# γδ T cells and more in HLA-haploidentical HSCT

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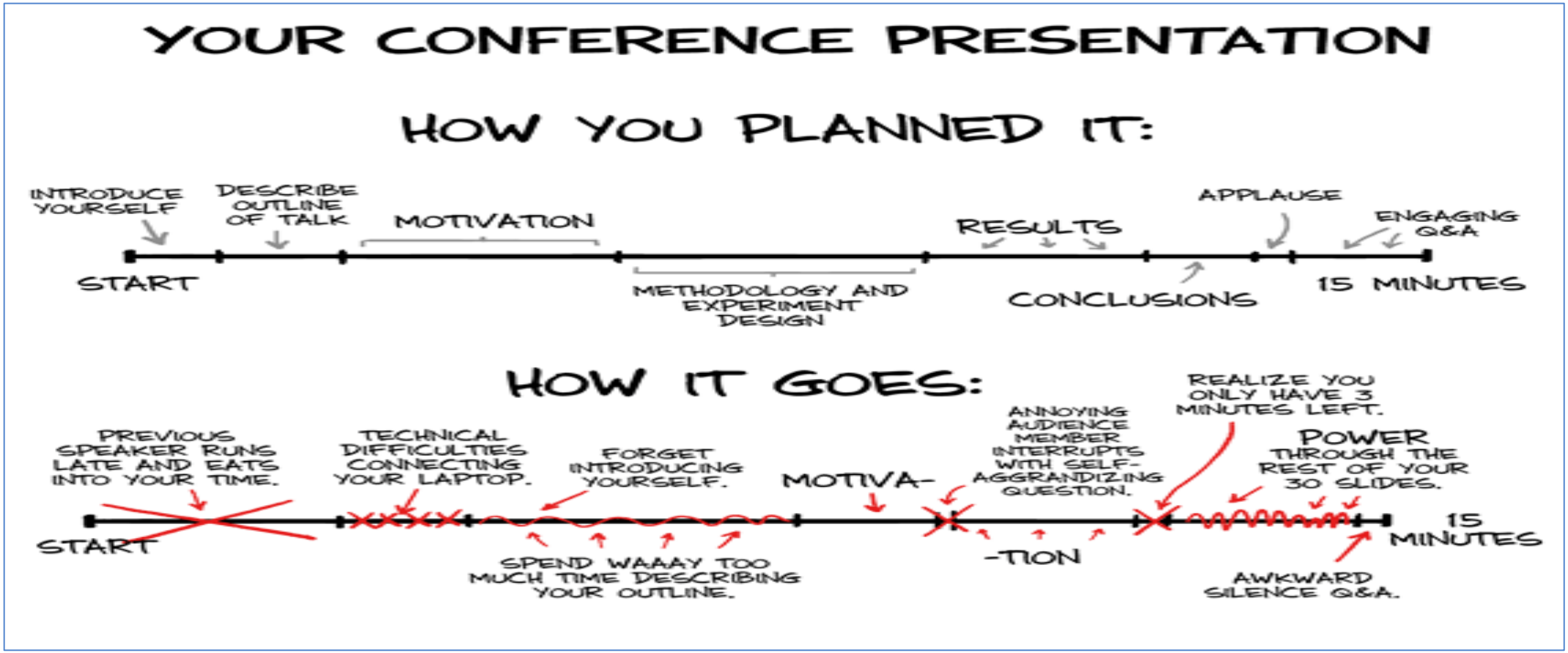
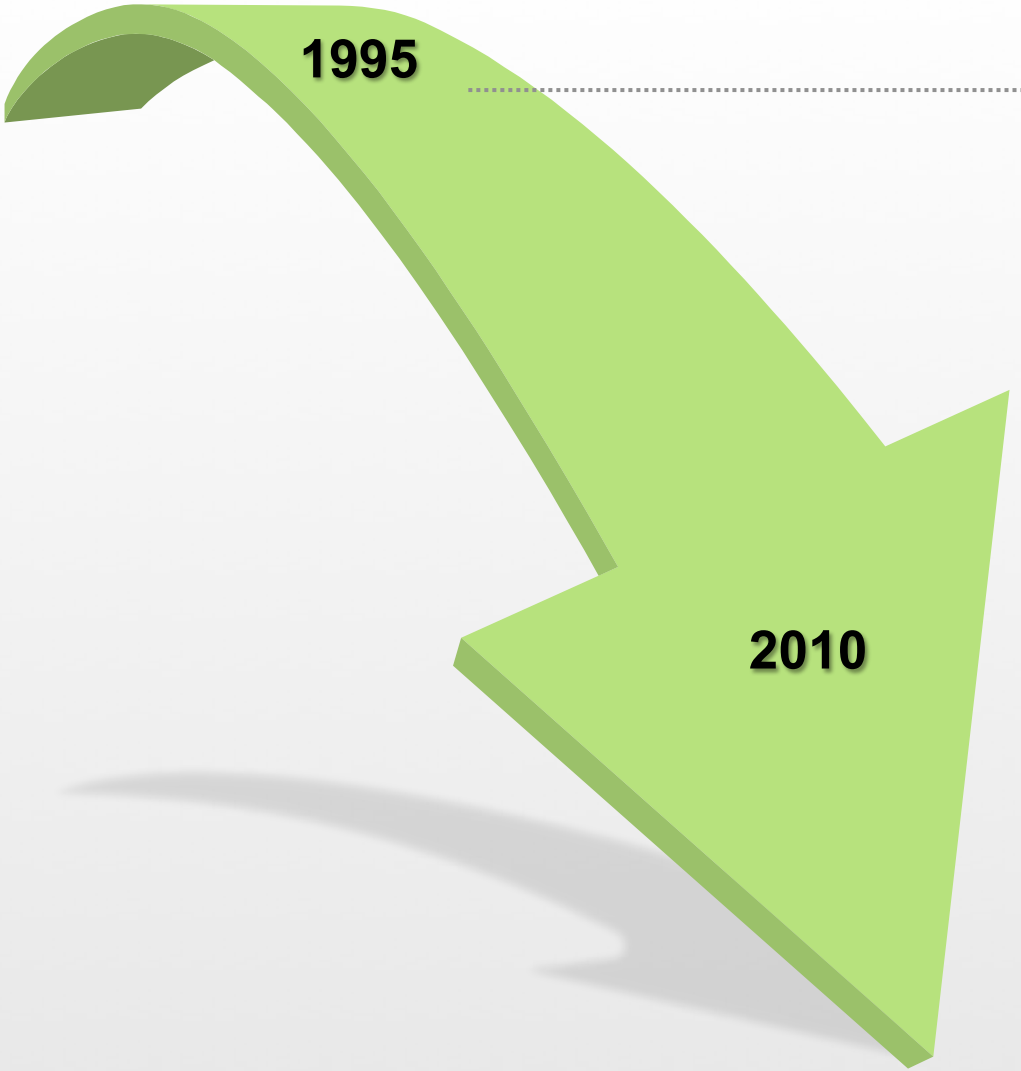
# Plan

- Historical perspective
- TCRαβ/CD19 depletion
- TCRγδ
- Clinical experience with BPX-501 cells



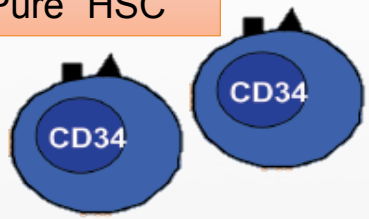

www.phdcomics.com

# Haploidentical donors: evolution of T-cell depletion strategy

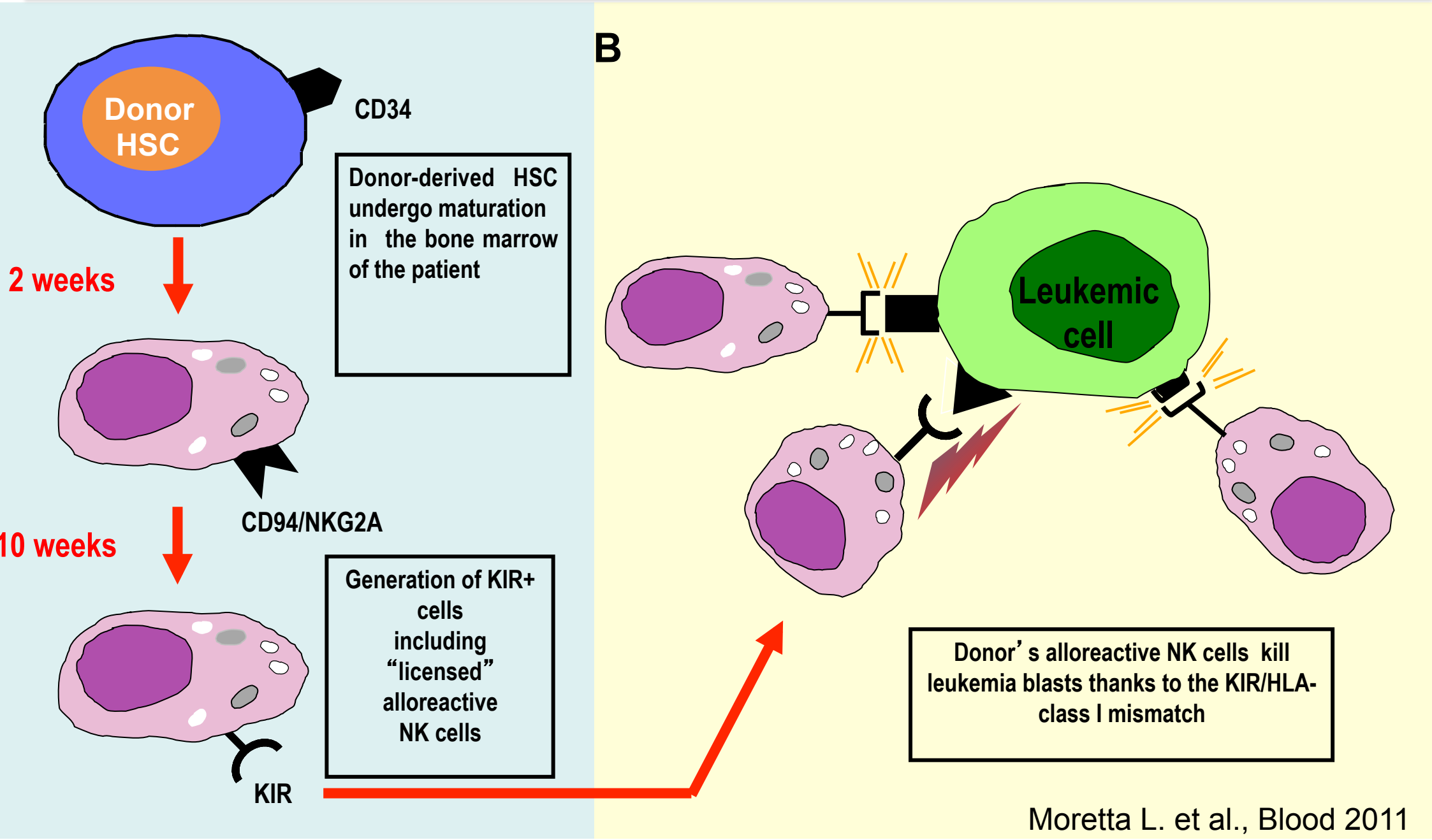



CD34+ selection

„Pure“ HSC

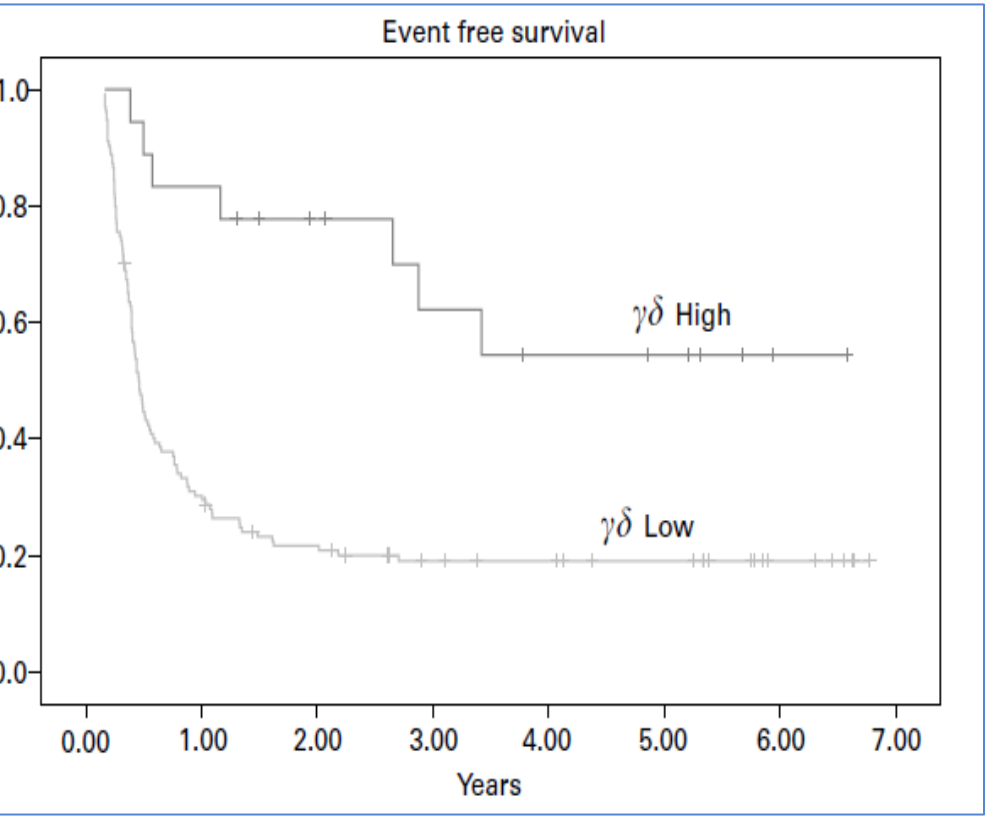


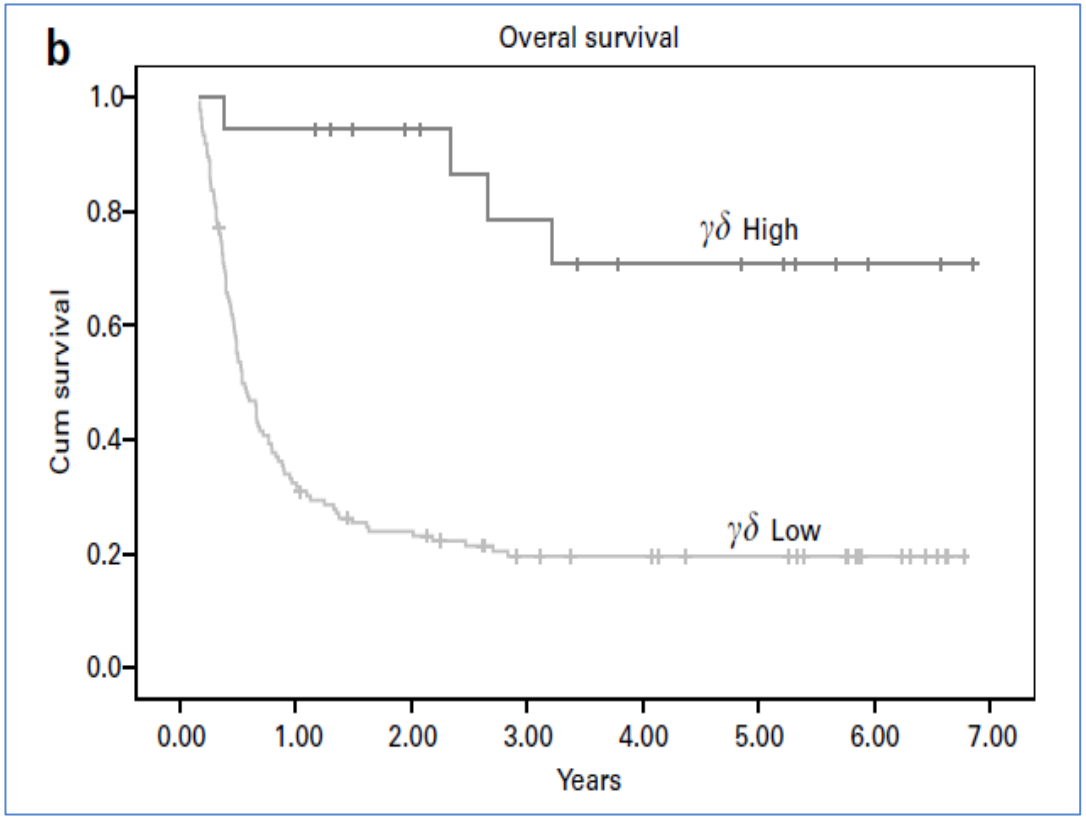
# Origin and differentiation of KIR+ alloreactive NK cells in haplo-HSCT




