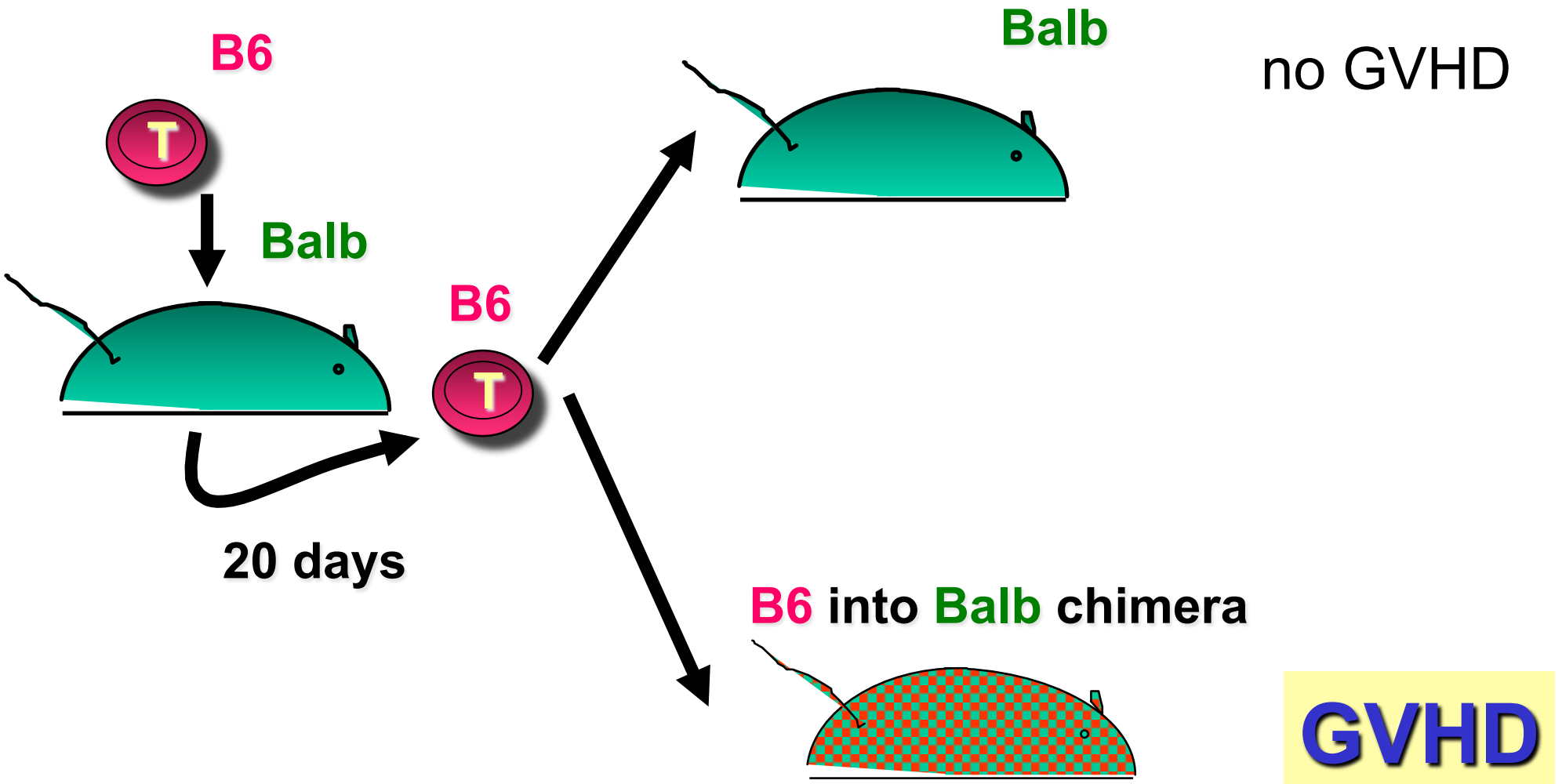
CD4+



control

no host APC

donor APC maintain allo-reactive T cells in CHRONIC GVHD

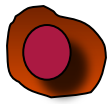


Tivol 2005

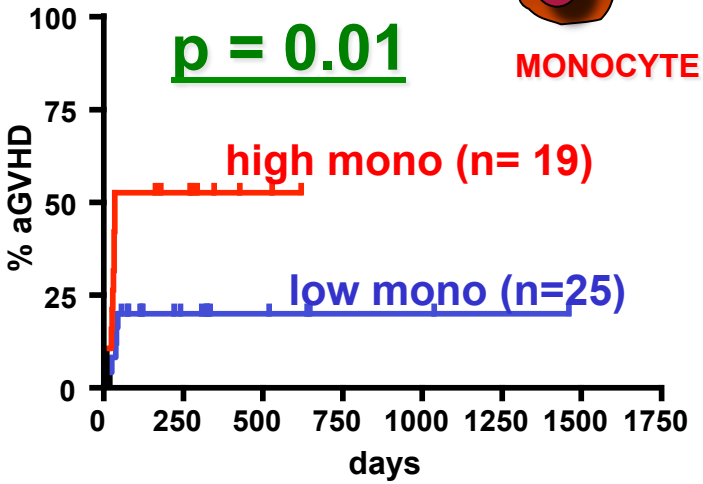
Evidence in humans

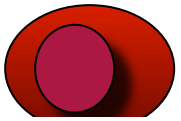
Higher Numbers of Blood CD14$^+$ Cells before Starting Conditioning Regimen Correlate with Greater Risk of Acute Graft-versus-Host Disease in Allogeneic Stem Cell Transplantation from Related Donors

Mario Arpinati,[1] Gabriella Chirumbolo,[1] Yogen Saunthararajab,[2] Marta Stanzani,[1] Francesca Bonifazi,[1] Giuseppe Bandini,[1] Michele Baccarani,[1] Damiano Rondelli[2]

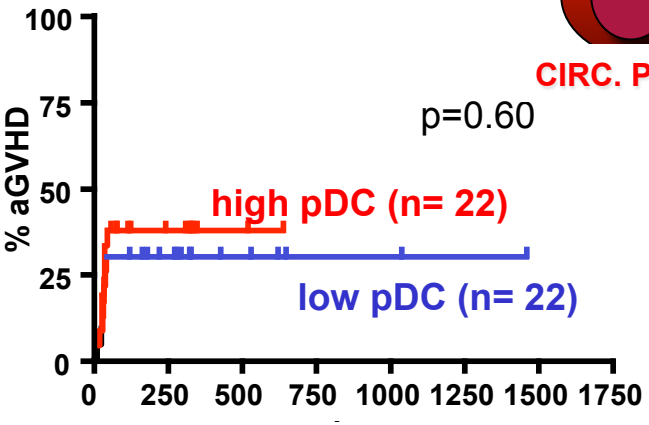


MONOCYTE





CIRC. PLASMACYTOID DC

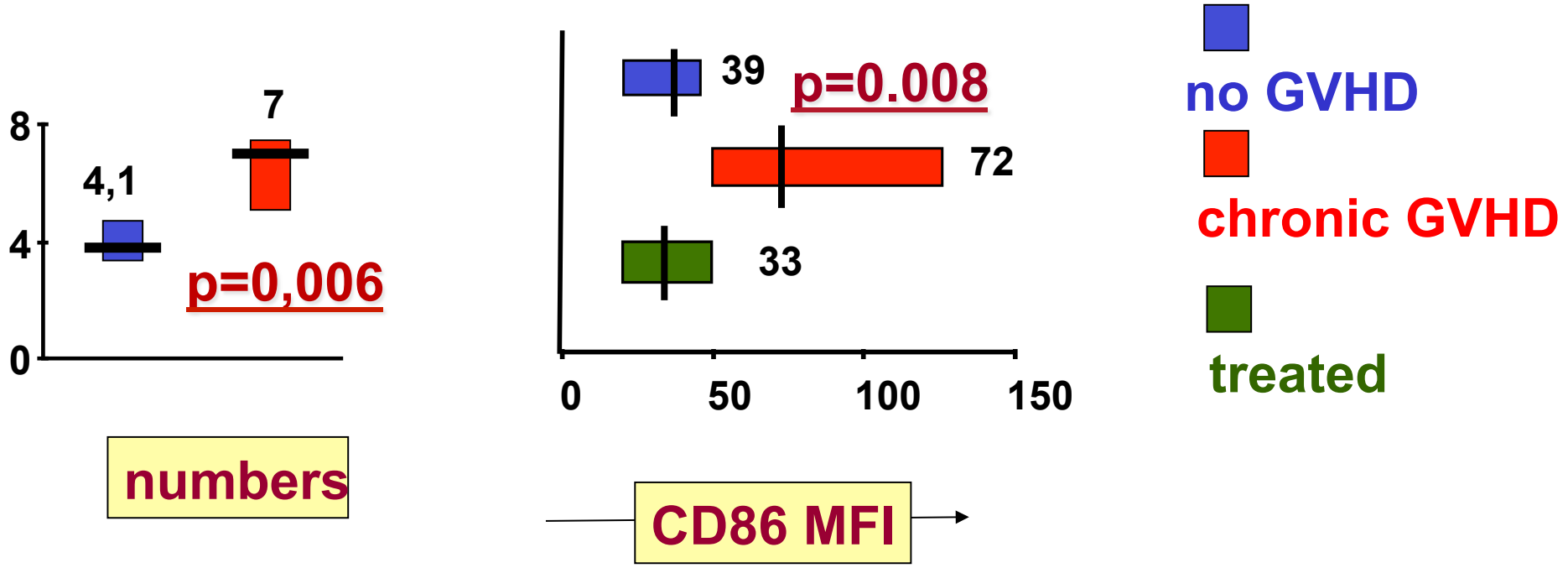


CIRC. MYELOID DC

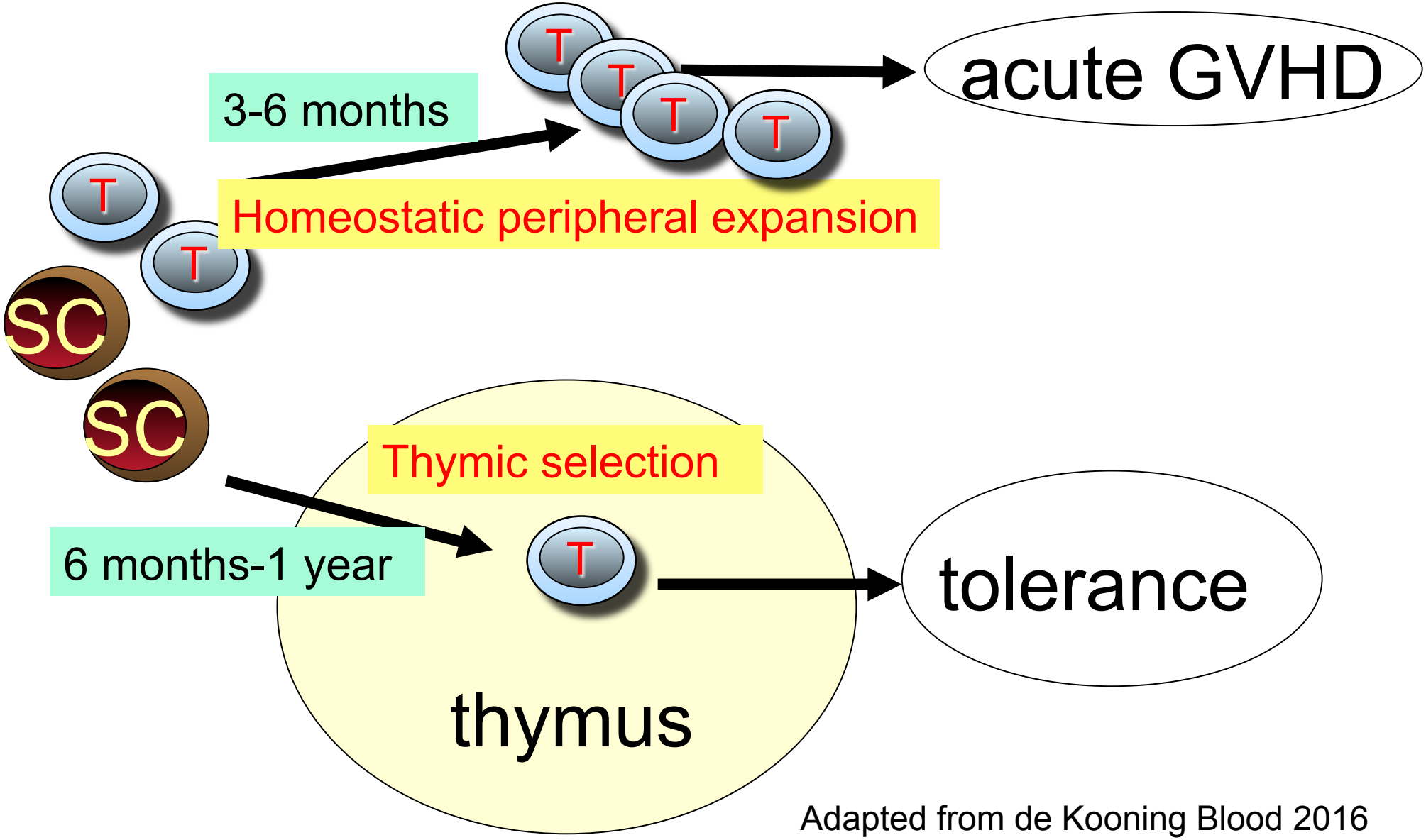
Increased Donor CD86+CD14+ Cells in the Bone Marrow and Peripheral Blood of Patients With Chronic Graft-Versus-Host Disease

