

***Treviso, November 21st 2022***

## **Highlights in Ematologia**

# **Monitoraggio dell'immunità post-trapianto allogenico**

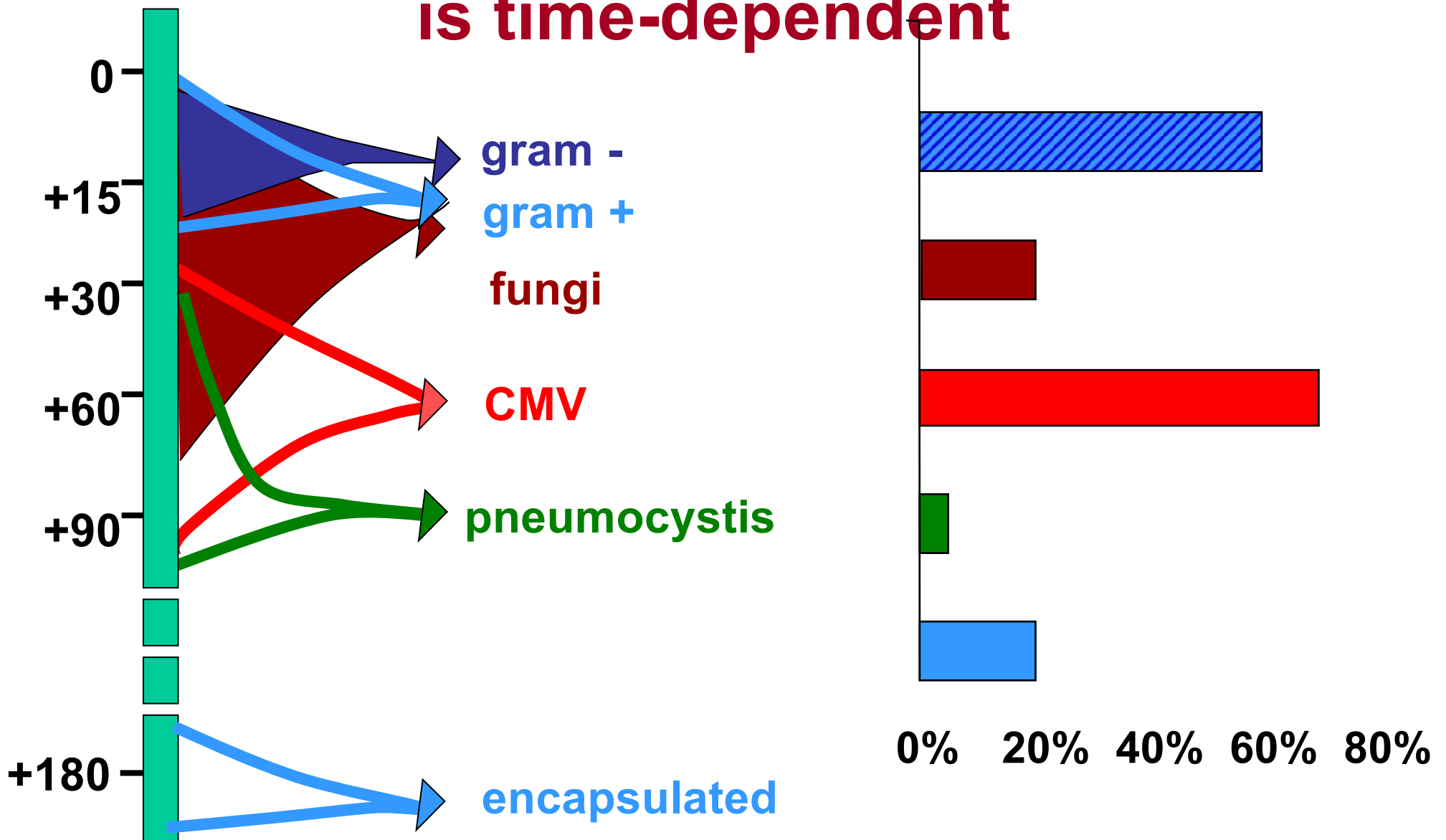
***M.Arpinati***

***Programma di Terapia Cellulare e Trapianto,***

***UO Ematologia,***

***IRCCS S.Orsola-Malpighi***

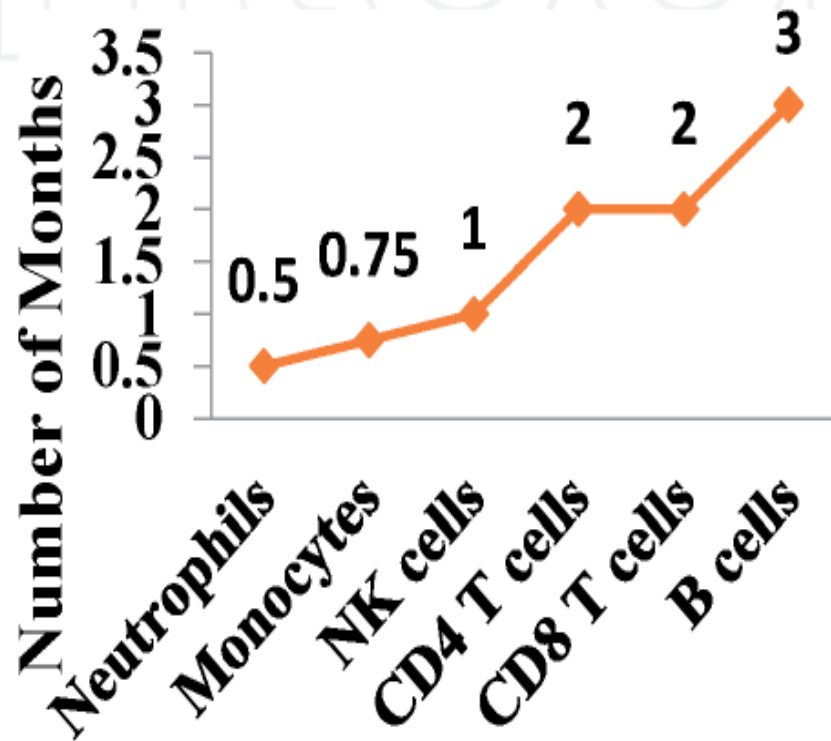
# The risk of infection post transplant is time-dependent



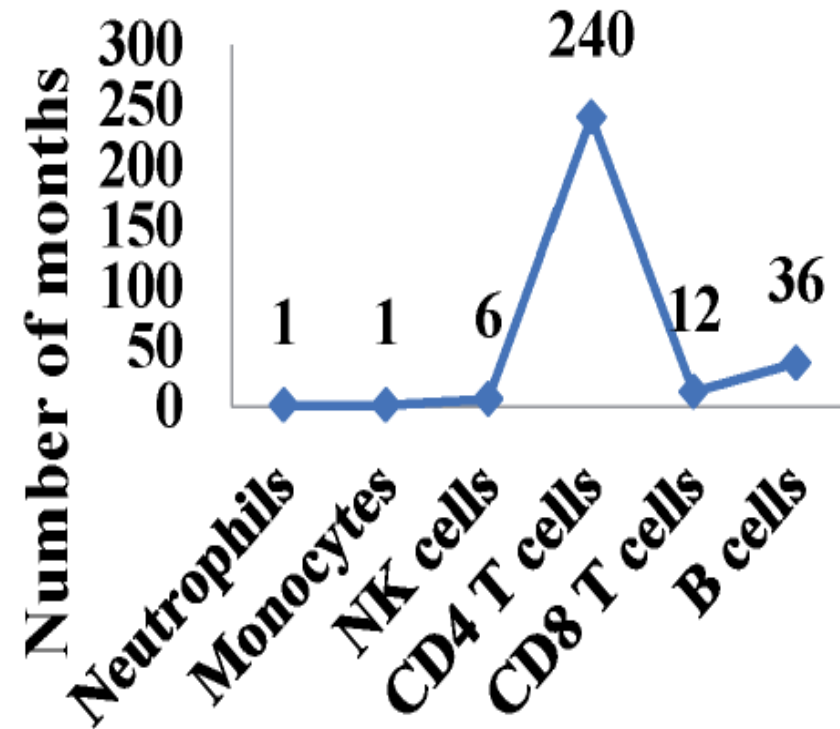
Adapted from Ogonek FI 2015

# Time to recovery of donor immunity

Time to appearance



Time to normal

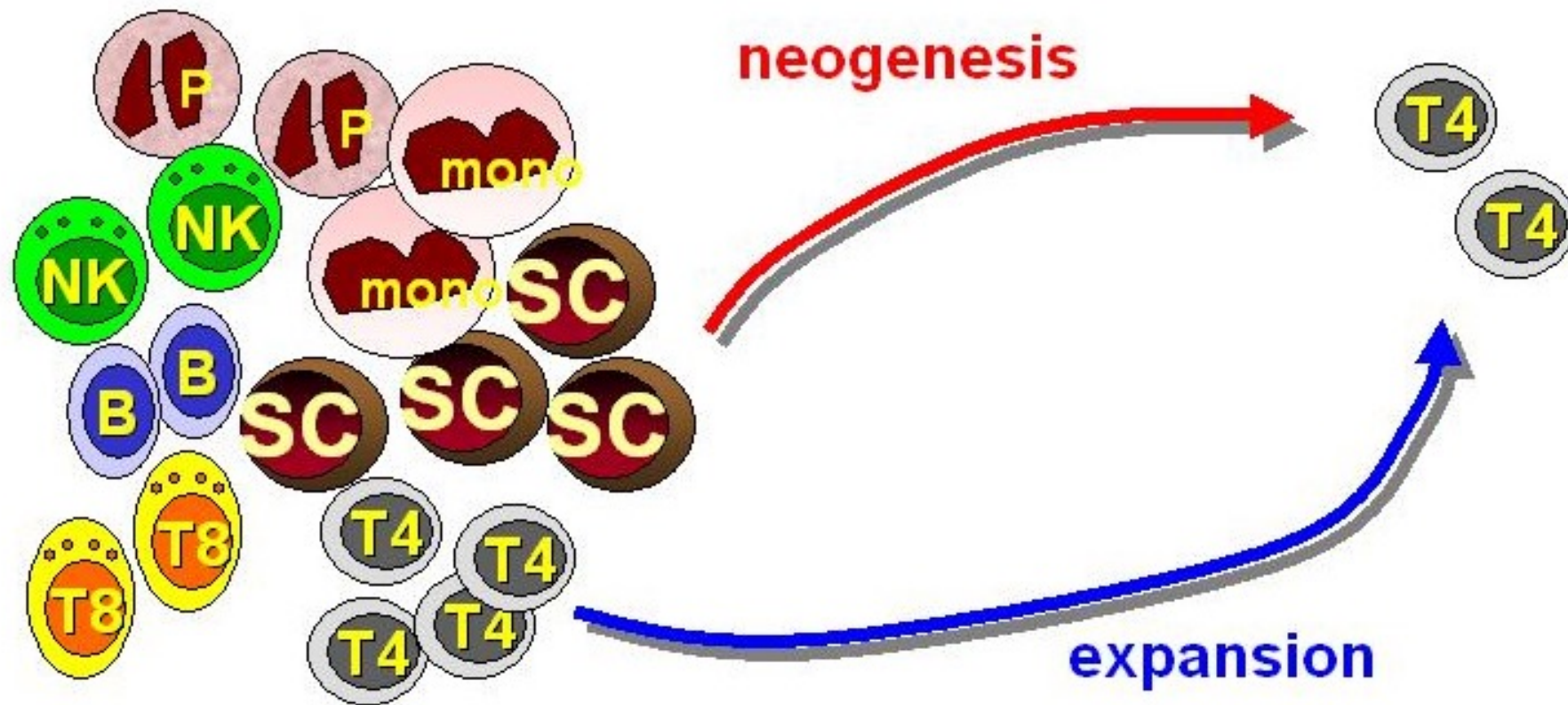


*Meenakshi Singh, Selma Z. D'Silva and Abhishweta Saxena*

*Assessment of Immune Reconstitution Following Hematopoietic Stem Cell Transplantation*

*DOI: <http://dx.doi.org/10.5772/intechopen.89198>*

# Mechanisms of Immune Recovery after Transplant

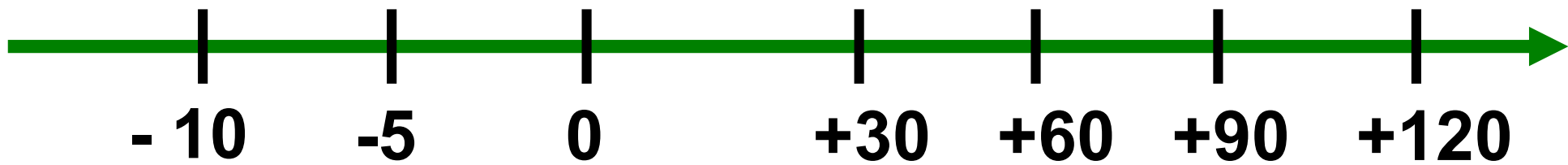
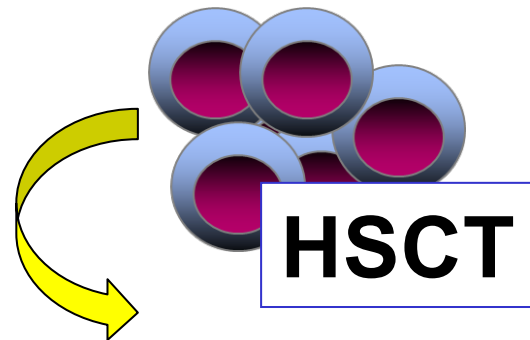




# **Factors contributing to immunity post transplant**

- 1) Conditioning regimen**
- 2) GVHD prophylaxis**
- 3) GVHD**
- 4) Graft source**
- 5) Thymic function**
- 6) Virus reactivation**

# 1) recipient immunity is destroyed before transplant



**Cyclophosphamide**

**Fludarabine**

**TBI**

**ATG**

**Campath**

## 2) Immunosuppressive drugs after HSCT

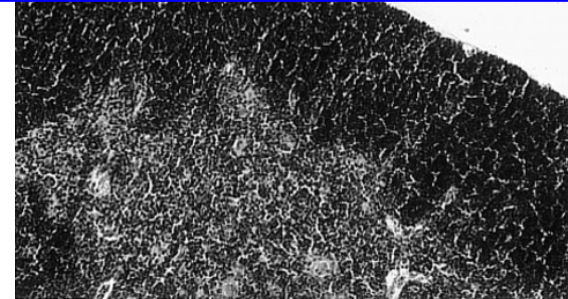
### DRUG

### MECHANISM

- 
- |                                |  |
|--------------------------------|--|
| • <b>CSA/FK-506</b>            | <b>blocks NFAT and IL2 transcription in activated T cells</b>  |
| • <b>Corticosteroids</b>       | <b>blocks function of APC. Apoptosis of T lymphocytes.</b>     |
| • <b>Methotrexate</b>          | <b>Blocks nucleotide synthesis by activated T lymphocytes.</b> |
| • <b>Mofetil Mycophenolate</b> | <b>Blocks nucleotide synthesis by activated T lymphocytes.</b> |
| • <b>Rapamycin</b>             | <b>Binds FKBP and blocks G1-S progression.</b>                 |
| • <b>Cyclophosphamide</b>      | <b>induces apoptosis of activated T lymphocytes.</b>           |

# 3) GVHD reduces immunity

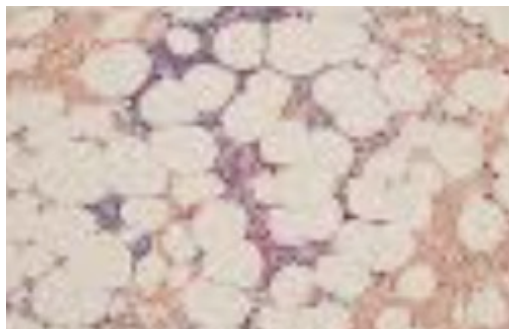
**thymic involution**



**hyposplenism**



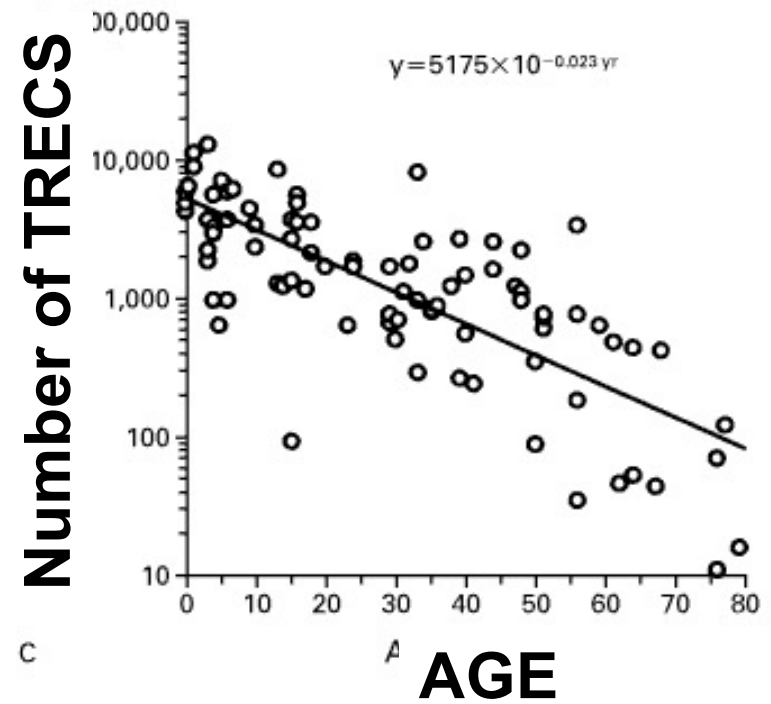
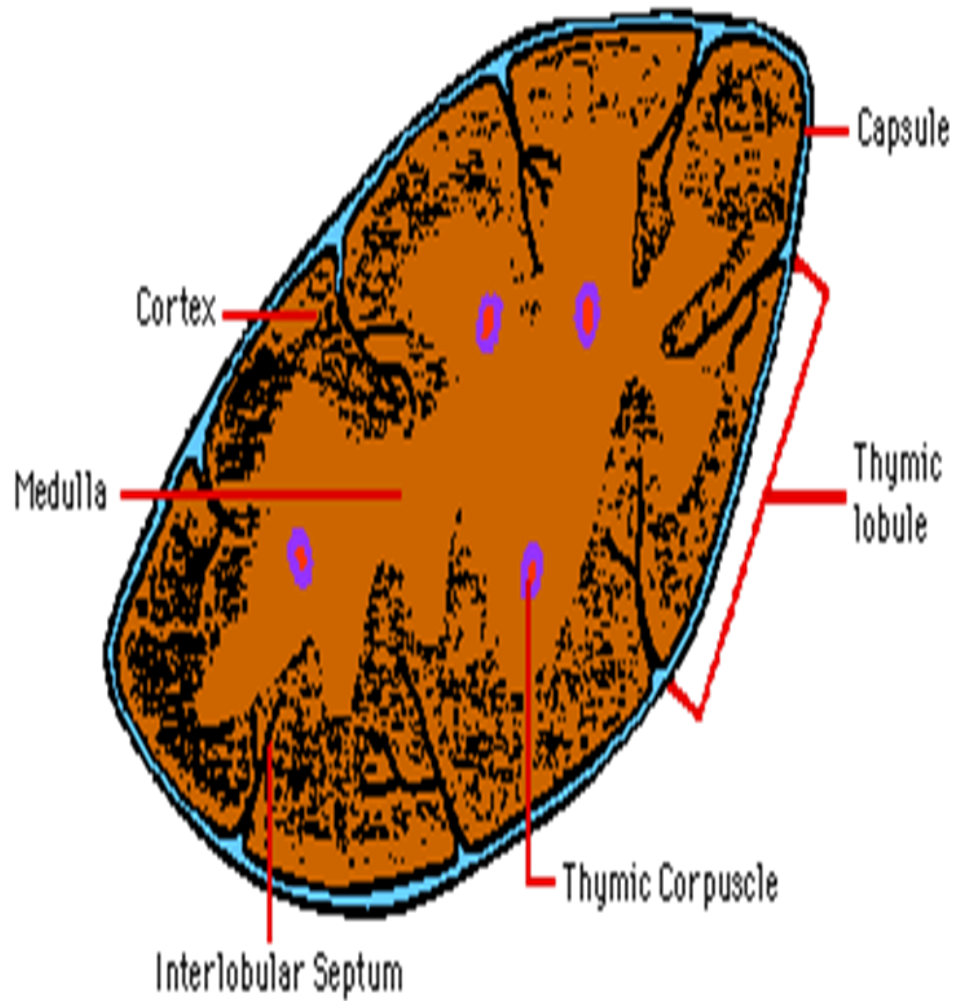
**BM suppression**



## 4) Composition of different grafts

	<b>PBSC</b> (n=121)	<b>MARROW</b> (n=81)	<b>CORD</b> (n=135)
<b>TNC</b>	<b>900 (670-1260)</b>	<b>290 (233-370)</b>	<b>32 (25-50)</b>
<b>CD34+</b>	<b>4.9 (3.8-6)</b>	<b>2.2 (1.4-2.6)</b>	<b>0.14 (0.1-0.25)</b>
<b>mDC</b>	<b>1.7 (1.2-2.4)</b>	<b>0.8 (0.5-1.5)</b>	<b>NV</b>
<b>pDC</b>	<b>2.5 (1.7-3.4)</b>	<b>0.7 (0.4-0.9)</b>	<b>NV</b>
<b>CD14+</b>	<b>211 (145-278)</b>	<b>6.6 (3.9-10.1)</b>	<b>1.4 (1.3-2)</b>
<b>CD19+</b>	<b>46 (30-64)</b>	<b>6.7 (4.5-9)</b>	<b>NV</b>
<b>CD56+</b>	<b>34 (25-48)</b>	<b>4.2 (2.8-7.2)</b>	<b>1.6 (1.2-2.5)</b>
<b>CD3+</b>	<b>201 (144-285)</b>	<b>24 (18-28)</b>	<b>3.1 (2.4-5.1)</b>
<b>CD4+</b>	<b>115 (87-165)</b>	<b>12 (9-15)</b>	<b>2.4 (1.5-4)</b>
<b>CD8+</b>	<b>80 (56-117)</b>	<b>12 (9-15)</b>	<b>1.6 (1.3-2.5)</b>

# 5) Thymic function



# 6) Virus reactivation

## Cytomegalovirus shapes long-term immune reconstitution after allogeneic stem cell transplantation

Raphael Itzykson,<sup>1,2</sup> Marie Robin,<sup>1</sup> Helene Moins-Teisserenc,<sup>2,3,4</sup> Marc Delord,<sup>2,5</sup> Marc Busson,<sup>3,4</sup> Aliénor Xhaard,<sup>1</sup> Flore Sicre de Fontebrune,<sup>1,2</sup> Régis Peffault de Latour,<sup>1</sup> Antoine Toubert,<sup>2,3,4</sup> and Gérard Socié<sup>1,2,4</sup>

Under-represented Population	FC	-log(P)
Memory B cells	-1.15	3.18
CD25+ activated CD8+ T cells	-1	1.69
CD25+ activated CD4+ T cells	-0.96	8.86
Naive CD8+ T cells	-0.9	2.66
Inducible Tregs	-0.83	5.77
Natural Tregs	-0.82	2.31
CD5+ B cells	-0.82	1.44
Central memory CD4+ T cells	-0.76	5.22
Naive B cells	-0.69	1.81
Early differentiated CD4+ T cells	-0.57	5.94

### CMV positive patients

Over-represented Population	FC	-log(P)
HLA-DR+ activated CD8+ T cells	1.17	12.63
Late effector memory CD8+ T cells	1.16	10.2
Effector memory CD8+ T cells	0.99	8.94
Memory CD8+ T cells	0.88	7.58



# Our data on Immune Recovery

**503 consecutive patients** transplanted in our institute between 2000 and 2014 had **PB samples collected at 1, 3, 6, 9 and 12 months after transplant.**

## Flow cytometry

Myeloid DC,  
Plasmacytoid DC,  
CD16 DC,  
CD16 mono,  
CD14 mono.  
CD3+ T lymphocytes  
CD4+ T lymphocytes  
CD8+ T lymphocytes  
T regulatory cells  
B lymphocytes,  
NK cells

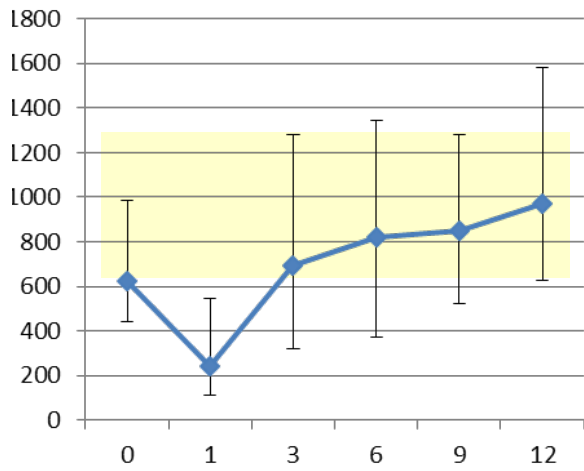
Ulbar et al. EBMT 2017  
Bonifazi et al. BMT 2018  
Bonifazi et al. BMT 2019