Moretta L. et al., Blood 2011

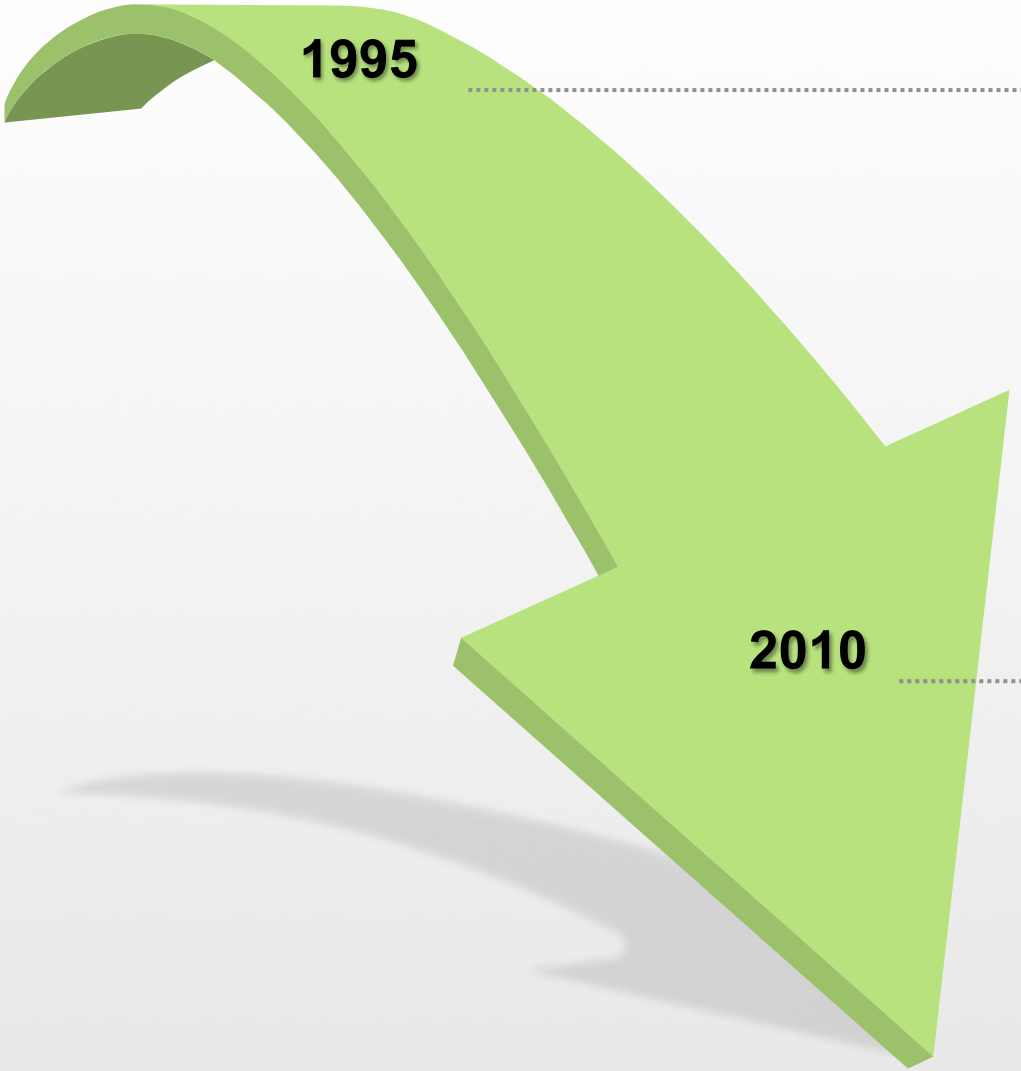
# Haploidentical donors: evolution of T-cell depletion strategy



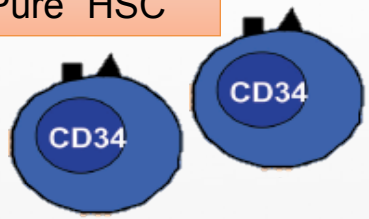

Godder K.T. et al., BMT 2007

# Haploidentical donors: evolution of T-cell depletion strategy



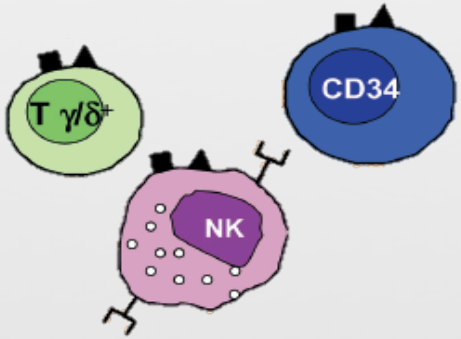

CD34+ selection

„Pure“ HSC

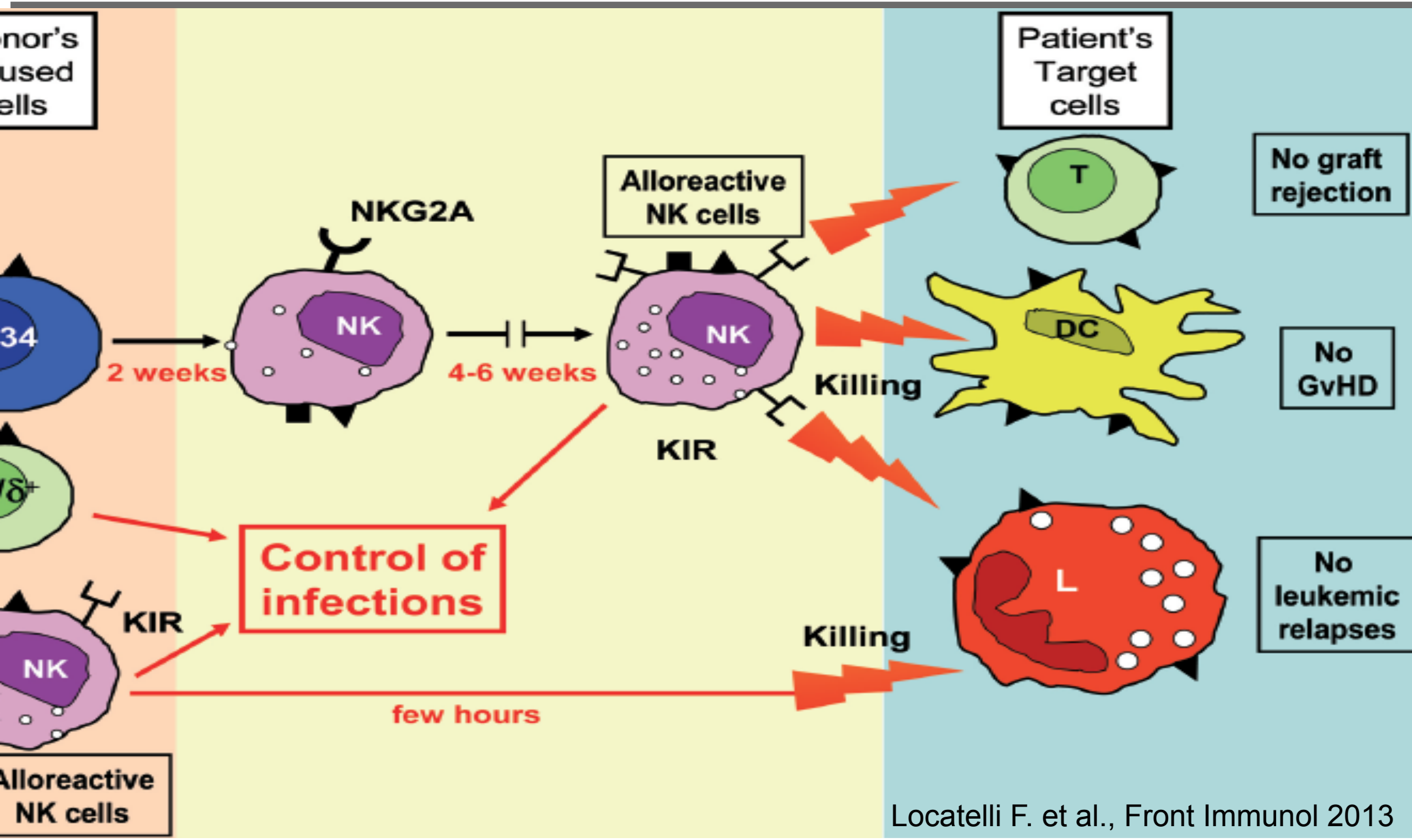



TCRαβ/CD19 depletion

HSC + effectors
(NK cells + γδ T cells)



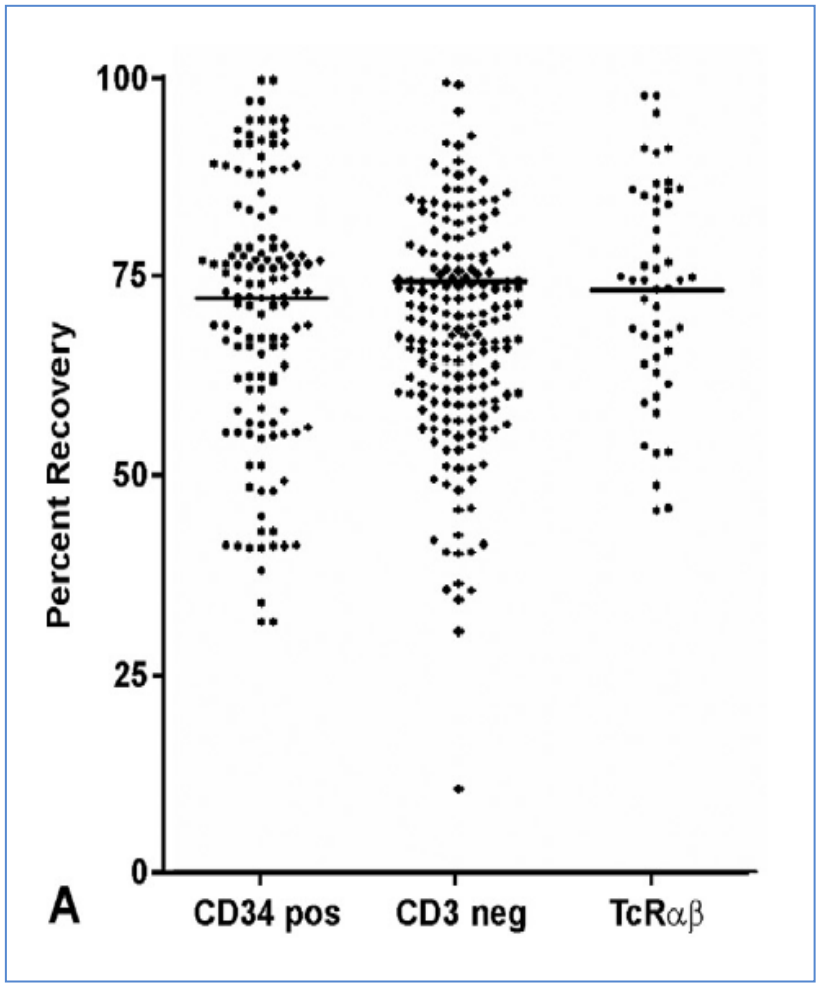
# T-cell depletion strategies

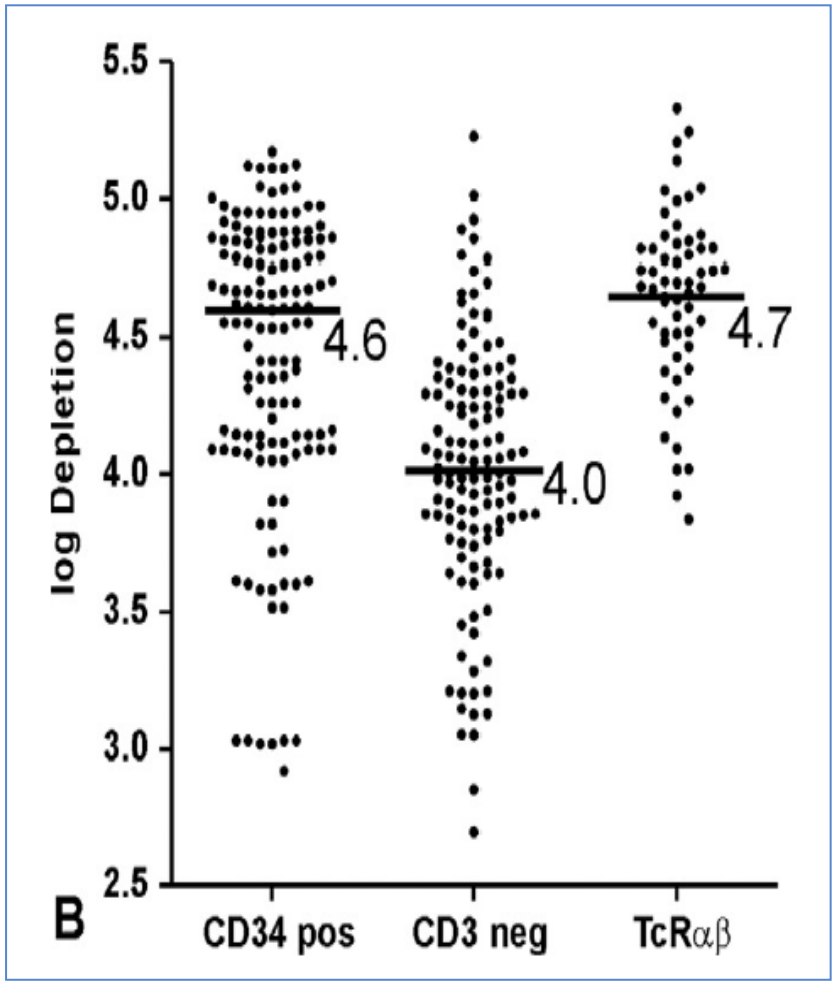



Locatelli F. et al., Front Immunol 2013

## Depletion of TCR $\alpha\ \beta$ + cells

### CD34+ recovery and T cell depletion

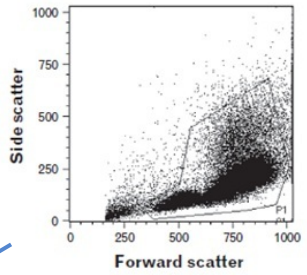


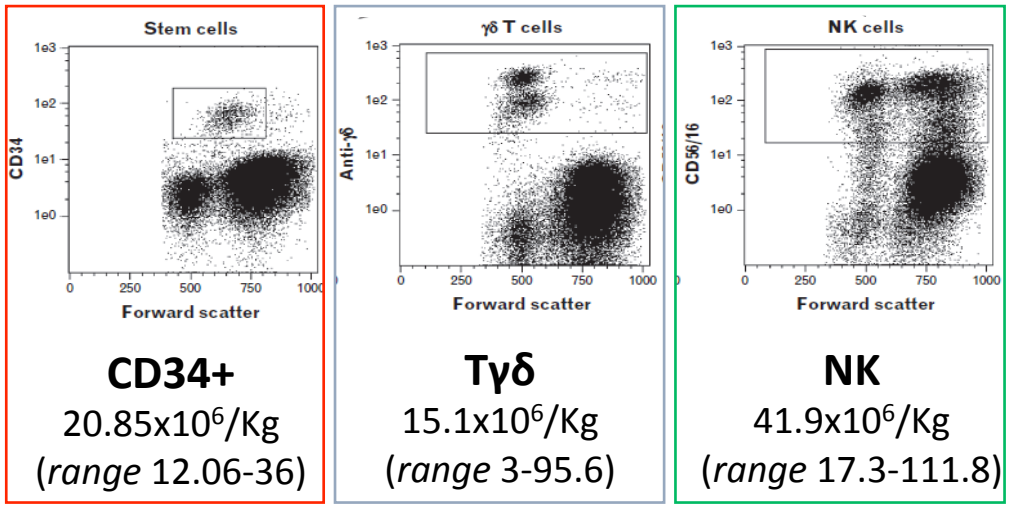




Handgretinger R., Sem Hematol 2012

# Depletion of TCR $\alpha$ $\beta$ + cells

**Nucleated Cells**
**1.3x10$^9$/Kg (range 0.71-2.43)**




**CD34+**
20.85x10$^6$/Kg
(*range* 12.06-36)

**Tγδ**
15.1x10$^6$/Kg
(*range* 3-95.6)

**NK**
41.9x10$^6$/Kg
(*range* 17.3-111.8)

| | |
|---|---|
| **Tαβ** | **4.25x10$^4$/Kg (*range* 0.4-9.4)** |
| **CD20+** | **3.65x10$^4$/Kg (*range* 1-12.3)** |

## IDEAL GRAFT COMPOSITION

- High CD34+
- High Tγδ
- High NK
- Low Tαβ
- Low CD20+

Adapted from Hangretinger, Sem Hematol 2012

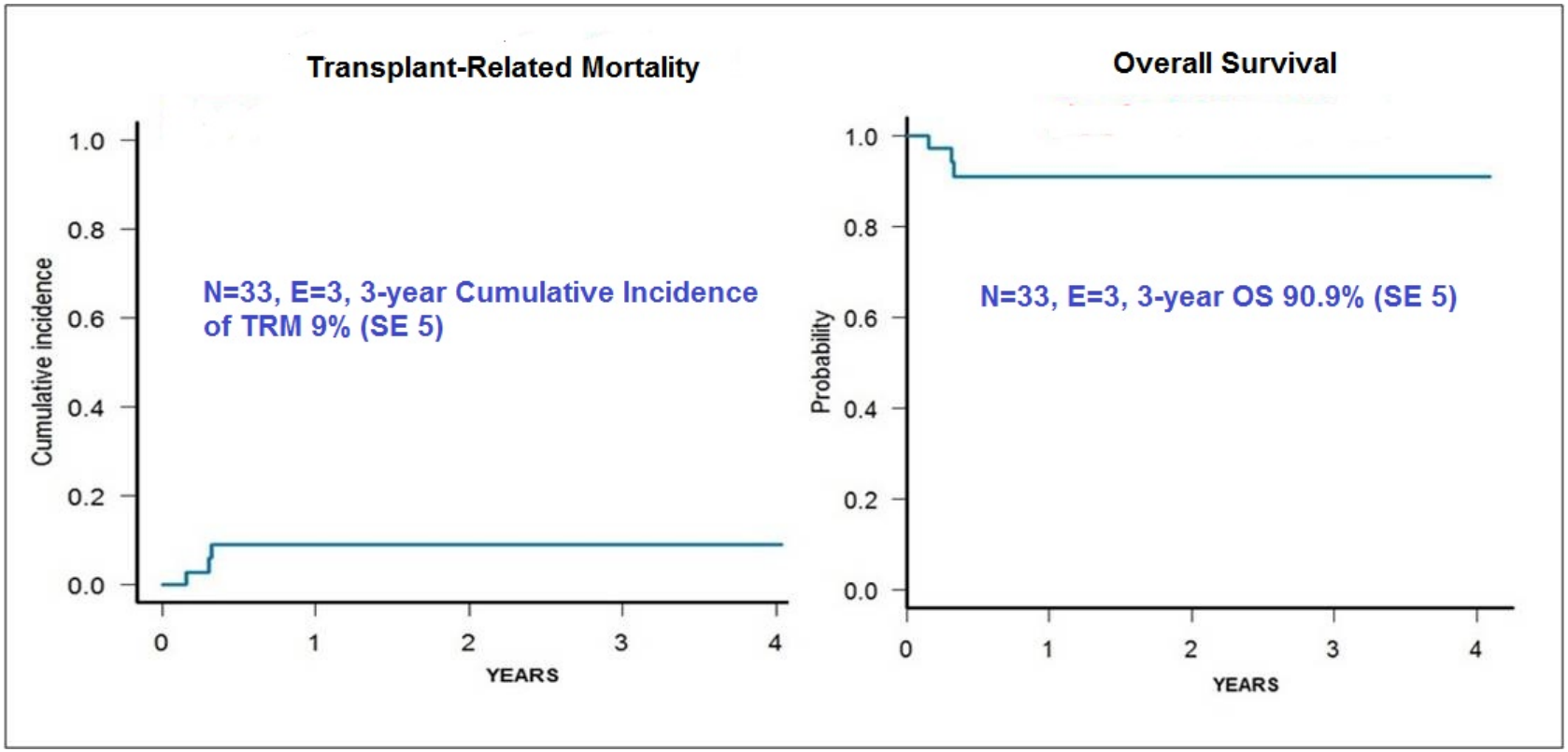
# Depletion of TCR $\alpha\ \beta$ + cells