Mario Arpinati,[1,3] Gabriella Chirumbolo,[1] Giulia Marzocchi,[1] Michele Baccarani,[1] and Damiano Rondelli[2]

(Transplantation 2008;85: 1826–1832)



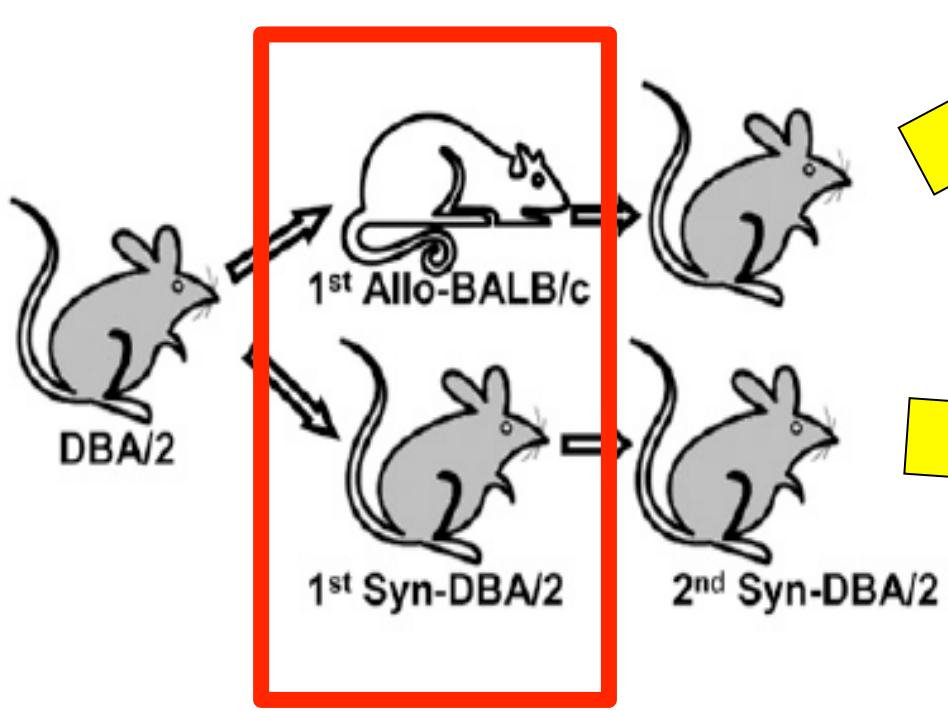
Specifity of BMT III:
HSC transplants should become tolerant



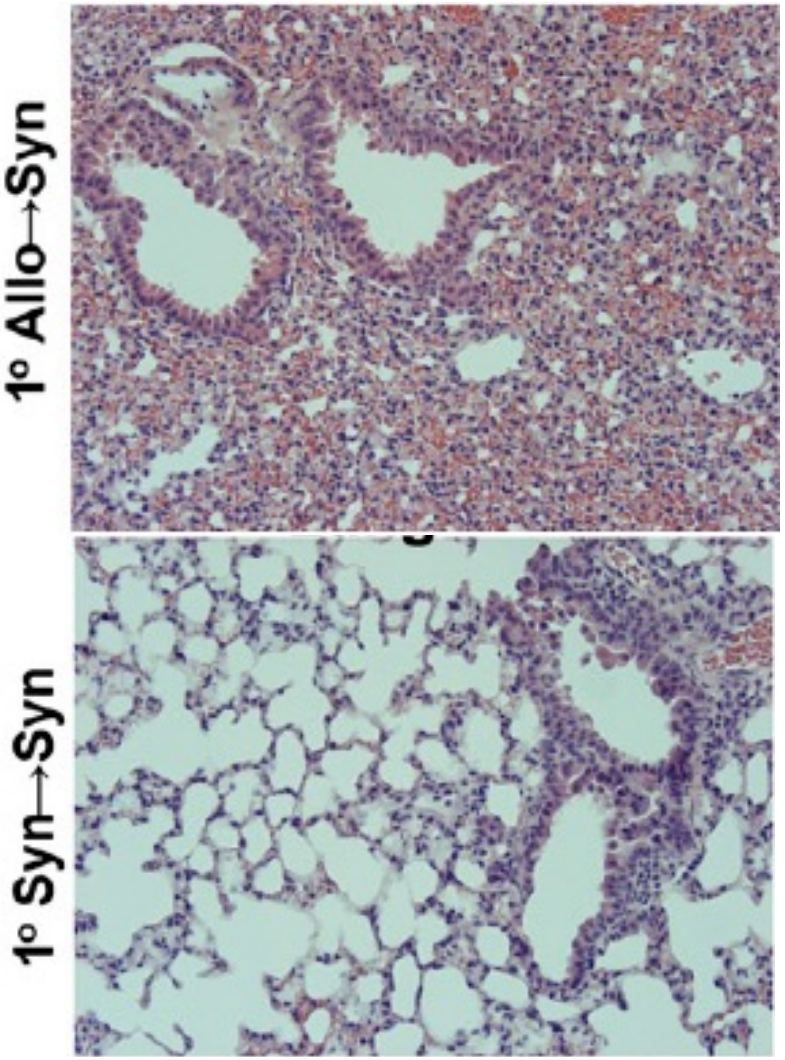
Adapted from de Kooning Blood 2016

However, the THYMUS…

- Deteriorates with age
- Is damaged by chemotherapy
- Is damaged by acute GVHD

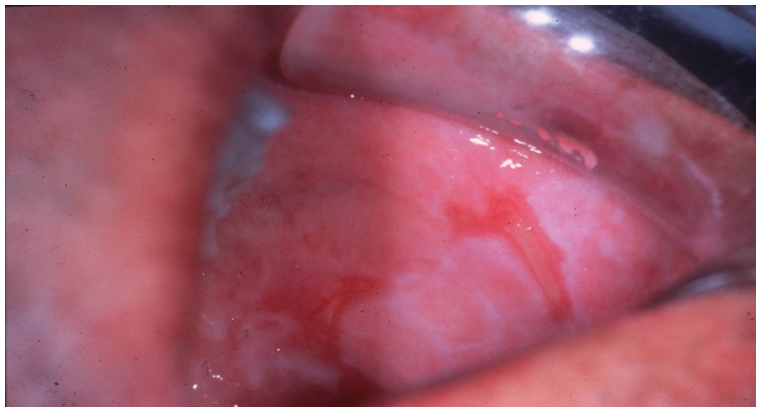


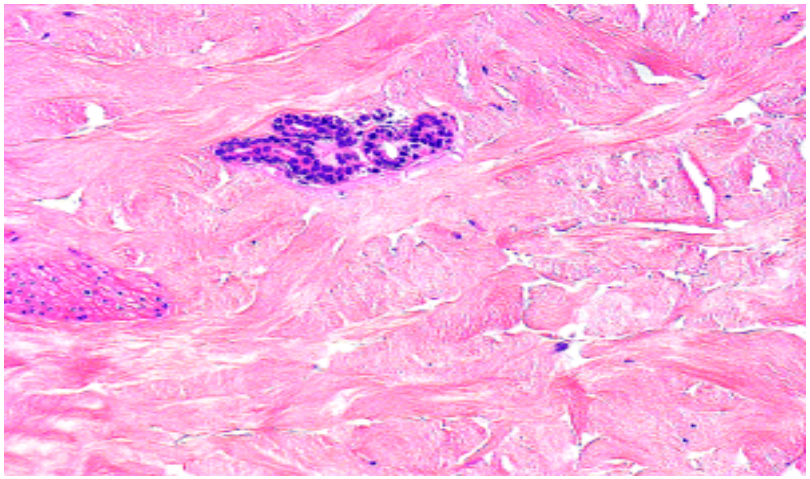
thymectomised



Zhao JI 2011

Chronic GVHD as an autoimmune syndrome





- Clinical (mimicking autoimmune diseases)
- Serological (autoantibodies)
- Histological (fibrosis)
- Immunological (B cell hyperplasia)