**recovery of immunity  
post transplant:  
T lymphocytes**

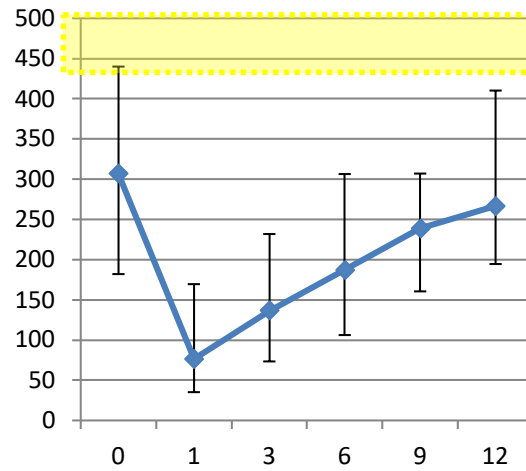
# recovery of T cells



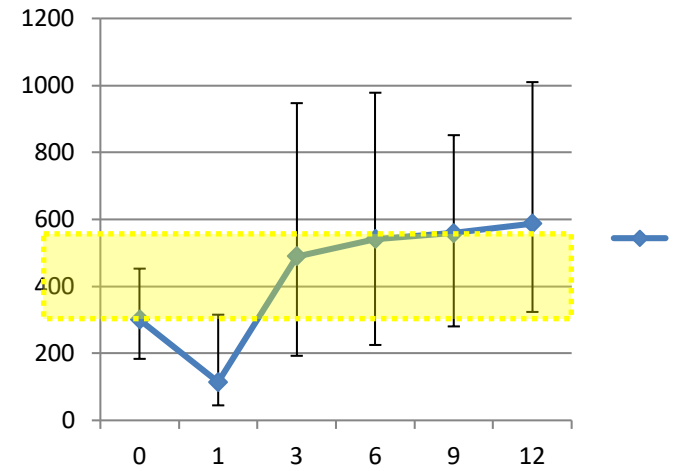
#CD3

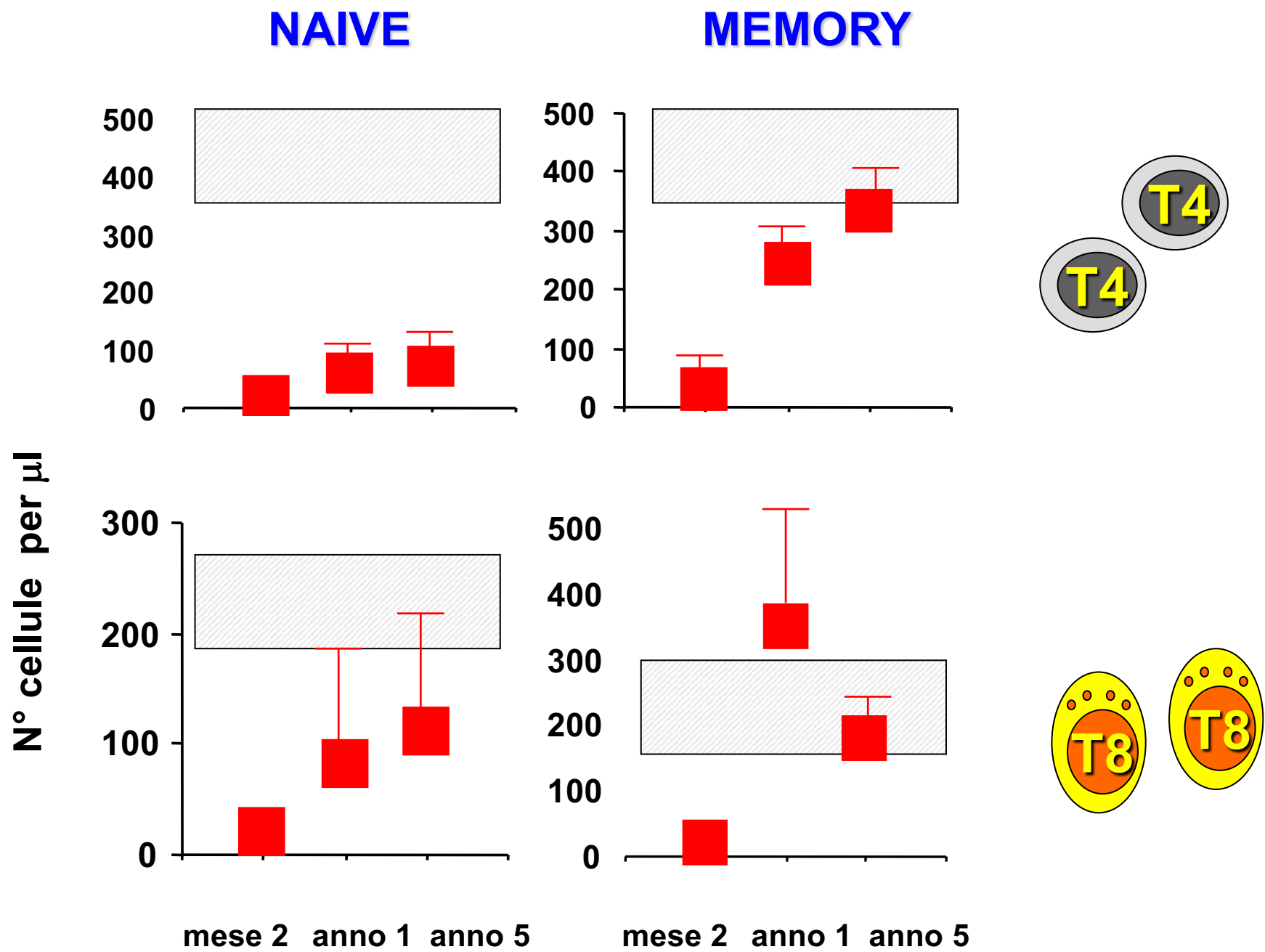


#CD4

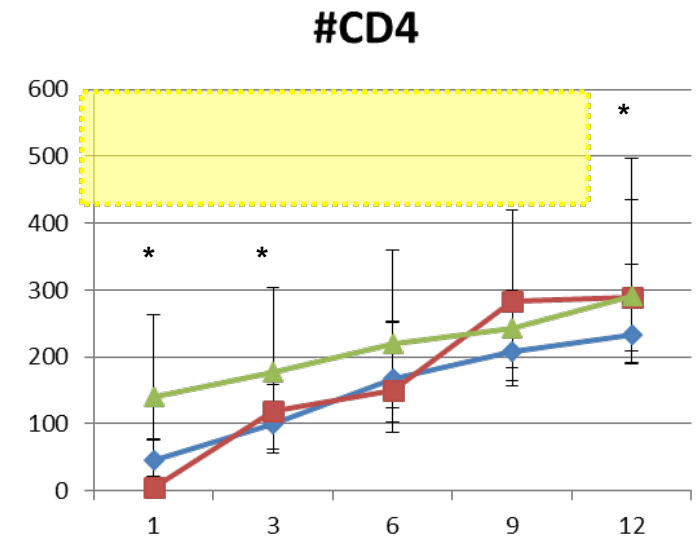
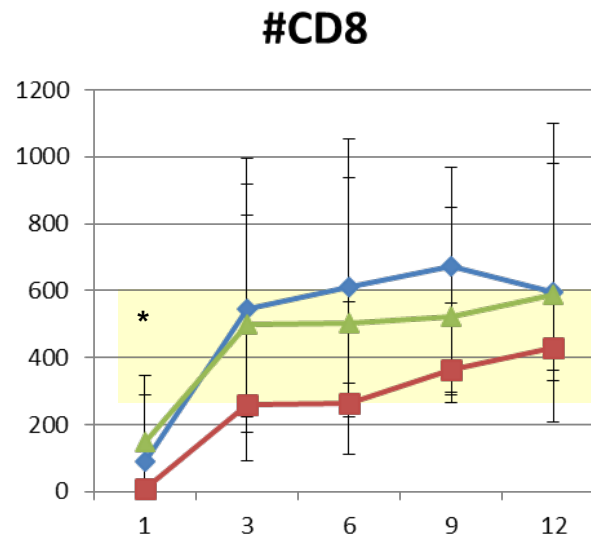
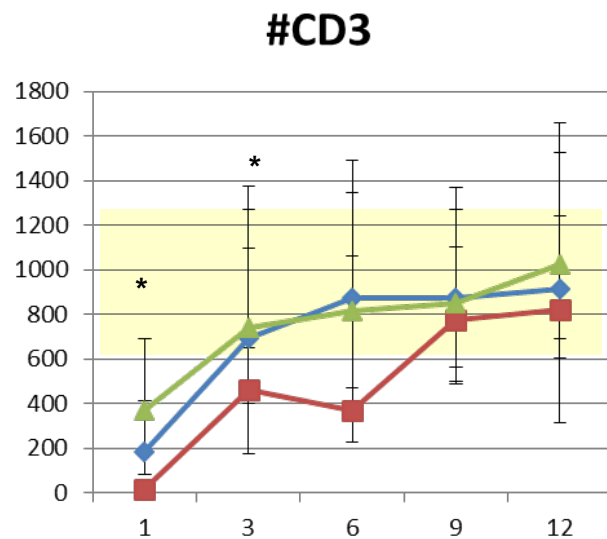
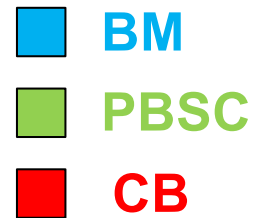


#CD8



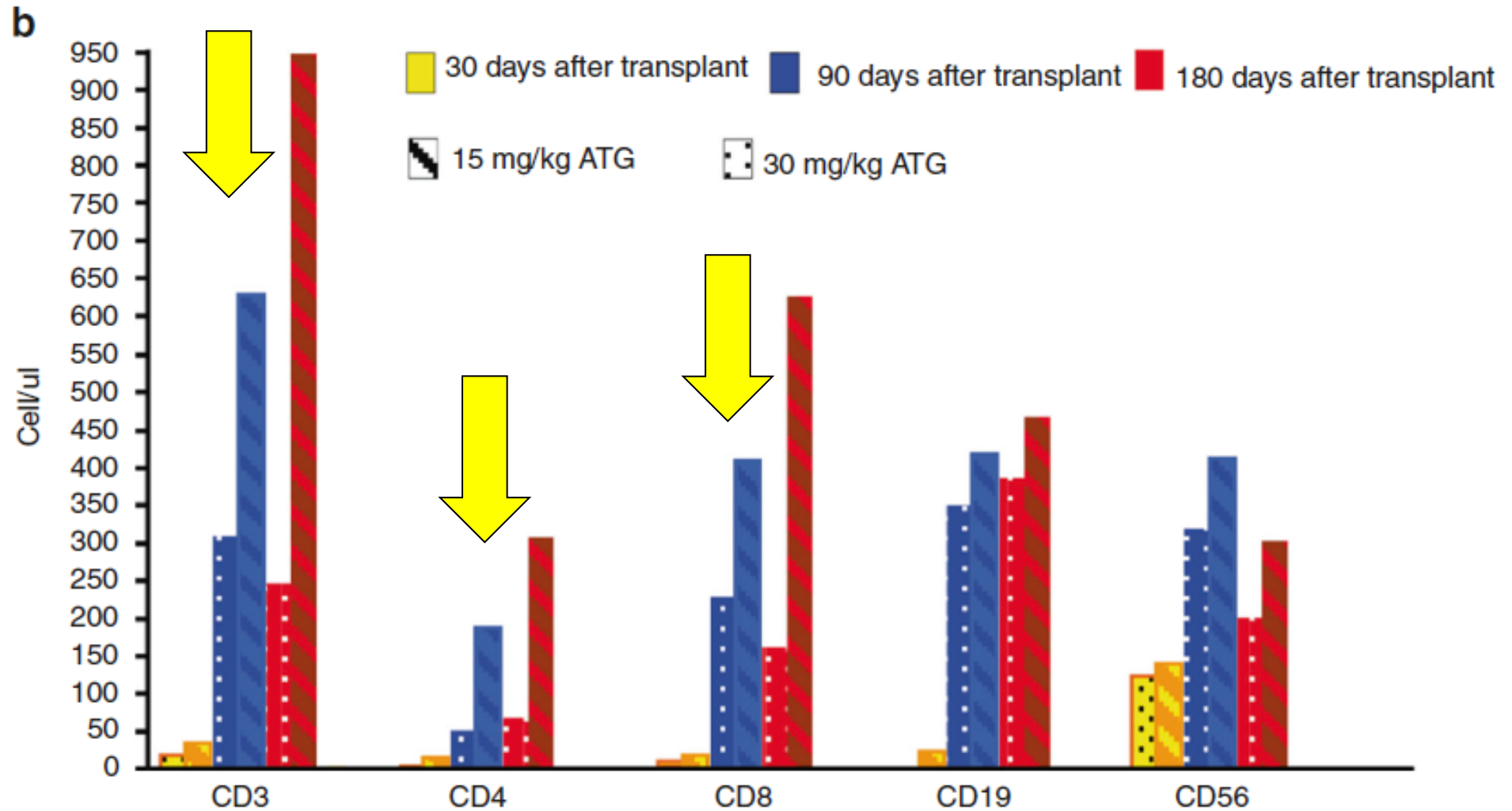


# T cell dose influences recovery



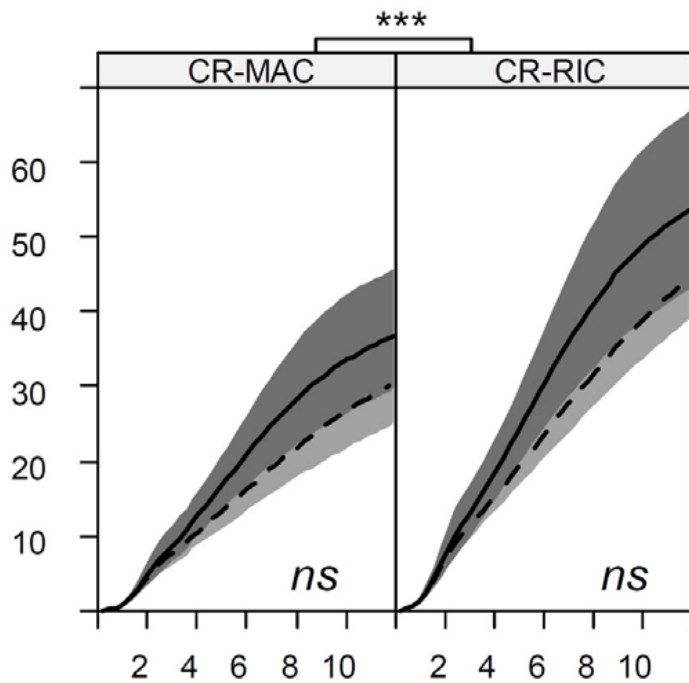
Ulbar et al. EBMT 2017  
Bonifazi et al. BMT 2018  
Bonifazi et al. BMT 2019

# T cell recovery after CBT and ATG dose

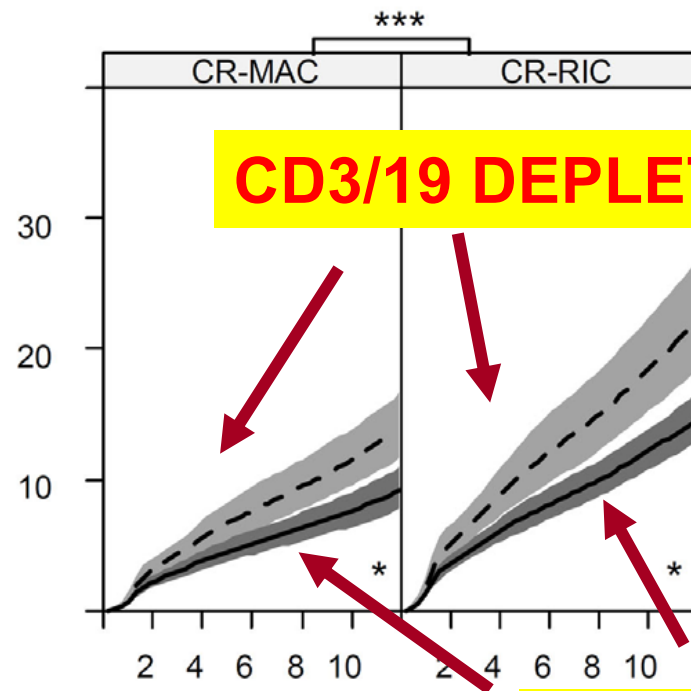


# Recovery of CD4 T cells after T cell depletion...in vitro

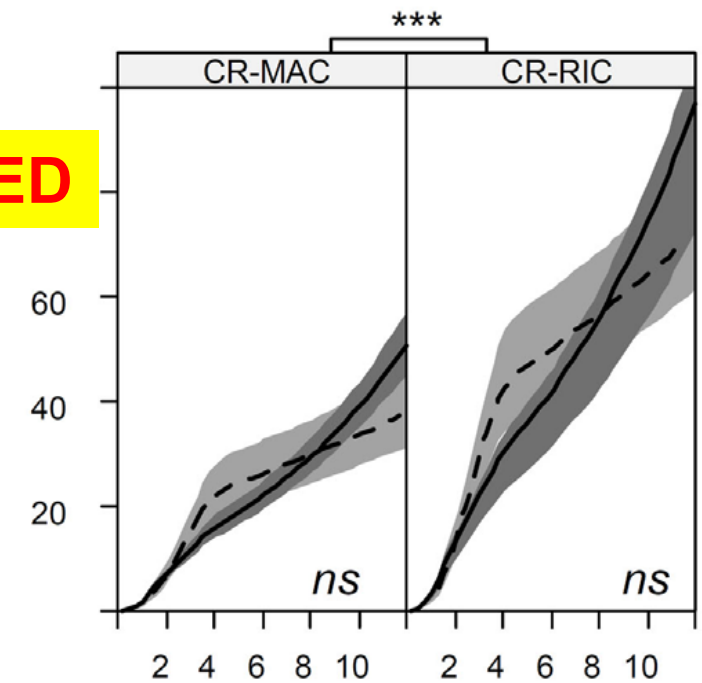
D T cells



E helper T cells



F cytotoxic T cells





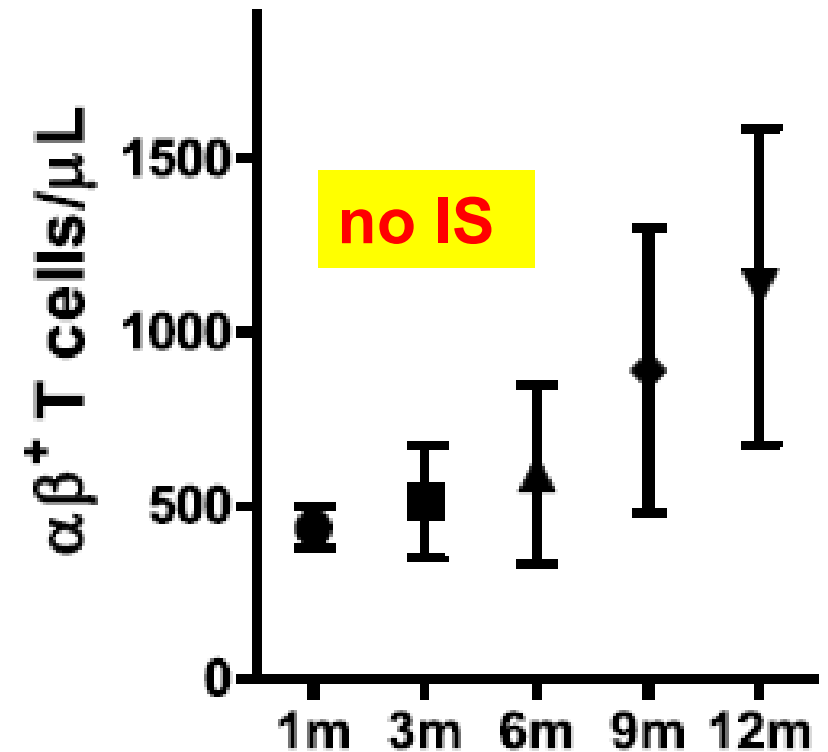
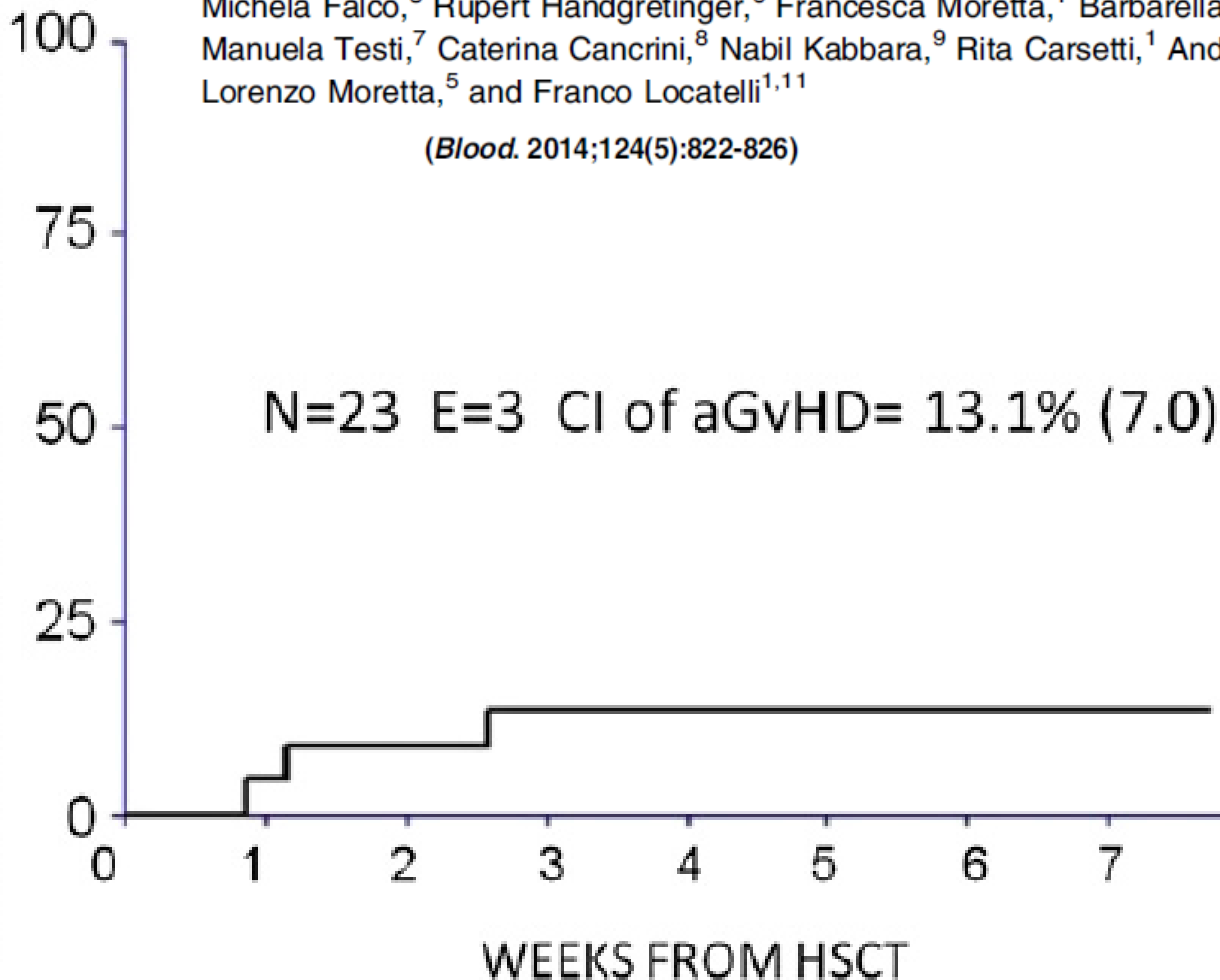
# Partial T depletion: alpha-beta T cells

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

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

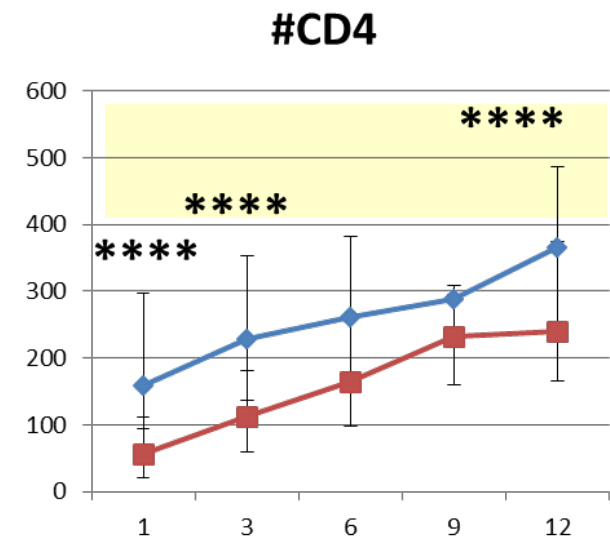
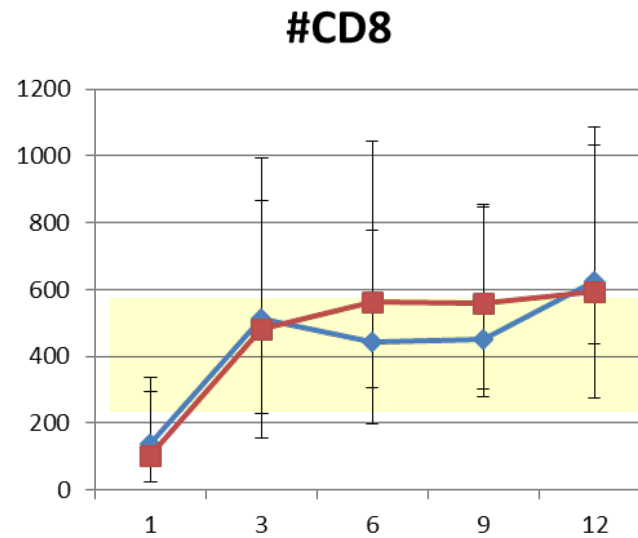
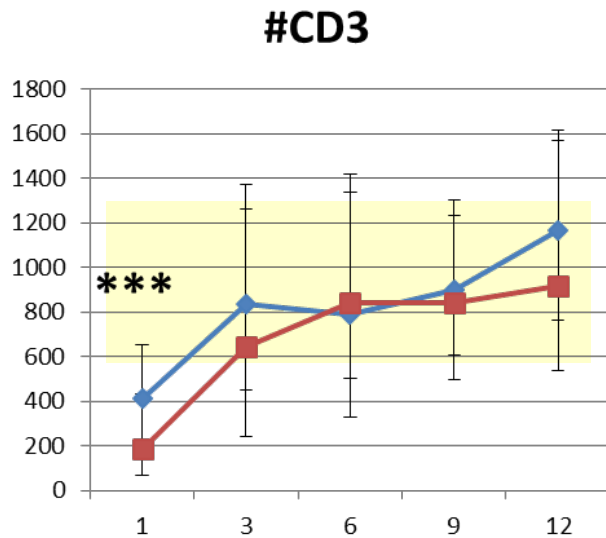
(*Blood*. 2014;124(5):822-826)