Brief Report

BLOOD, 31 JULY 2014 • VOLUME 124, NUMBER 5

## 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]



## Depletion of TCR $\alpha\beta$ + cells

Regular Artic

# Outcome of chi
# HSCT after αβ

Franco Locatelli,[1,2] Pietro I lli,[5]
Barbarella Lucarelli,[1] Letizi
Mattia Algeri,[1] Rita Maria F
Alessandro Moretta,[8] Alice

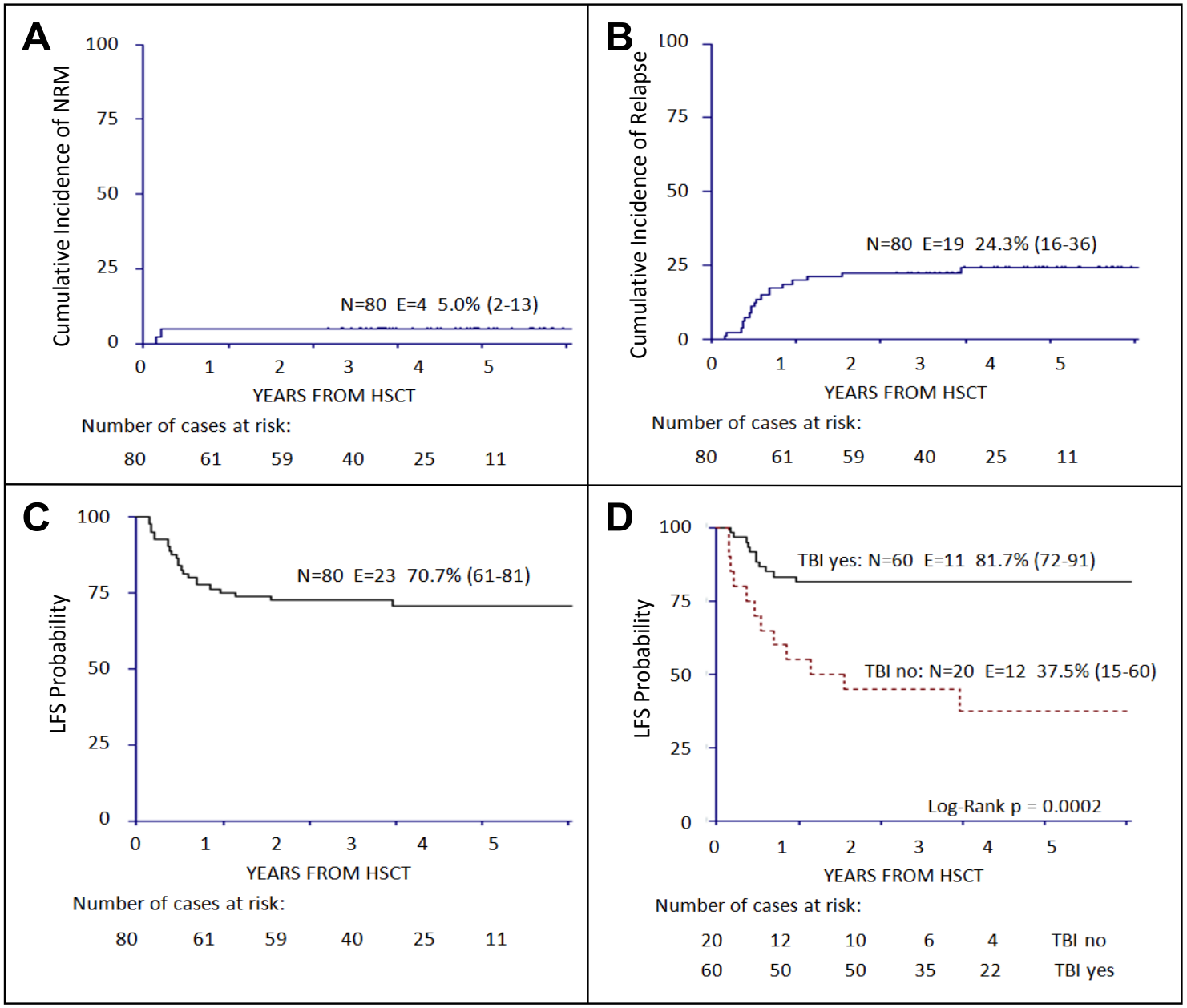
**Table 1. Patient, donor, and transplantation characteristics**

| Patients | (n = 80) |
|---|---|
| **Sex** | |
| Male | 55 (69%) |
| Female | 25 (31%) |
| Median (range) age at diagnosis, y | 6.6 (0.4-16.8) |
| Median (range) age at transplantation, y | 9.7 (0.9-20.9) |
| **Disease** | |
| ALL | 56 (70%) |
| AML | 24 (30%) |
| **ALL phenotype** | |
| BCP | 41 (73%) |
| T | 15 (27%) |
| **ALL recurrent molecular lesions** | |
| t(4;11)(AF4/MLL) | 3 |
| t(9;22)(BCR/ABL) | 2 |
| SIL-TAL | 1 |
| t(12;21)(TEL/AML1) | 2 |
| Hypodiploid | 1 |
| **AML recurrent molecular/cytogenetic lesions** | |
| MLL/FLT3-ITD | 5 |
| 7- | 1 |
| Complex karyotype | 3 |
| inv(16) (MYH11-CBFB) | 2 |
| Other | 1 |
| **Disease status at transplantation** | |
| ALL | |
| CR1* | 15 (19%) |
| CR2† | 37 (46%) |
| ≥CR3 | 4 (5%) |
| AML | |
| CR1‡ | 16 (20%) |
| CR2 | 8 (10%) |

**Key Points**

ldren with AL given haplo-
CT after αβ T- and B-cell
letion are exposed to a
risk of acute and chronic
HD and NRM.
leukemia-free, GVHD-
survival of patients given
type of allograft is
nparable to that of HLA-
tched donor HSCT
pients.

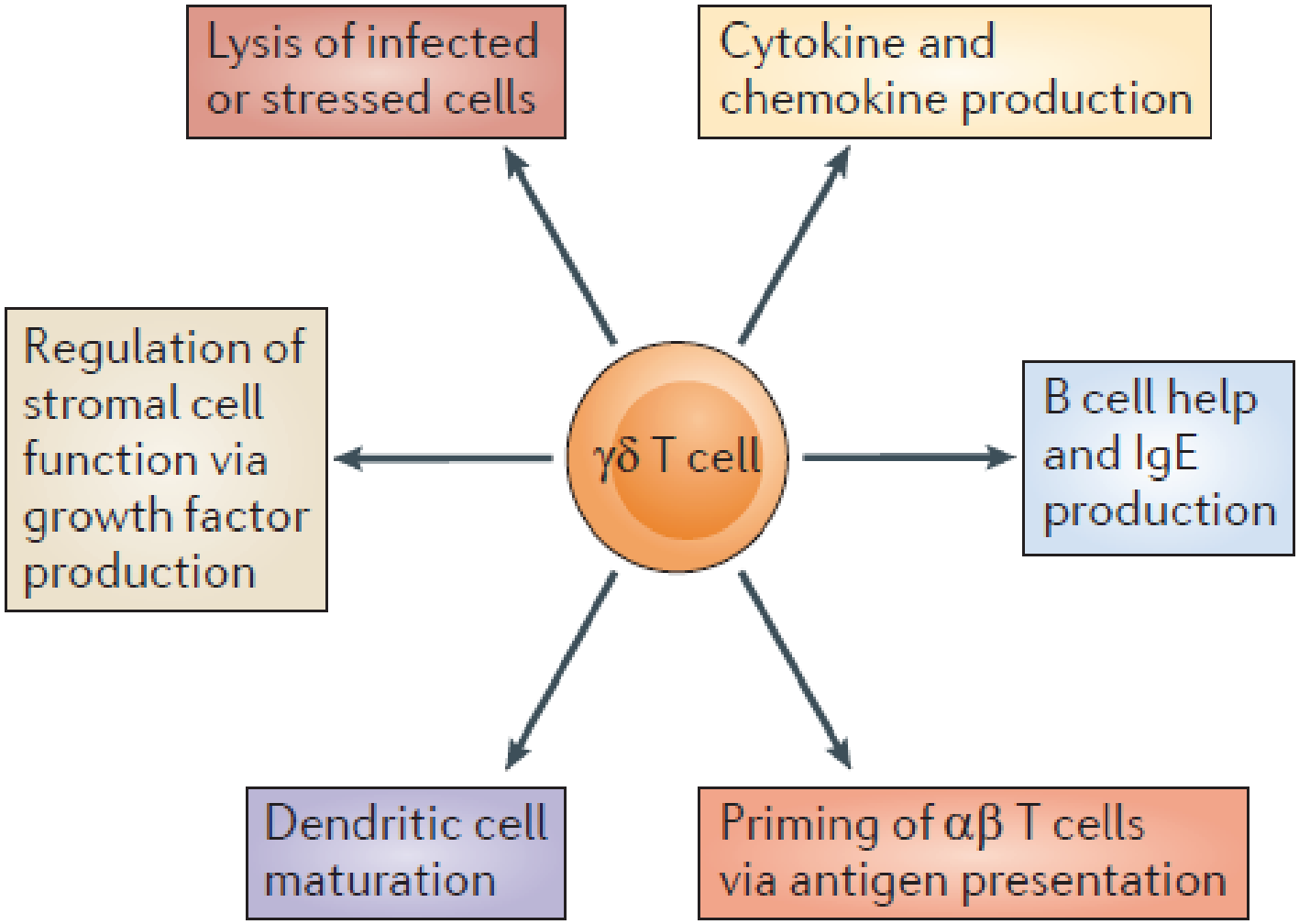
# Depletion of TCR $\alpha\ \beta$ + cells




Locatelli F, et al. Blood 2017

# γ δ T-cell

on-alloreactive, “innate-like”, T
mphocyte subpopulation;
ormally accounting for 1-10%
circulating T lymphocytes;
pable of recognizing targets in
MHC-independent manner
rough several activating
ceptors, like γδ-TcR, NKG2D
d TLRs;
fferent functions, such as anti-
ective and anti-tumor activity




Vantourot, Nat Rev Immunol 2012

## Human γ δ T-cell subsets

### Vδ2 (Vγ9)

Predominant circulating γδ T-cell subset

Recognition of phospho-antigens
(TCR e NKG2D)

*In vitro* activation upon
zoledronic acid stimulation
(± IL-2)

### Vδ1

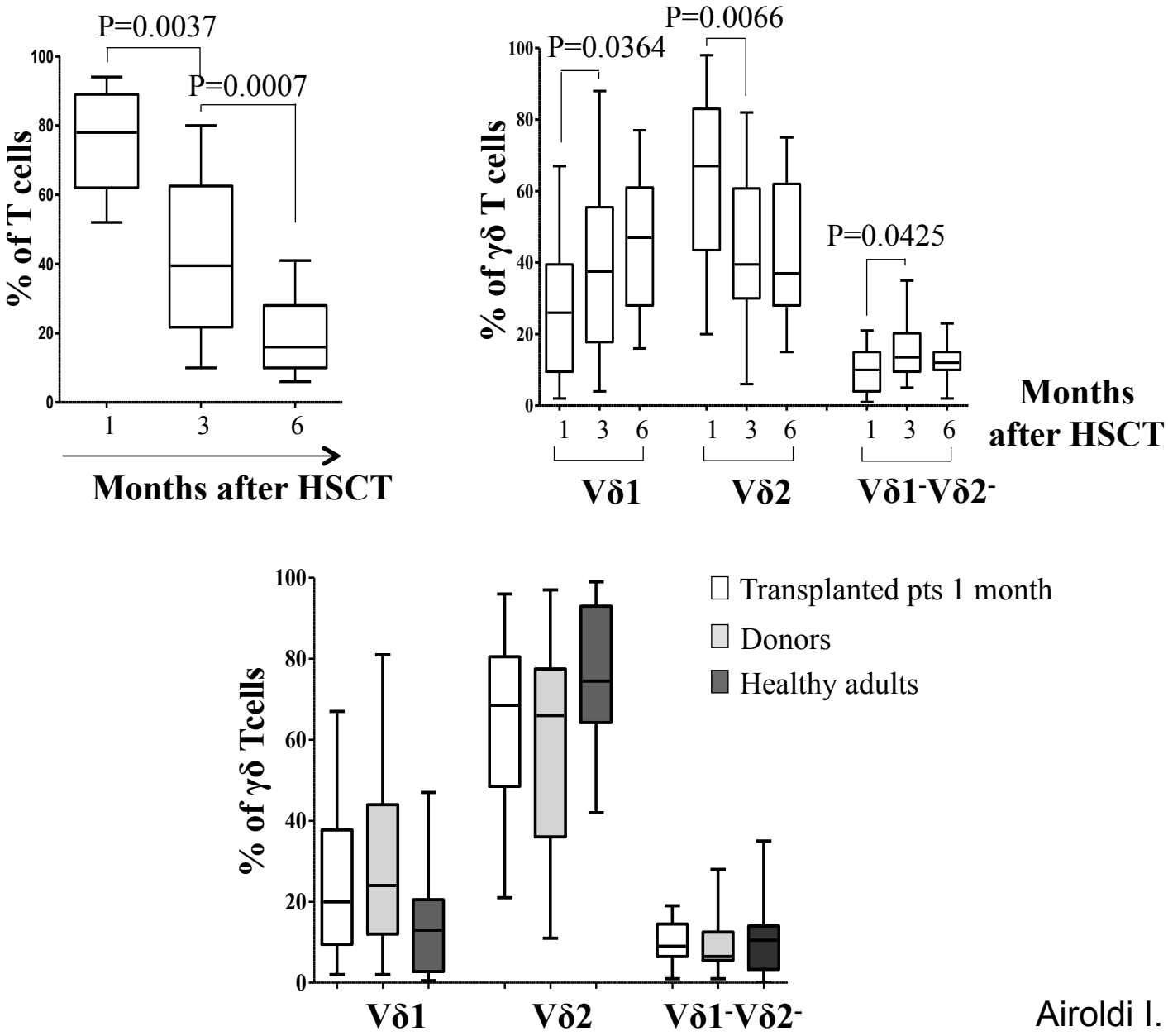
Predominant γδ T-cell subset in the tissues

Recognition of antigens (?) and ligands
(TCR, NKG2D, NKp30, DNAM-1,
CD1c, CD1d)