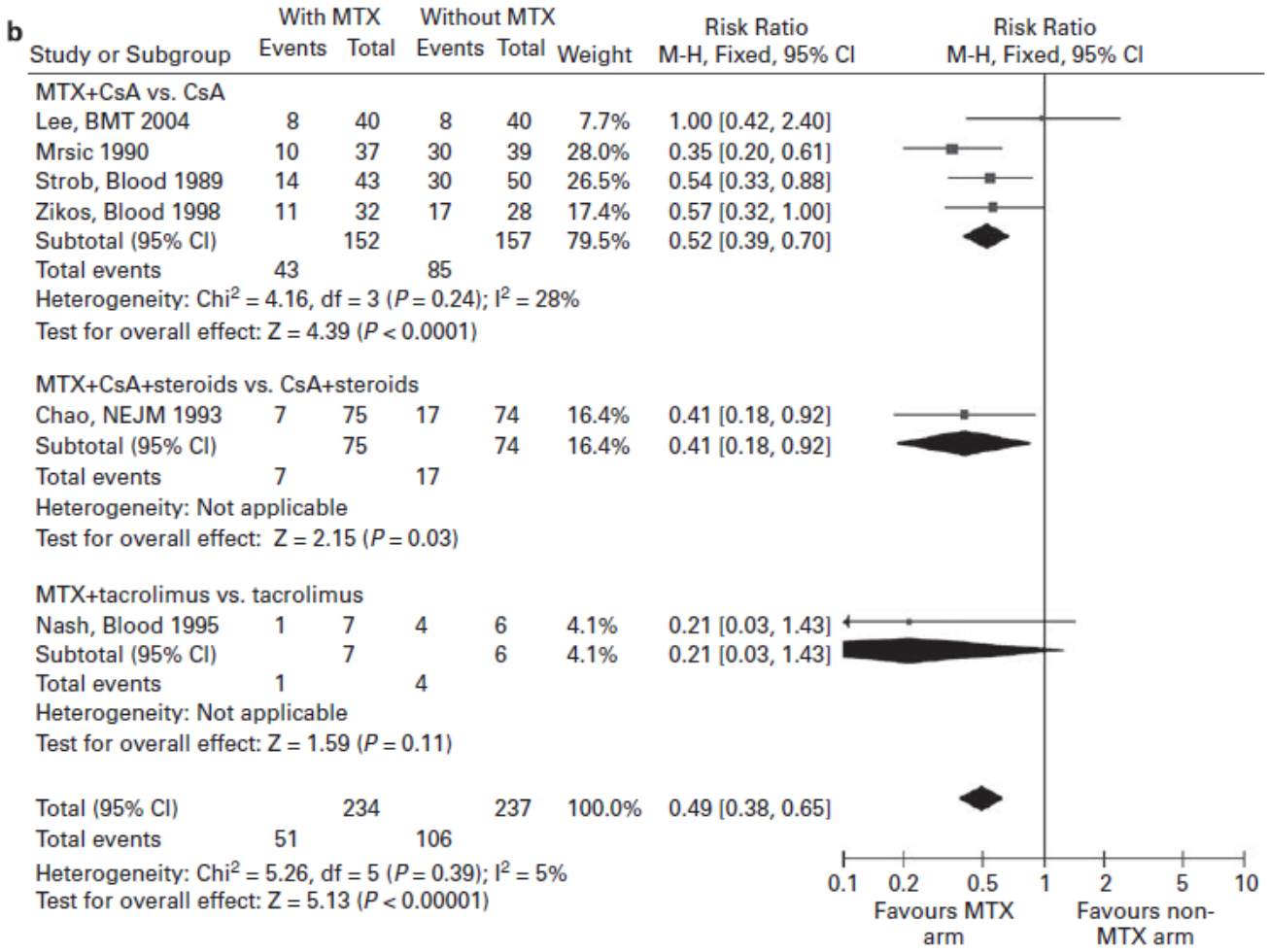
Sociè and Ritz Blood 2014

Standard Prophylaxis of GVHD

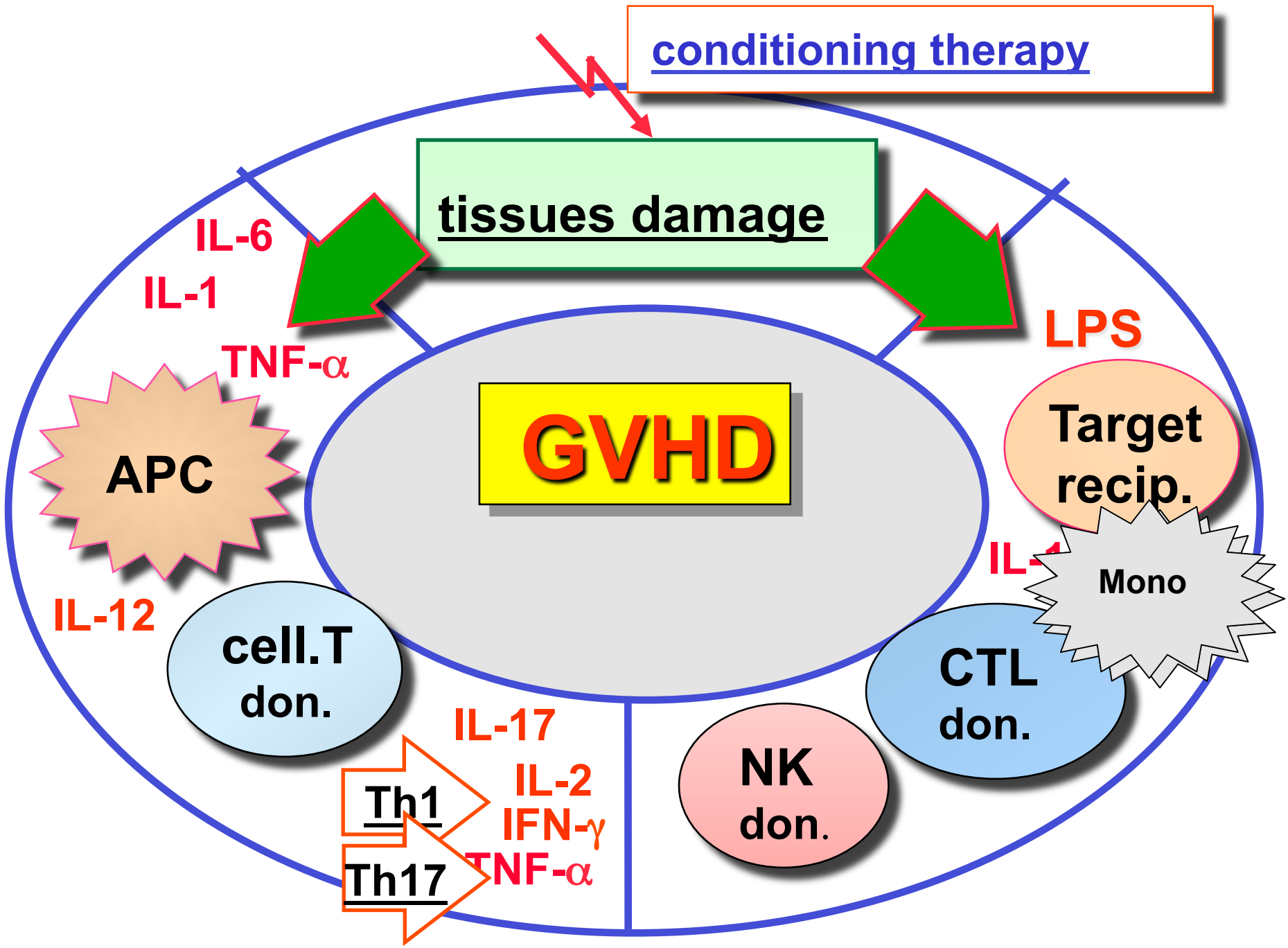
0 +30 +60 +90 +120 +150 +180

calcineurin inhibitor

MTX or MMF



Ram BMT 2009

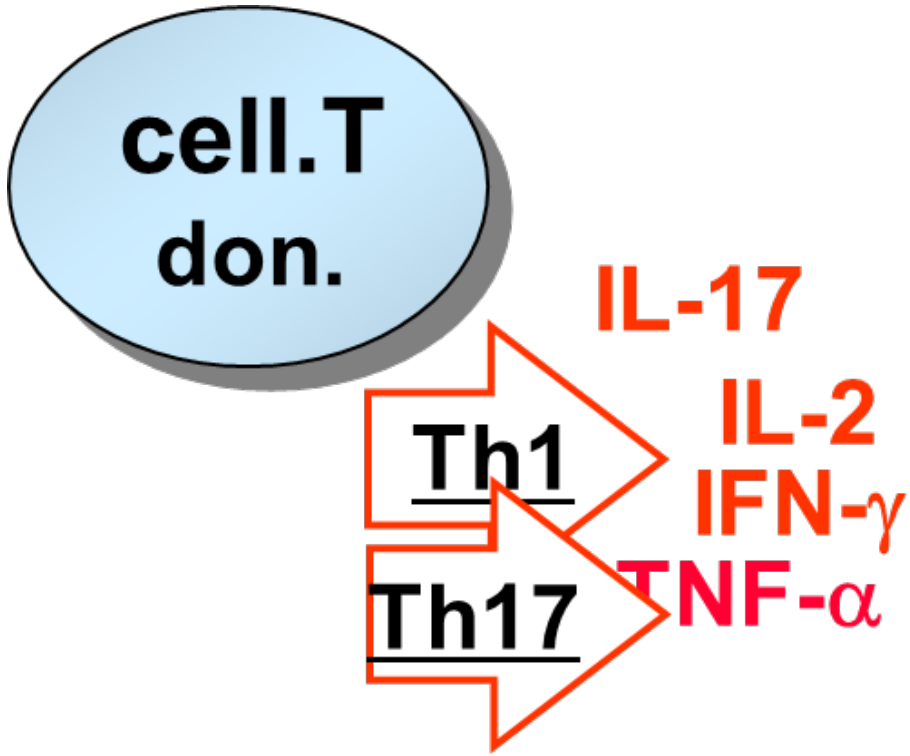

conditioning therapy
tissues damage
IL-6
IL-1
TNF-α
LPS
GVHD
APC
Target recip.
IL-12
cell.T don.
Mono
CTL don.
IL-17
Th1
IL-2
IFN-γ
TNF-α
Th17
NK don.