N=23 E=3 CI of aGvHD= 13.1% (7.0)



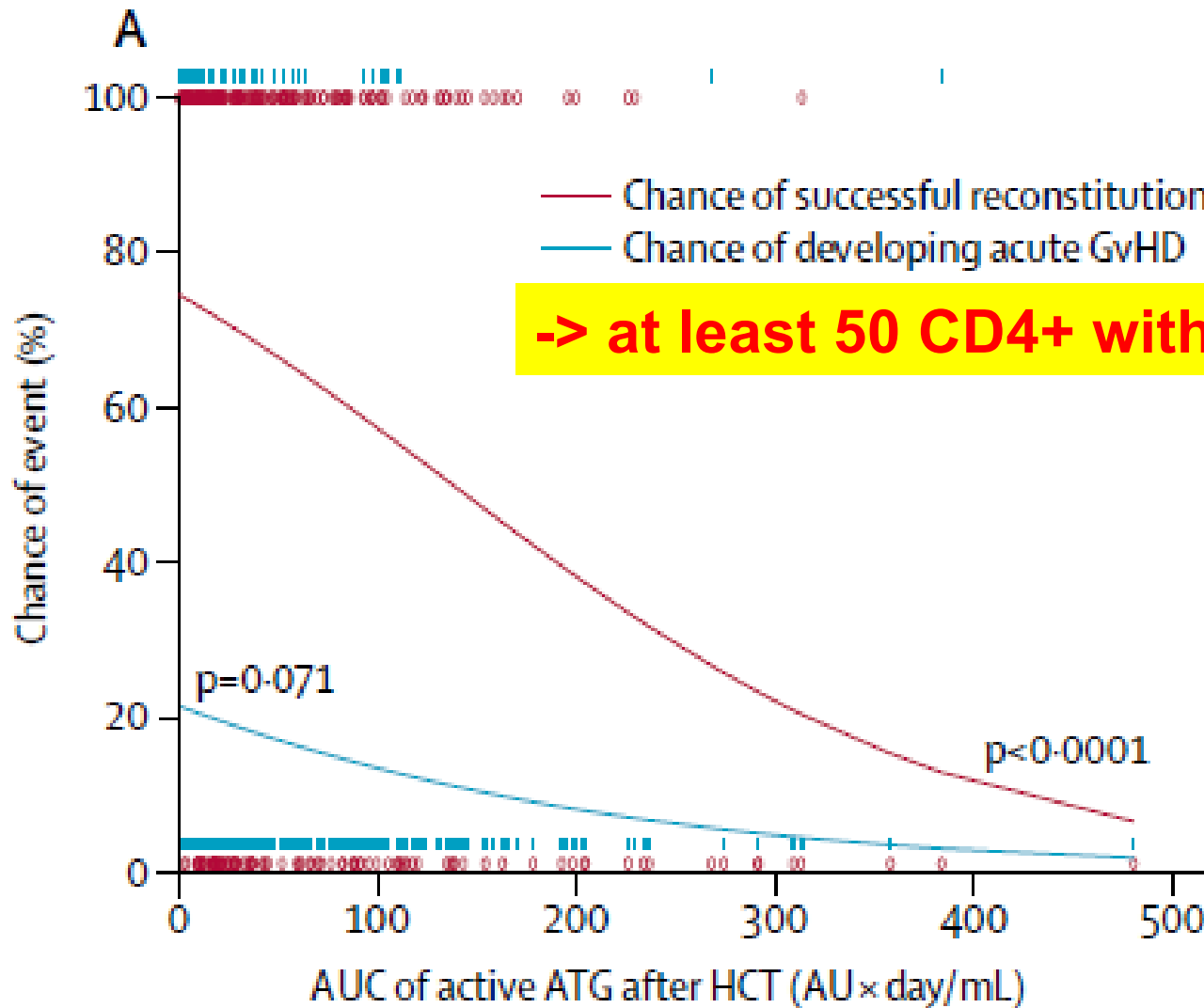
# Recovery of CD4 T cells after T cell depletion....in vivo

■ NO ATG  
■ YES ATG



Ulbar EBMT 2017  
Bonifazi BMT 2018

# Importance of ATG exposure

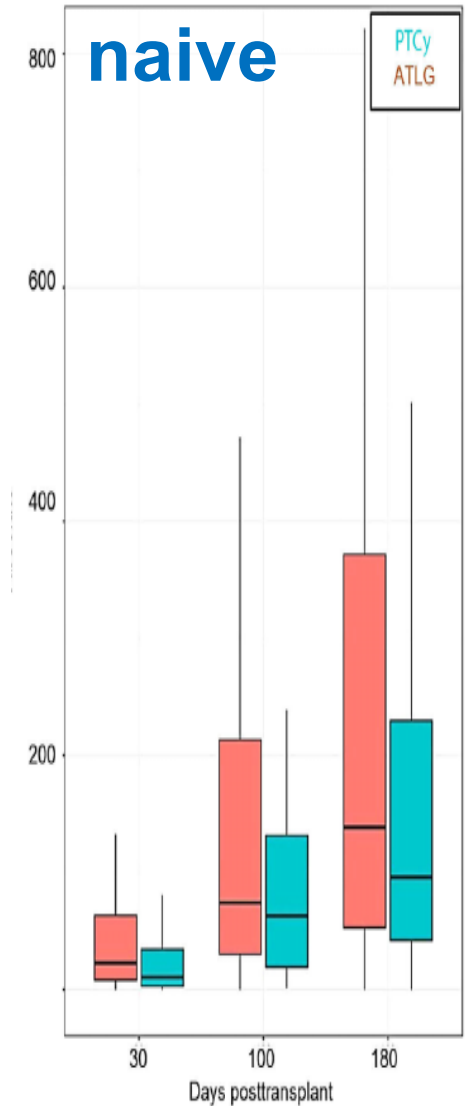
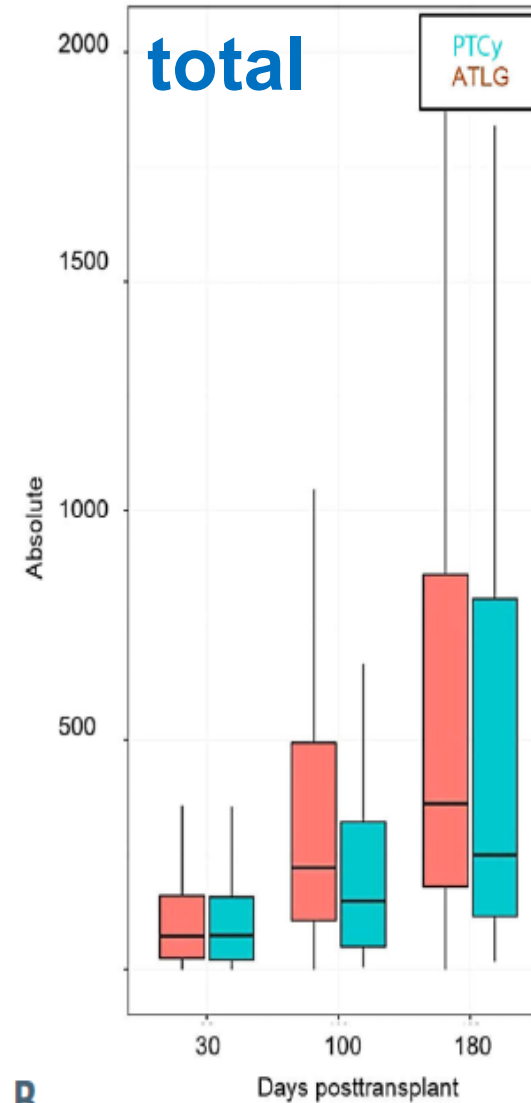
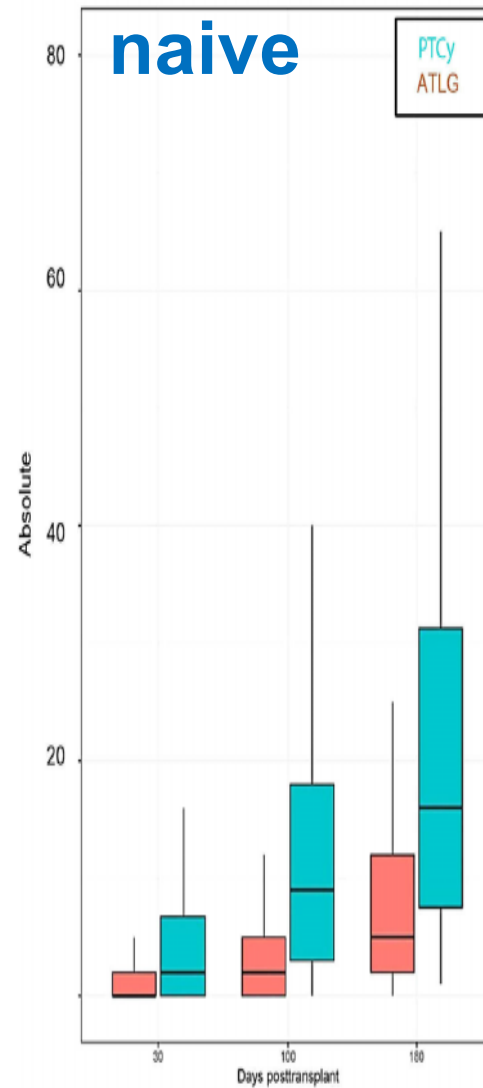
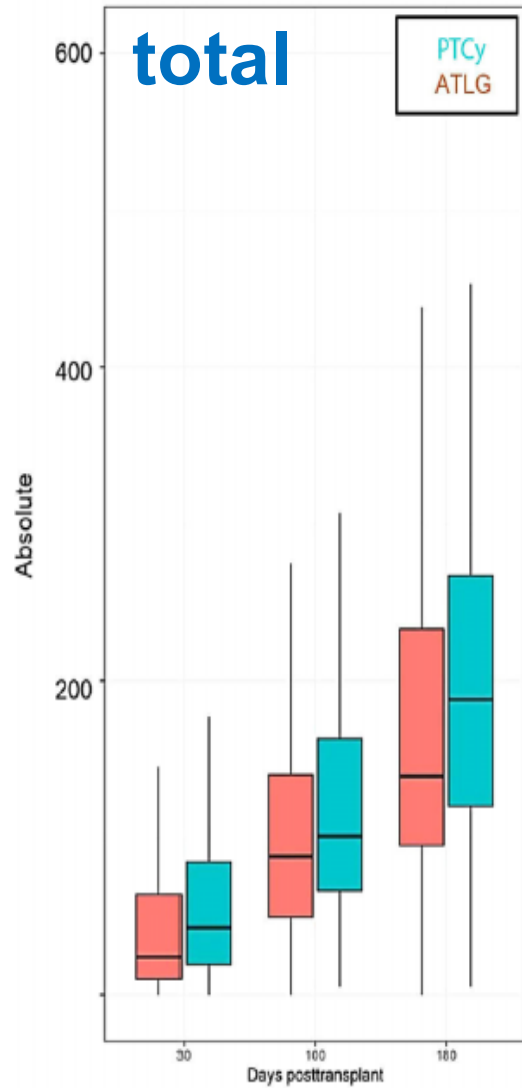


# Better recovery with pTCy?

Massoud Haematologica 2022

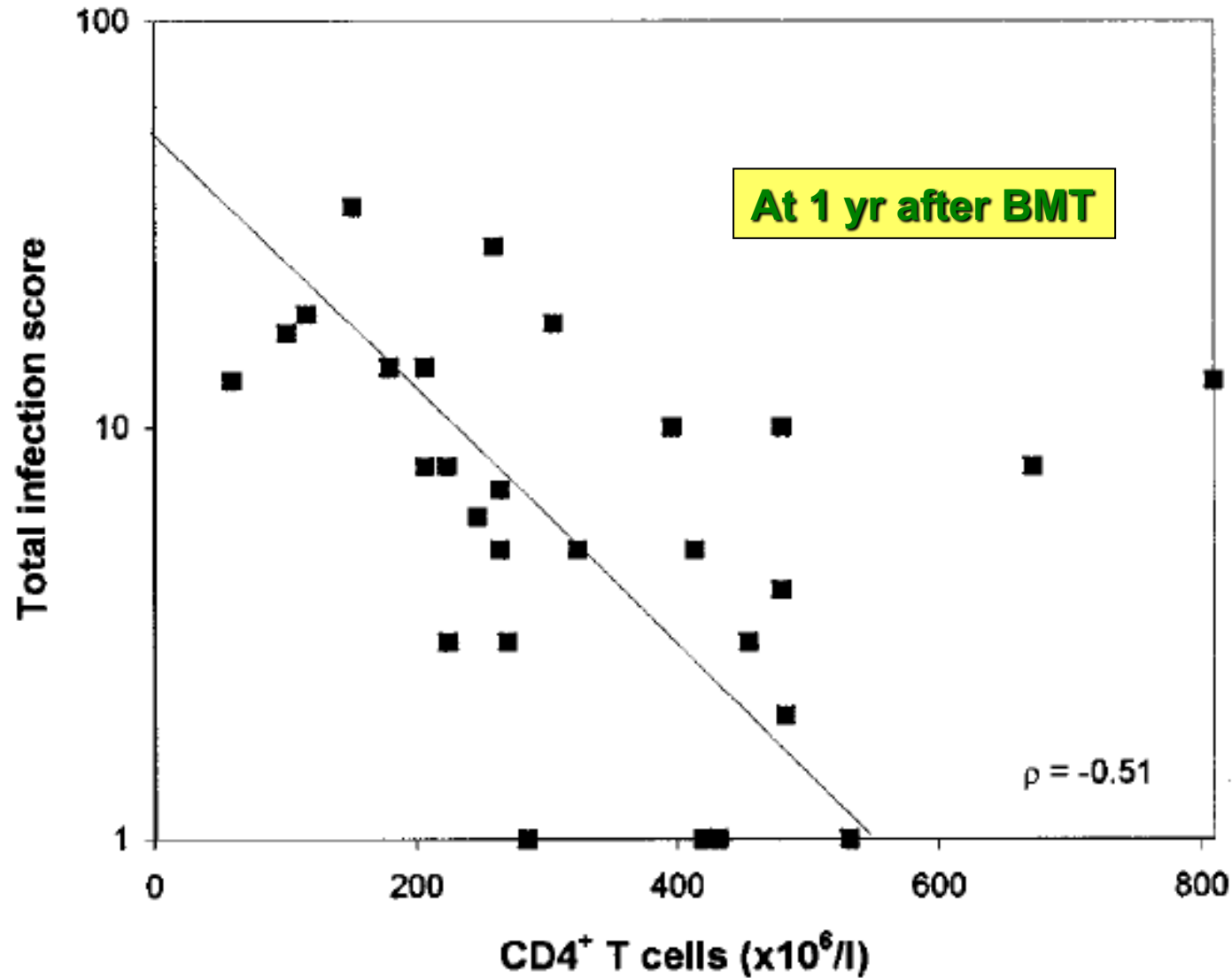
**CD4+**

**CD8+**



D

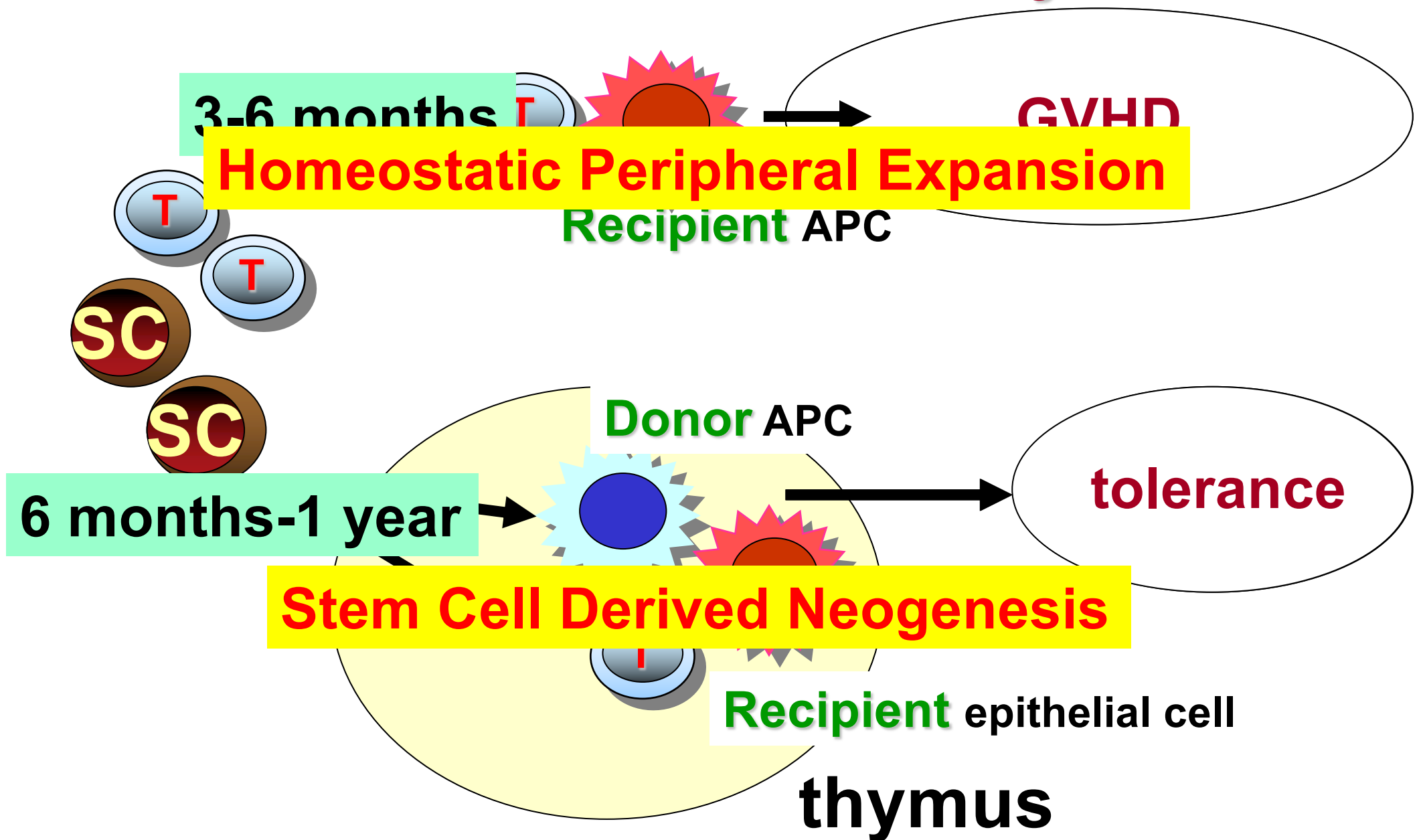
# CD4+ T and Risk of Infection



# Clinical impact of T cell recovery

Savani 2006	lymphocytes	day 30	>300
Kim 2006	CD4+ T cells	3 months	>200
Koehl 2007	CD8+ T cells	Within 1 year	>5th percentile
Berger 2008	CD4+ T cells	35 days	>86
Servais 2016	CD4+ T cells	3 months	>200
De Koning 2021	CD4+ T cells	Within 100 days	>50

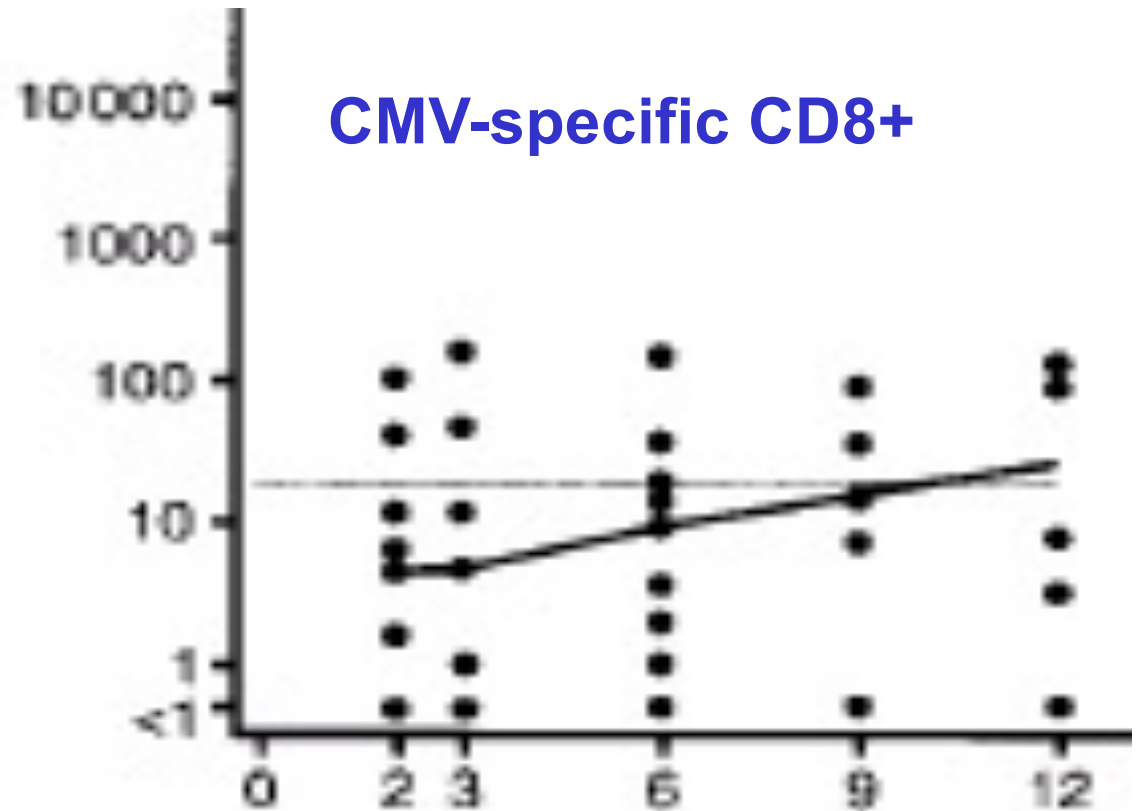
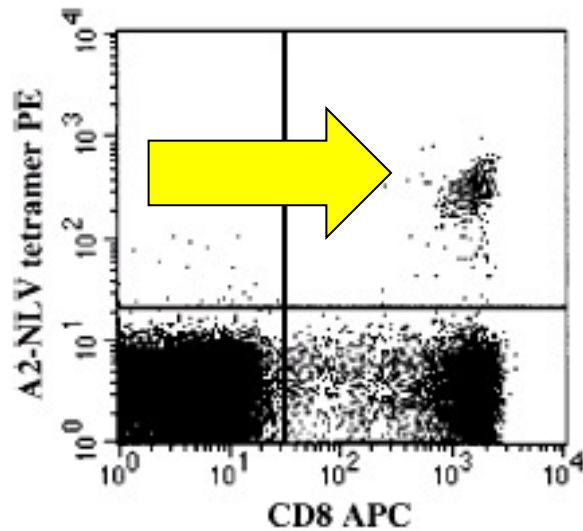
# a model of T cell recovery