*In vivo* expansion upon
CMV reactivation

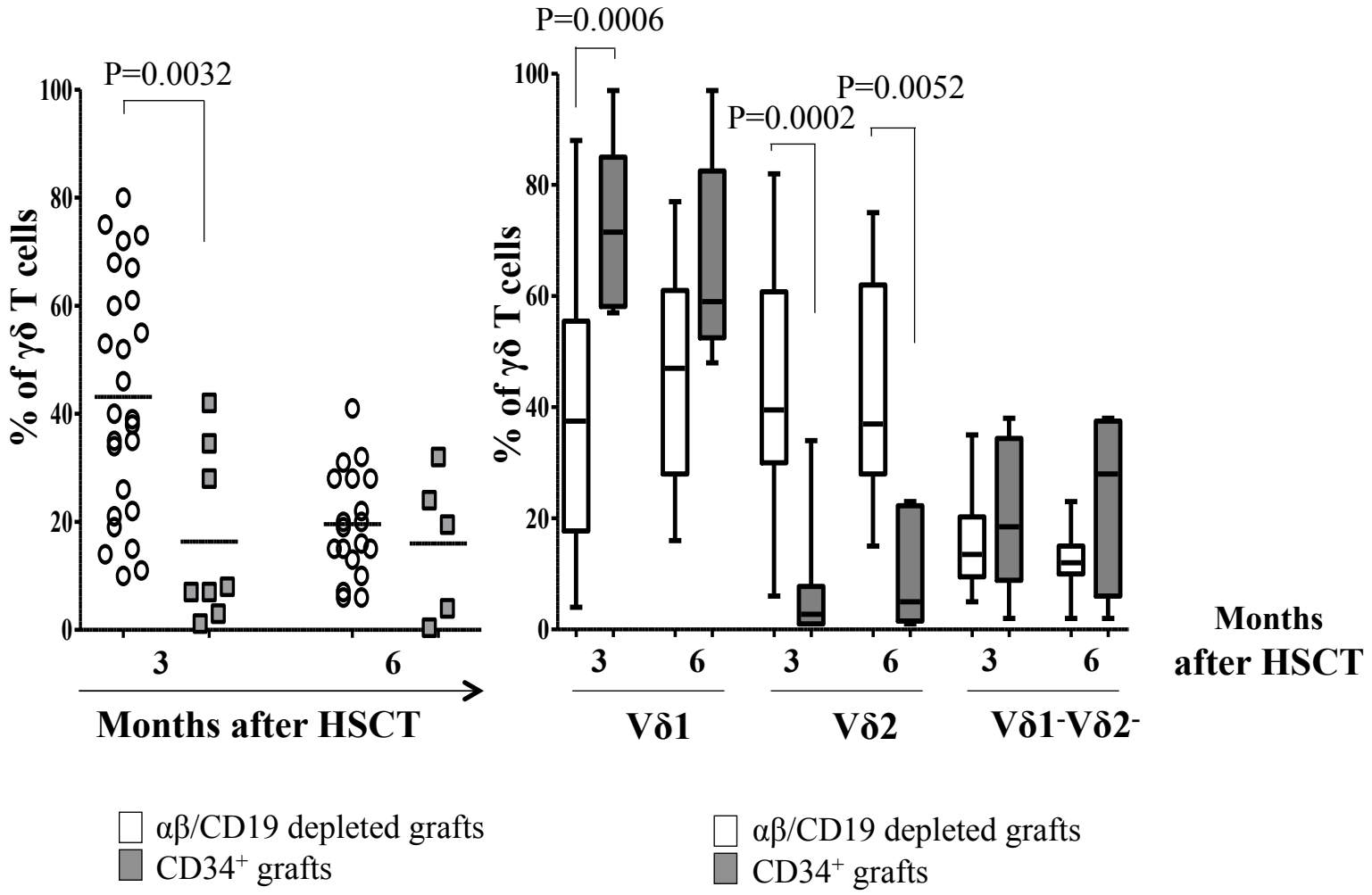
**Anti-tumor activity**

# Phenotype of $\gamma\ \delta$ T cells emerging in recipients of haplo-HSCT



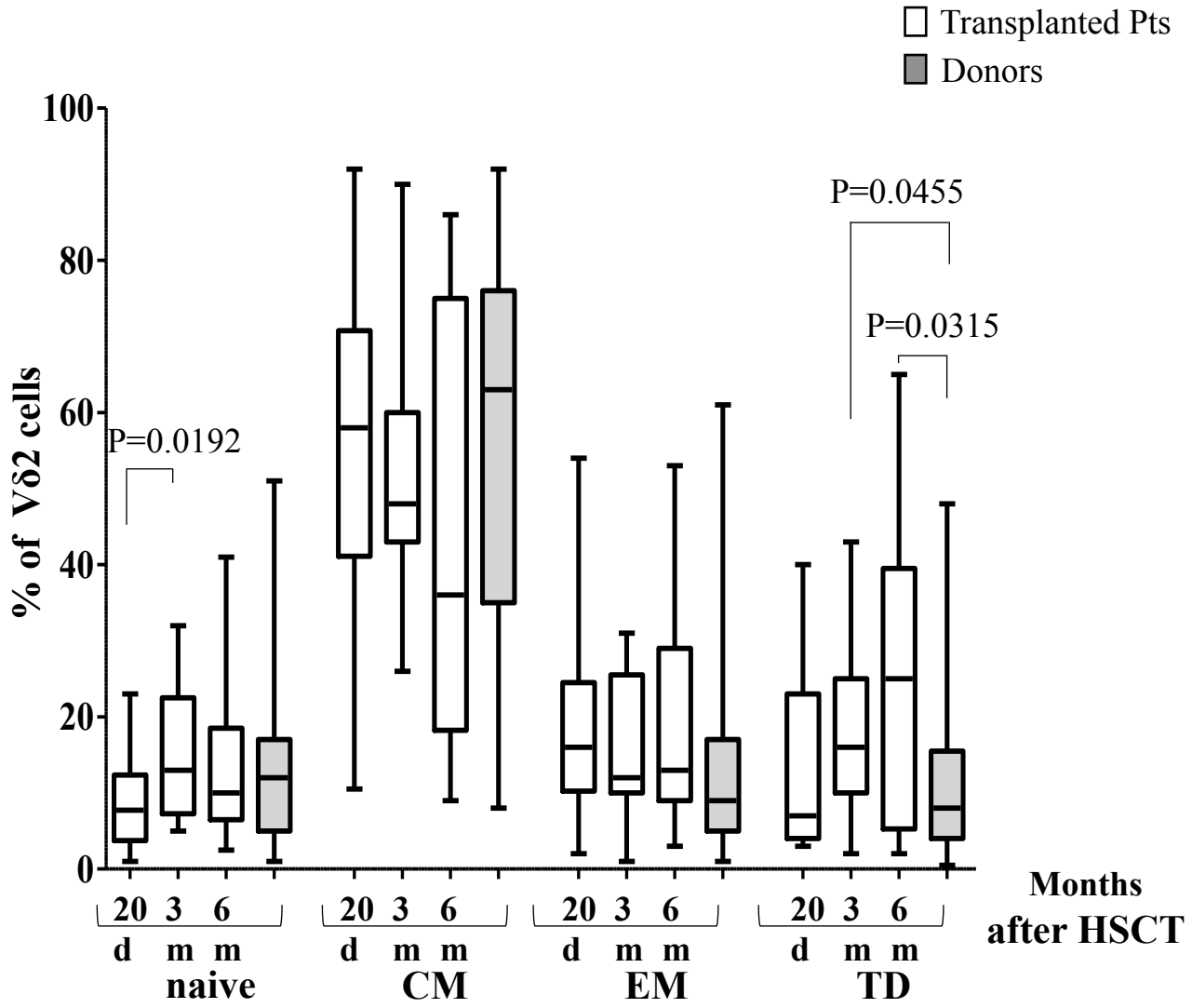

Airoldi I. et al., Blood 2015

# Circulating γ δ T cells in patients receiving α β /CD19 depleted grafts or CD34+ cells



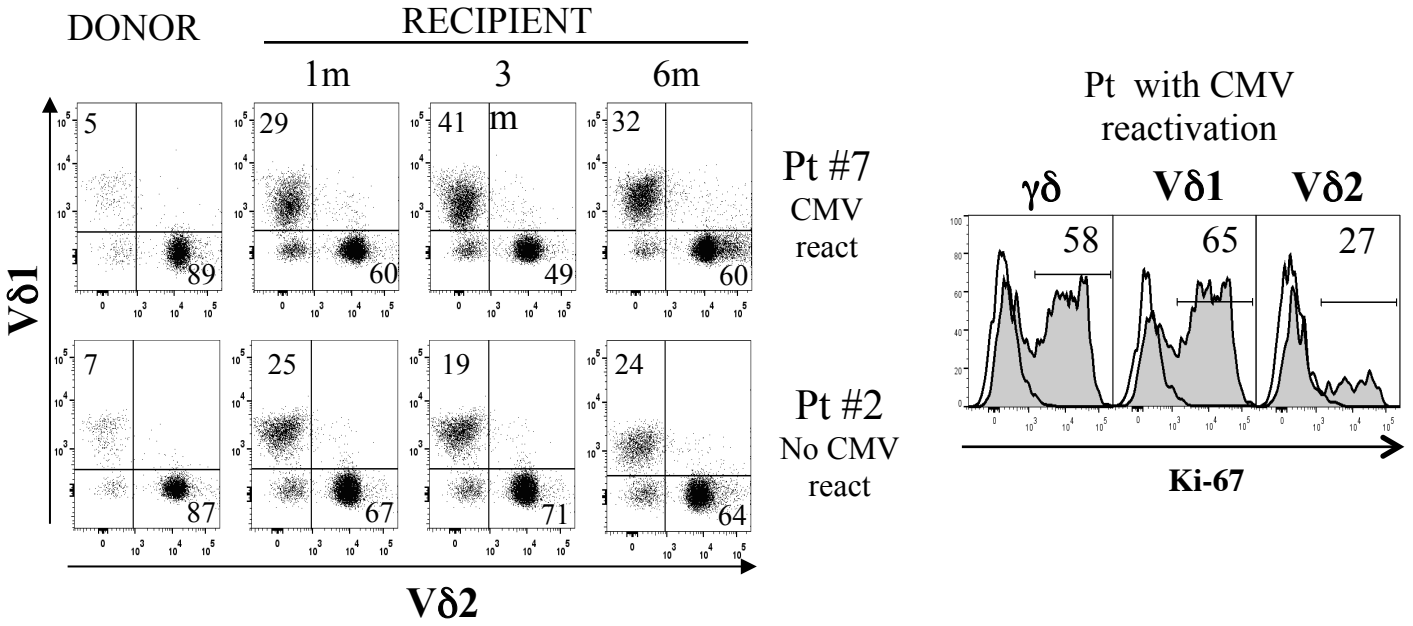

Airoldi I. et al., Blood 2015

# Differentiation status of emerging V δ 2 cells after HSCT



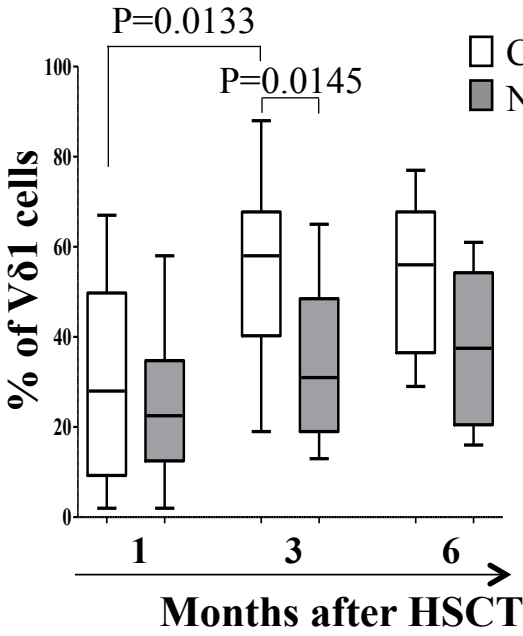

Airoldi I. et al., Blood 2015

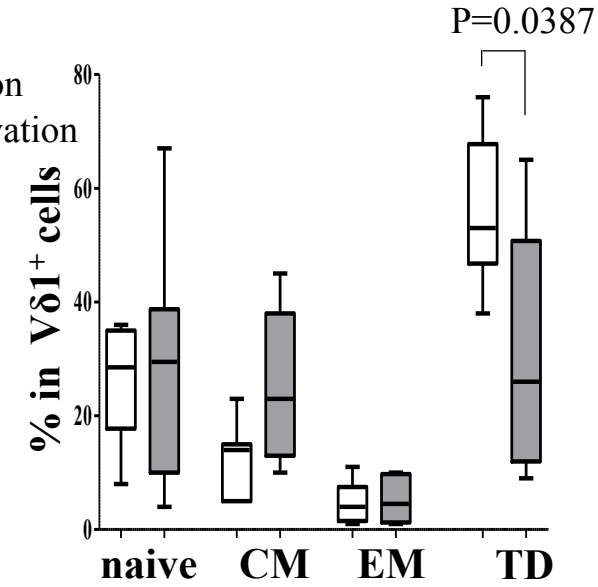
# Peculiarities of γ δ T cells in patients experiencing CMV reactivation