Discovery-based prophylaxis: Modulating T cell function

In vivo T depletion

Partial T depletion

Regulatory T cells

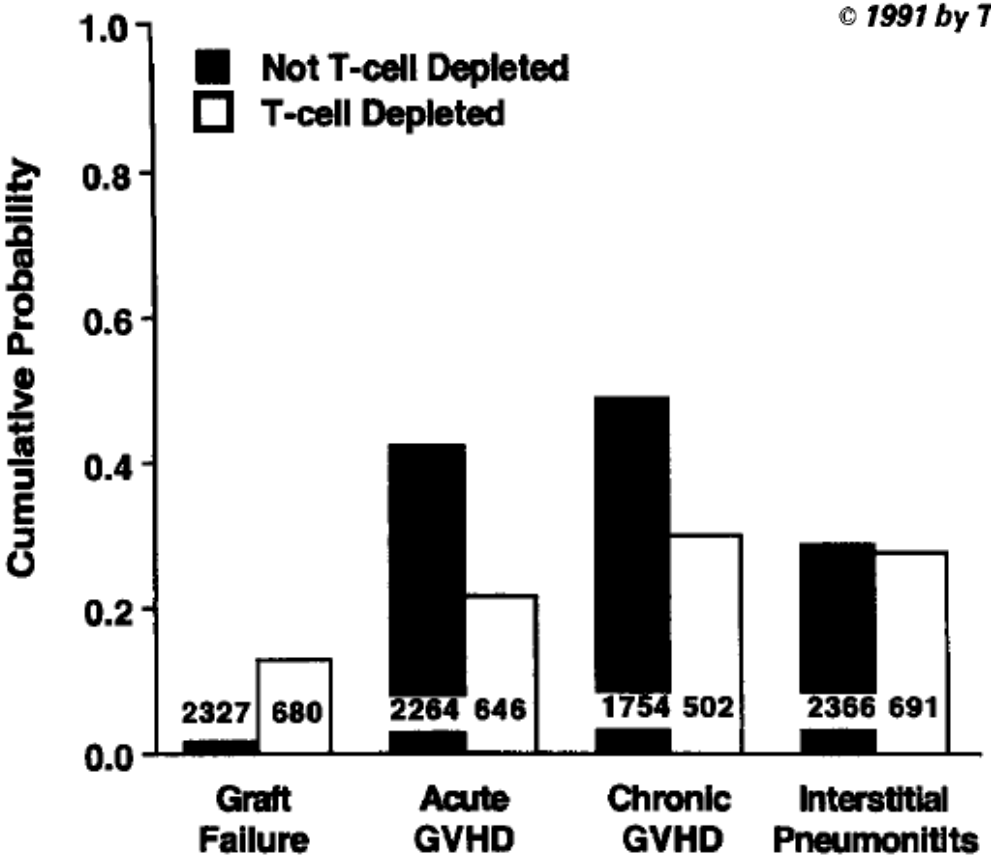


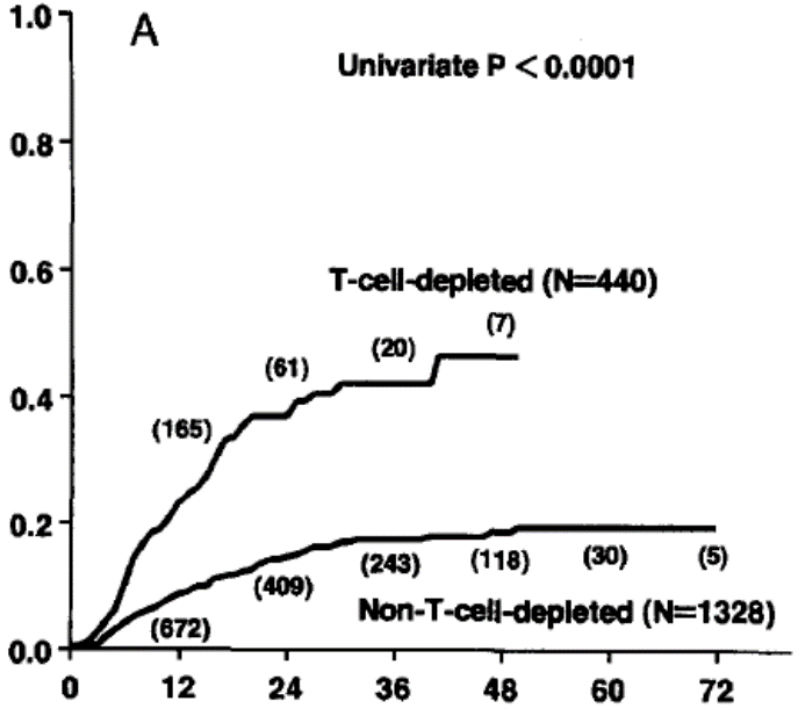
Holtan Blood 2014

Full in vitro T cell depletion increases relapse

T-Cell Depletion of HLA-Identical Transplants in Leukemia

By Alberto M. Marmont, Mary M. Horowitz, Robert Peter Gale, Kathleen Sobocinski, Robert C. Ash, Dirk W. van Bekkum, Richard E. Champlin, Karel A. Dicke, John M. Goldman, Robert A. Good, Roger H. Herzig, Richard Hong, Tohru Masaoka, Alfred A. Rimm, Olle Ringdén, Bruno Speck, Roy S. Weiner, and Mortimer M. Bortin