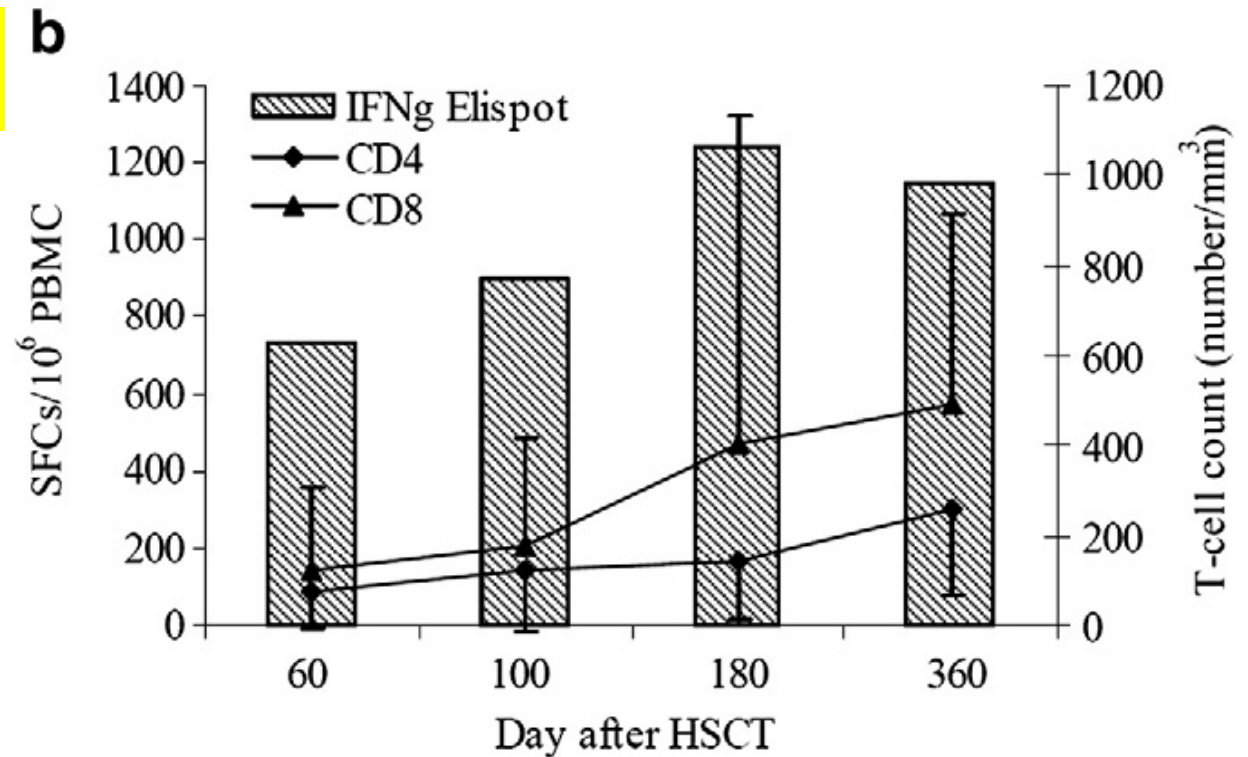
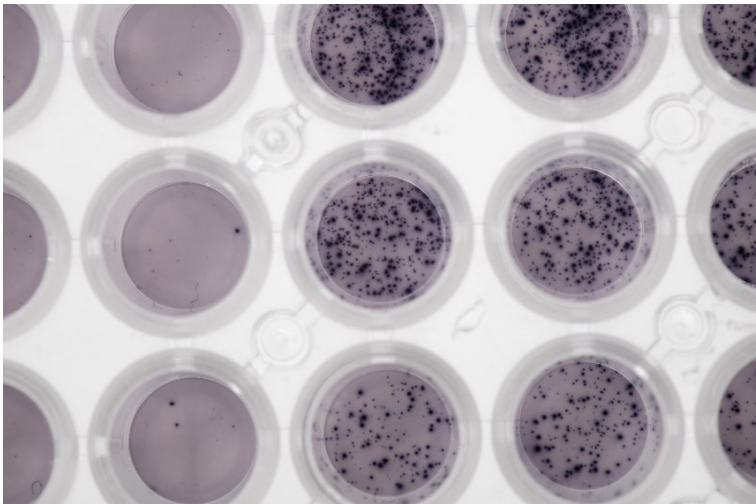
# infection-induced HPE: CMV

## Tetramer-based FCM



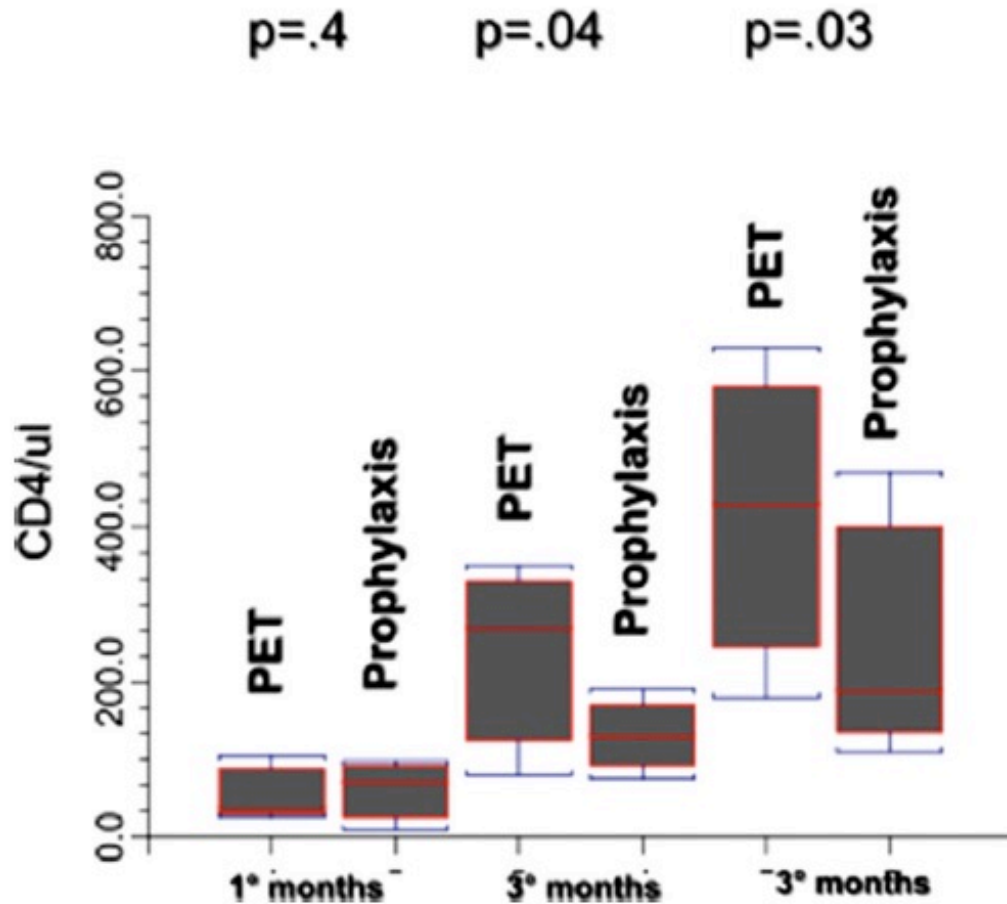
# infection-induced HPE: EBV

## IFN $\gamma$ -based ELISPOT

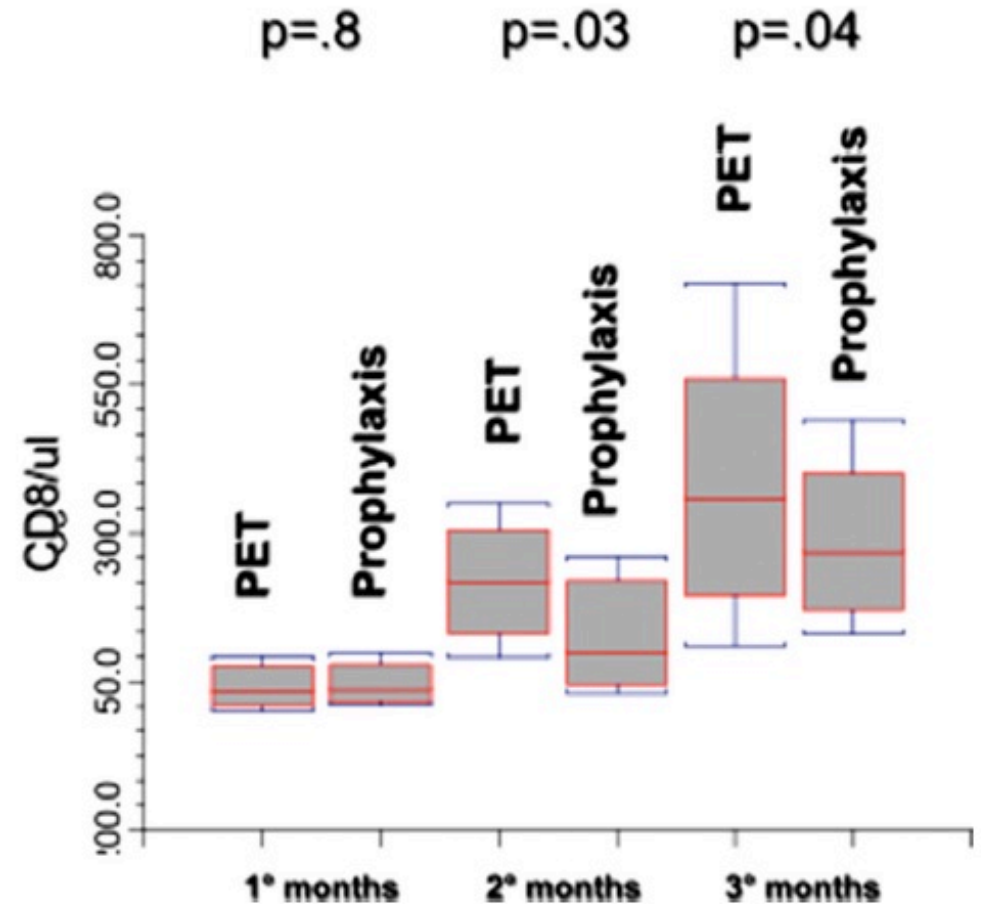


# CMV prophylaxis delays T cell recovery

A. CD4 T cell reconstitution

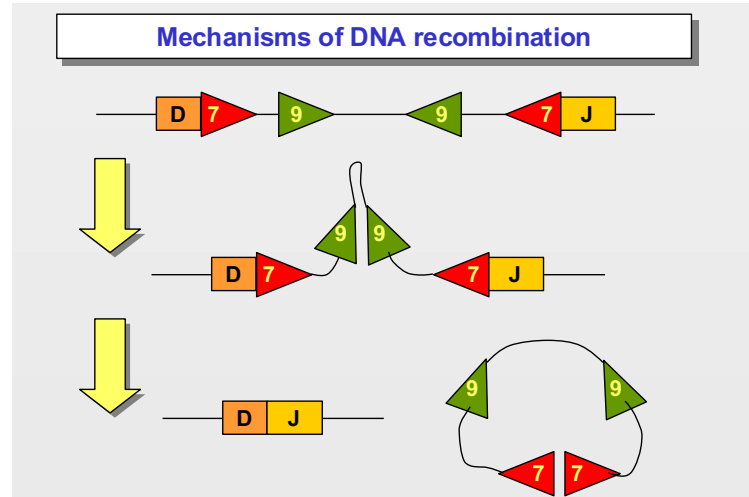


B. CD8 T cell reconstitution

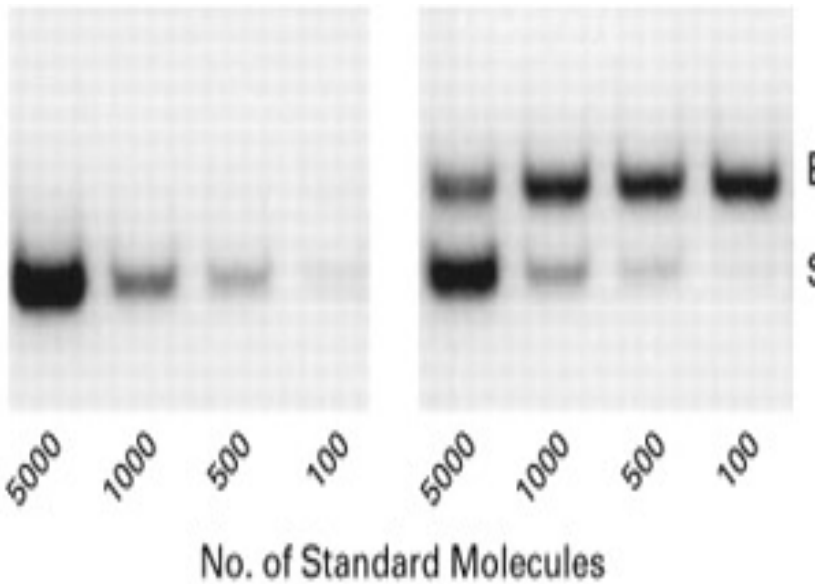
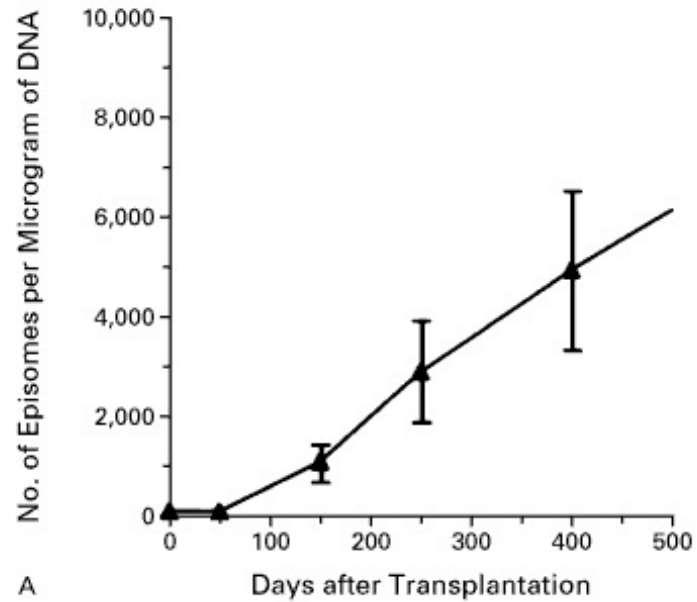