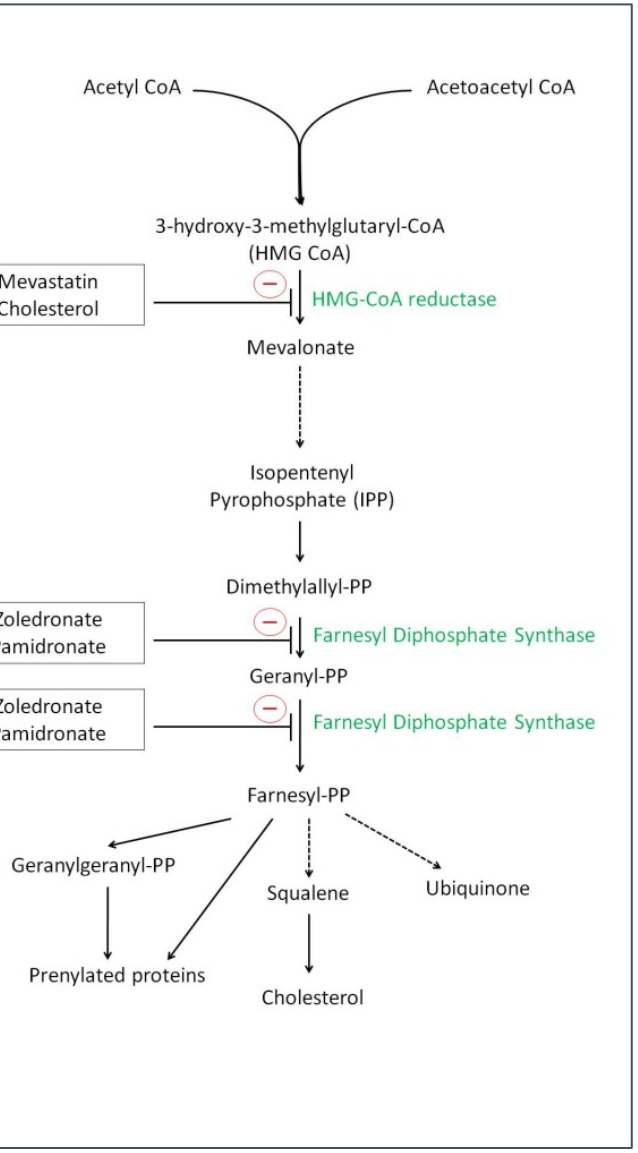


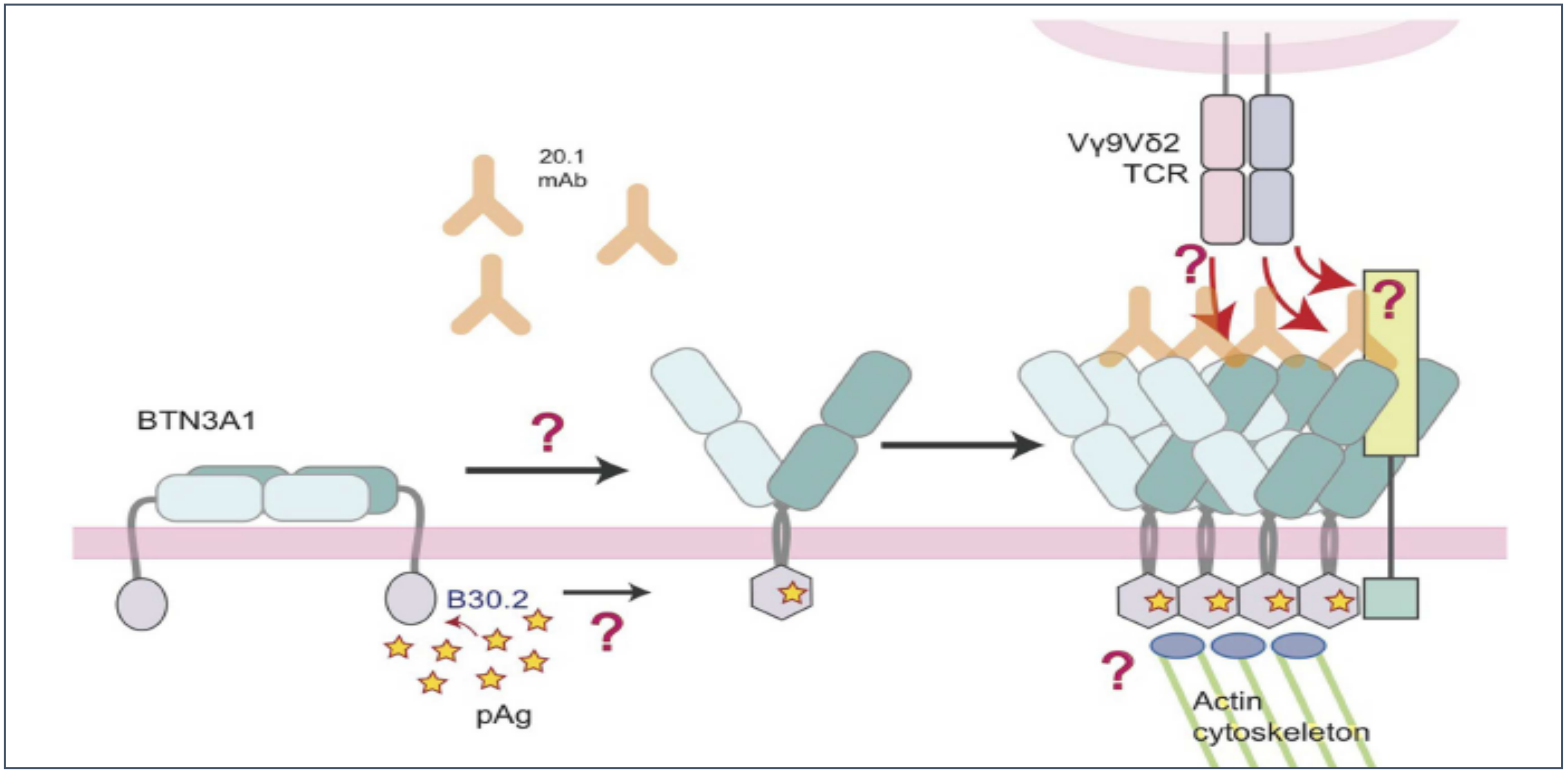


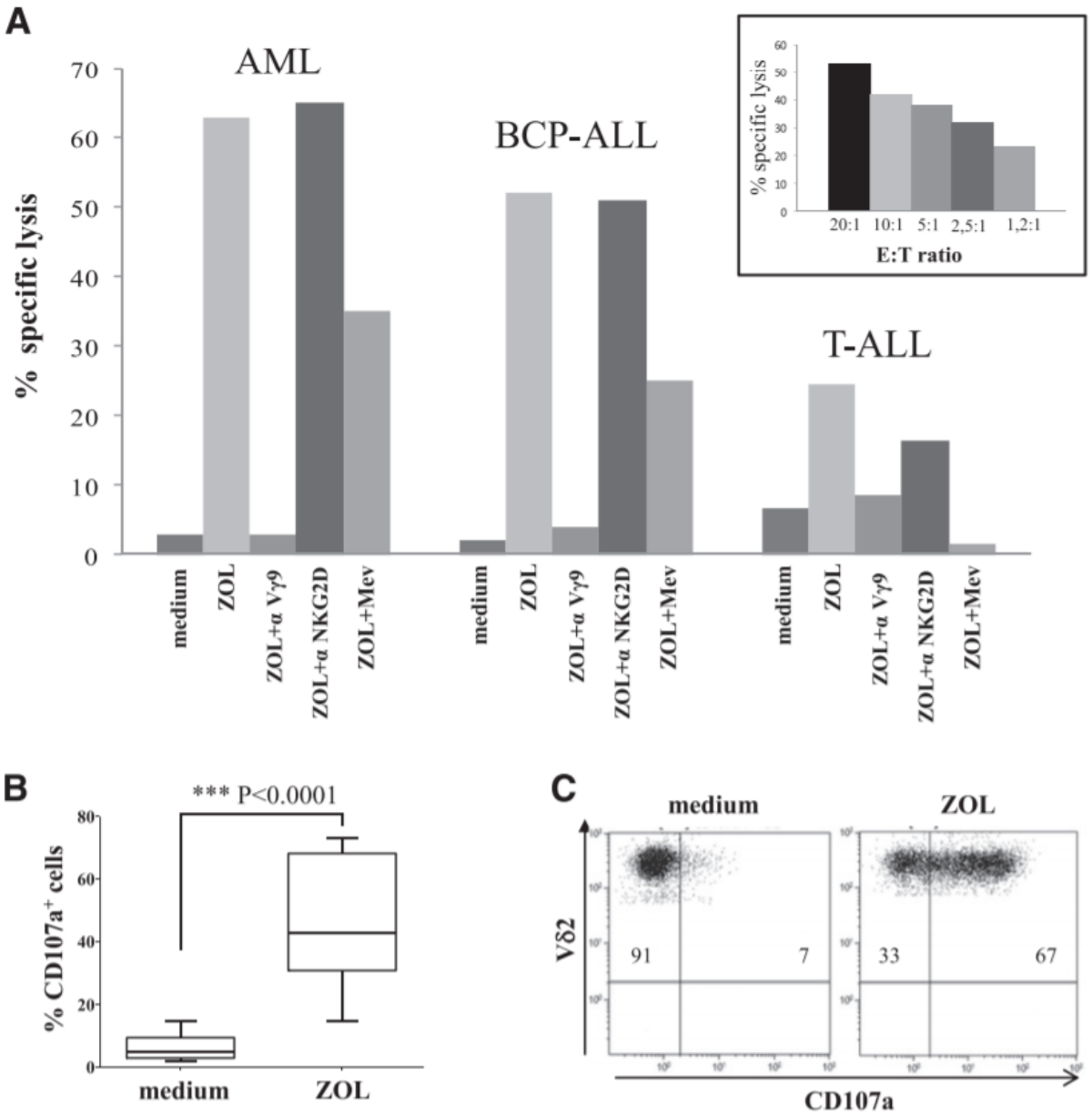

Airoldi I. et al., Blood 2015

# Bisphosphonates administration



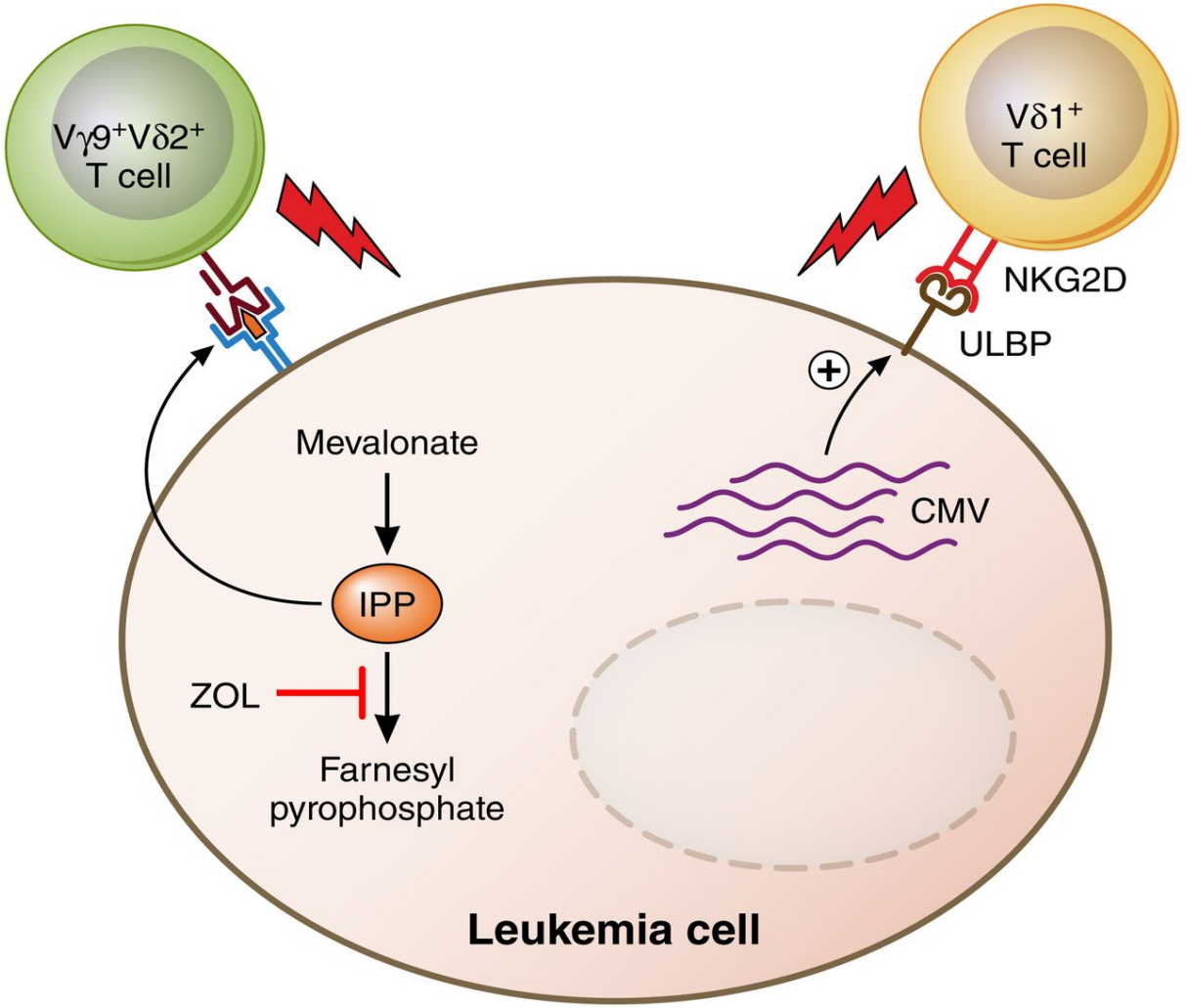



# Lysis of leukemia cells




Airoldi I. et al., Blood 2015

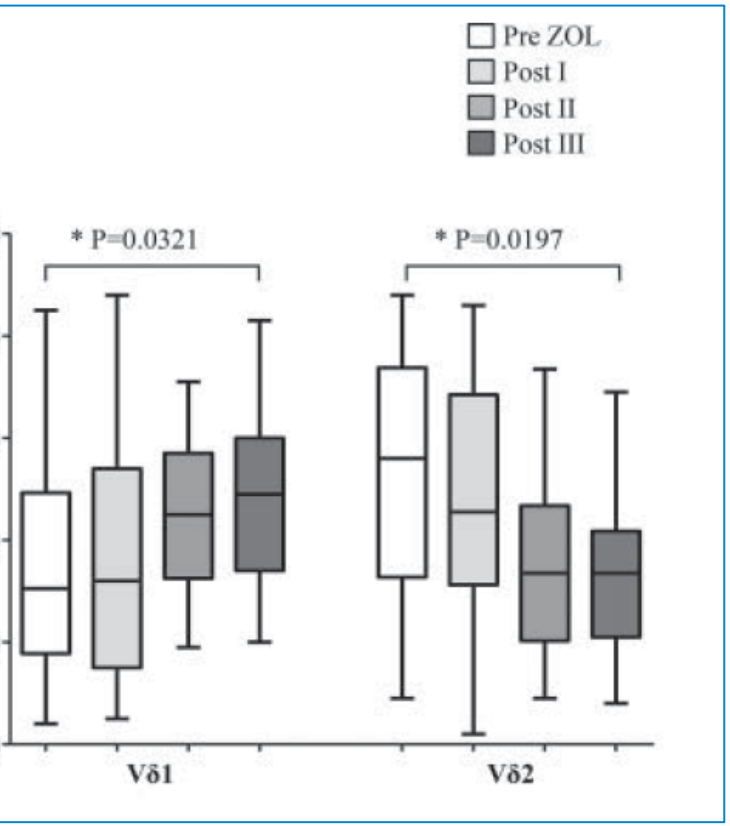
# Putative antileukemia anti-CMV activities of human γ δ TCR-bearing T cells

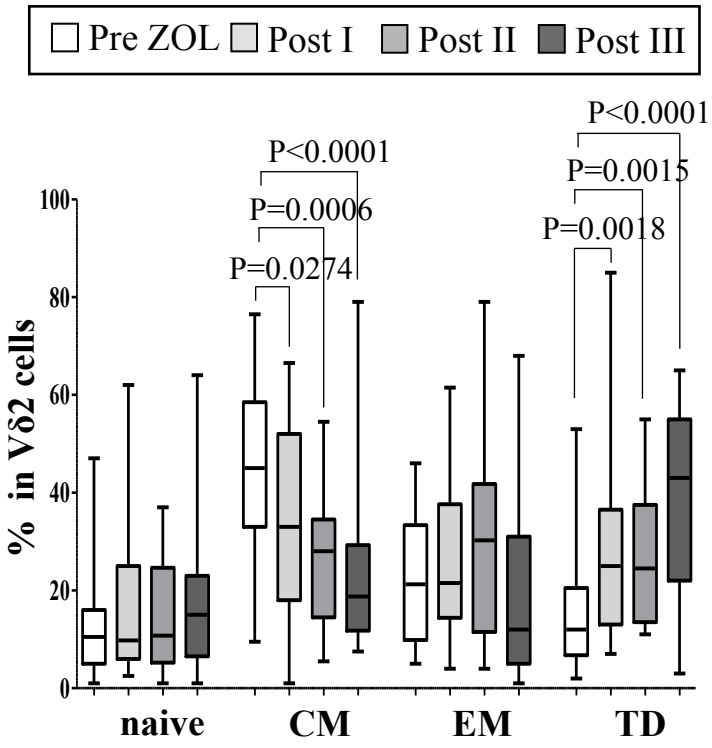


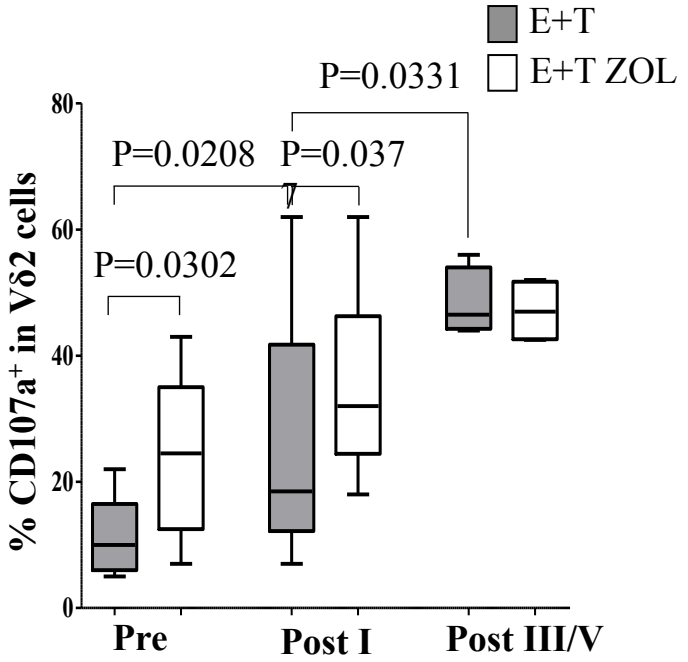
# In-vivo ZOL administration

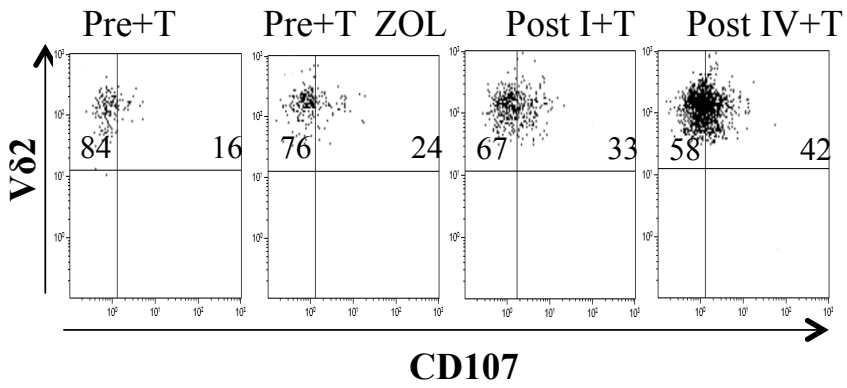
| | Number | Percentage (%) | Median | Range |
|---|---|---|---|---|
| Total | 46 | 100 | | |
| Gender | | | | |
| Male | 33 | 72 | | |
| Female | 13 | 28 | | |
| Age at diagnosis, years | | | 9.2 | 0.6-22 |
| Age at transplant, years | | | 10.9 | 1-22.2 |
| Disease | | | | |
| BCP-ALL | 26 | 57 | | |
| T-ALL | 7 | 15 | | |
| AML | 11 | 24 | | |
| MPAL | 2 | 4 | | |
| Phase of disease | | | | |
| CR1 | 13 | 28 | | |
| CR2 | 23 | 50 | | |
| >CR2 | 8 | 18 | | |
| active disease | 2 | 4 | | |
| Genetic abnormalities | | | | |
| t(9;22) | 1 | 2 | | |
| complex karyotype | 1 | 2 | | |
| FLT3-ITD | 2 | 4 | | |
| cytogenetic abnormalities involvong 11q23* | 3 | 7 | | |
| Previous HSCT | 7 | 15 | | |