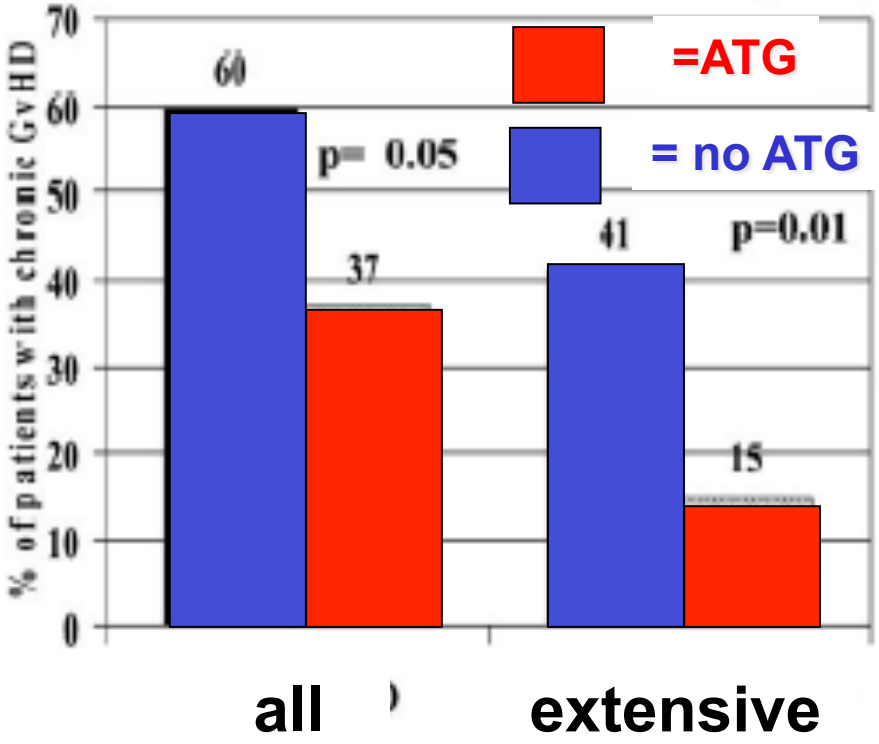




As well as infections and graft failure

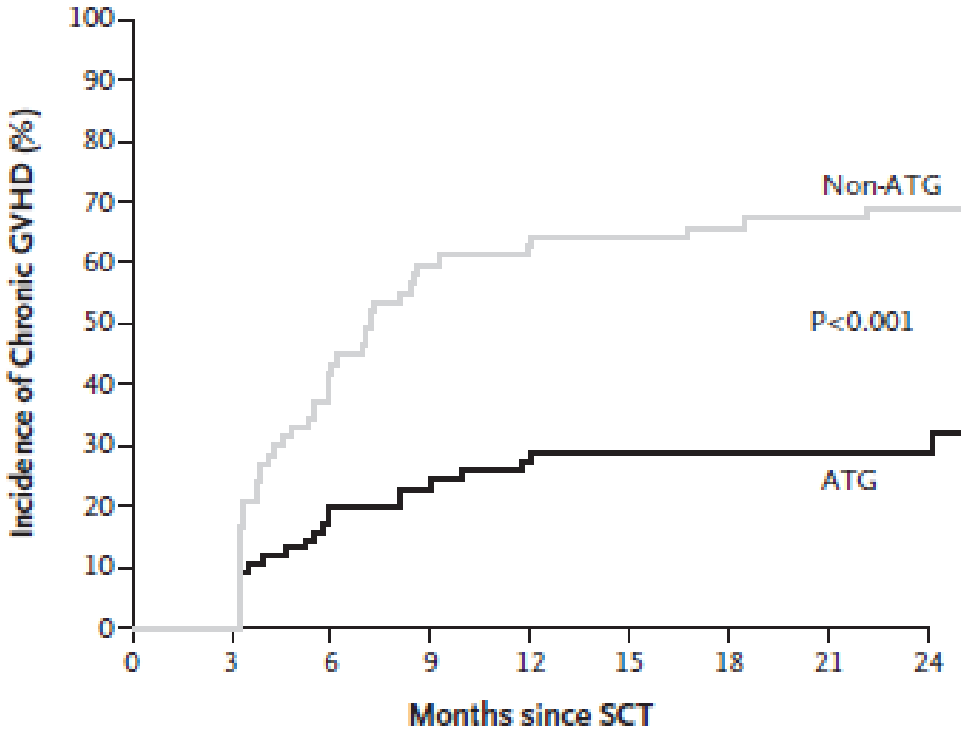
In vivo T depletion: ATG

Bacigalupo BBMT 2006



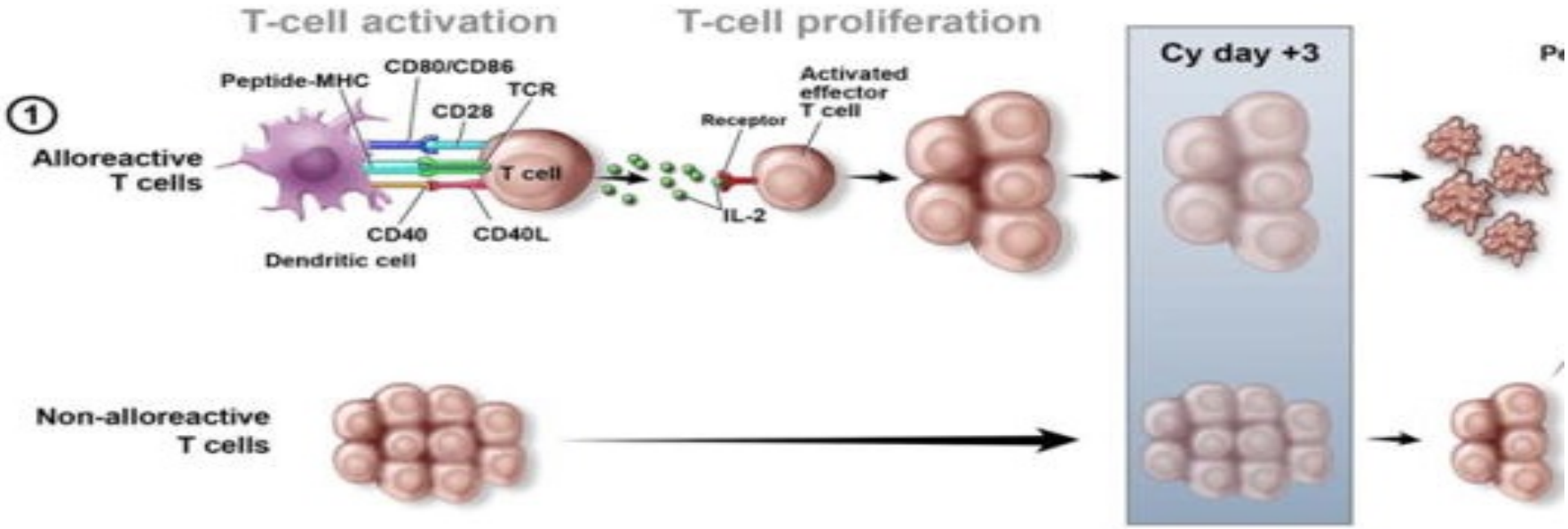
UNRELATED

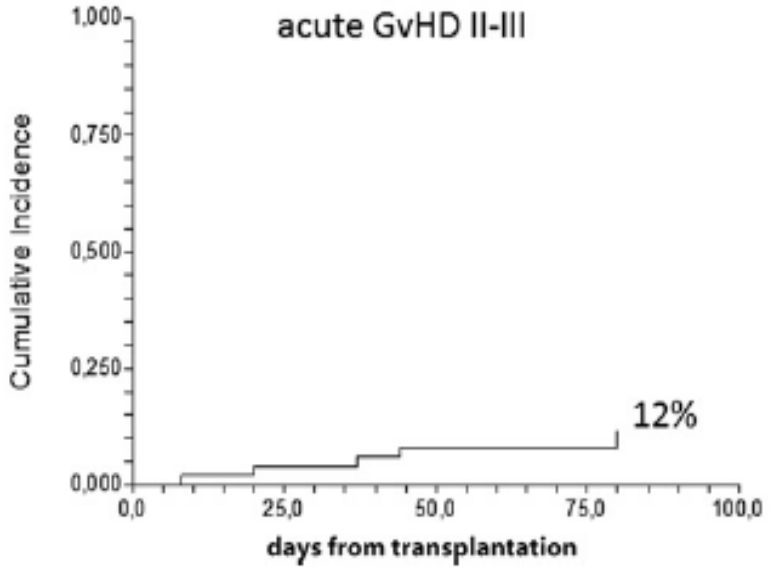
Kroger and Bonifazi NeJM 2016

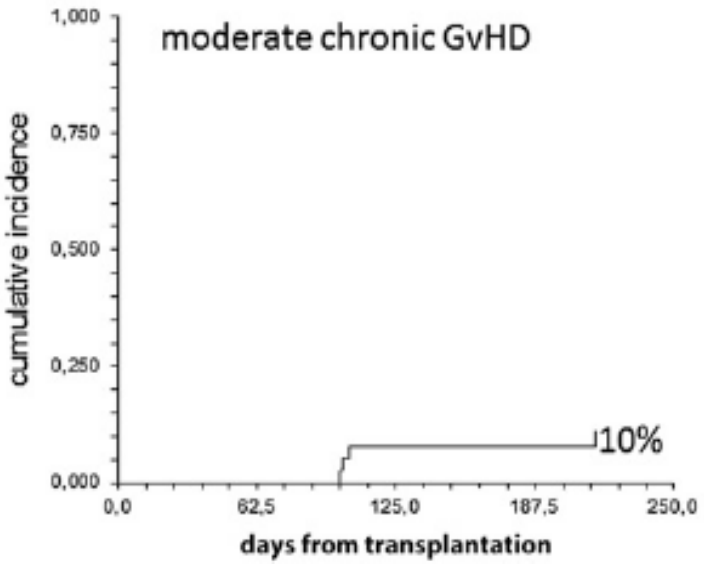


FAMILY

In vivo T depletion: cyclophosphamide







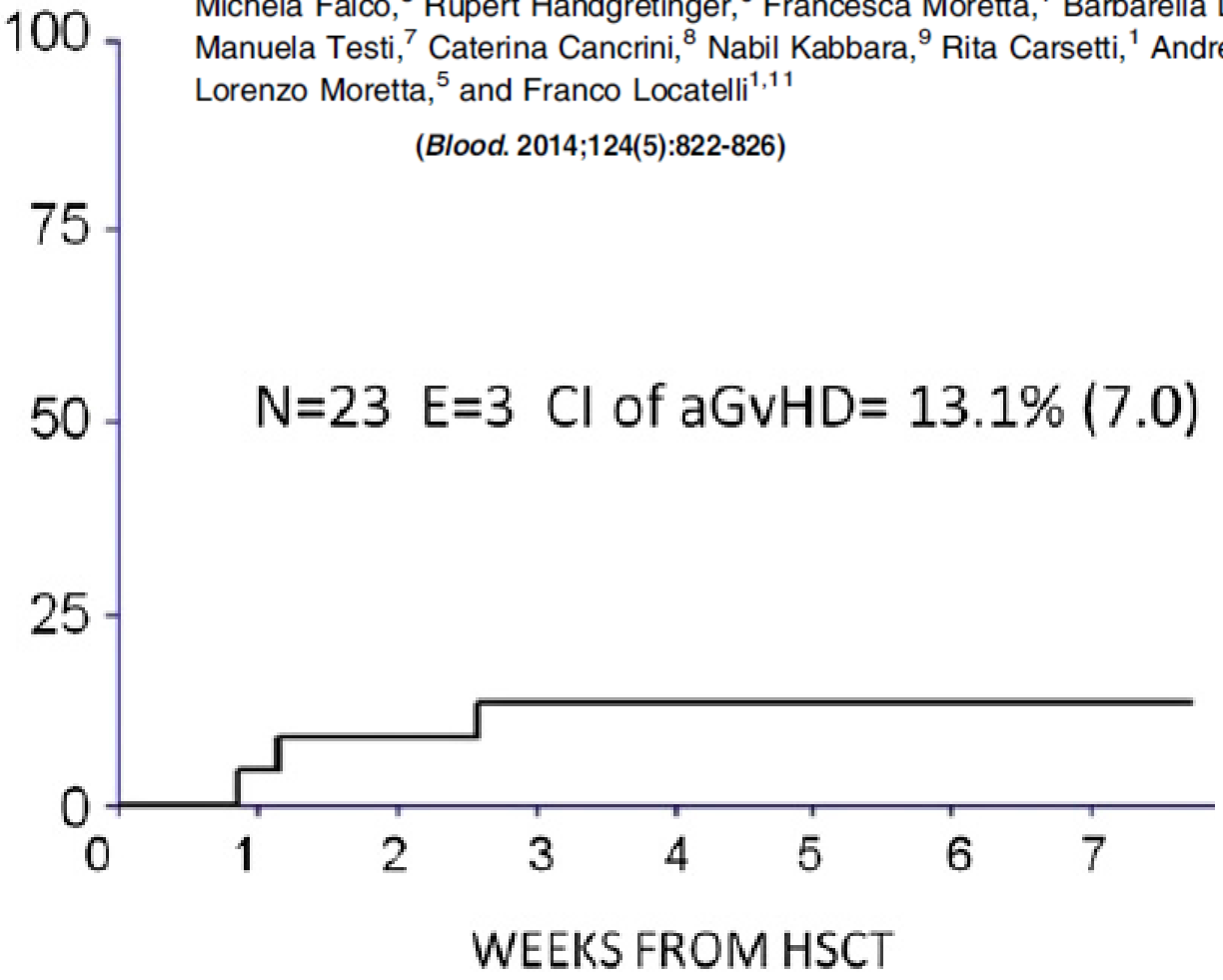
Luznik Sem Oncol 2012
Raiola BBMT 2013

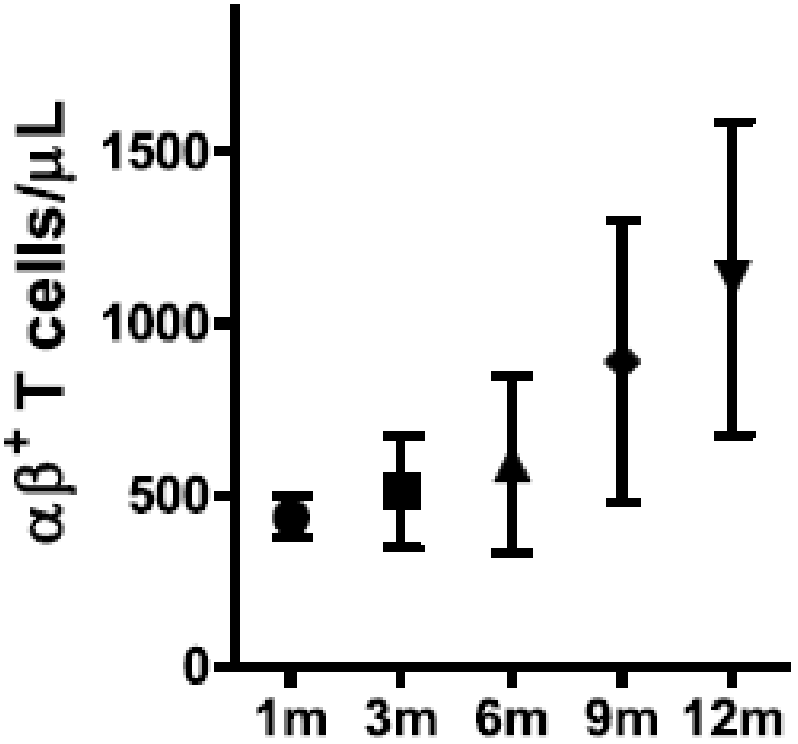
Partial T depletion: alpha-beta T cells

HLA-haploidentical stem cell transplantation after removal of $\alpha\beta^+$ T and B cells in children with nonmalignant disorders

Alice Bertaina,[1] Pietro Merli,[1] Sergio Rutella,[1,2] Daria Pagliara,[1] Maria Ester Bernardo,[1] Riccardo Masetti,[3] Daniela Pende,[4] Michela Falco,[5] Rupert Handgretinger,[6] Francesca Moretta,[1] Barbarella Lucarelli,[1] Letizia P. Brescia,[1] Giuseppina Li Pira,[1] Manuela Testi,[7] Caterina Cancrini,[8] Nabil Kabbara,[9] Rita Carsetti,[1] Andrea Finocchi,[8] Alessandro Moretta,[10] Lorenzo Moretta,[5] and Franco Locatelli[1,11]

(*Blood*. 2014;124(5):822-826)





TCD with modified T cell add back

Toxicity of conditioning regimen
Viral context

HS-tk-expressing T donor lymphocytes

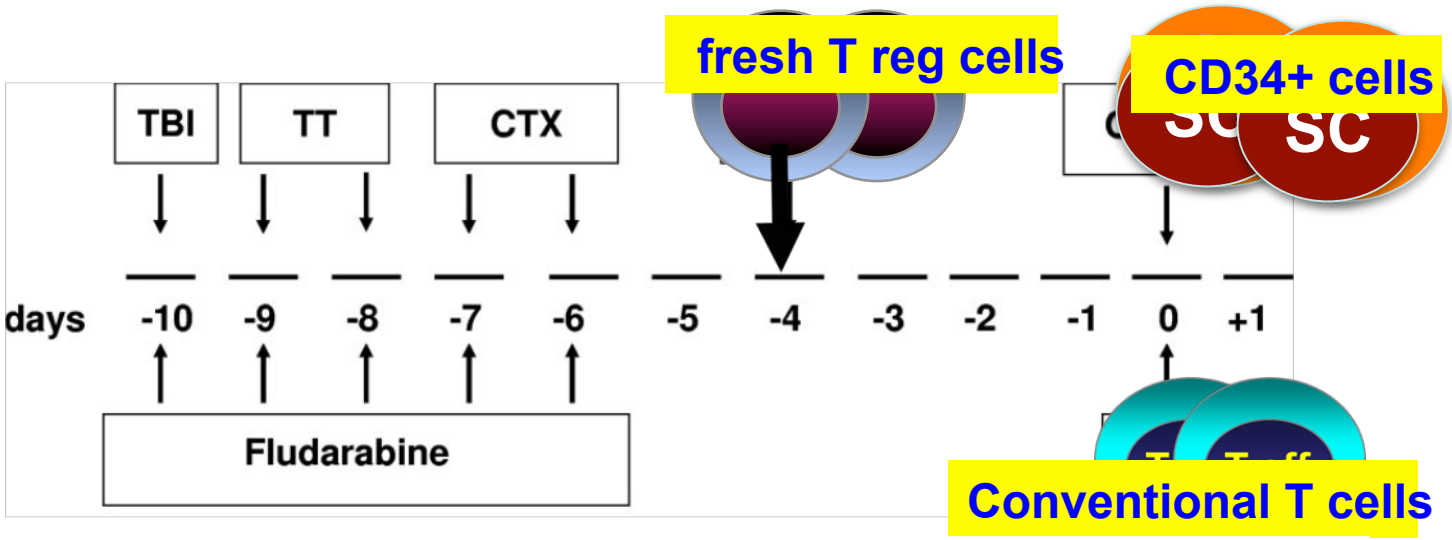
TABLE 1 | Clinical trials of TK-suicide gene therapy in allogeneic HSCT.

Clinical application	Vector (suicide gene/marker gene)	Days of culture	N° of treated patients	Clinical response (n° of patients)	Incidence of GvHD n° pts	Complete response of GvHD to GCV
To treat disease relapse occurring after HLA-identical allogeneic HSCT	RV (HSV-TK/ΔLNGFr)	14	23	11[a]	4	3/3[b]
	RV (HSV-TK/NeoR)	Ne	23	6[a]	0	Ne
	RV (HSV-TK/NeoR)	Ne	3	1[a]	1	Ne
	RV (HSV-TK/NeoR)	24–48	9	2[a]	1	1/1
	RV (HSV-TK/ΔLNGFr)	9–11	5	4[a]	2	2/2
Day 0 in TCD allogeneic HSCT	RV (HSV-TK/NeoR)	12	12	4[a]	5	5/5[c]
	RV (HSV-TK/NeoR)	–	3	1[a]	1	1/1
Day 60 in TCD allogeneic HSCT	RV (HSV-TK/ΔLNGFr)	10	9	7[a]	1	1/1
Day 42 in TCD haploidentical HSCT	RV (HSV-TK/ΔLNGFr)	14	8	3[d]	1	1/1
	RV (HSV-TK/ΔLNGFr)	10	28	22[d]	11	10/10[e]
	RV (TKmut2/ΔLNGFr)	10	4	4[d]	0	Ne
Total			127	61	27	24/24

Greco, Bonini e Ciceri Front Immunol 201

T regs prevent GVHD in HLA-haplo transplantation.