# measurement of thymic function: **TRECS**

Patel NEJM 2001

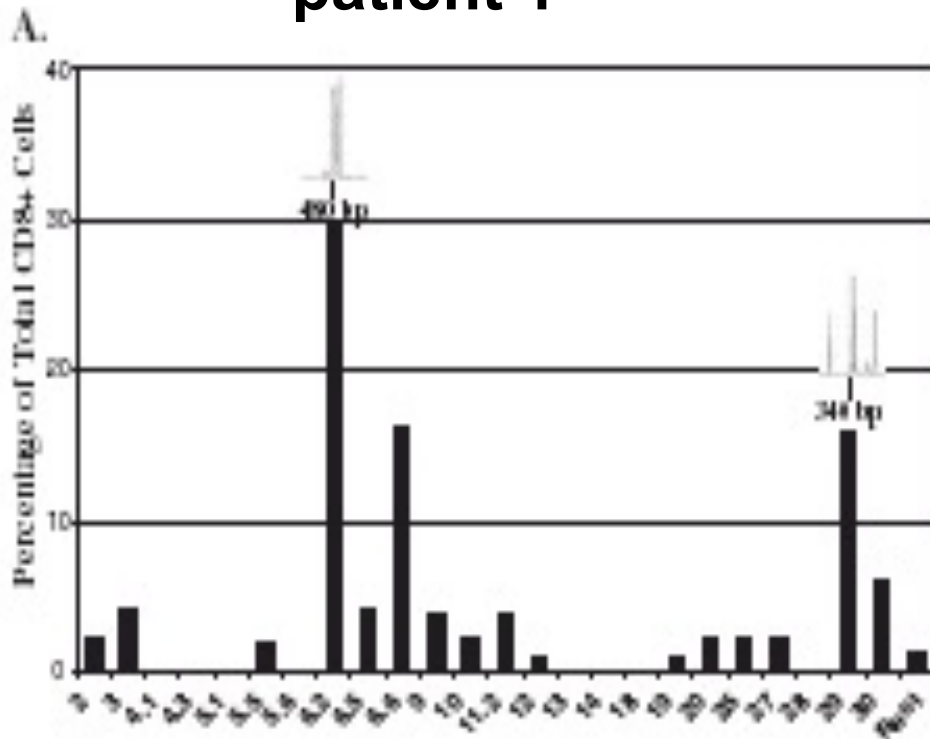


Patients with Severe Combined Immunodeficiency

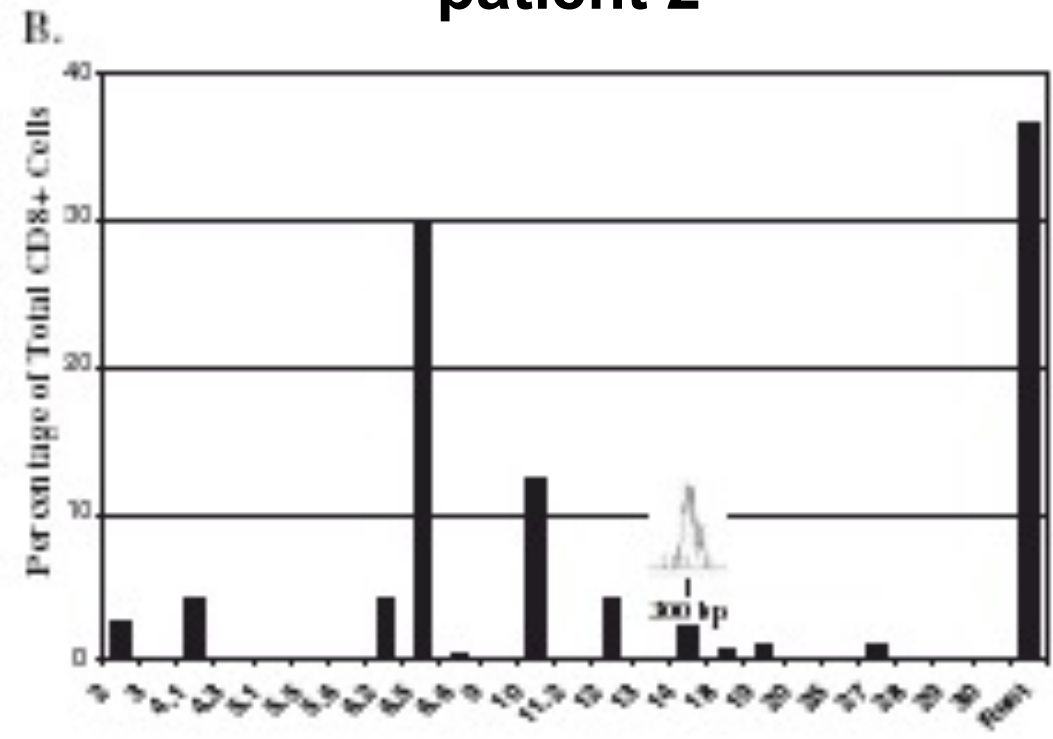


# T cell repertoire: **FLOW CYTOMETRY**

patient 1



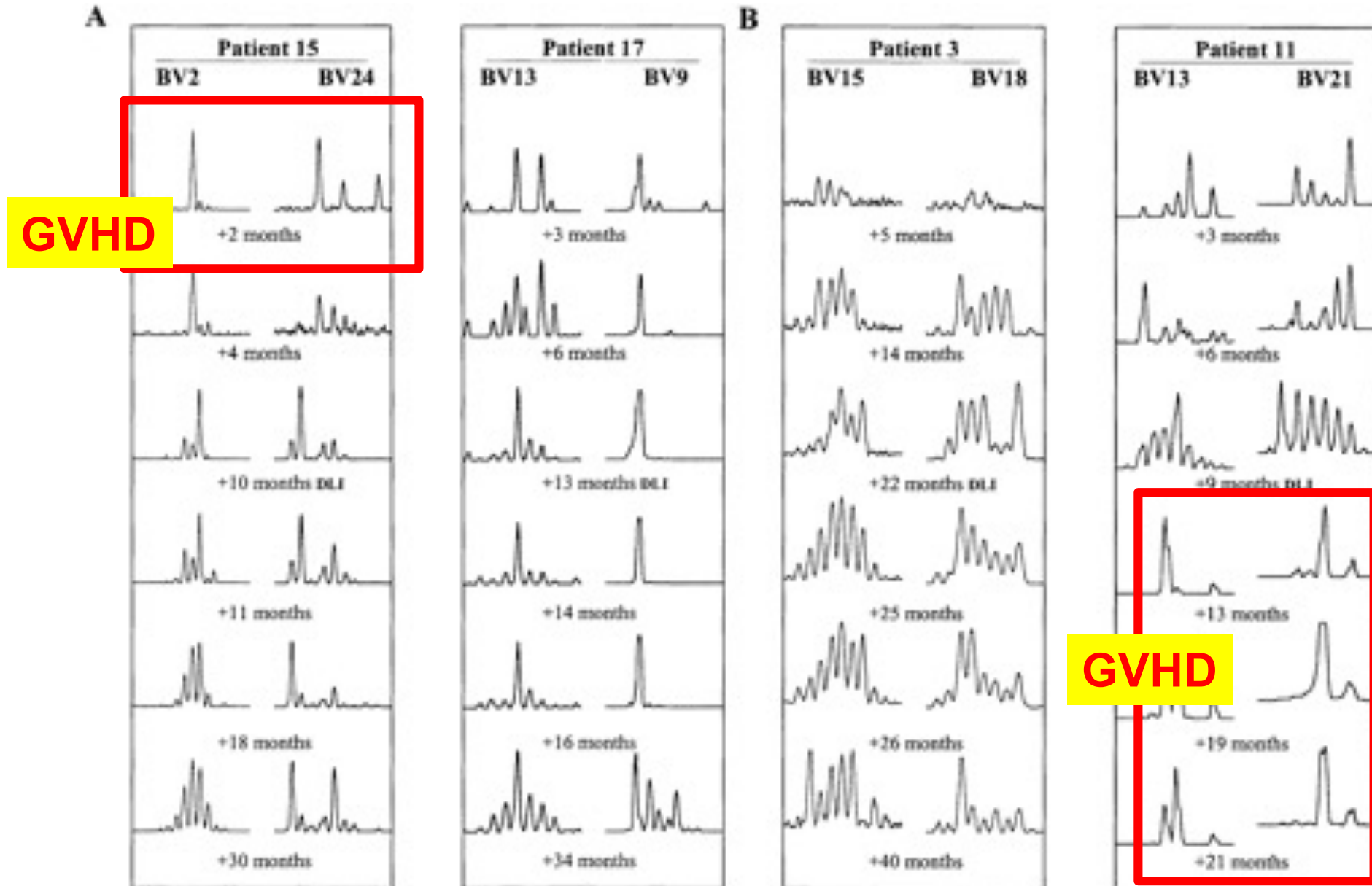
patient 2



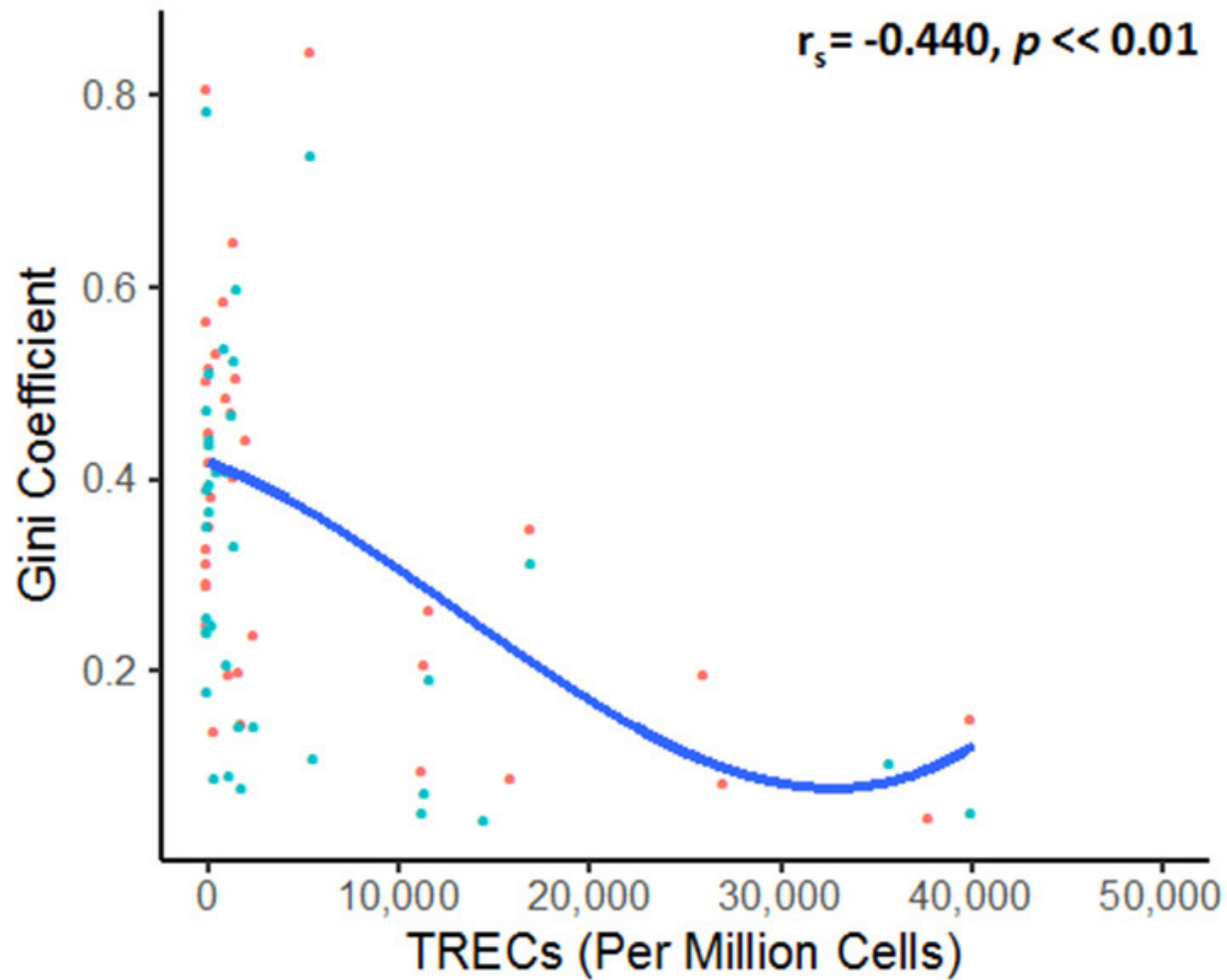
Vβ Family

# T cell repertoire: **SPECTRATYPING**

Verfuert Blood 2000

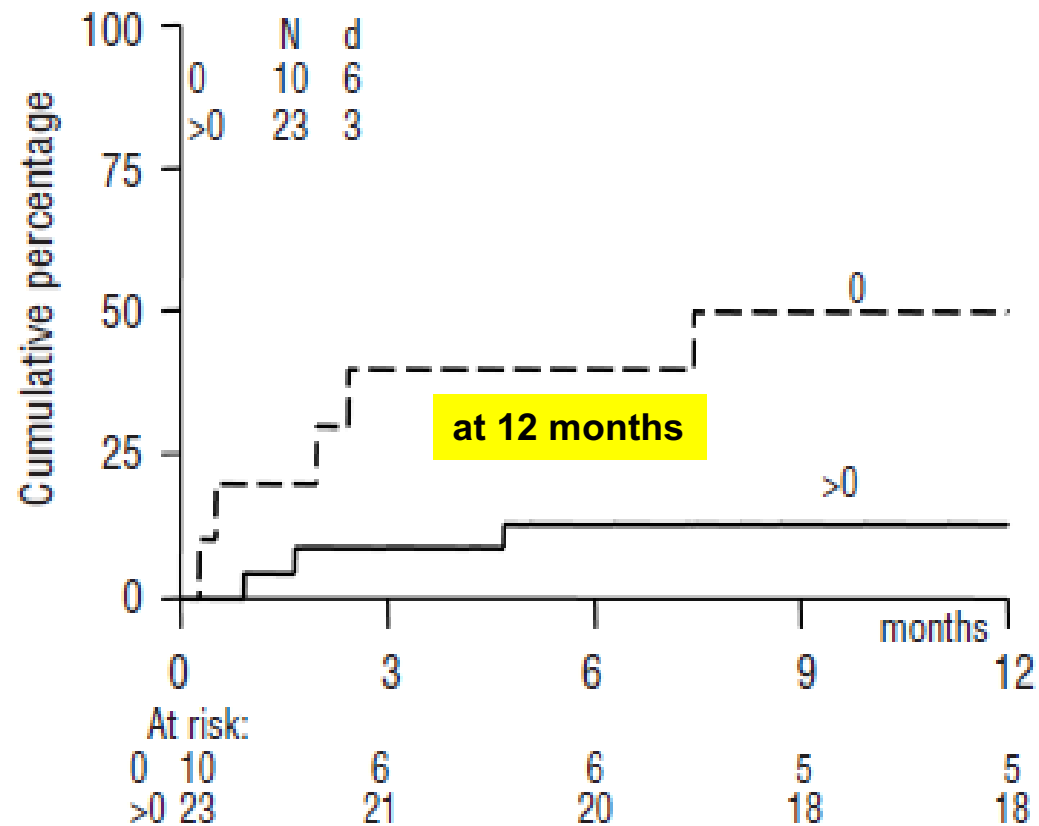
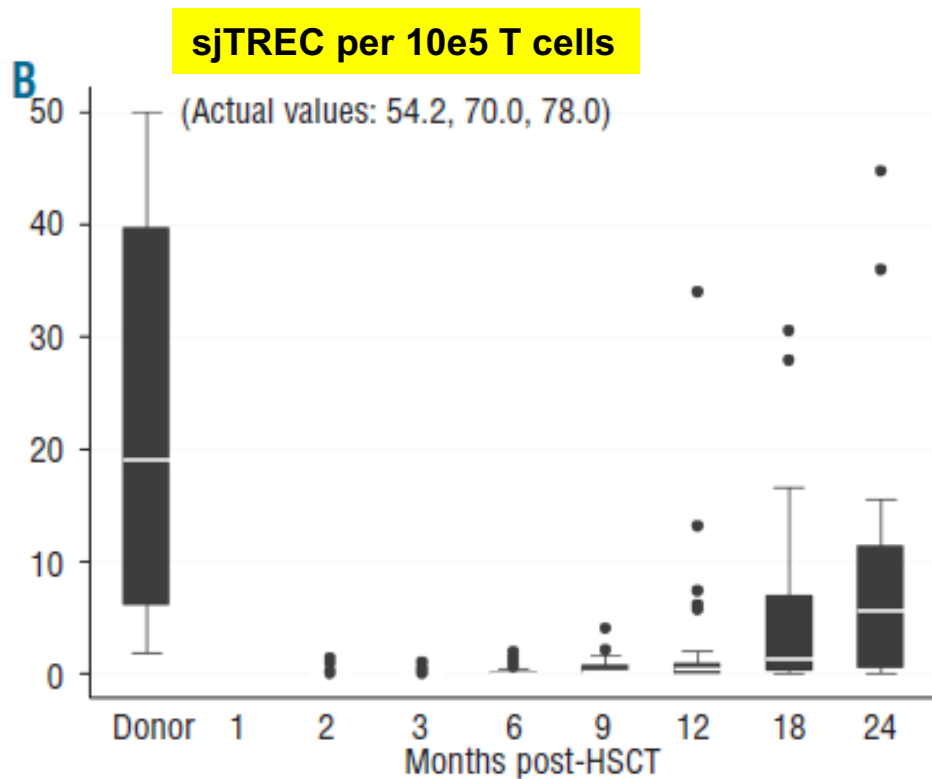


# T cell repertoire: **NGS**

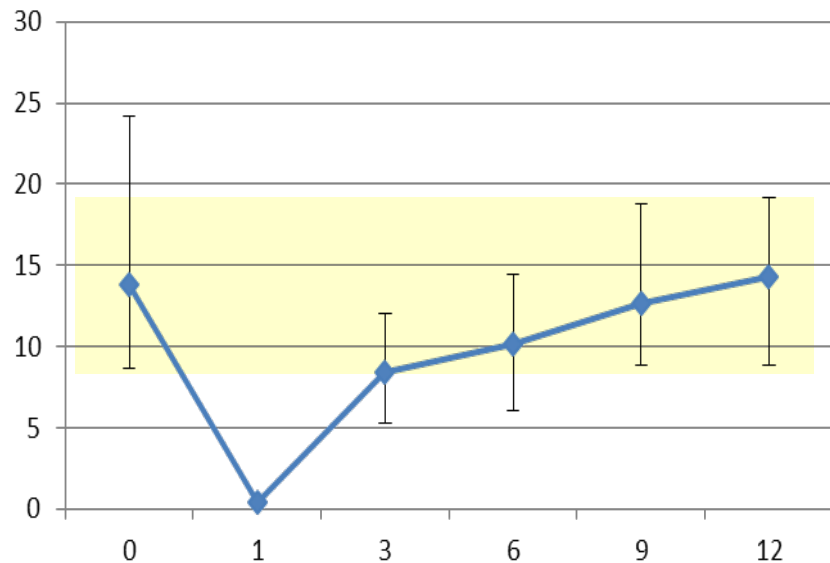




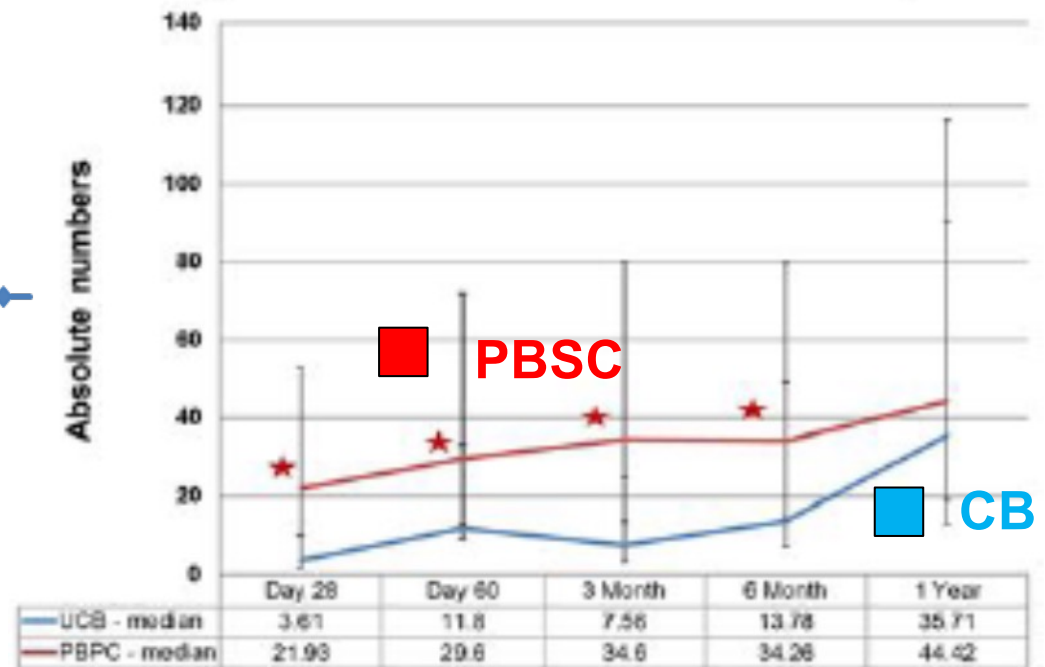
# thymic function predicts infection



# Recovery of T reg cells



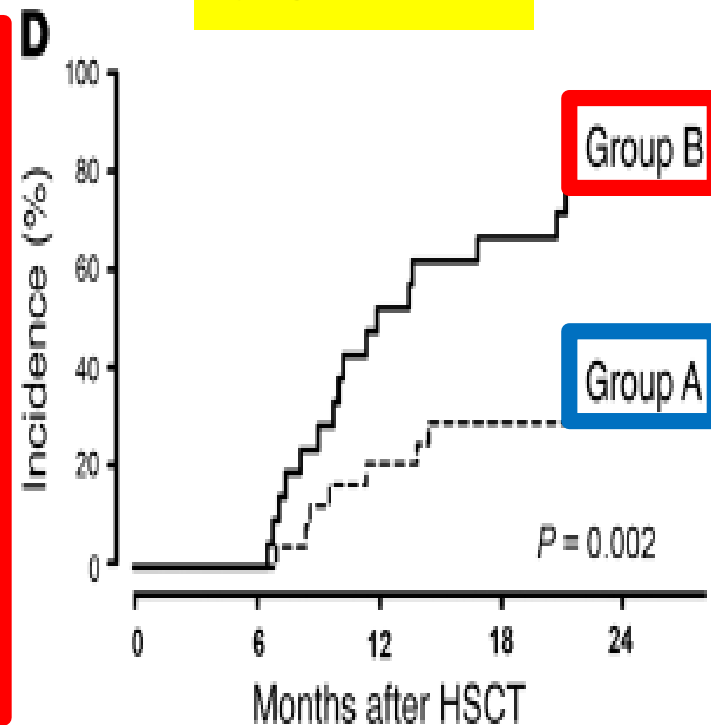
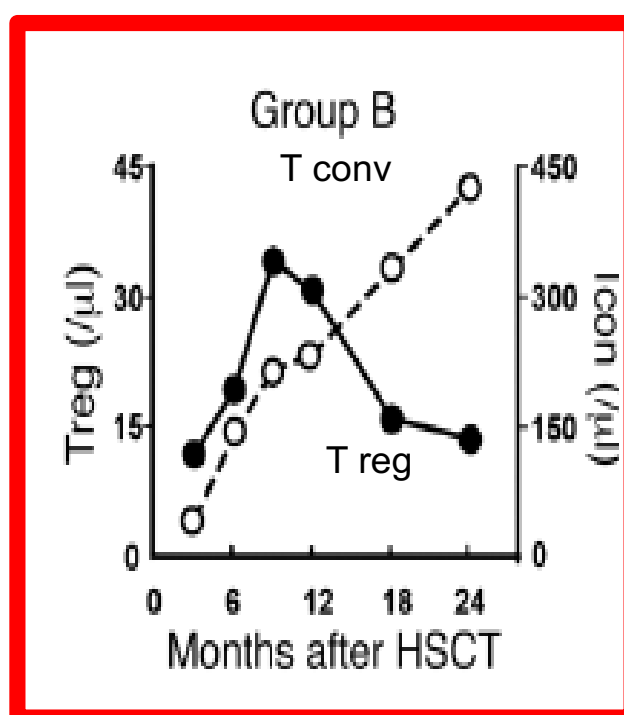
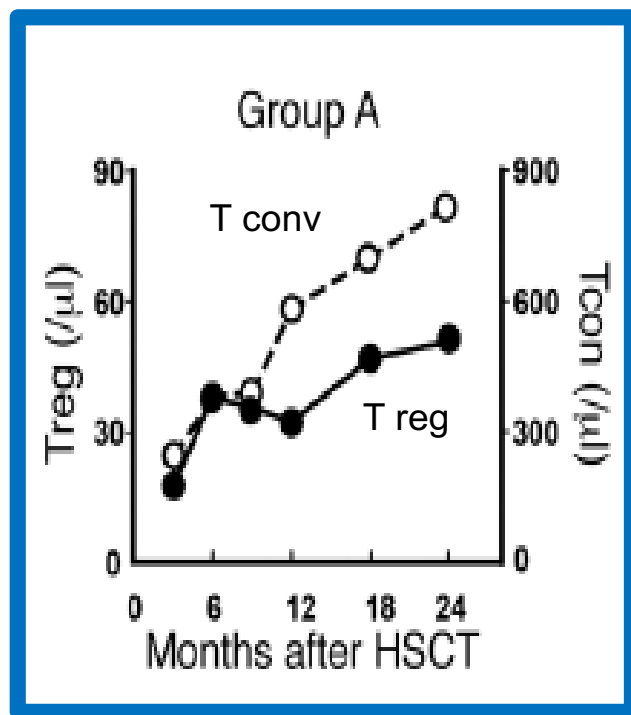
## Tregs (CD4+CD25<sup>bright</sup>CD127<sup>-</sup>)



Mehta ASH 2016  
Ulbar EBMT 2017

# Loss of Treg recovery in cGVHD

**cGVHD**

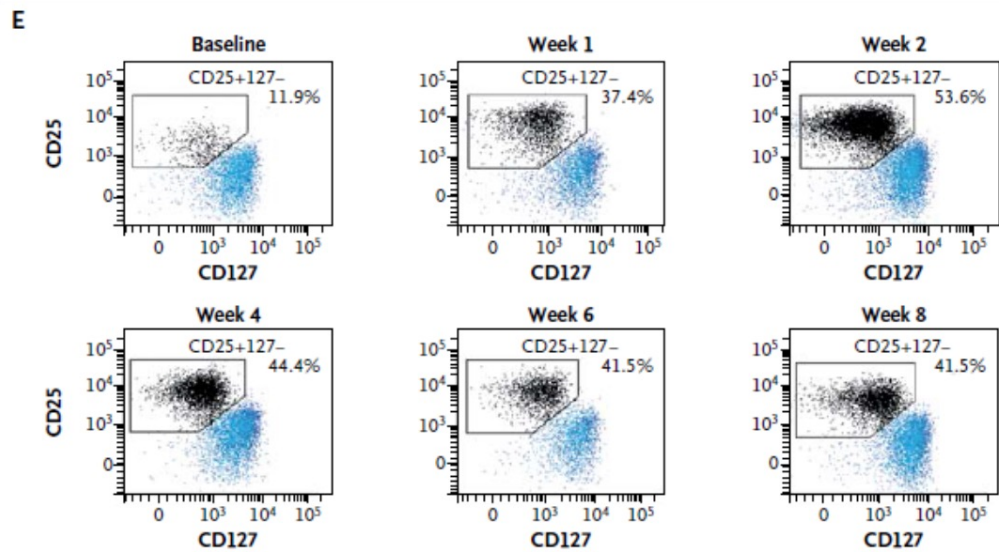


# Increasing T reg numbers leads to improved GVHD



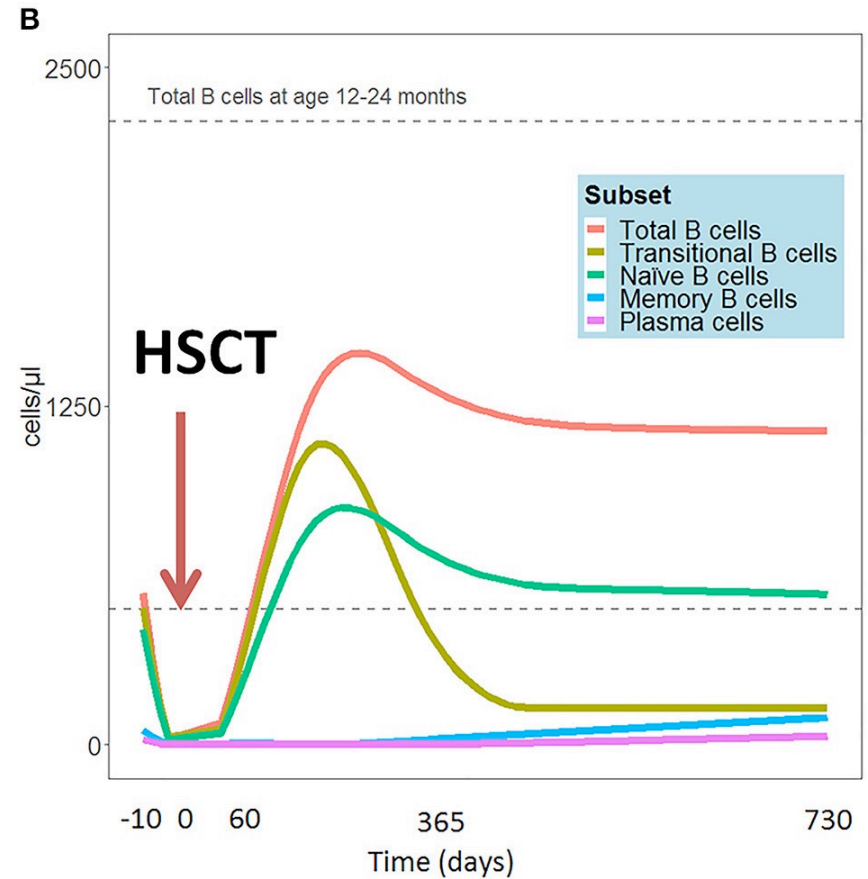
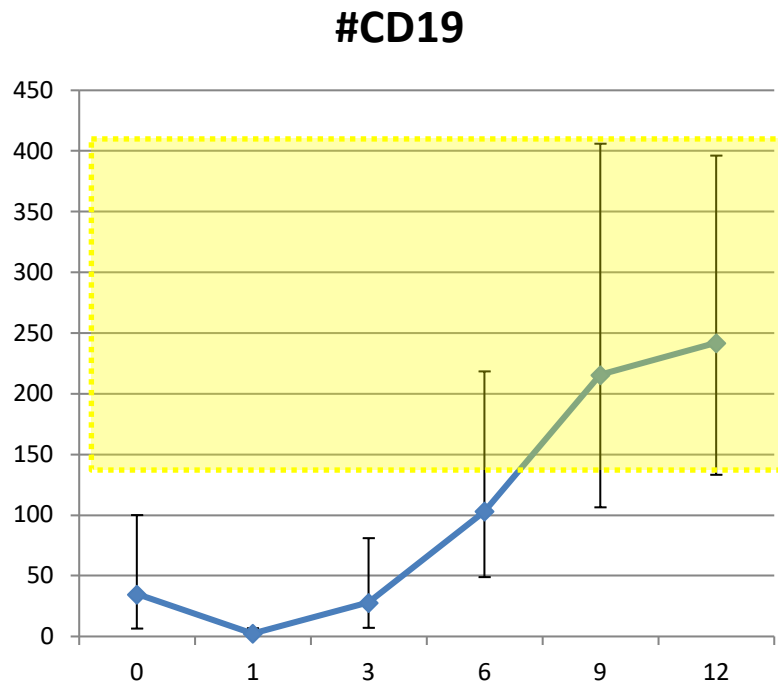
## Interleukin-2 and Regulatory T Cells in Graft-versus-Host Disease

John Koreth, M.B., B.S., D.Phil., Ken-ichi Matsuoka, M.D., Ph.D., Haesook T. Kim, Ph.D., Sean M. McDonough, M.S., Bhavjot Bindra, B.S., Edwin P. Alyea III, M.D., Philippe Armand, M.D., Ph.D., Corey Cutler, M.D., M.P.H., Vincent T. Ho, M.D., Nathaniel S. Treister, D.M.D., D.M.Sc., Don C. Bienfang, M.D., Sashank Prasad, M.D., Dmitrios Tzachanis, M.D., Ph.D., Robin M. Joyce, M.D., David E. Avigan, M.D., Joseph H. Antin, M.D., Jerome Ritz, M.D., and Robert J. Soiffer, M.D.



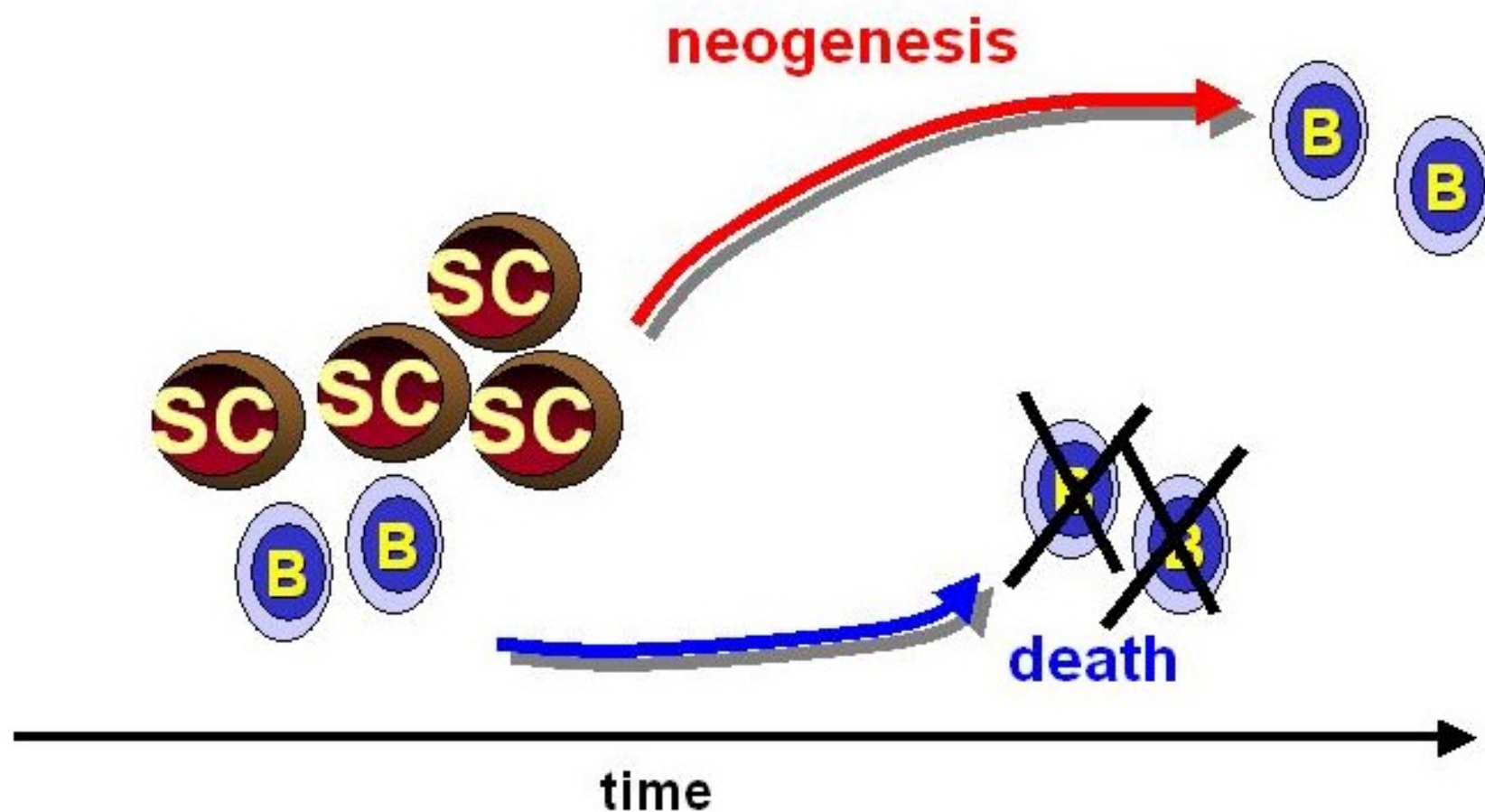
**recovery of immunity  
post transplant:  
B lymphocytes**

# recovery of B cells is slower...



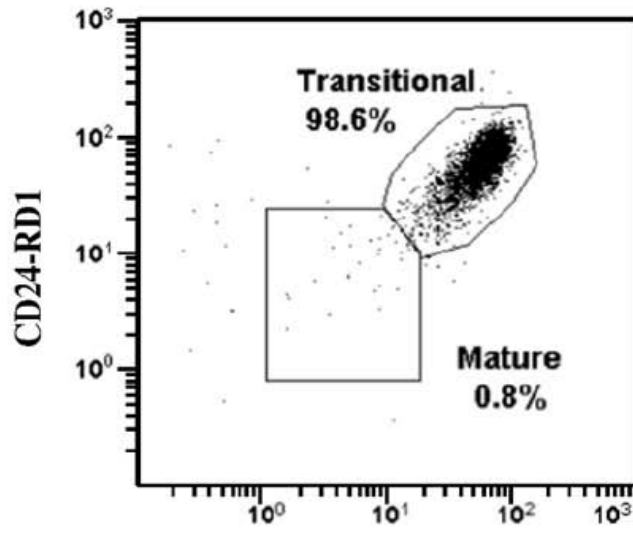
**...but complete (or more...)**

# B Cell Neogenesis is Prevalent after Allogeneic HSCT

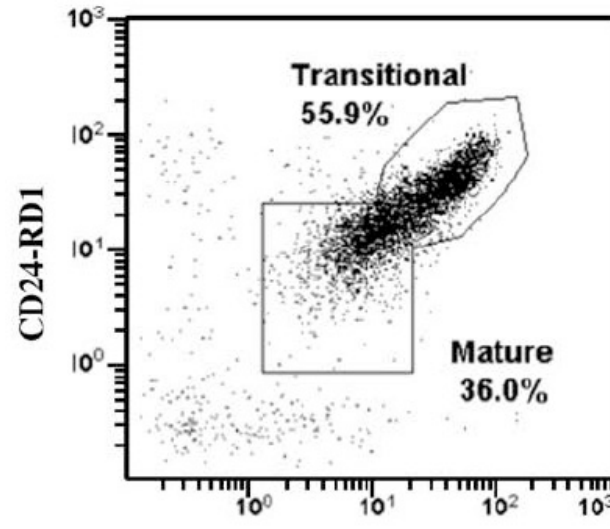




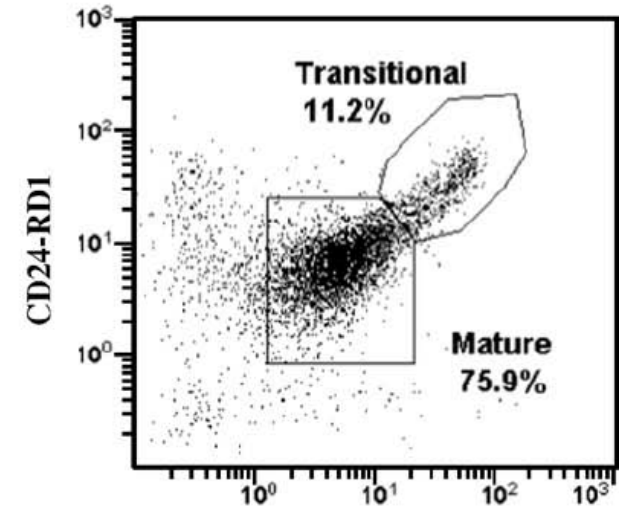
# First appearance of transitional B cells