# ZOL infusion induces differentiation, increases the percentage of V δ 1 cells and cytotoxicity of V δ 2 cells *in-v*



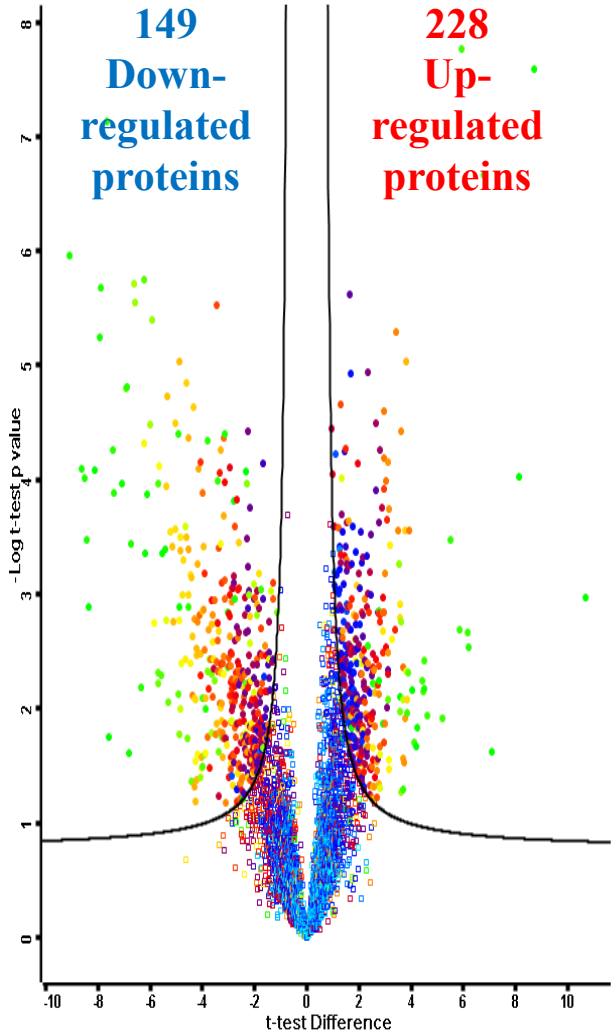



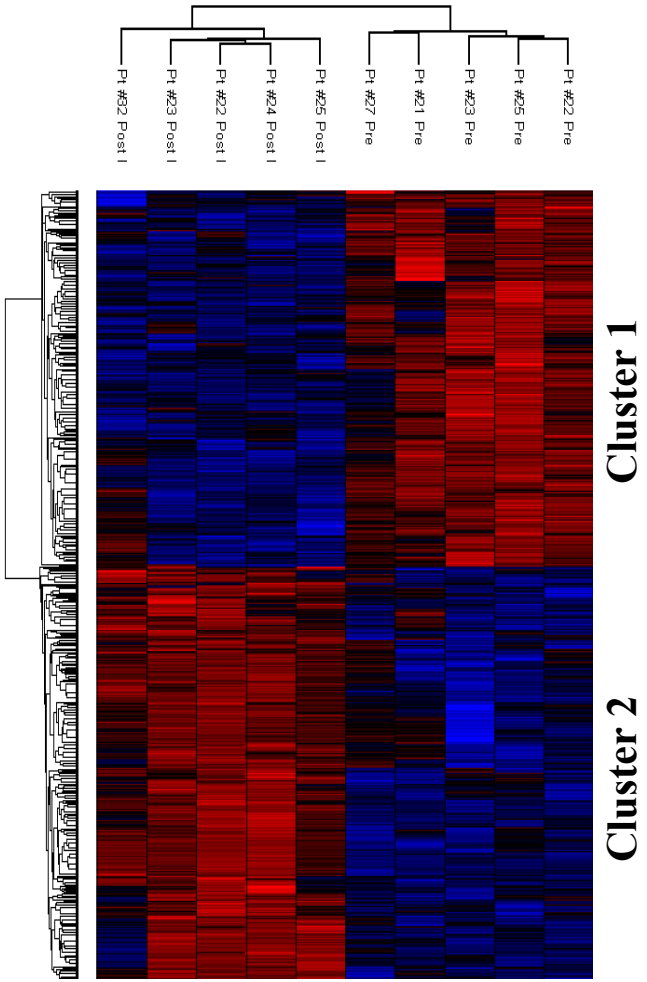




Bertaina A. et al., OncoImmunology 2017

# The first ZOL infusion modulates γδ T cell proteome profile

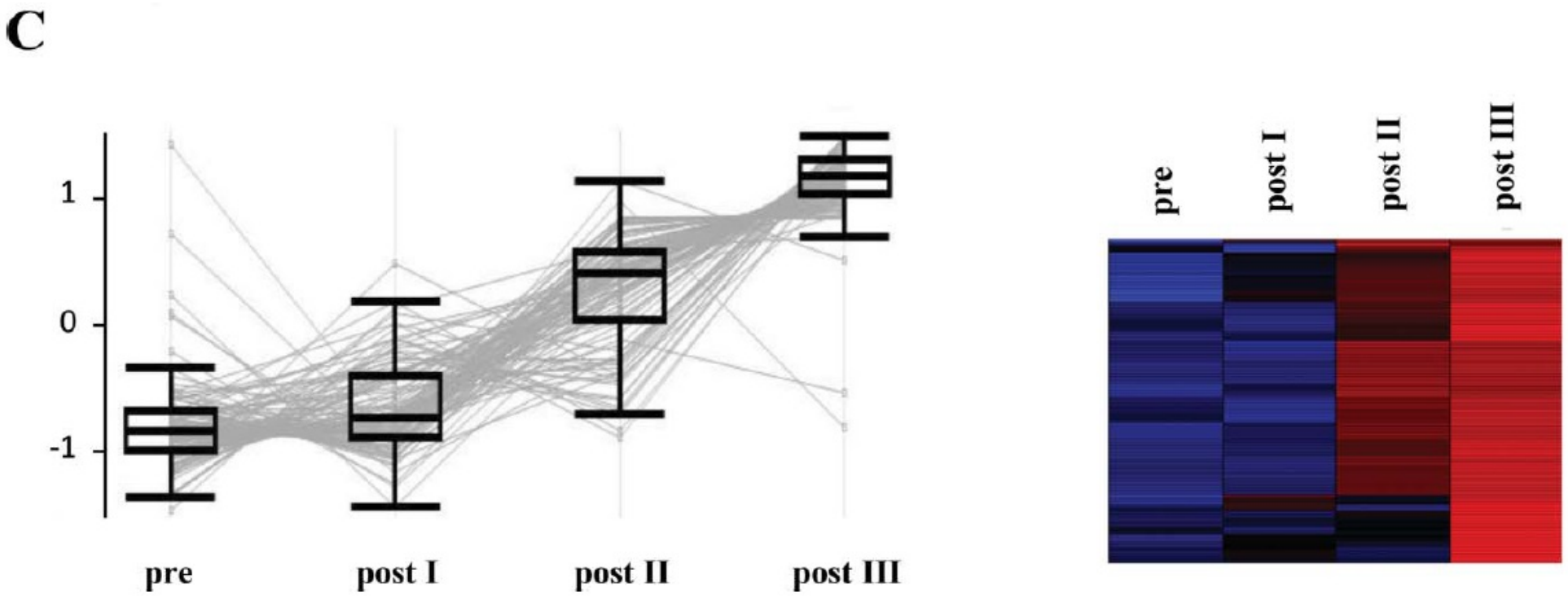
**4722 proteins identified**




Bertaina A. et al., OncoImmunology 2017

## Proteomic analysis




Bertaina A. et al., OncoImmunology 2017

## In-vivo Zoledronic acid administration – Toxicity

- 138 infusions

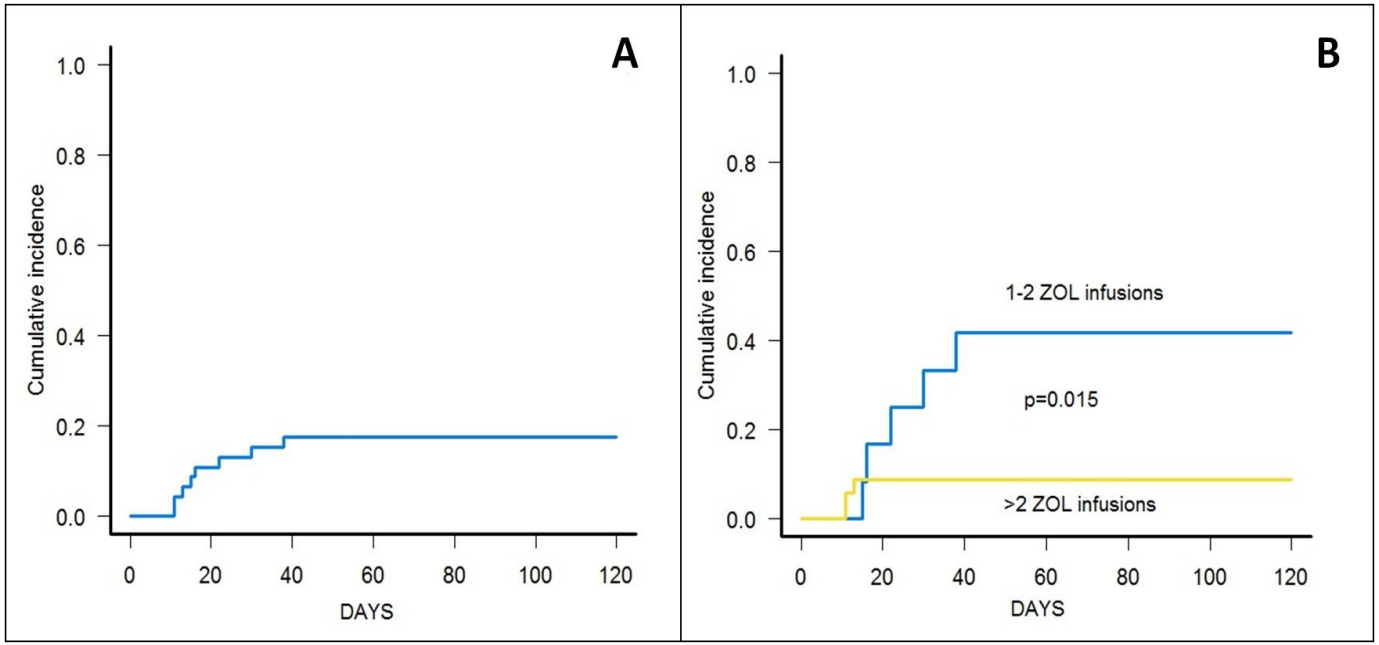
- mean of 3 infusions per patient (range 1-5)

| Dosage | (0.05-0.1 mg/kg) |
|---|---|
| <10 kg | 1 mg |
| 10-30 Kg | 2 mg |
| >30 kg | 4 mg |

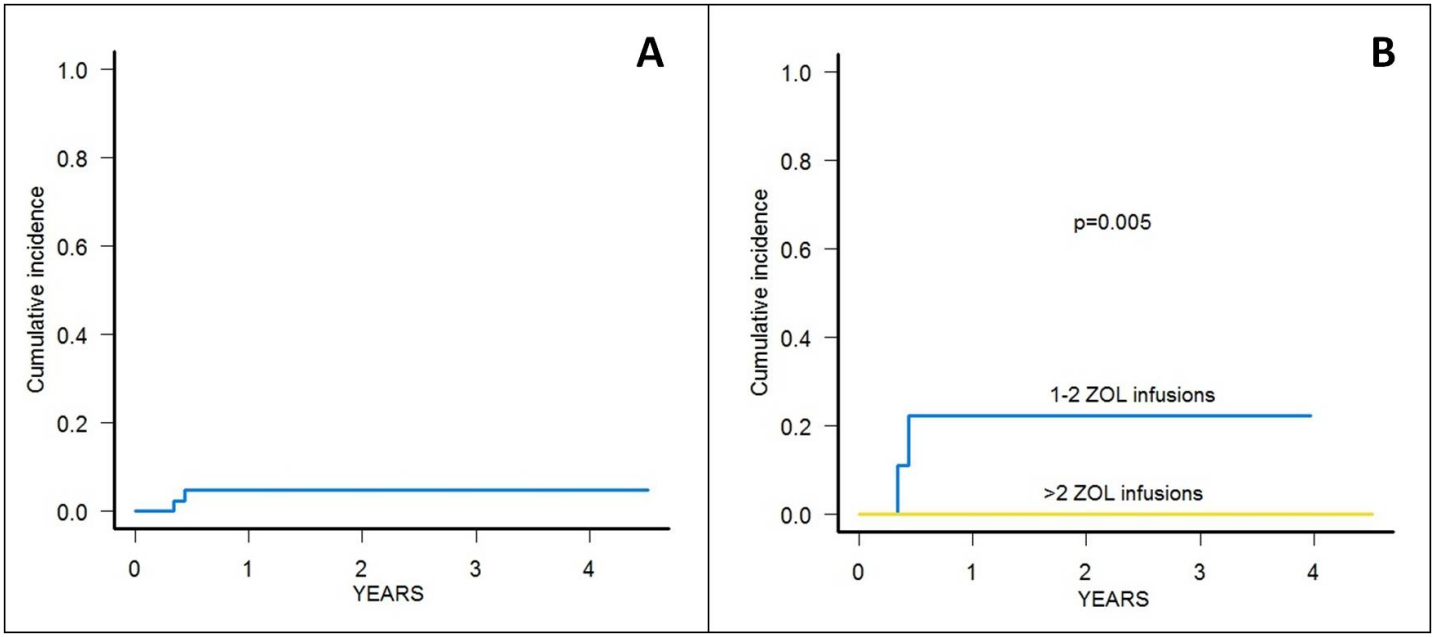
| Effect | Number (%) |
|---|---|
| Symptomatic hypocalcemia | 1 (2%) |
| Fever/acute phase reaction | 8 (17%) |
| Transient decrease of WBC and/or PLT | 3 (7%) |
| Arthralgia | 1 (2%) |
| Serious (e.g., aseptic necrosis of bone of jaw) | 0 |