Di Ianni et al. Blood 2011



2 out 26 acute GVHD II

0 out of 26 chronic GVHD

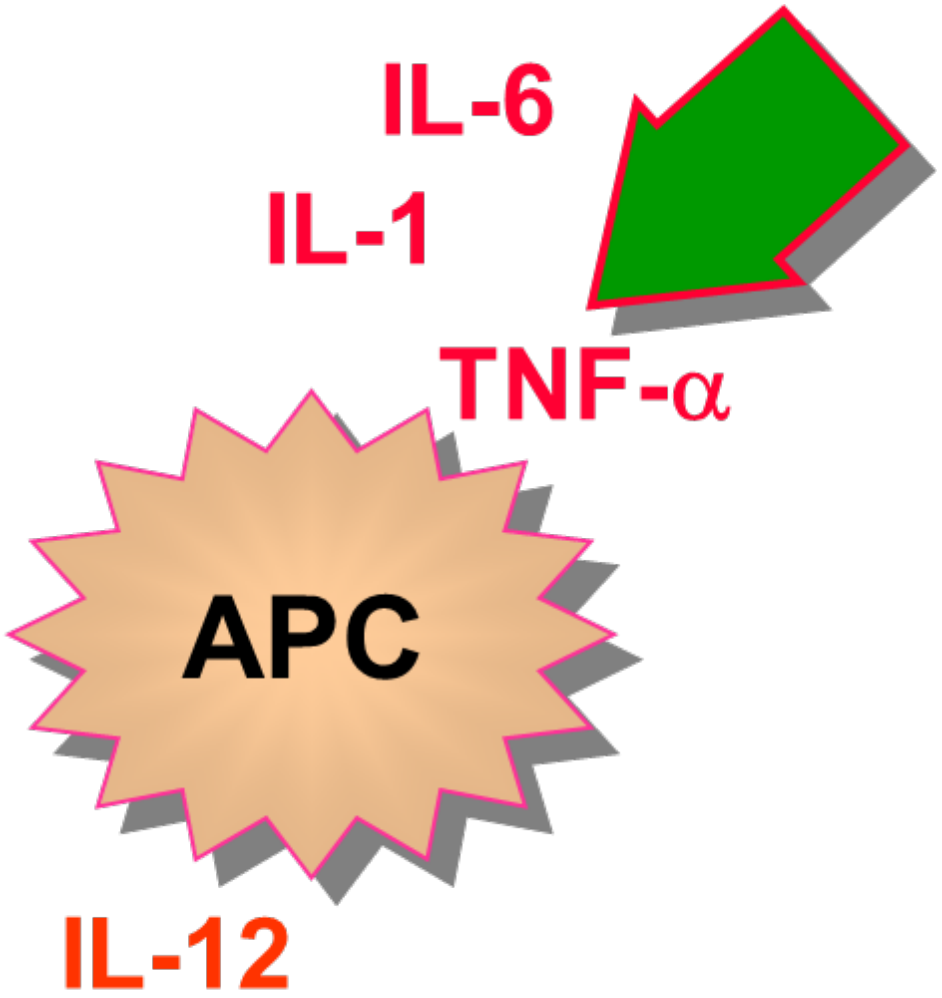
Effective GVHD prevention

Discovery-based prophylaxis: Modulating APC function

Cells
e.g. donor NK cells

Antibodies
e.g. Campath

Drugs
e.g. rapamycin
bortezomib
HDAC inhibitors

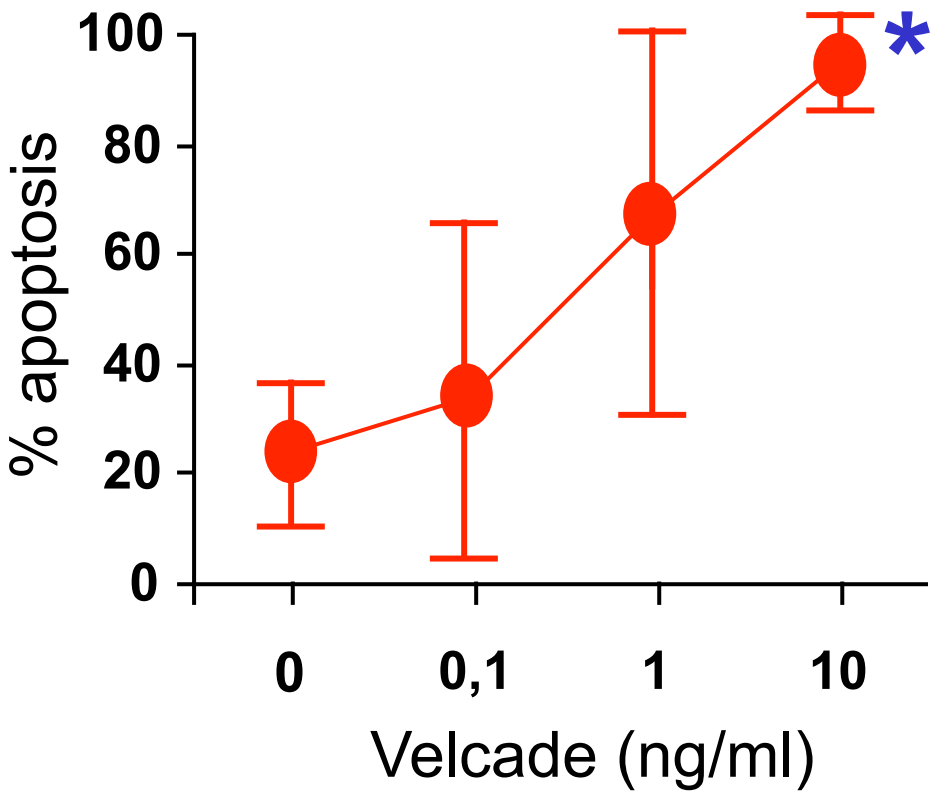


Holtan Blood 2014

Bortezomib kills APC in vitro and prevents GVHD in vivo

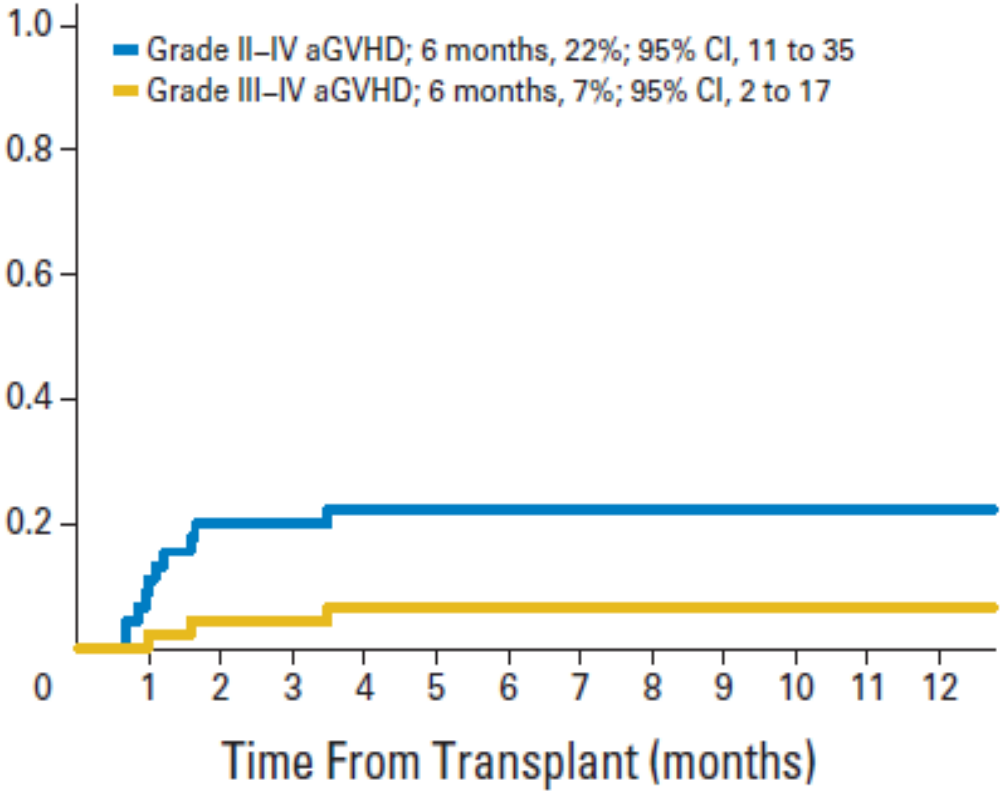
In Vitro

Arpinati BMT 2008



In Vivo

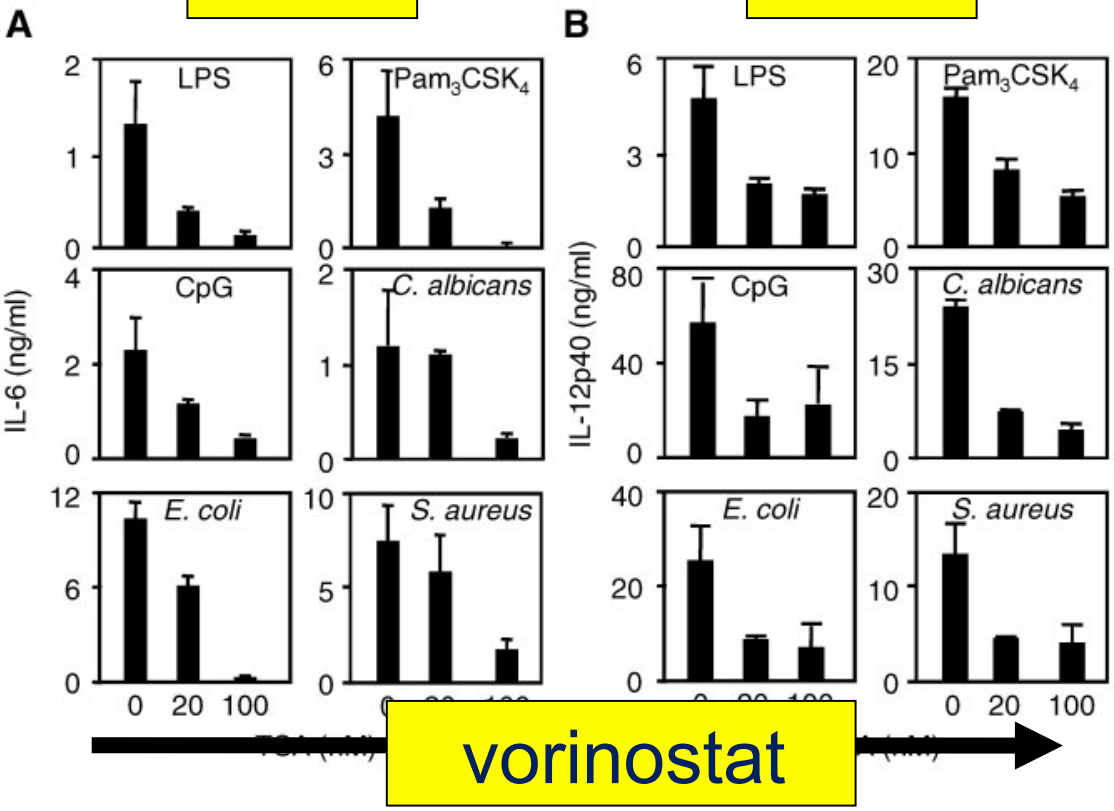
Koreth JCO 2012



Vorinostat kills APC in vitro and prevents GVHD in vivo

IL-6

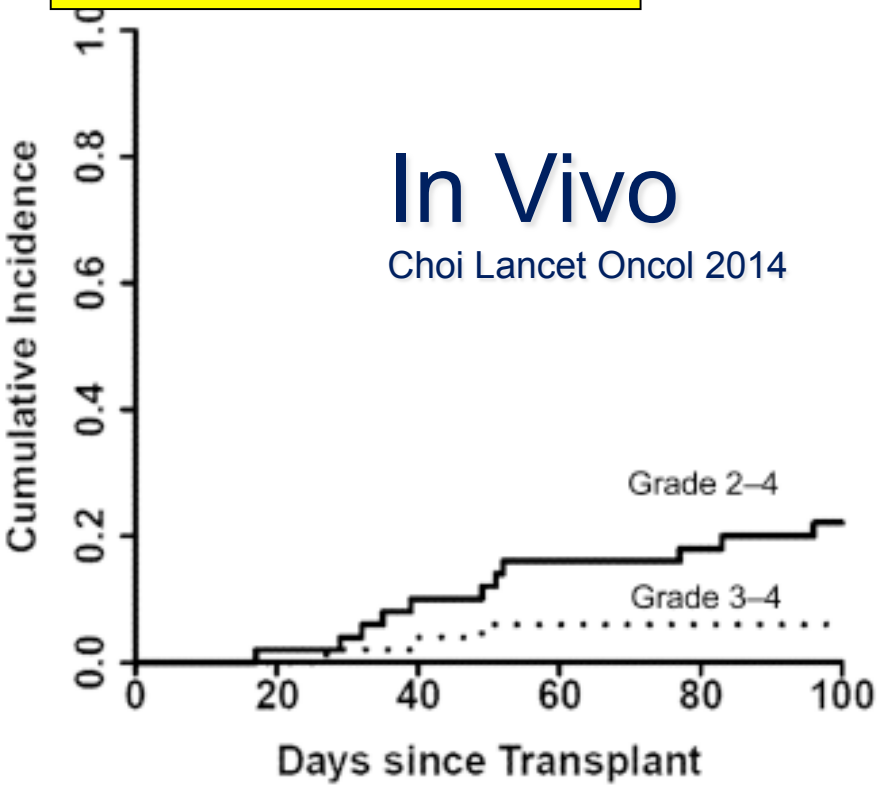
IL-12



In Vitro

Roger Blood 2011

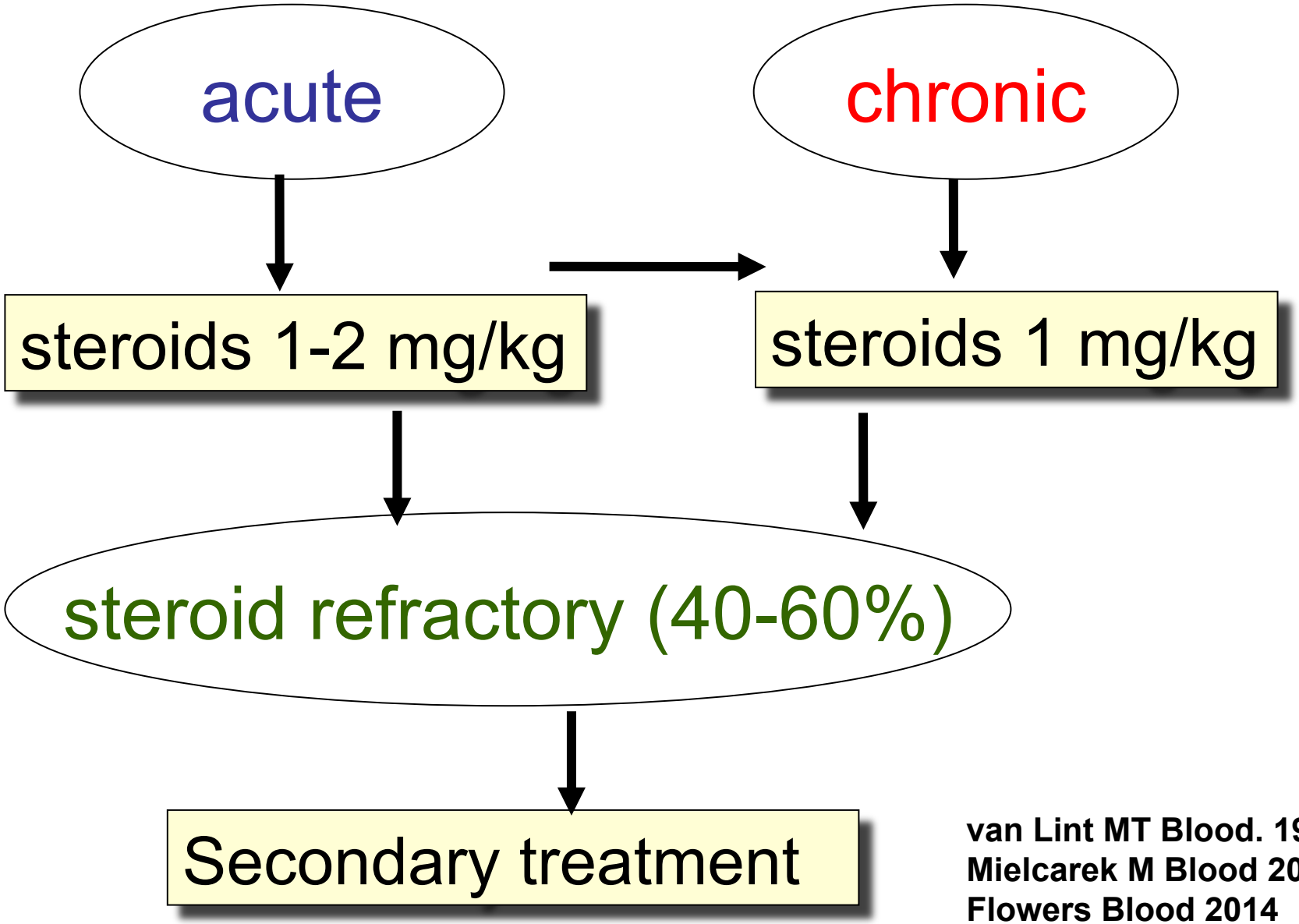
Acute GVHD



In Vivo

Choi Lancet Oncol 2014

Standard treatment of GVHD



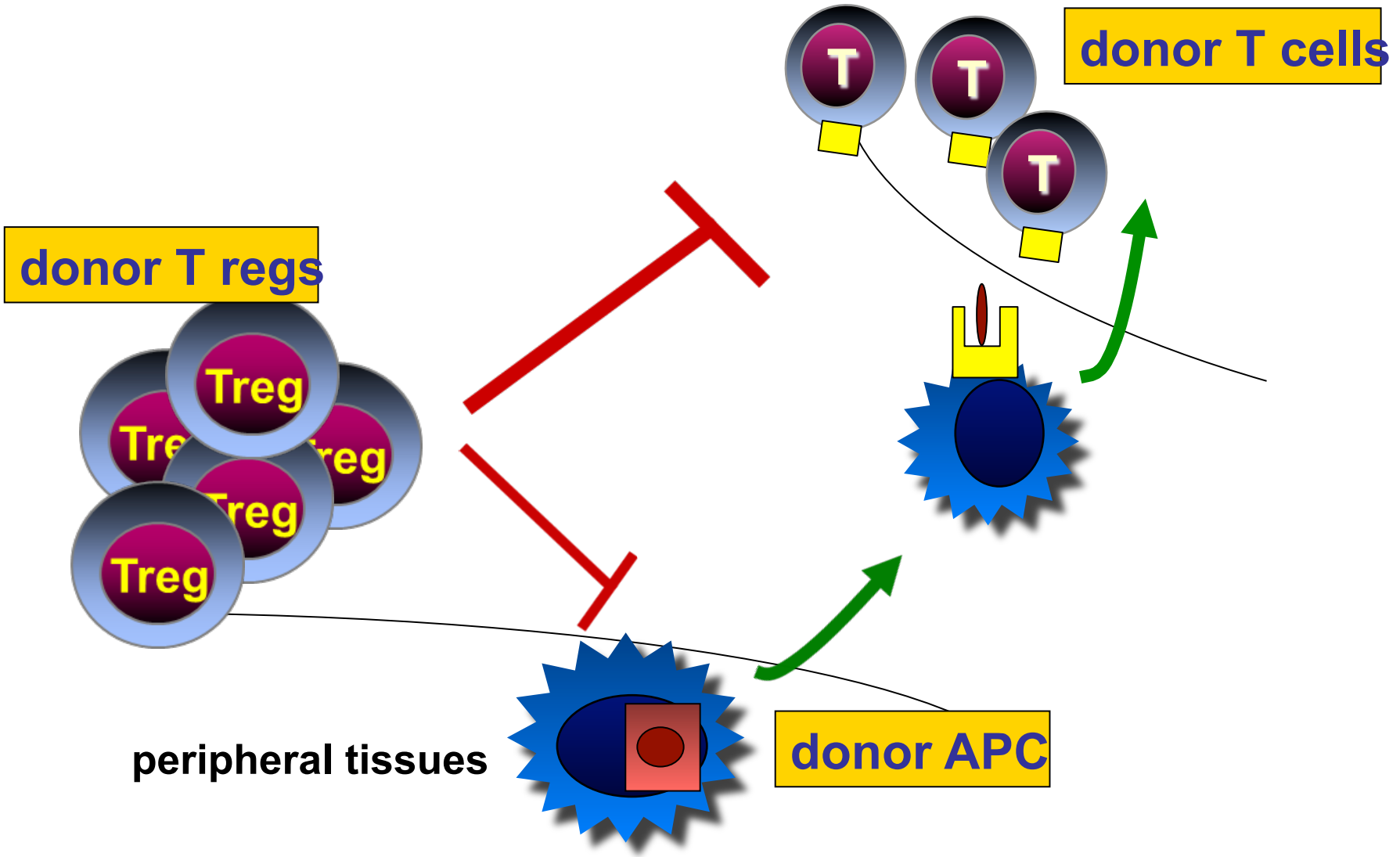
van Lint MT Blood. 1998
Mielcarek M Blood 2009
Flowers Blood 2014

Biologic treatment of GVHD

Cell-based therapies

MSCs	Suppress immune effector functions, secrete cytokines/growth factors for tissue repair and angiogenesis, can be obtained from related donors or third party	Phase 3, not yet reported in peer-reviewed literature (NCT00366145)	124,143
MAPCs	No expression of classical HLA class I markers (distinct from MSC), suppress T-cell activation via prostaglandin E2 synthesis, but only if colocalized with T cells at sites of activation	Preclinical (mouse)	144,145
Tregs	Expanded from umbilical cord blood, reduced aGVHD grade II-IV incidence from 61% to 43% in double UCB HCT (historical control); in haploidentical-related donors, Tregs reduced GVHD and enhanced immune reconstitution	Phase 1	54,146
$TRAIL^+$ T cells	Cytolytic mechanism against both tumor cells and alloreactive T cells	Preclinical (mouse)	147
NKs	GVHD protection only conferred if infusion was derived from Ly49-mismatched donor	Preclinical (mouse)	148
NKTs	Invariant NKTs attenuated murine GVHD in association with increased IL-2, IL-4, and IL-5 levels	Preclinical (mouse)	149
DCs	Tolerogenic DCs enhanced immunosuppressive cytokines in circulation, increased Tregs	Preclinical (mouse)	150
MDSCs	L-arginine depletion, contact-dependent immunosuppression	Preclinical (mouse)	59

Infuse T regs in GVHD?