CD38-PC5  
Months 1.7 after HSCT



CD38-PC5  
Months 4 after HSCT

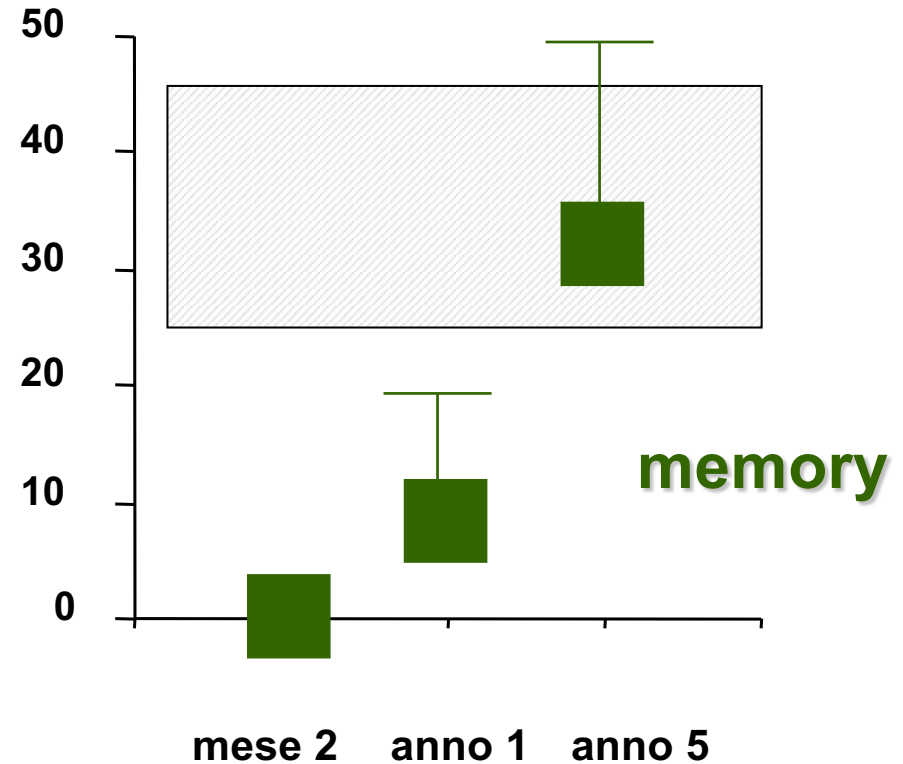
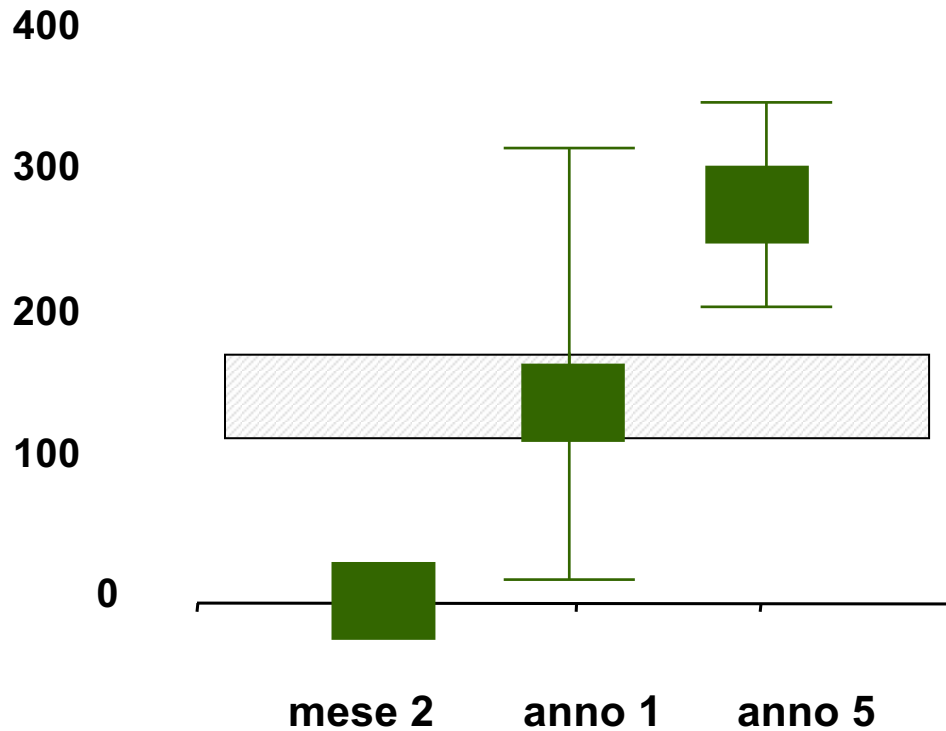


CD38-PC5  
Months 9 after HSCT

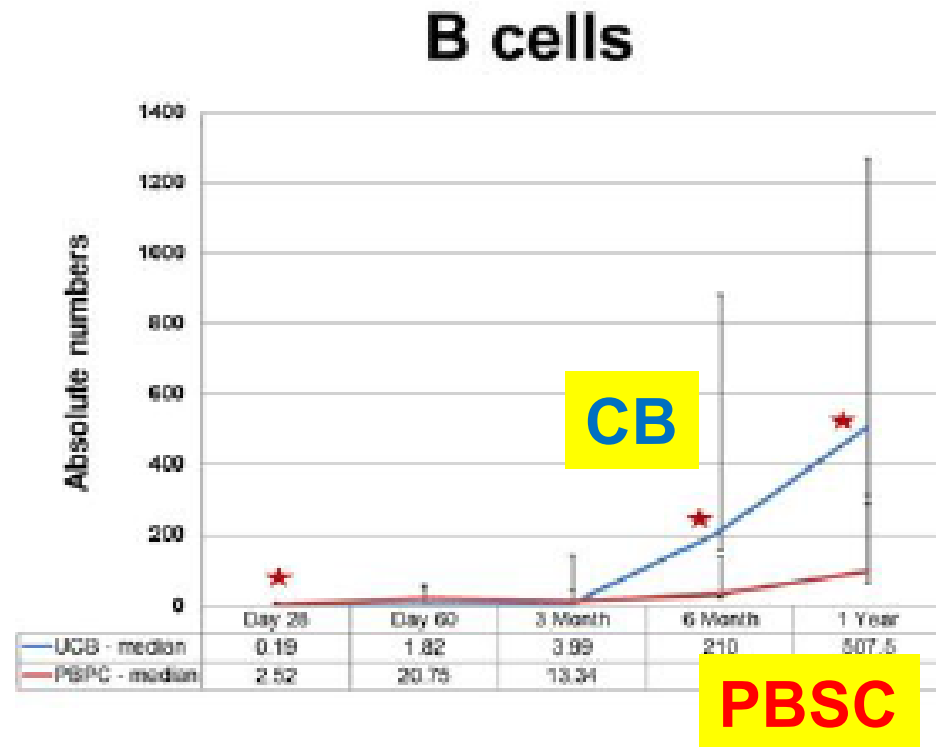
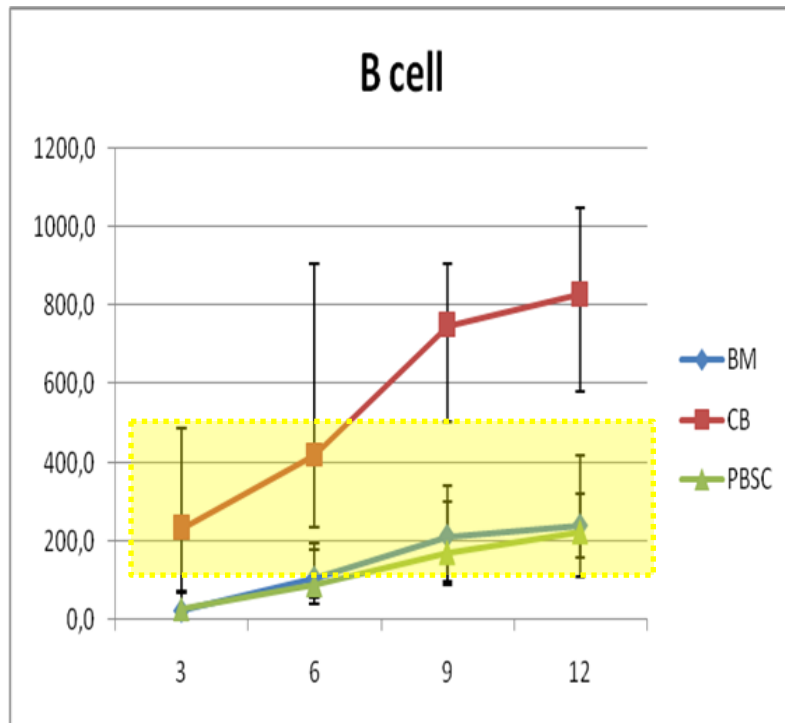


# Recovery of B cell subsets

naive

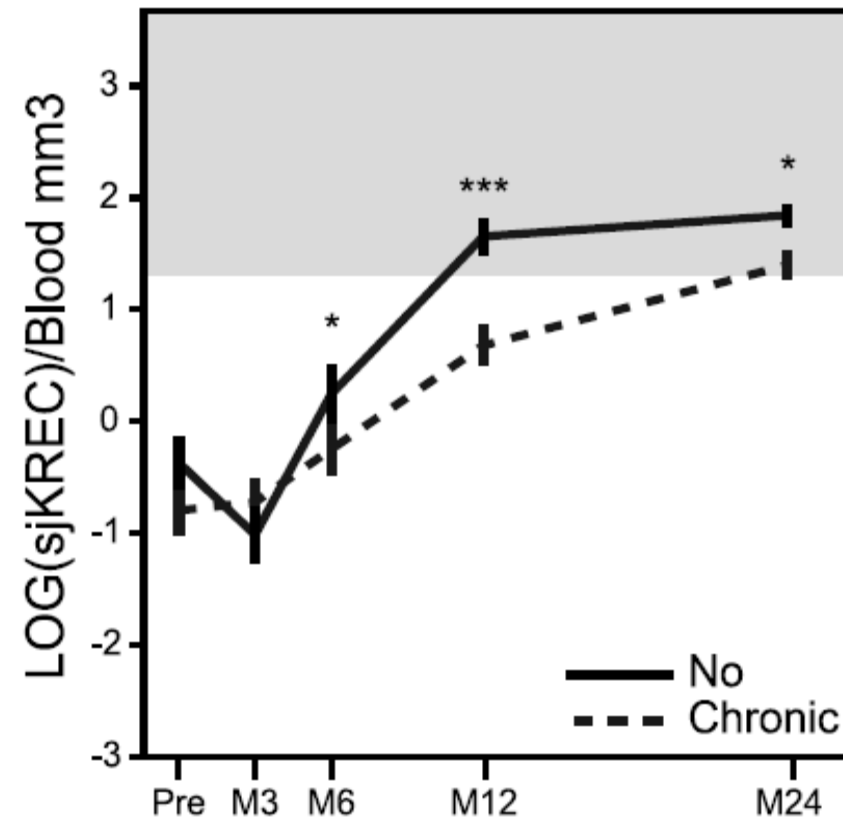
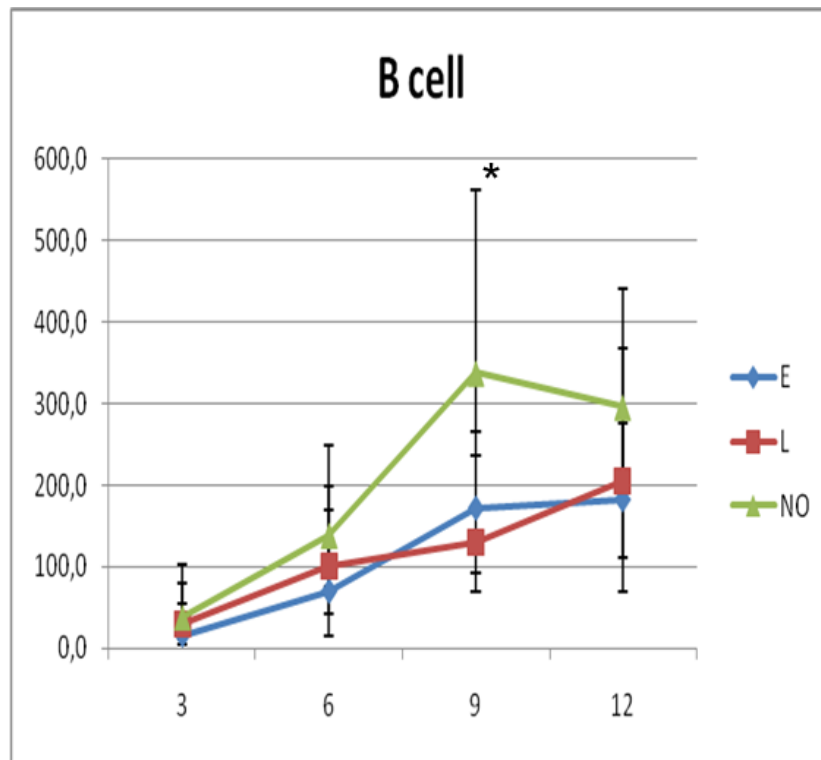


# Faster B cell neogenesis after CBT



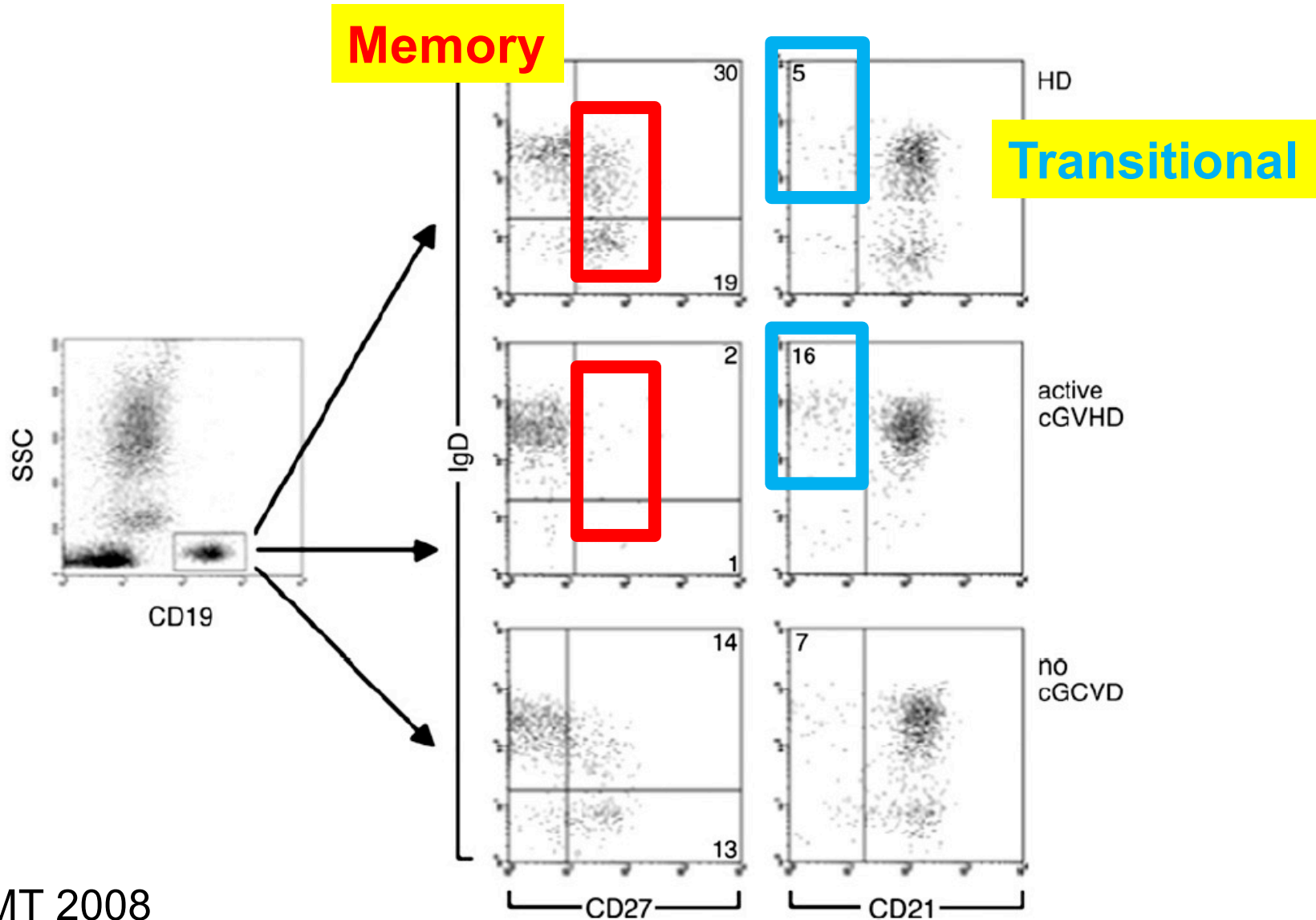
Mehta ASH 2016  
 Ulbar EBMT 2017  
 Bonifazi BMT 2019

# Reduced B cell neogenesis in cGVHD



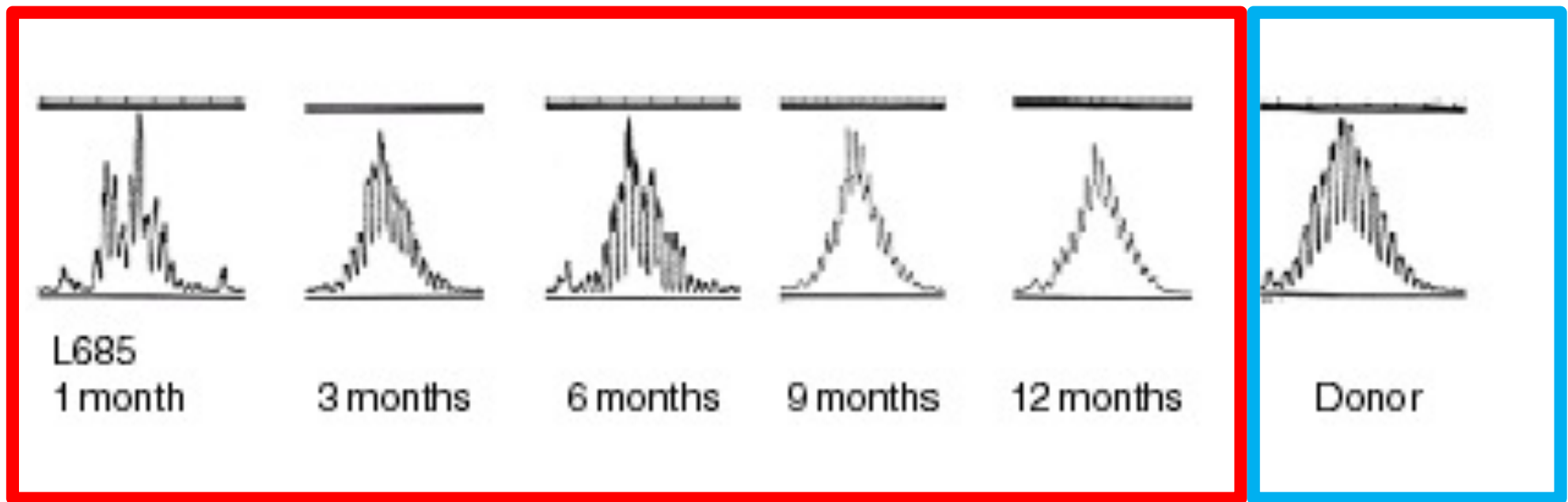
Ulbar EBMT 2017  
Glauze Blood 2014

# Loss of Memory B cells generation in cGVHD



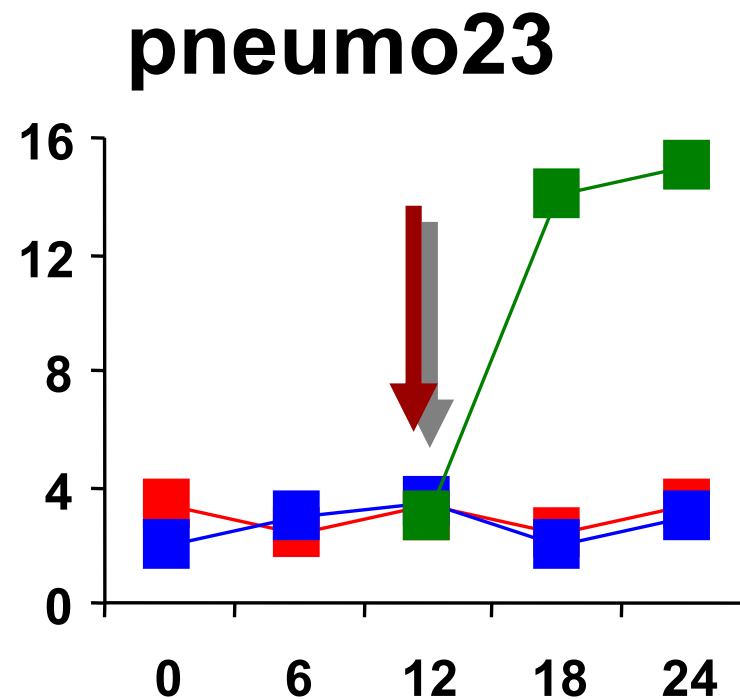
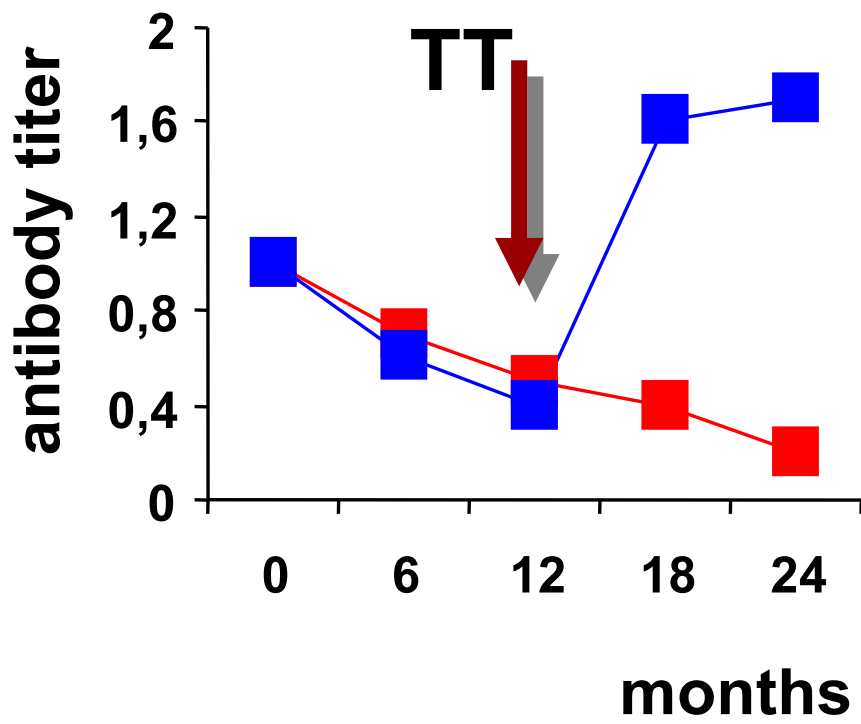
# Normal B cell repertoire after transplant