# In-vivo Zoledronic acid administration – GVHD

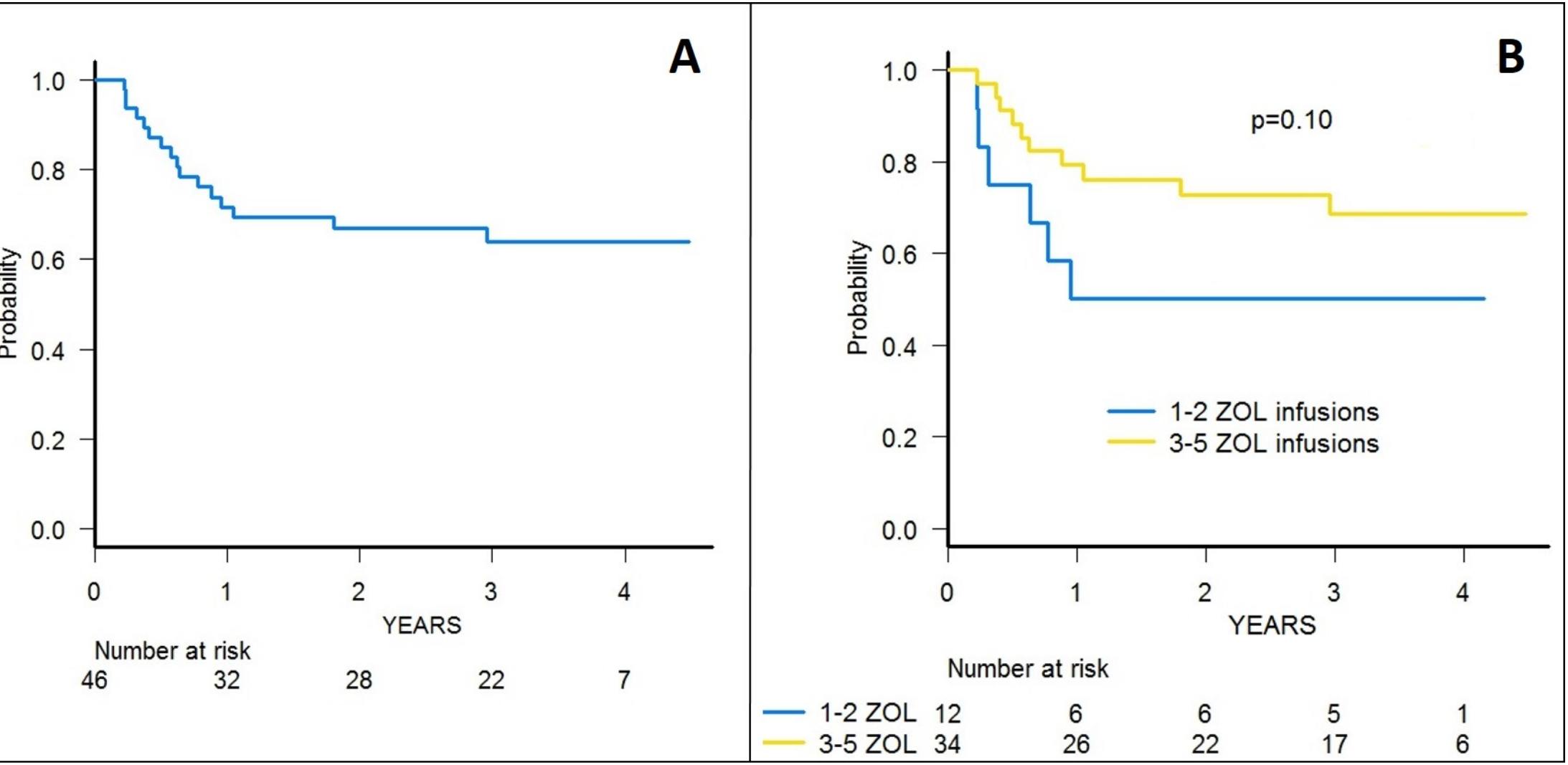
Acute GVHD



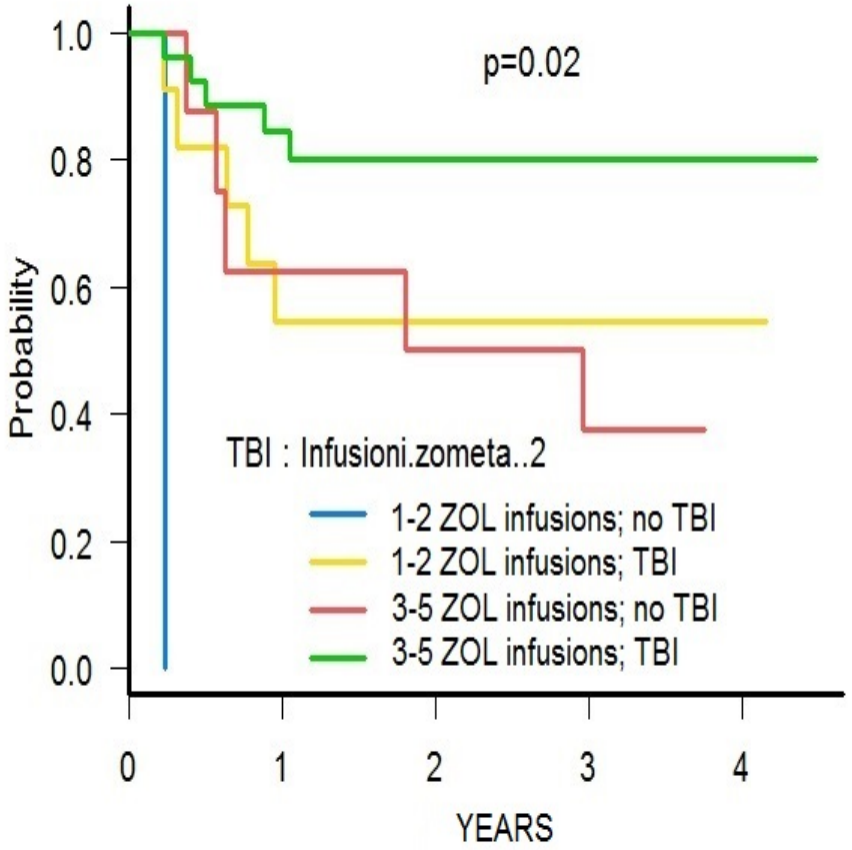

Chronic GVHD



# In-vivo Zoledronic acid administration – Outcome

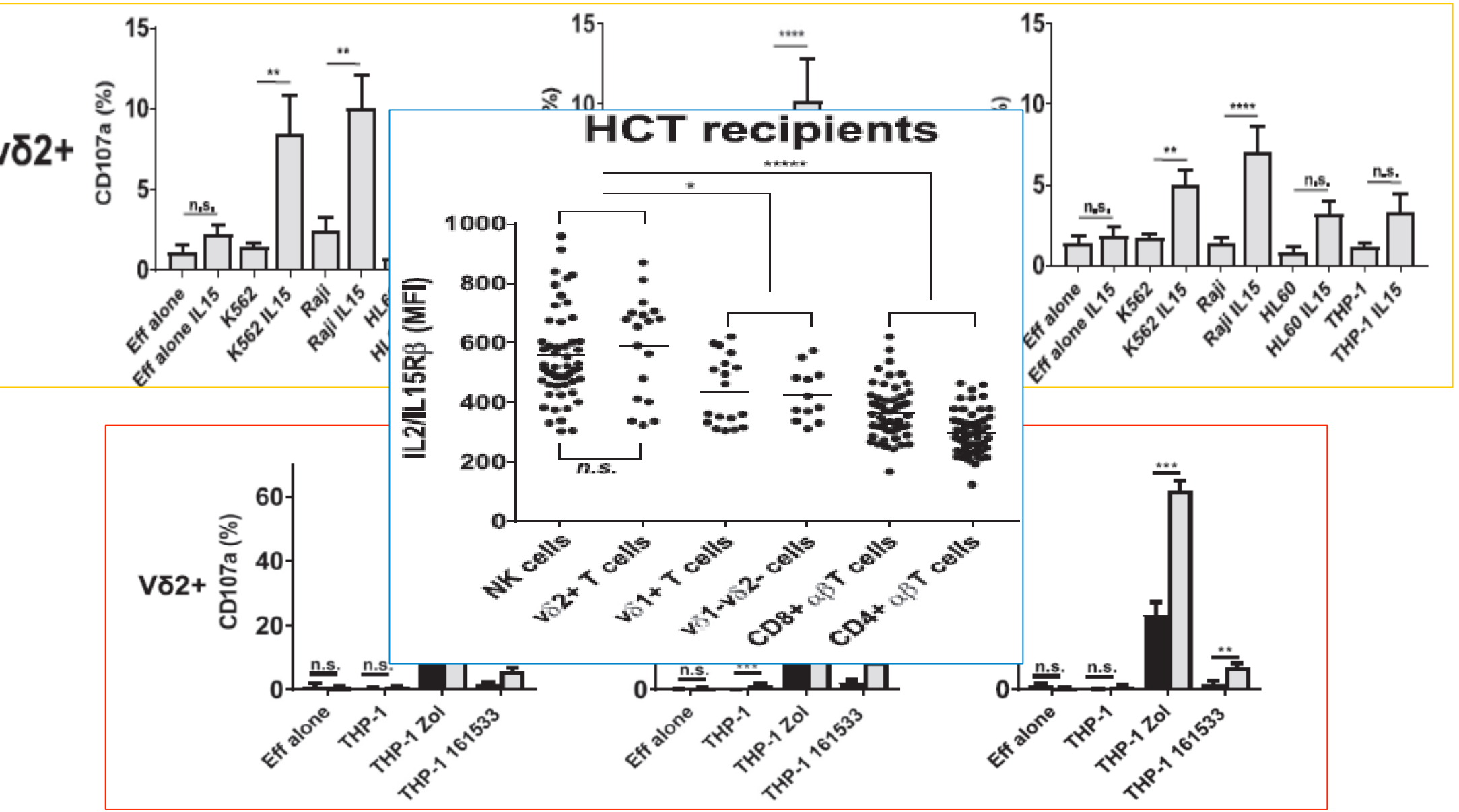


# In-vivo Zoledronic acid administration – Outcome



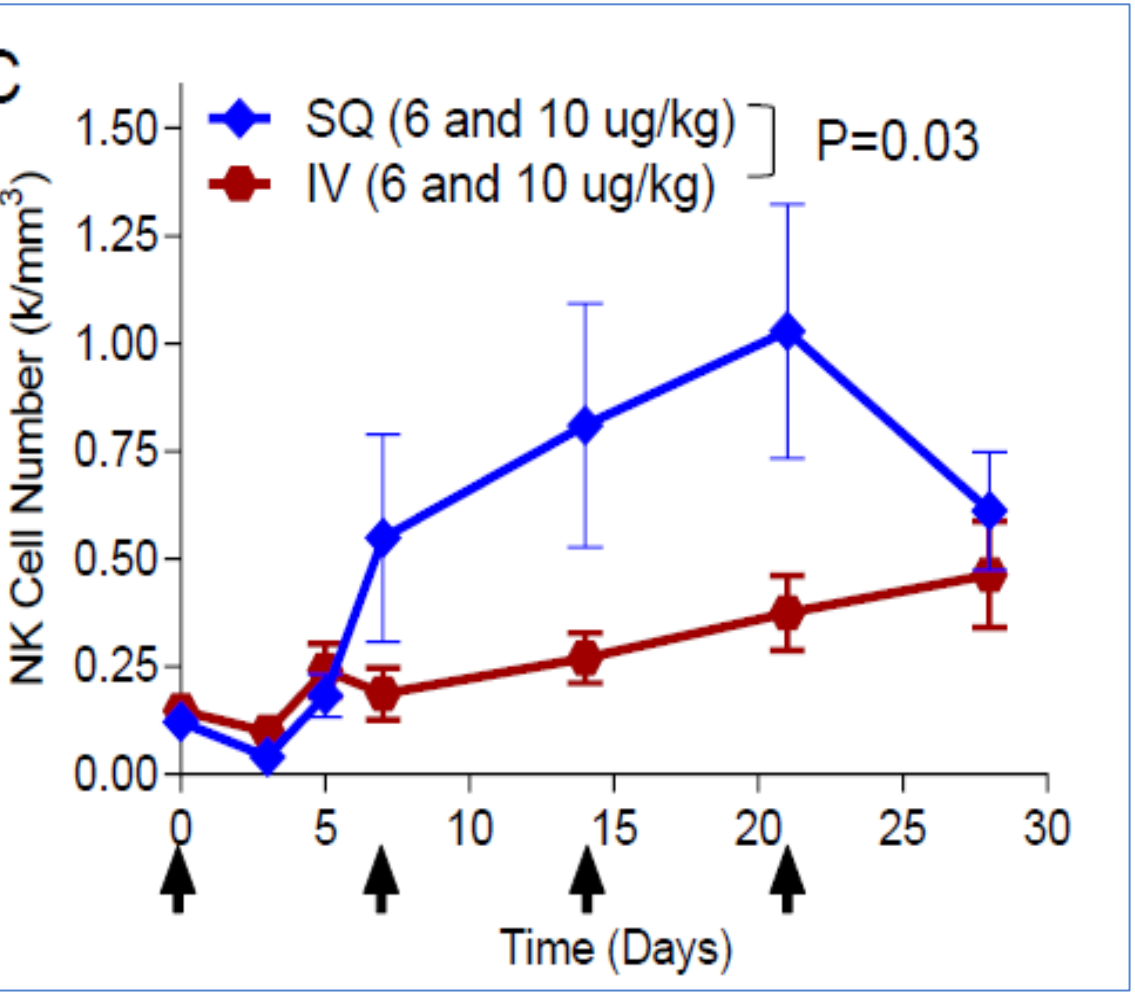

| Variable | Hazard ratio | Lower 95% CI | Upper 95% CI | p value |
|---|---|---|---|---|
| Use of TBI | 0.2172 | 0.0672 | 0.7019 | 0.010 |
| Zoledronic Acid infusions > 2 | 0.2890 | 0.0895 | 0.9324 | 0.037 |

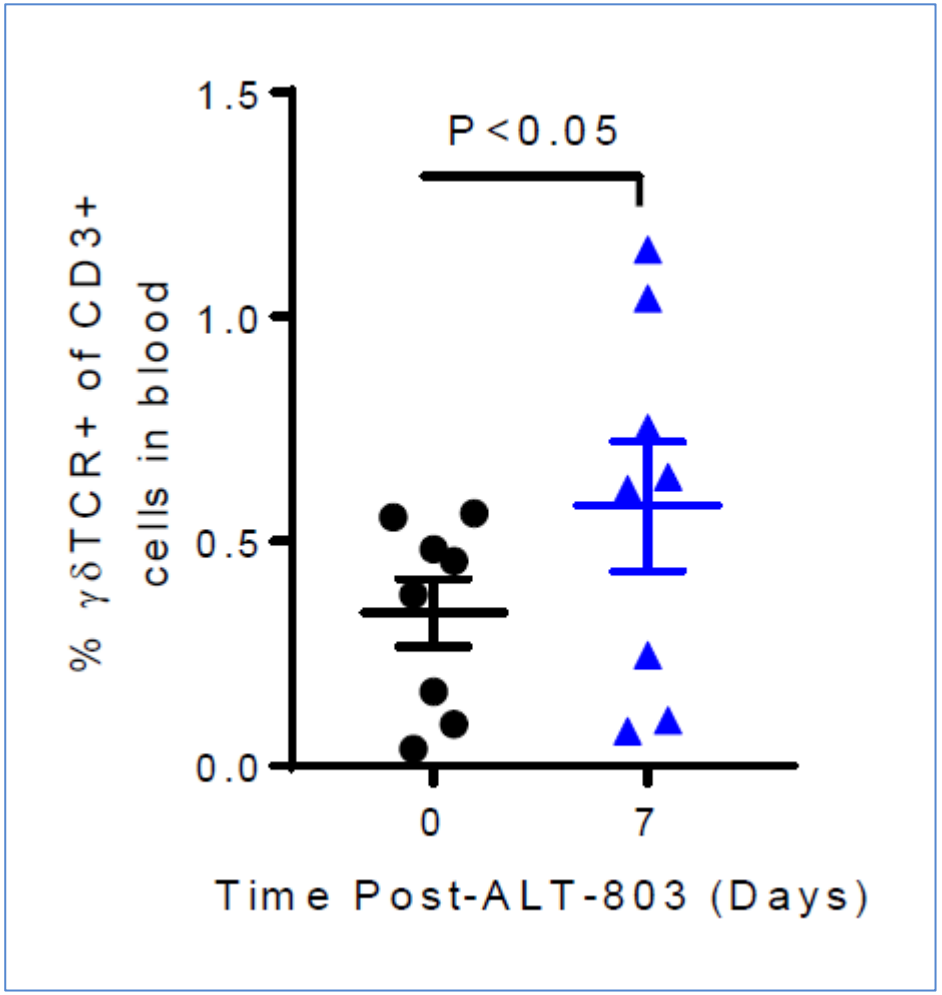
# IL-15: in-vitro experience




De Witte M.A. et al., BBMT 2018

# IL-15R agonists: in-vivo experience




Romee R. et al., Blood 2018

# Strategies to accelerate immune recovery

**A.**

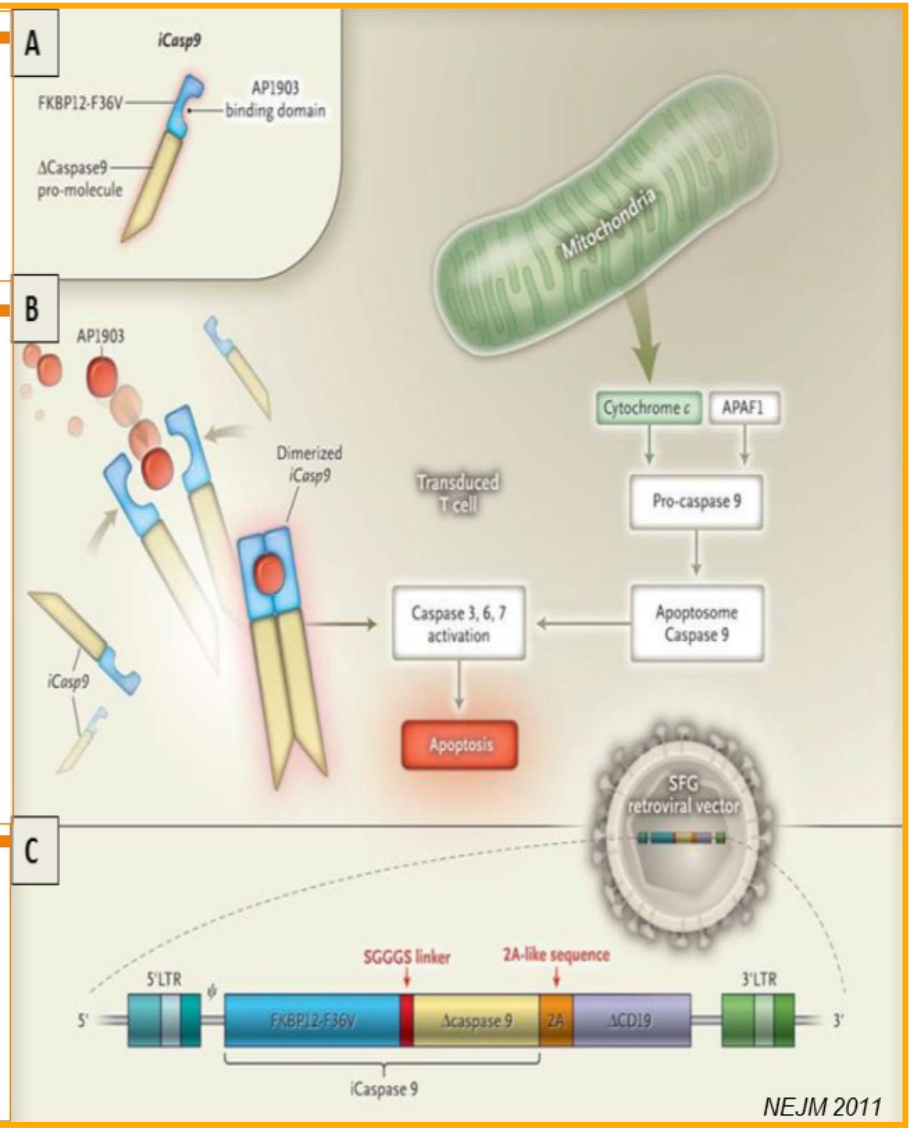
- Caspase 9 gene with FKBP mutant-with rimiducid binding domain (iCasp9)
- Rimiducid specifically designed to bind FKBP variant

**B.**

- Rimiducid induces dimerization of the FKBP-caspase 9
- Caspase cascade activated
- Resulting in rapid apoptosis and cell-death (clinical symptoms start to resolve in 1 hour, generally resolved in 24 hours)

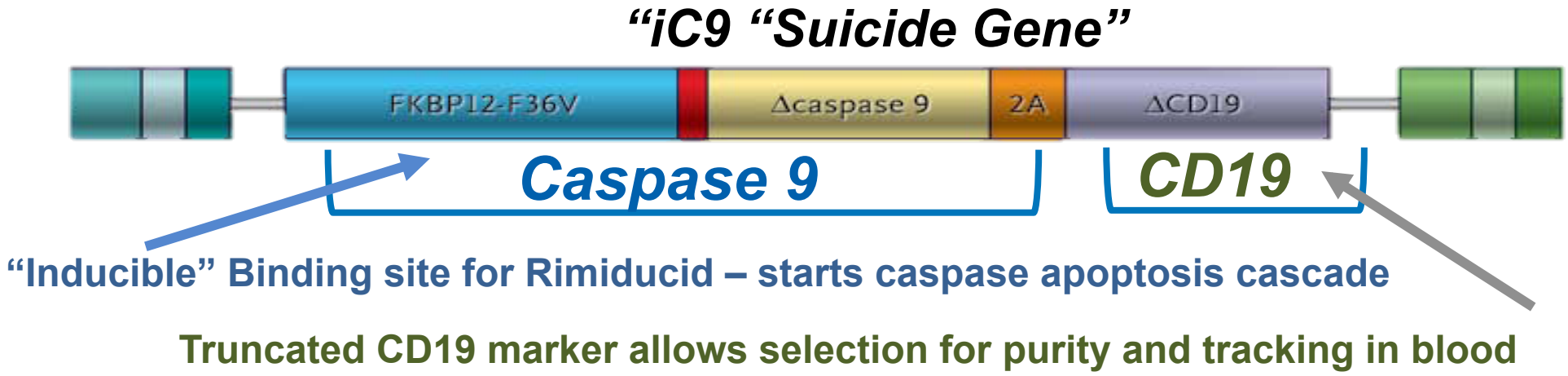
**C.**

- iCasp9 gene containing truncated CD19 marker, integrated into T cells through retroviral vector




Di Stasi A, et al. New Eng J Med 2011

# BPX-501 CaspaCide T cells




- **From normal donor leukapheresis -- GMP facilities US / Europe**
- **Activated and expanded in culture, transduced with the iC9 suicide gene and selected for CD19+ cells**
- **Cryopreserved and stored in liquid nitrogen**
- **Maintain characteristics of normal T cells**
  - Broad T-cell repertoire
  - Antiviral and antigen specific activity