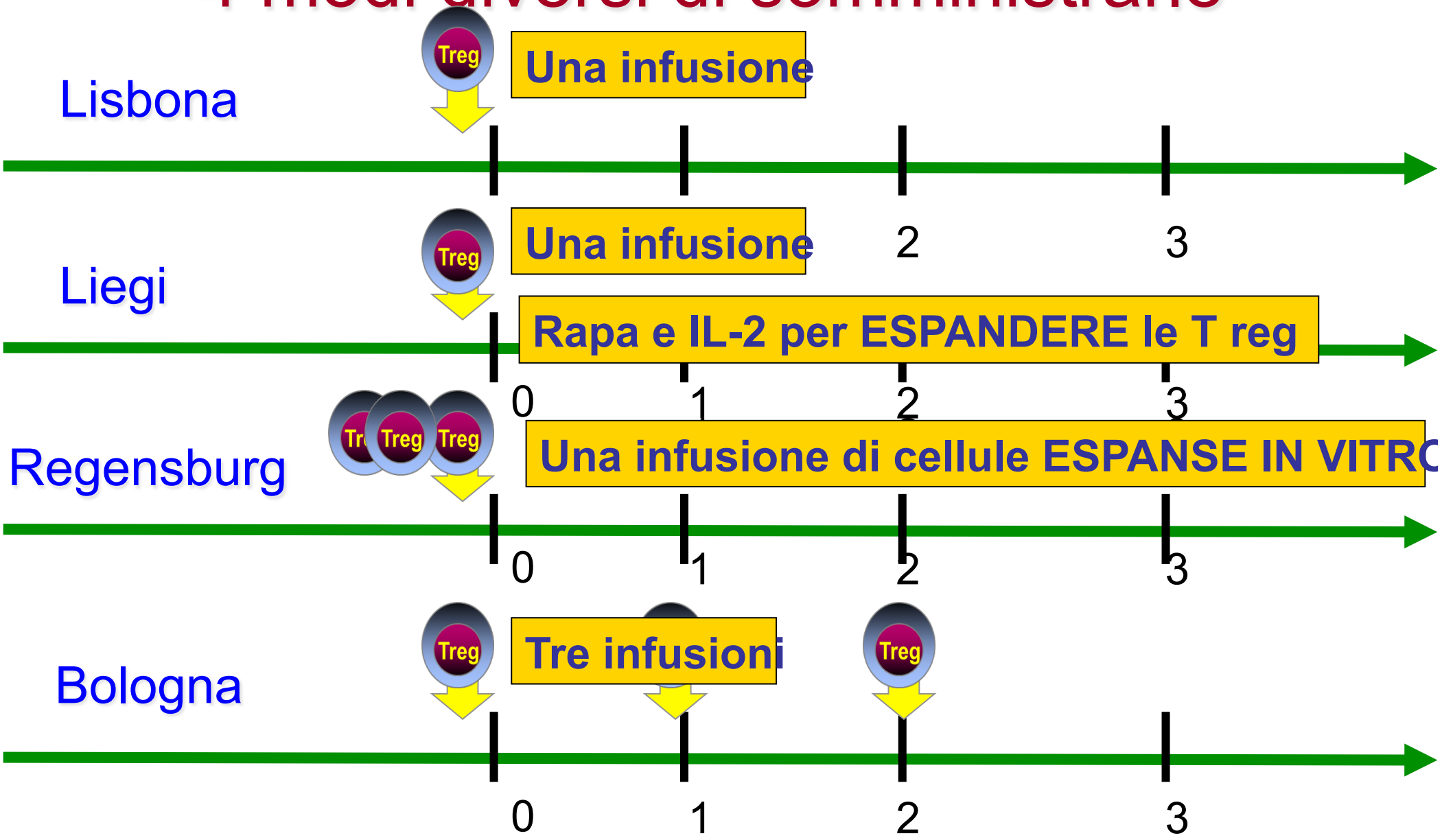
Adapted from Bruce R. Blazar et al Nature Reviews Immunology 12, 443-458 (June 2012)

Multiple donor regulatory T cell (Treg) infusions (T reg DLI) for severe refractory chronic Graft Versus Host Disease (GVHD) after allogeneic Hematopoietic Stem Cell Transplantation (HSCT).

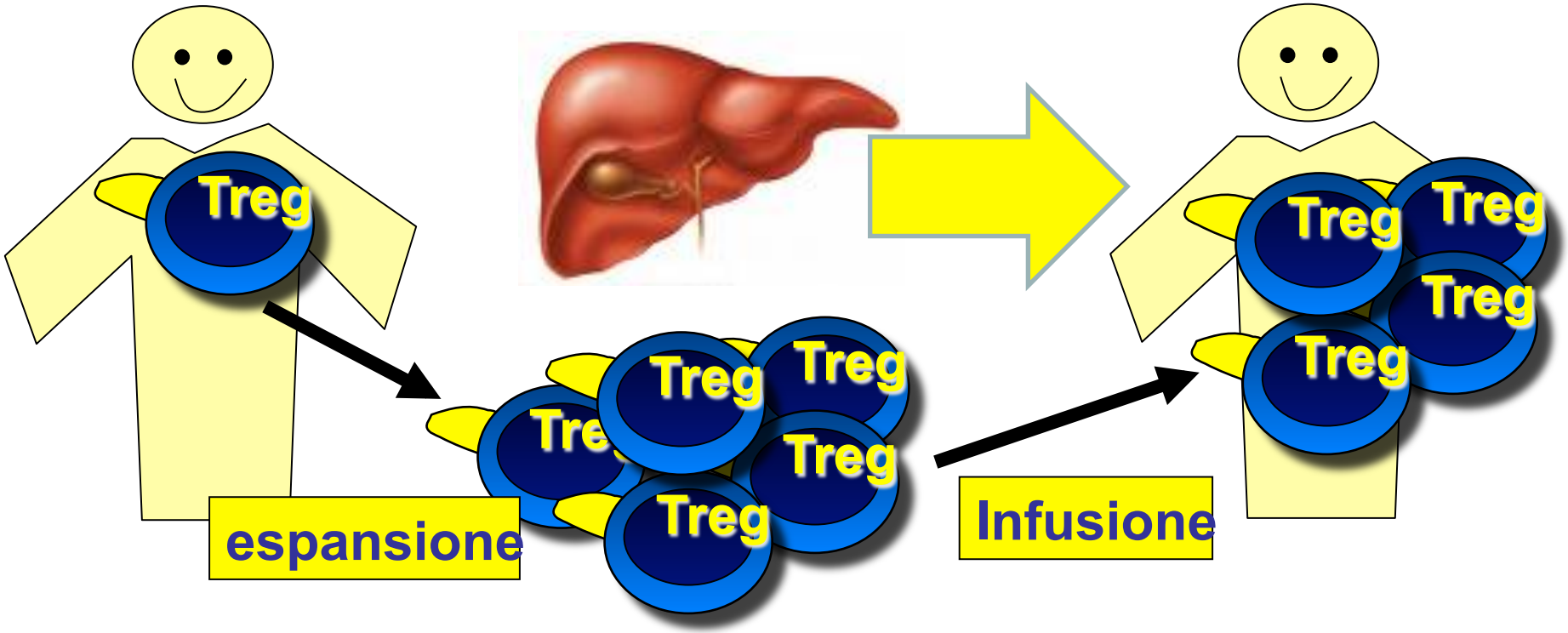
N.	Proposer name	Country
1	INSTITUTO DE MEDICINA MOLECULAR	PT
2	KLINIKUM DER UNIVERSITAET REGENSBURG	DE
3	UNIVERSITE DE LIEGE	BE
4	AZIENDA OSPEDALIERO UNIVERSITARIA POLICLINICO S. ORSOLA MALPIGHI	IT
5	MAX PLANCK GESELLSCHAFT ZUR FOERDERUNG DER WISSENSCHAFTEN E.V.	DE
6	ALACRIS THERANOSTICS GMBH	DE
7	THE UNIVERSITY OF LIVERPOOL	UK
8	GABO:MI GESELLSCHAFT FUR ABLAUFORGANISATION:MILLIARIUM MBH & CO KG	DE

TREGeneration has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 643776

4 modi diversi di somministrarle



Positive selection, expansion and transplantation of regulatory T cells to prevent cellular rejection and to induce tolerance in solid organ transplantation



PI: RM Lemoli/L Catani **RF-2011-02346763**

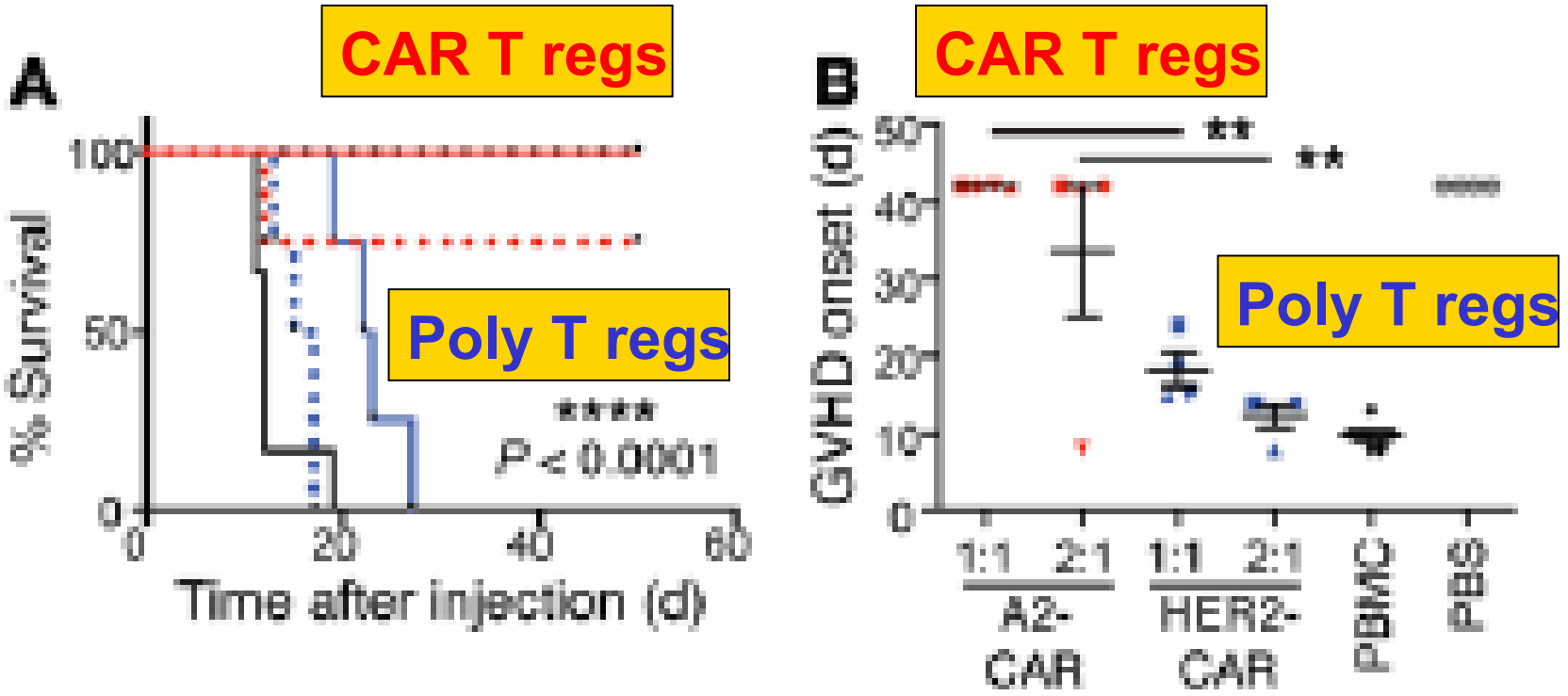
A jump to the future: CAR T-regs?

The Journal of Clinical Investigation RESEARCH ARTICLE

Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor

Katherine G. MacDonald,[1] Romy E. Hoeppli,[1] Qing Huang,[2] Jana Gillies,[1] Dan S. Luciani,[1] Paul C. Orban,[1] Raewyn Broady,[2] and Megan K. Levings[1]

[1]Department of Surgery and [2]Department of Medicine, University of British Columbia, and Child and Family Research Institute, Vancouver, British Columbia, Canada.



La fine del rigetto (GVHD)?