## CDR3 SPECTRATYPING

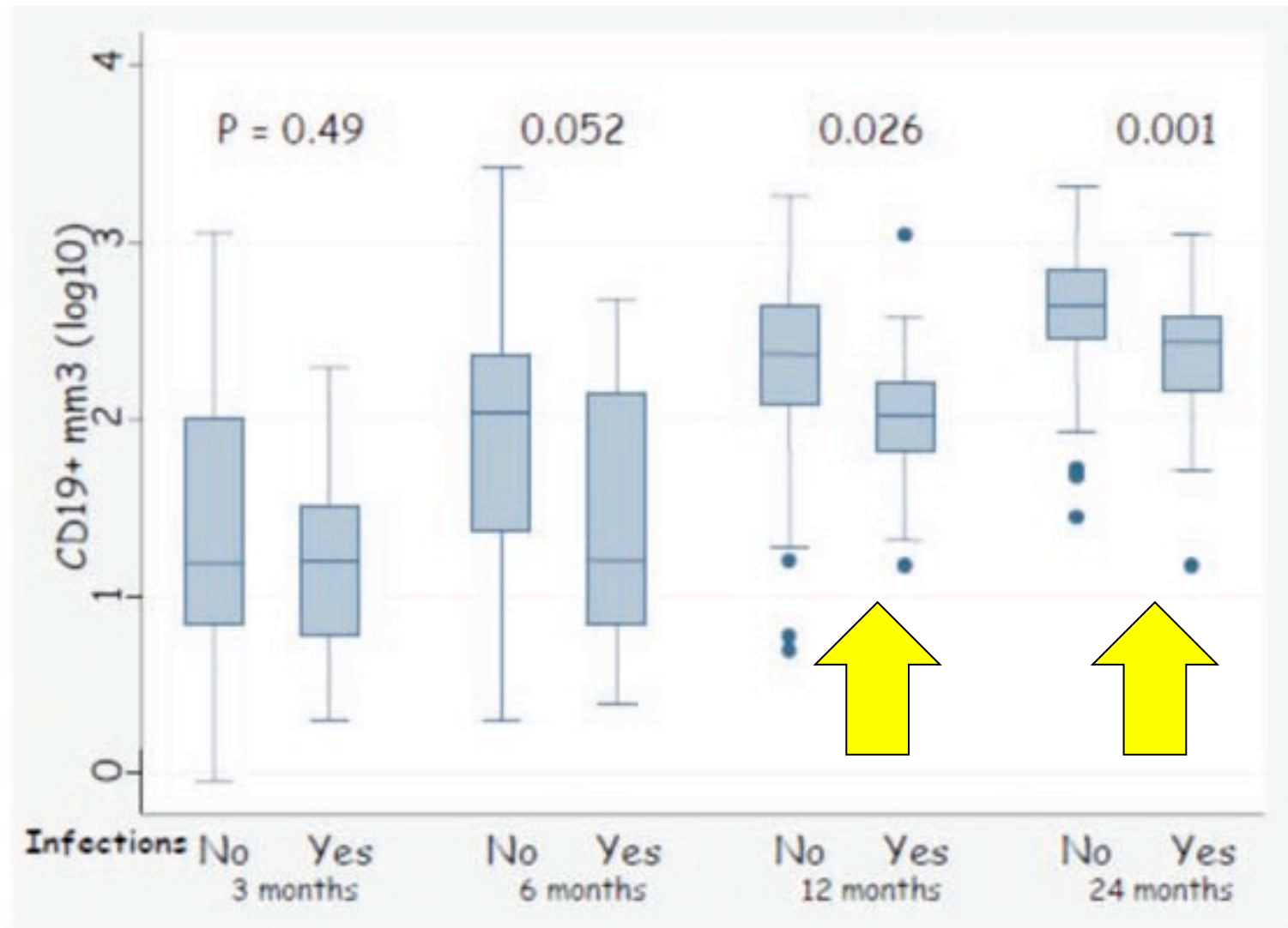


# Response to specific antigens

- chronic GVHD
- no GVHD
- normal



# Impact of B cell recovery on late infections



**recovery of innate  
immunity:  
NK  
gamma/delta  
DC**

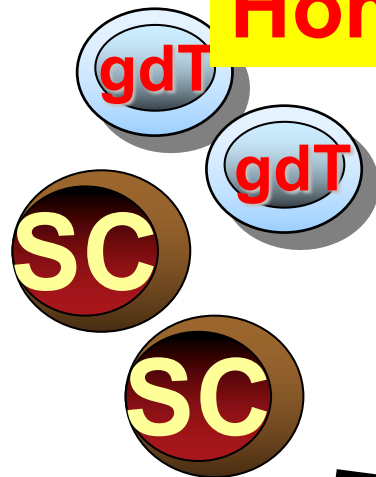


# NK and g/d T cell Recovery

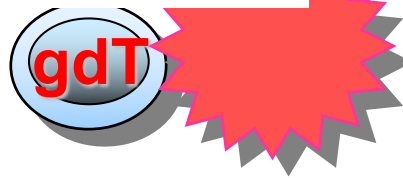
g/d T cells

1-2 months

Homeostatic Peripheral Expansion



Viruses



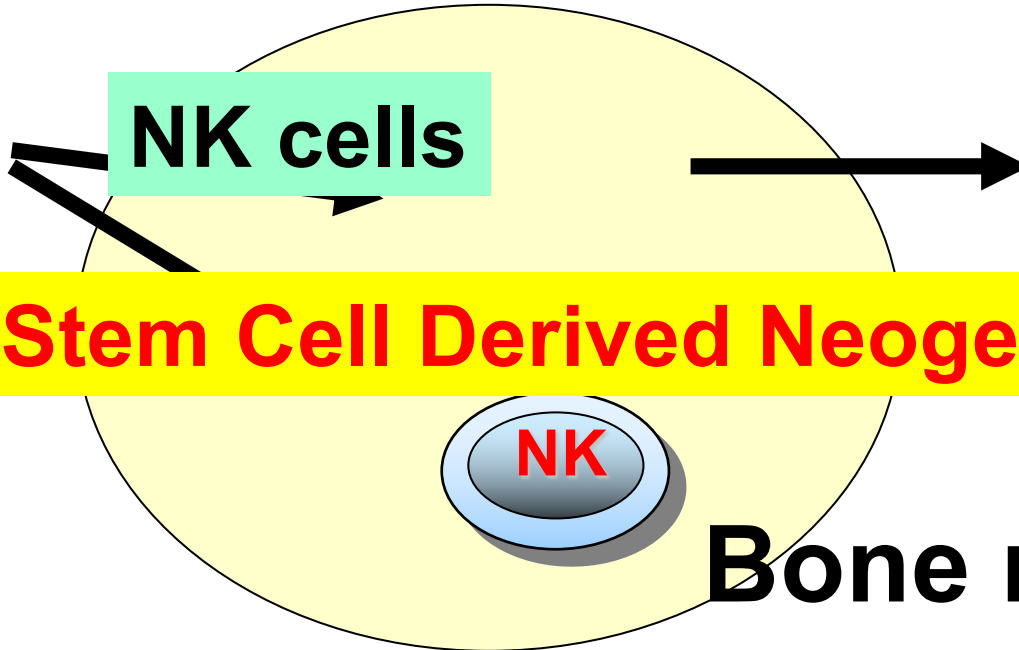
NK cells

1-2 months

Stem Cell Derived Neogenesis

NK

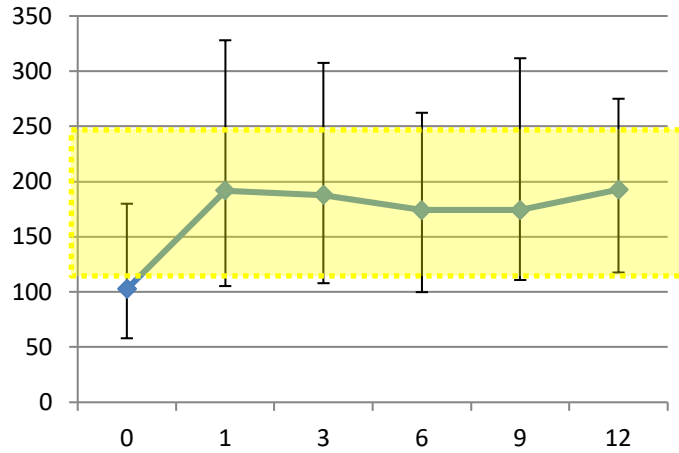
Bone marrow



# recovery of NK cells

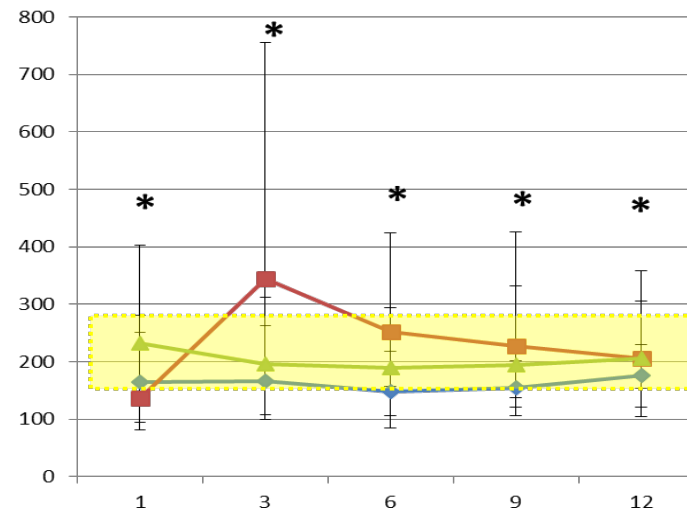


#NK



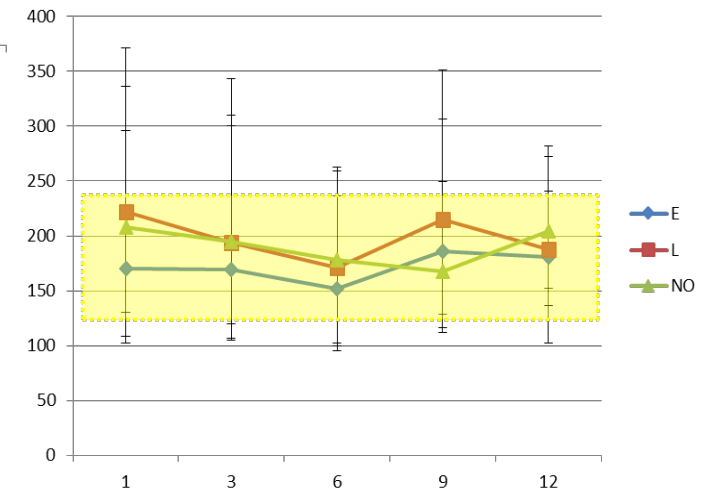
◆

#NK



◆ BM  
 ■ CB  
 ▲ PBSC

#NK



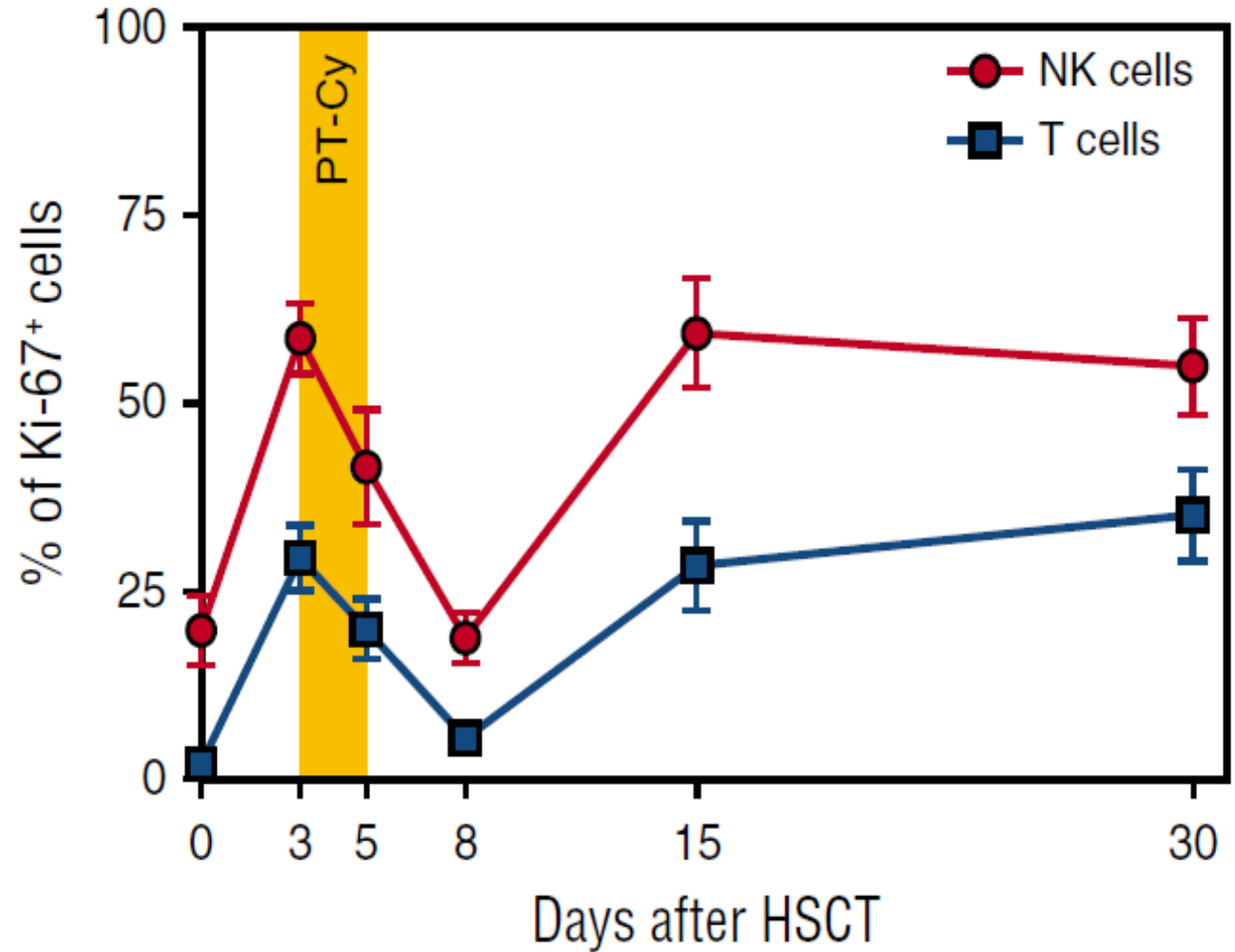
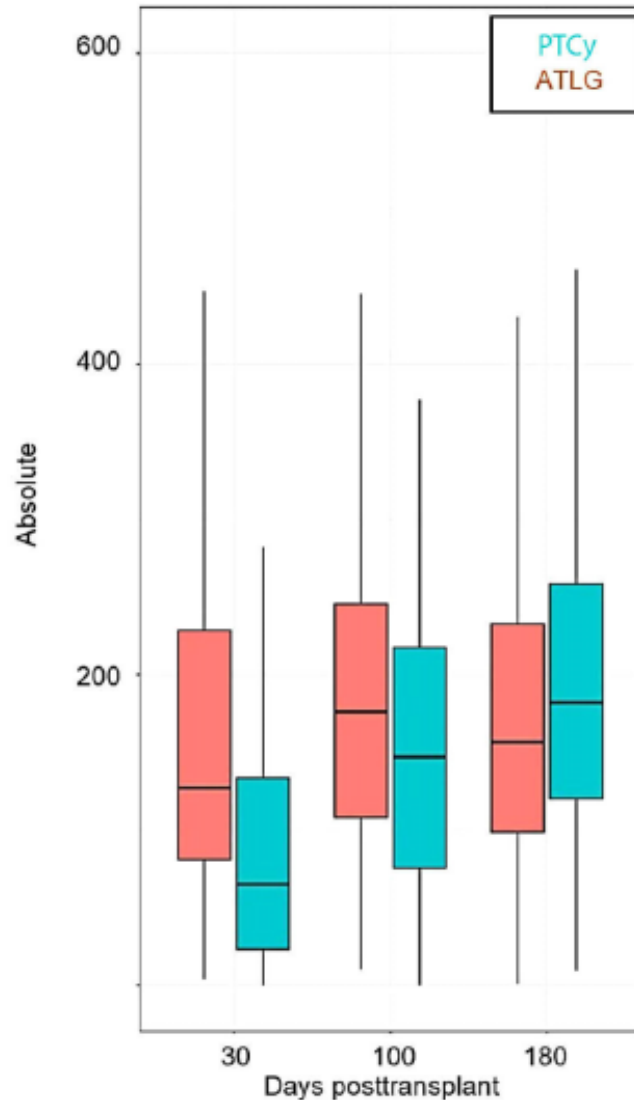
◆ E  
 ■ L  
 ▲ NO

Ulbar EBMT 2017  
 Bonifazi BMT 2018  
 Bonifazi BMT 2019

# NK RECOVERY delayed by pTCy

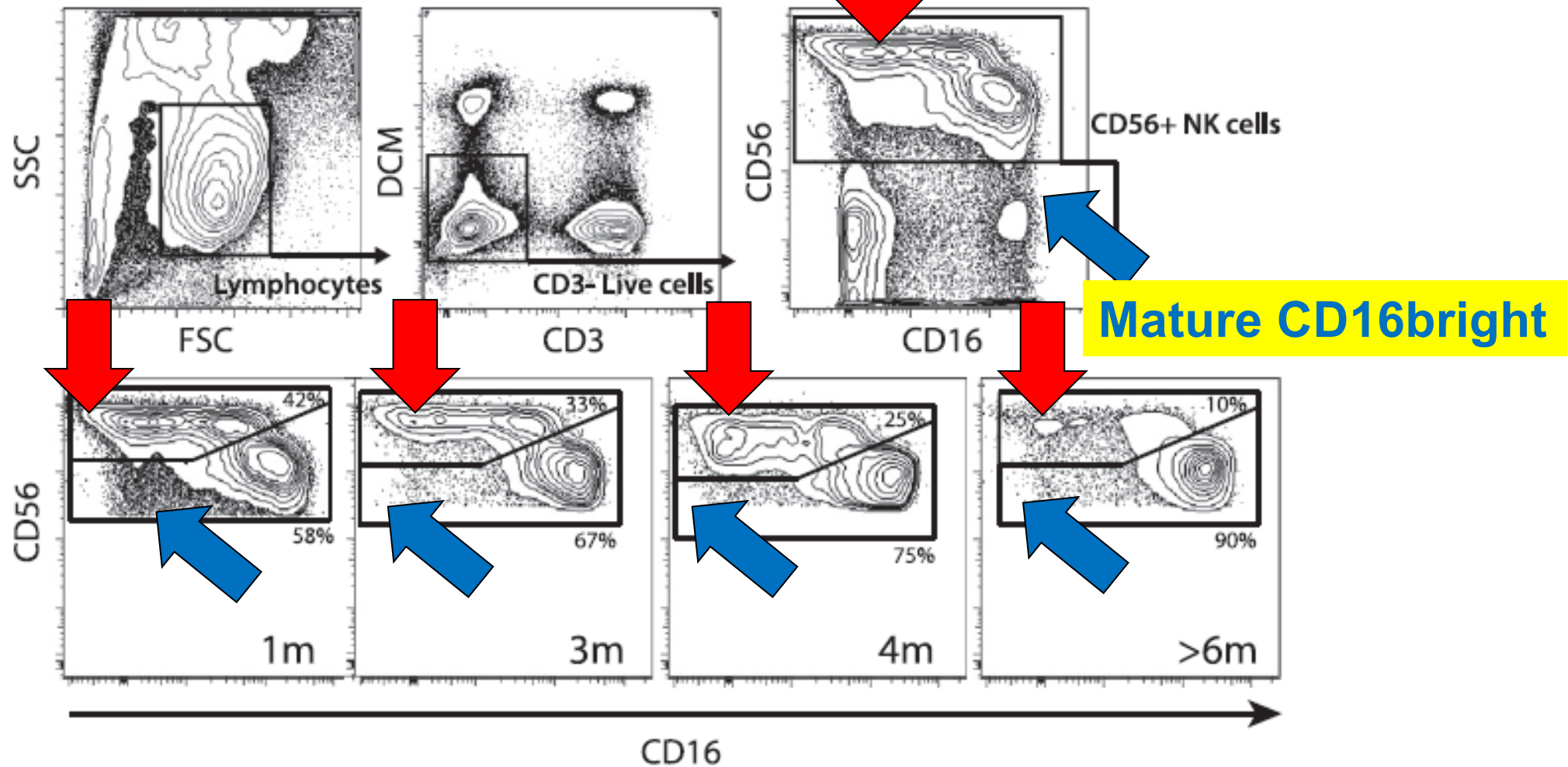
Massoud Haematologica 2022  
Russo Blood 2018

A



# Kinetics of NK Recovery

Immature CD56bright

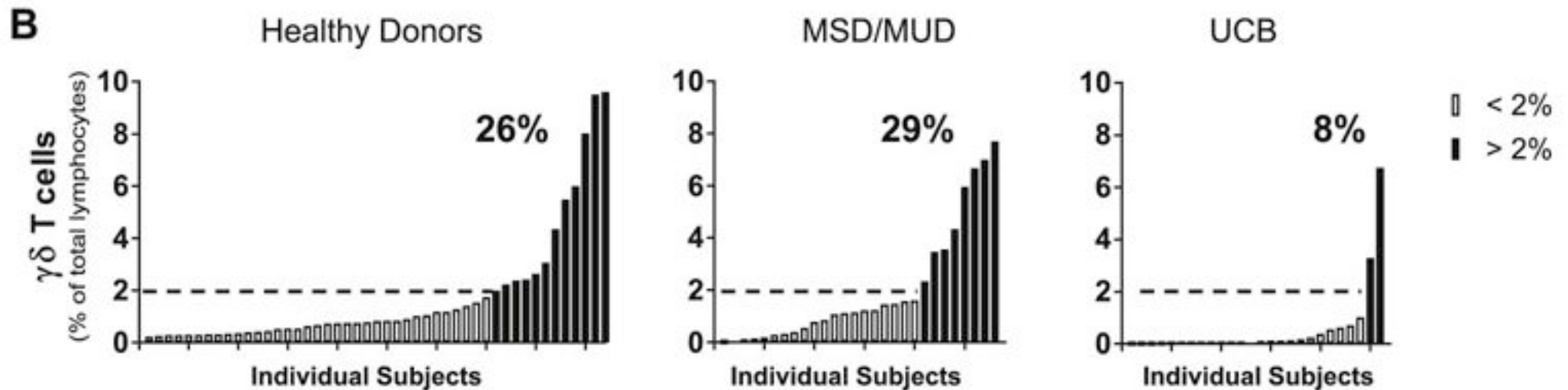


# Recovery of gamma/delta T cells

Early reconstitution of NK and  $\gamma\delta$  T cells and its implication for the design of post-transplant immunotherapy

Moniek A. de Witte<sup>1,2</sup>, Dhifaf Sarhan<sup>1</sup>, Zachary Davis<sup>1</sup>, Martin Felices<sup>1</sup>, Daniel A. Vallera<sup>3</sup>, Peter Hinderlie, Julie Curtsinger<sup>4</sup>, Sarah Cooley<sup>1</sup>, John Wagner<sup>5</sup>, Jurgen Kuball<sup>2,6</sup>, and Jeffrey S. Miller<sup>1</sup>

At 3 months

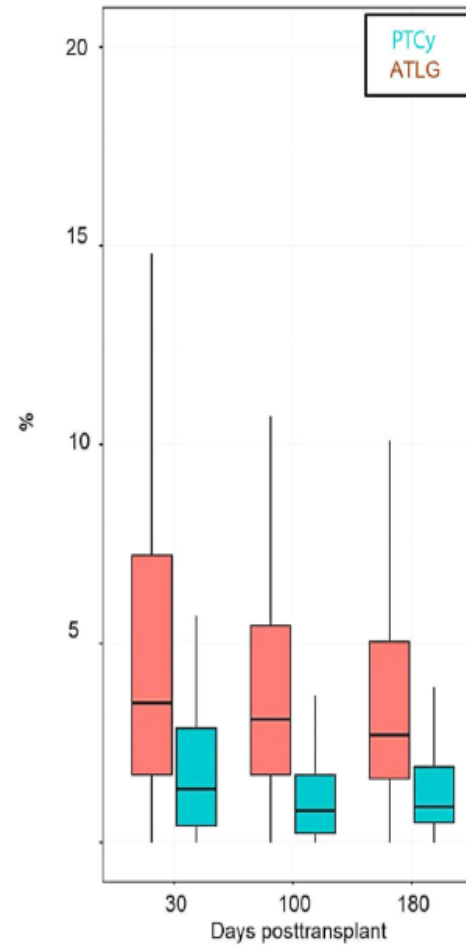
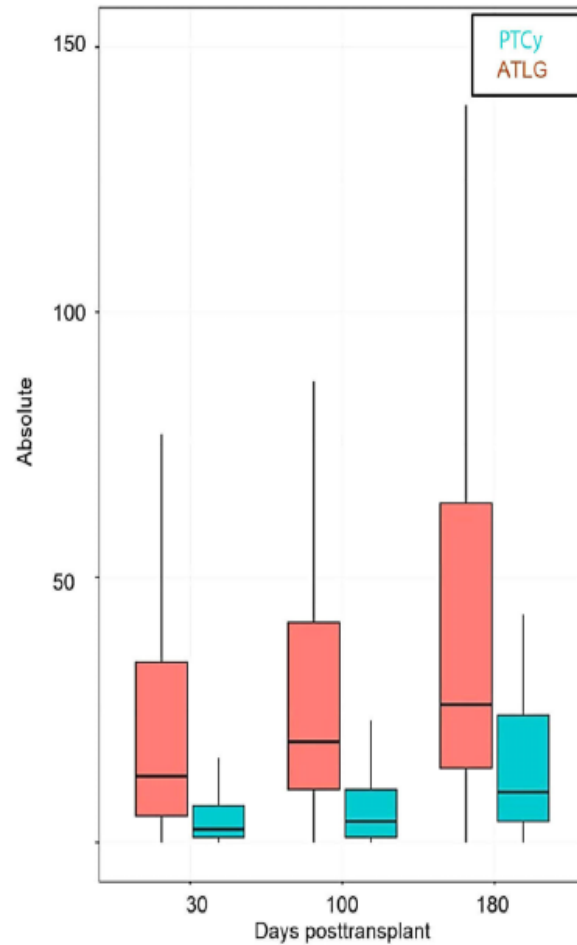


# pTCy reduces recovery of gamma/delta T

Massoud Haematologica 2022

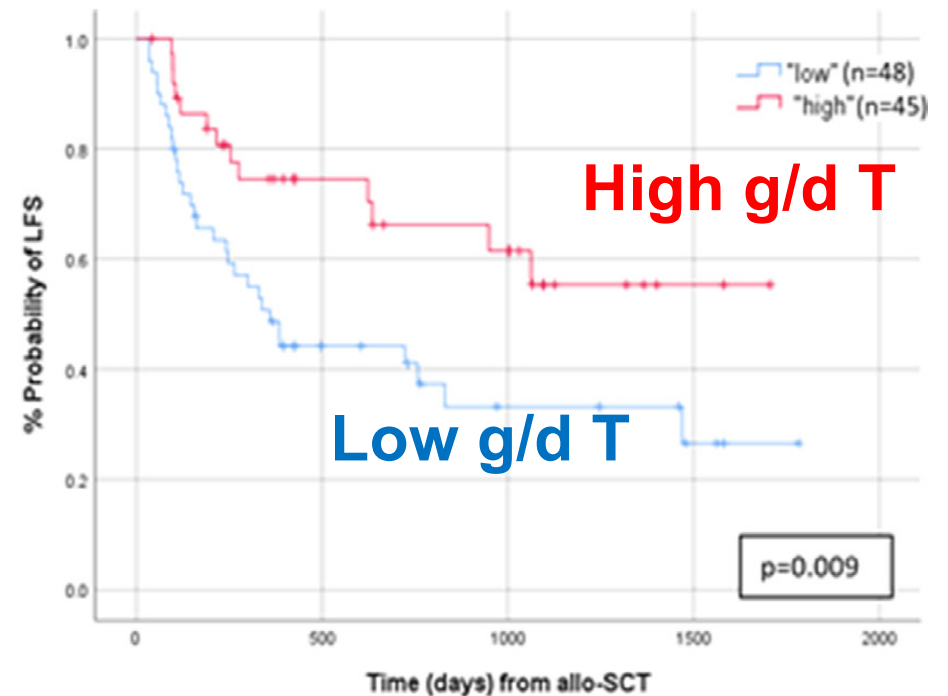
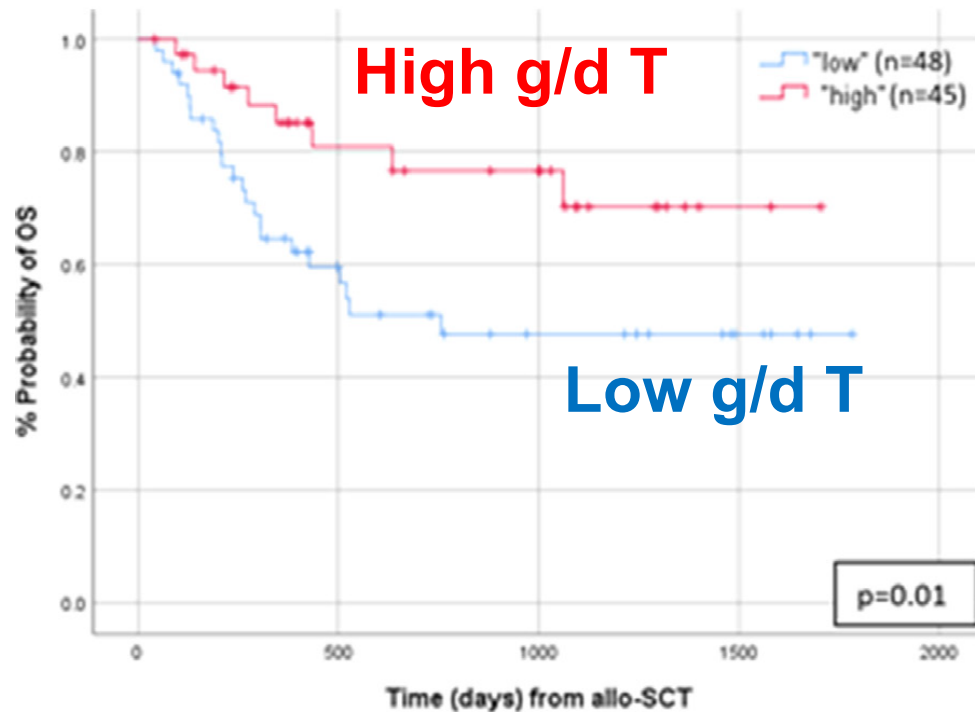
C

$\gamma\delta$  T cells



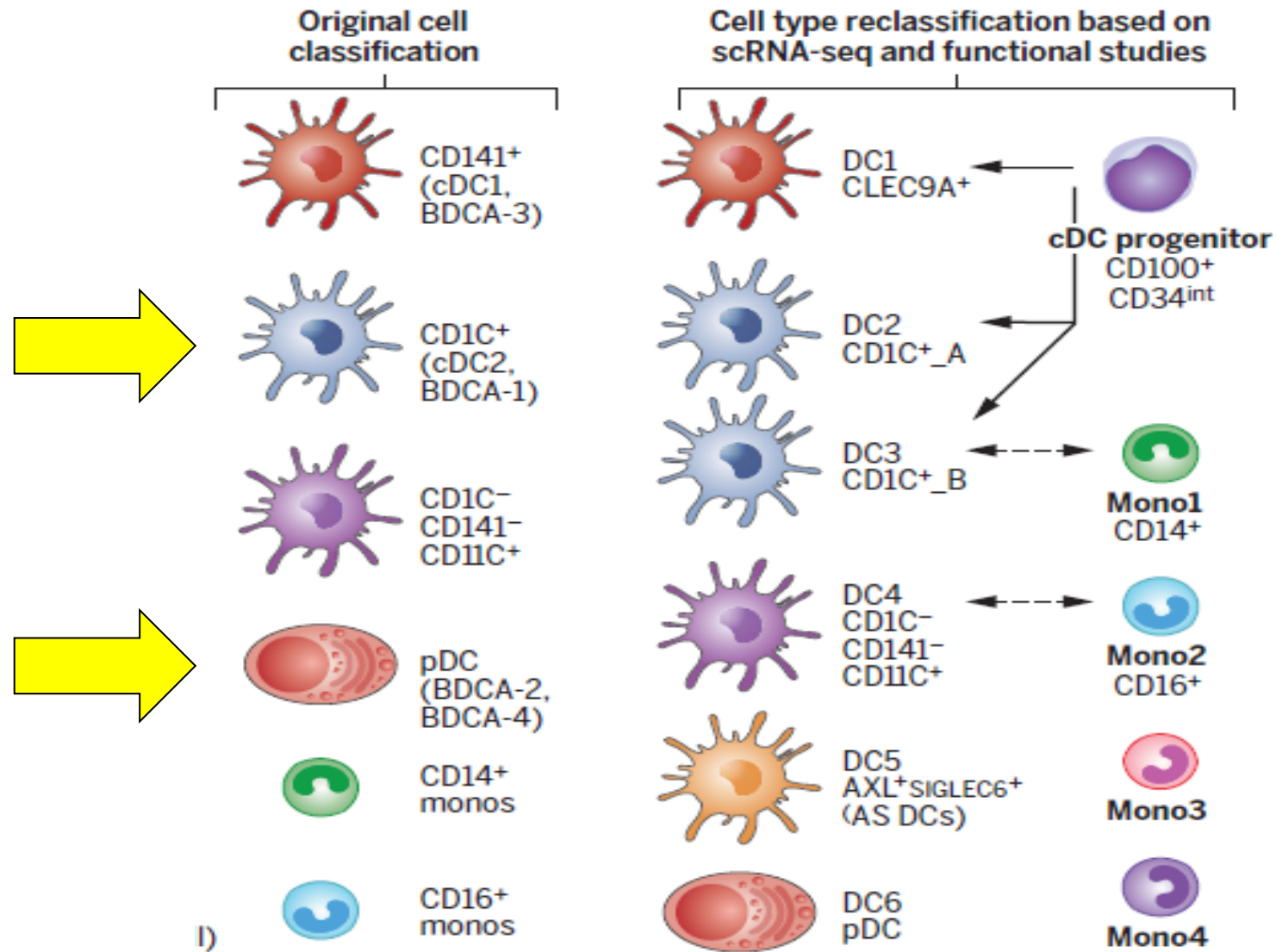
# Gamma/delta T cells mediated GVL

At 30 days



# The human DC monocyte system

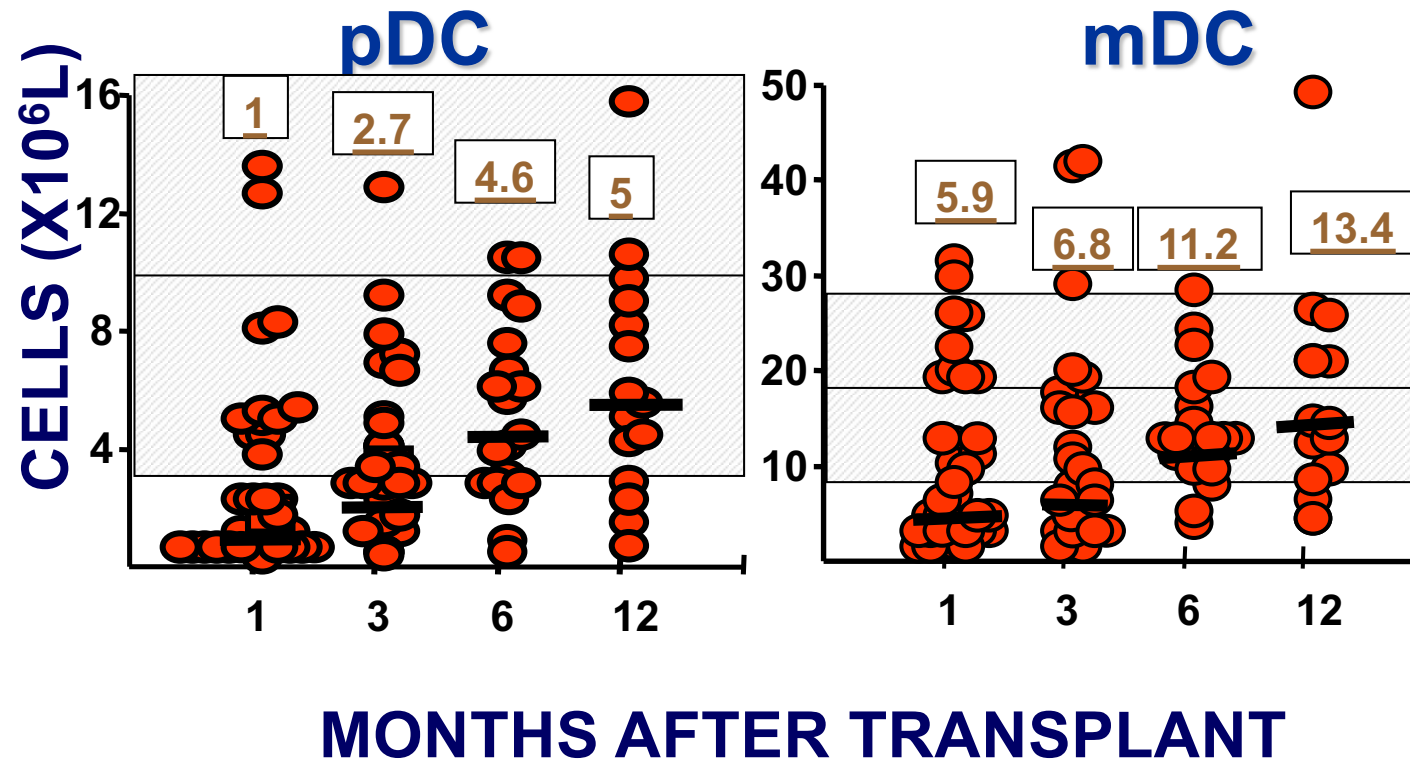
Villani Science 2017





# DC Reconstitution post Transplant

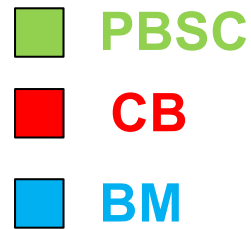
ARPINATI ET AL. BBMT 2004



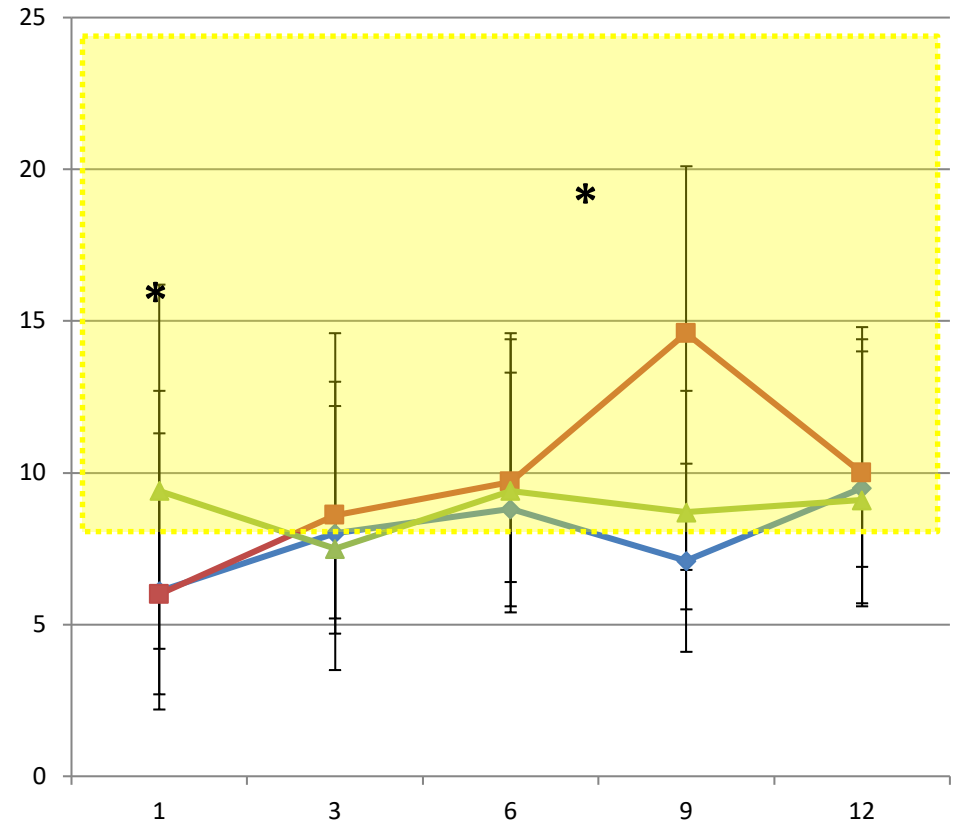
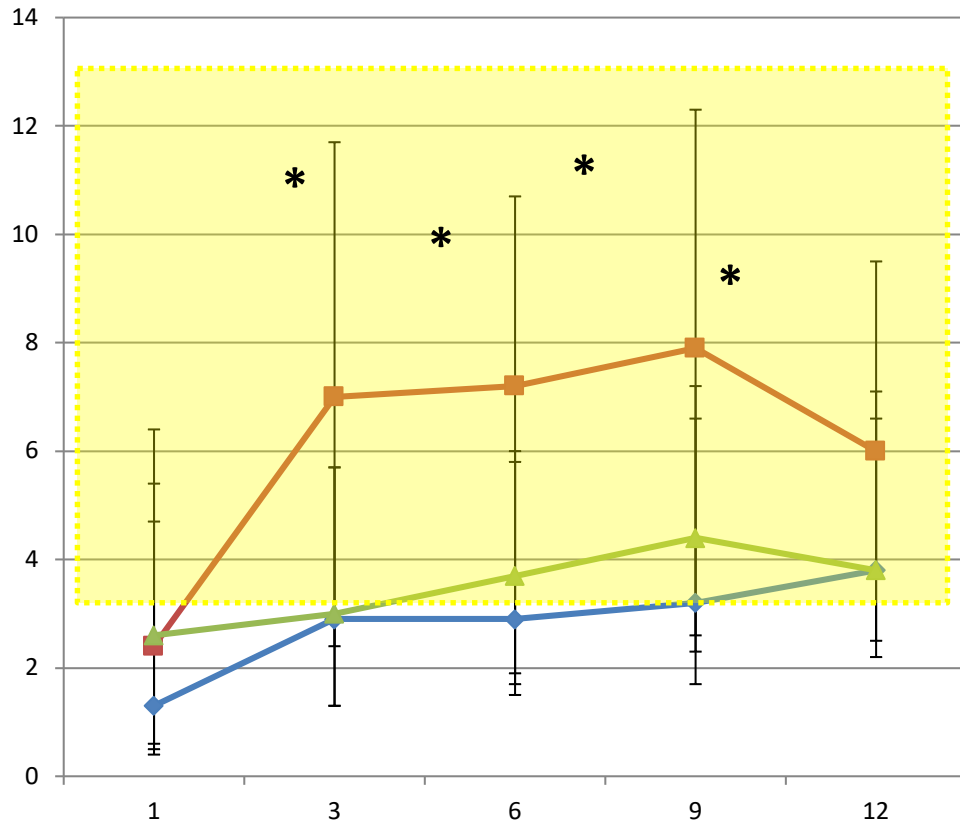
# Better recovery of pDC in CBT

Ulbar EBMT 2017

pDC



mDC

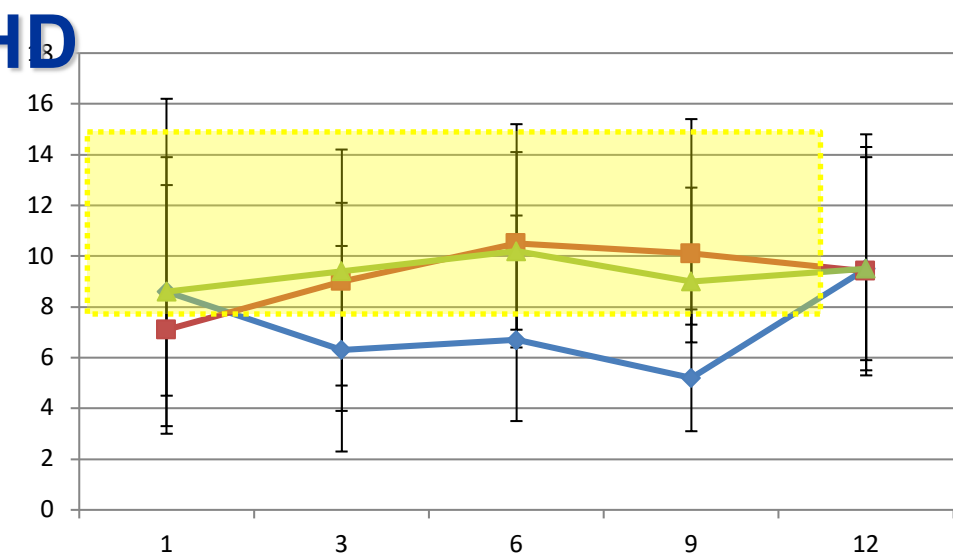
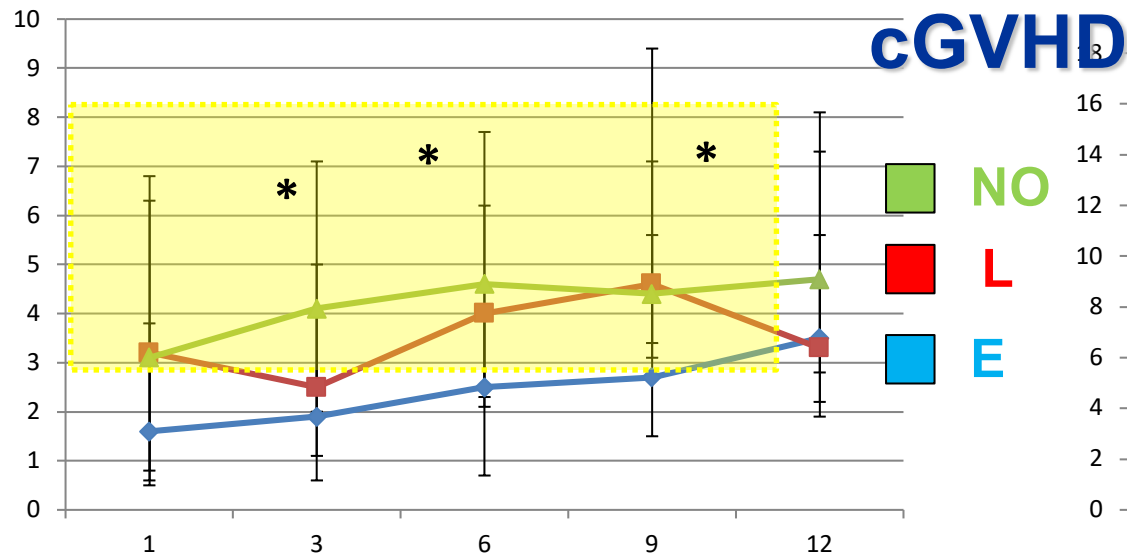
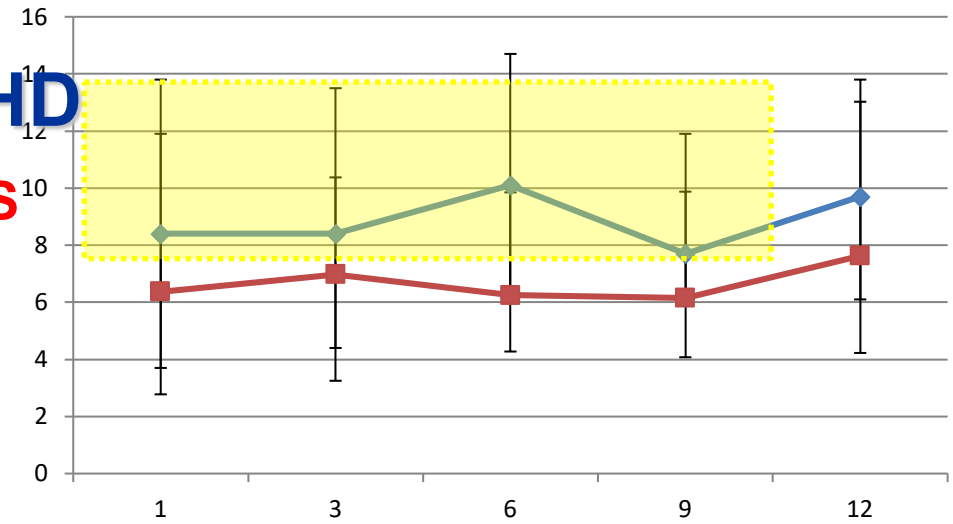
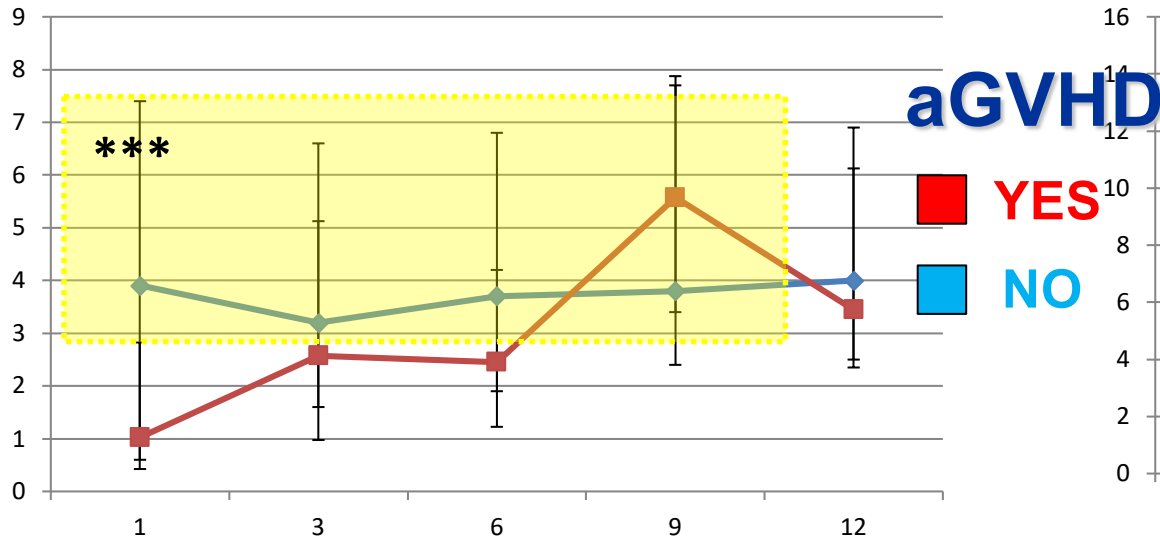


# Loss of pDC in chronic GVHD

Ulbar EBMT 2017  
Chirumbolo 2022 submitted

pDC

mDC



# **Clinical significance of Immune Monitoring?**

# Recommended Assessment of Immune Recovery

## T cells:

- CD4+ and CD8+ counts
- naive T cell counts
- TCR repertoire analysis
- antigen-specific responses (e.g. CMV)

 : recommended

 : usually performed

 : experimental

## B cells:

- CD19+ cell counts
- B cell subset counts
- Serologic response to vaccines

## Innate immunity:

- NK and gamma/delta T cell counts
- DC counts

## **Immunology Lab**

**GABRIELLA CHIRUMBOLO**

**MARTINA BARONE**

## **Immunotherapy Program**

**FRANCESCA BONIFAZI**

**ENRICO MAFFINI**

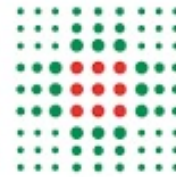
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**MICHELE DICATALDO**

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## **IRCCS S.Orsola-Malpighi**



**SERVIZIO SANITARIO REGIONALE  
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IRCCS Istituto di Ricovero e Cura a Carattere Scientifico

## **University of BOLOGNA**