# BP-004 Study Design

**se I portion:** **Classical 3+3 design** | **Phase II portion:** **MTD/RD**

**X $10^5$, 5 X $10^5$ & 1 X $10^6$ BPX-501 T Cells/kg** | **1 X $10^6$ BPX-501 T Cells/kg**

**EU BP-004 centers: OBPG-Rome, GOSH-London, Great Northern-Newca**

**Haploidentical donor (usually a parent)**

**Non-mobilized apheresis for BPX-501 product**

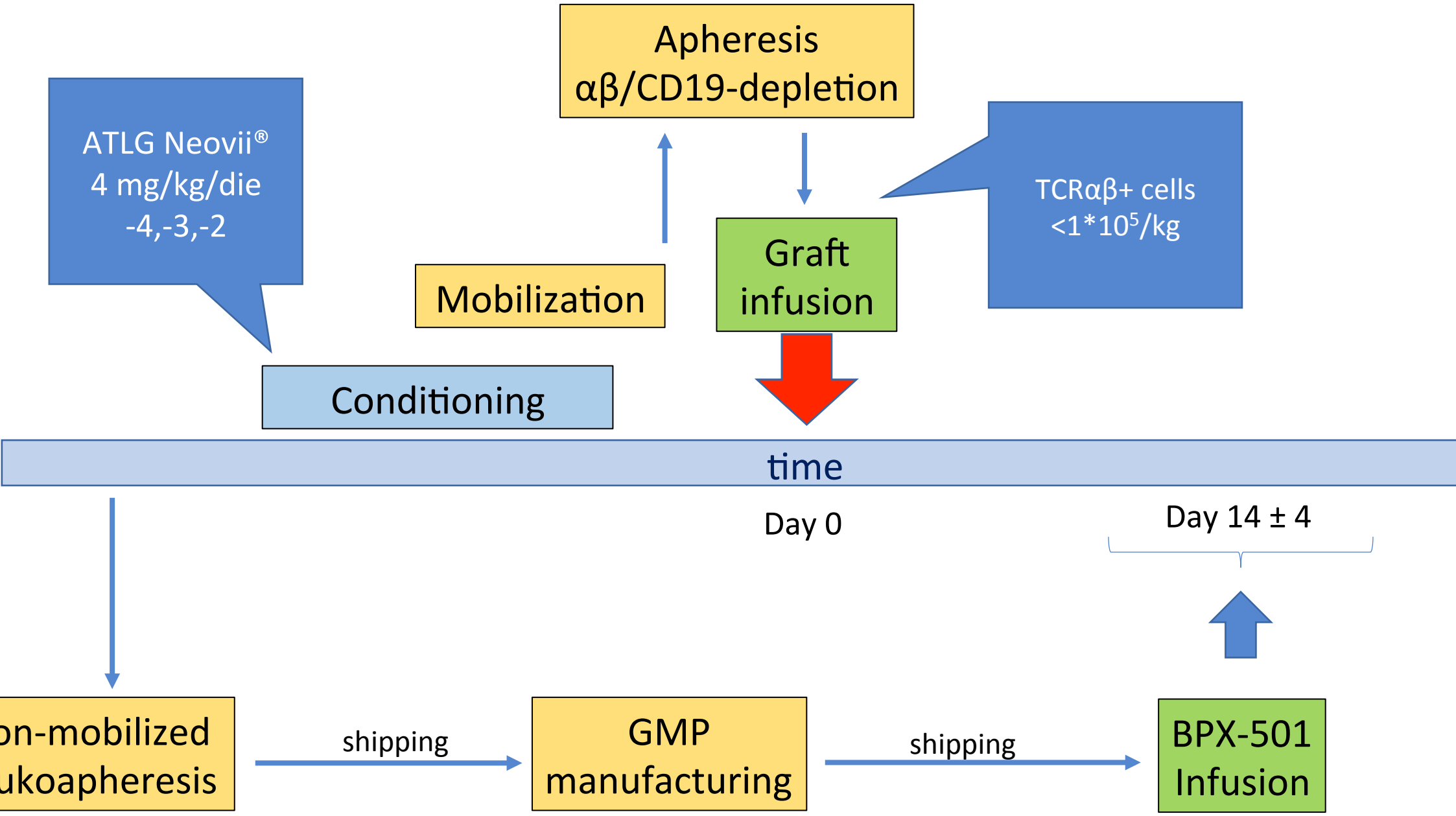
**TCRαβ/CD19 B-cell Depleted Allograft**

**BPX-501 T cells Infused Day 14 ± 4 post Tx**

**No Post-Transplant GVHD Prophylaxis**

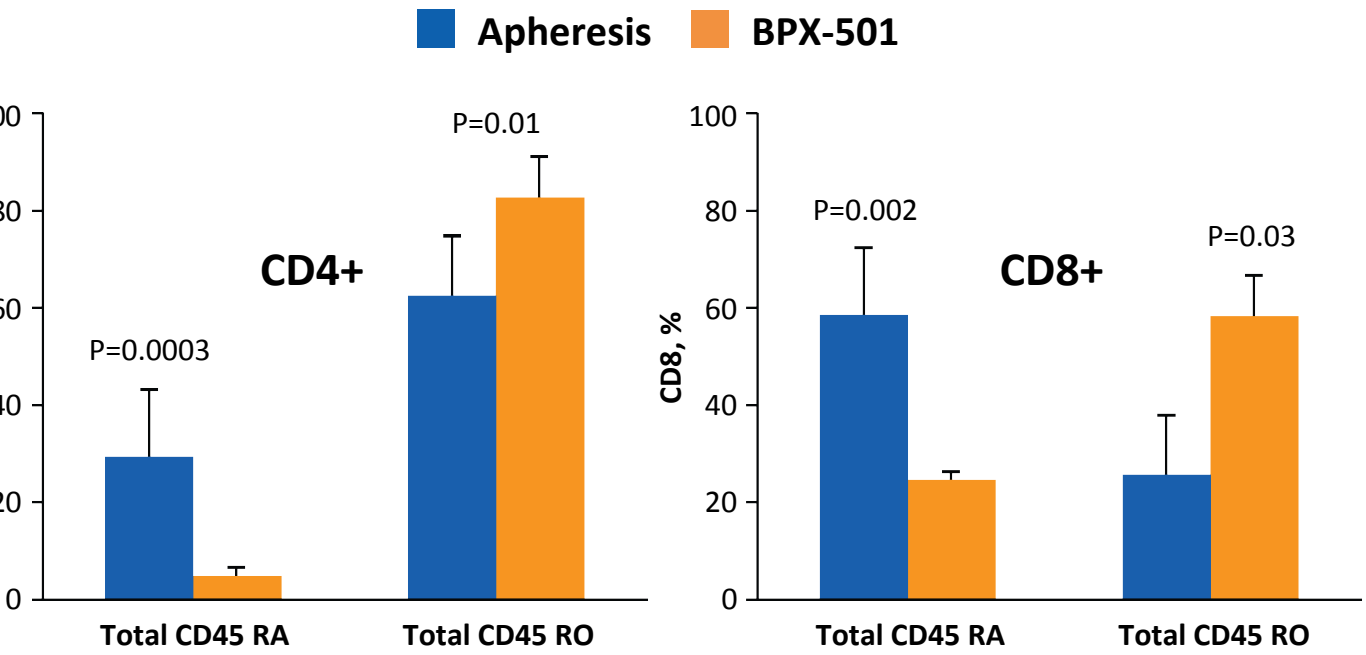
**Rimiducid (AP1903) Used for Uncontrollable GVHD**

# BP-004 Study Design

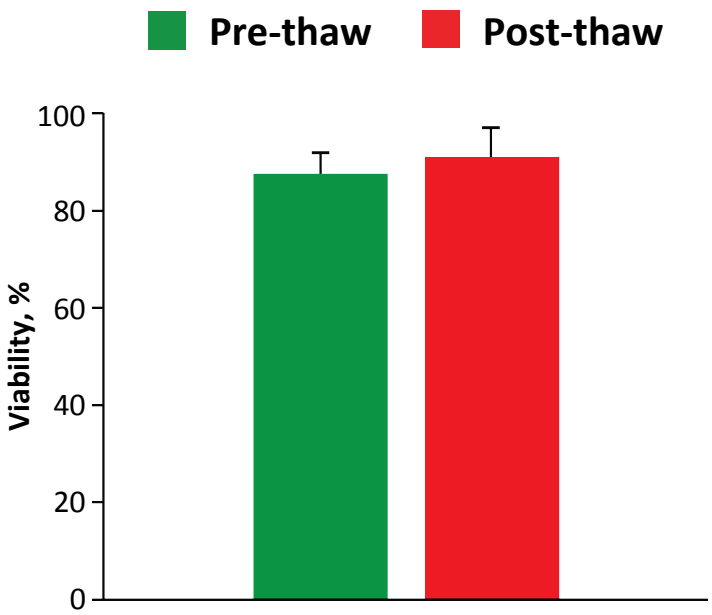


# aracterization of BPX-501 T-cells

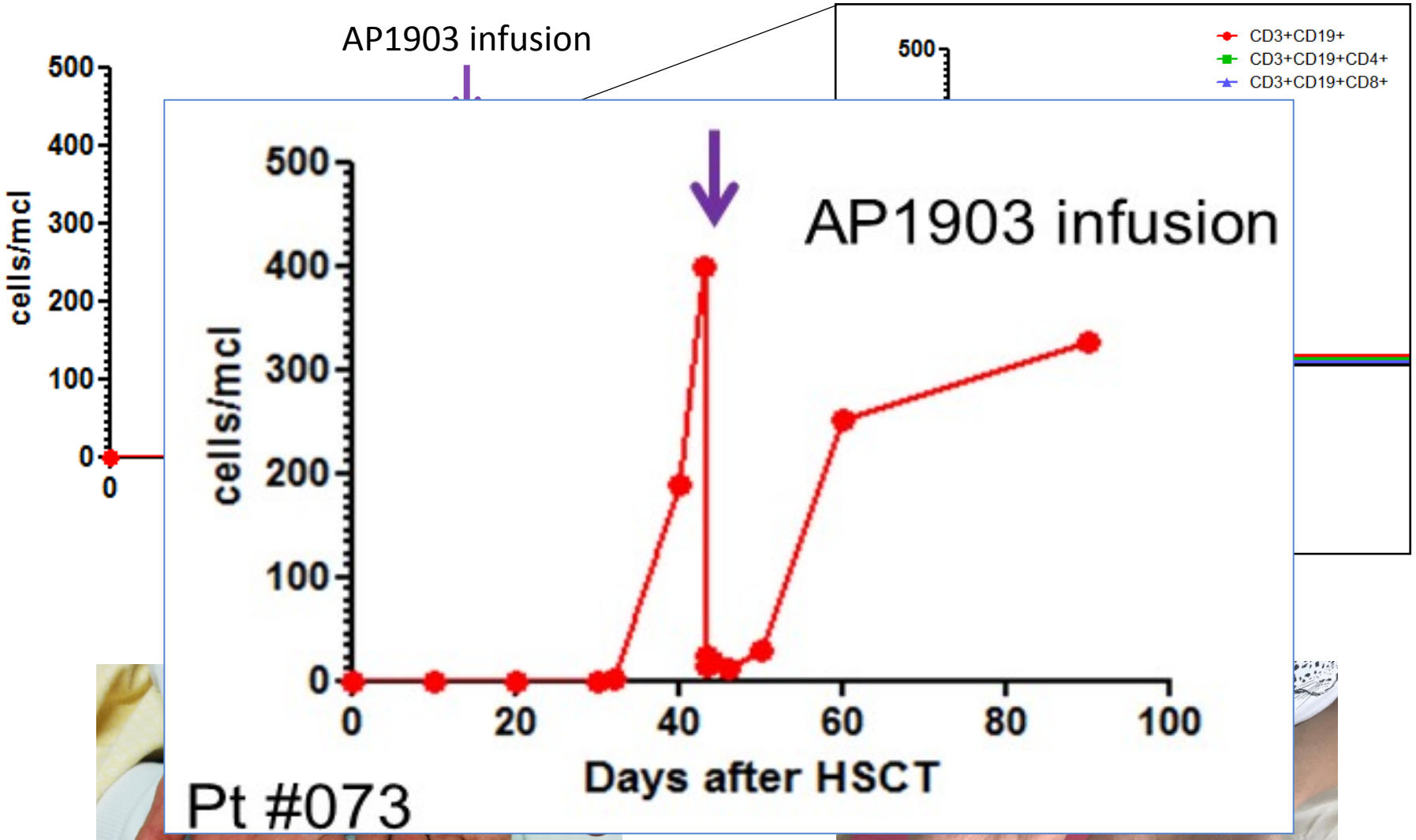
## After expansion and transduction, BPX-501 T-cells have increased memory effector phenotype in both CD4 and CD8 subsets

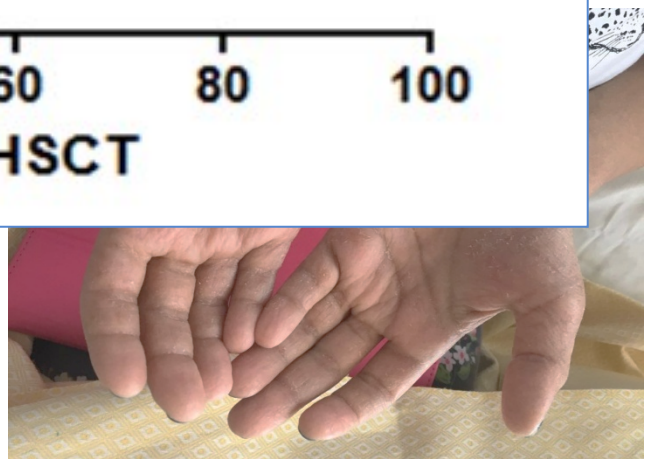



## BPX-501 T-cells retain high viability after cryopreservation and thaw



# X-501 cells – use of dimerizing agent



73

# -004-011 - Patient Demographics

**Single center experience on all patients follow-up > 60 days enrolled in the study**

| | N (=144) | % |
|---|---|---|
| **Acute leukemia** | **67** | **(47%)** |
| **ALL** | **39** | **(58%)** |
| **AML** | **28** | **(42%)** |
| **Erythroid disorders** | **28** | **(19%)** |
| **PIDs** | **31** | **(21%)** |
| **Fanconi Anemia** | **8** | **(6%)** |
| **Other diseases*** | **10** | **(7%)** |

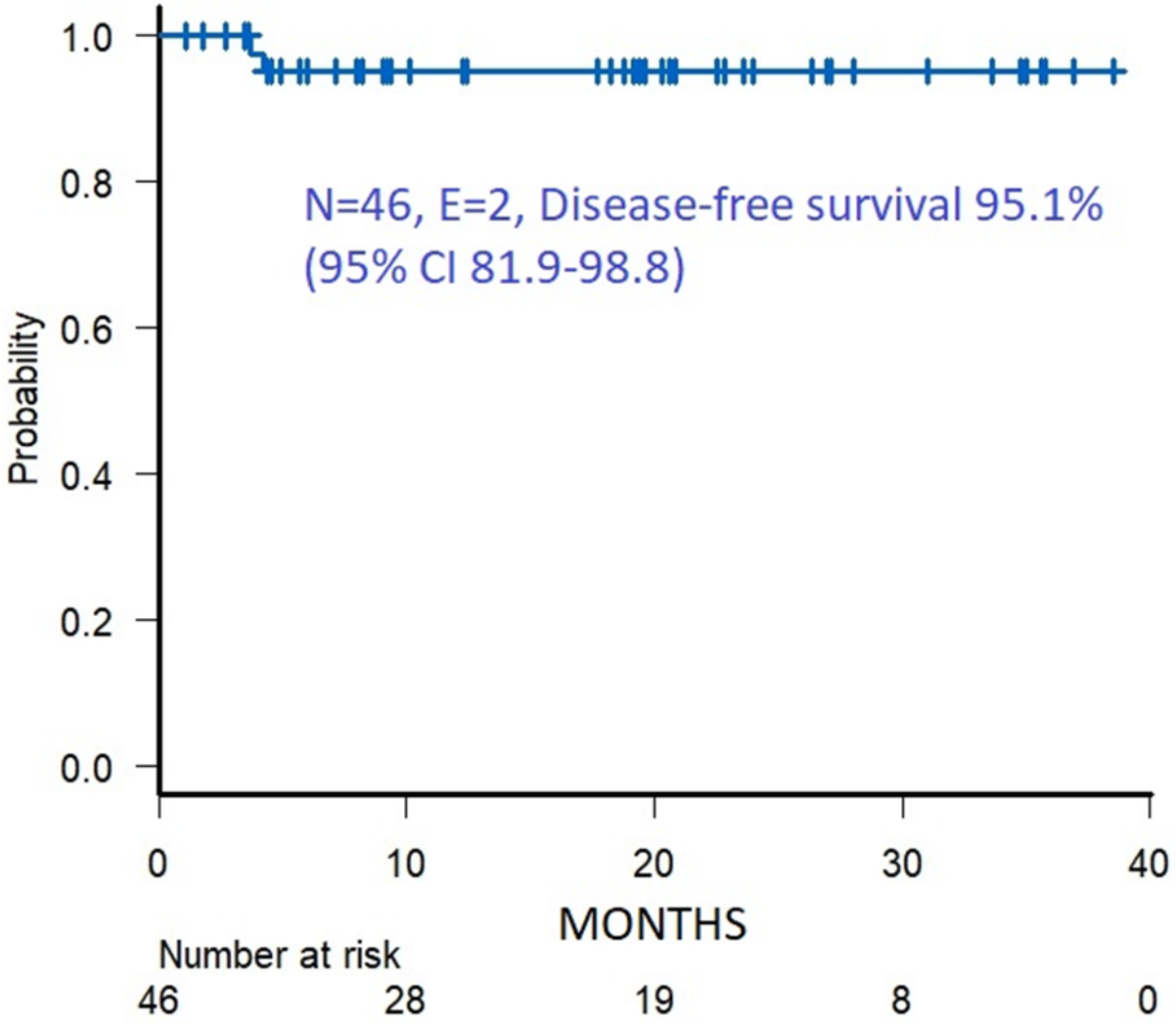
*NHL, MDS, Osteopetrosis, CAMT

# Overall and disease-free survival

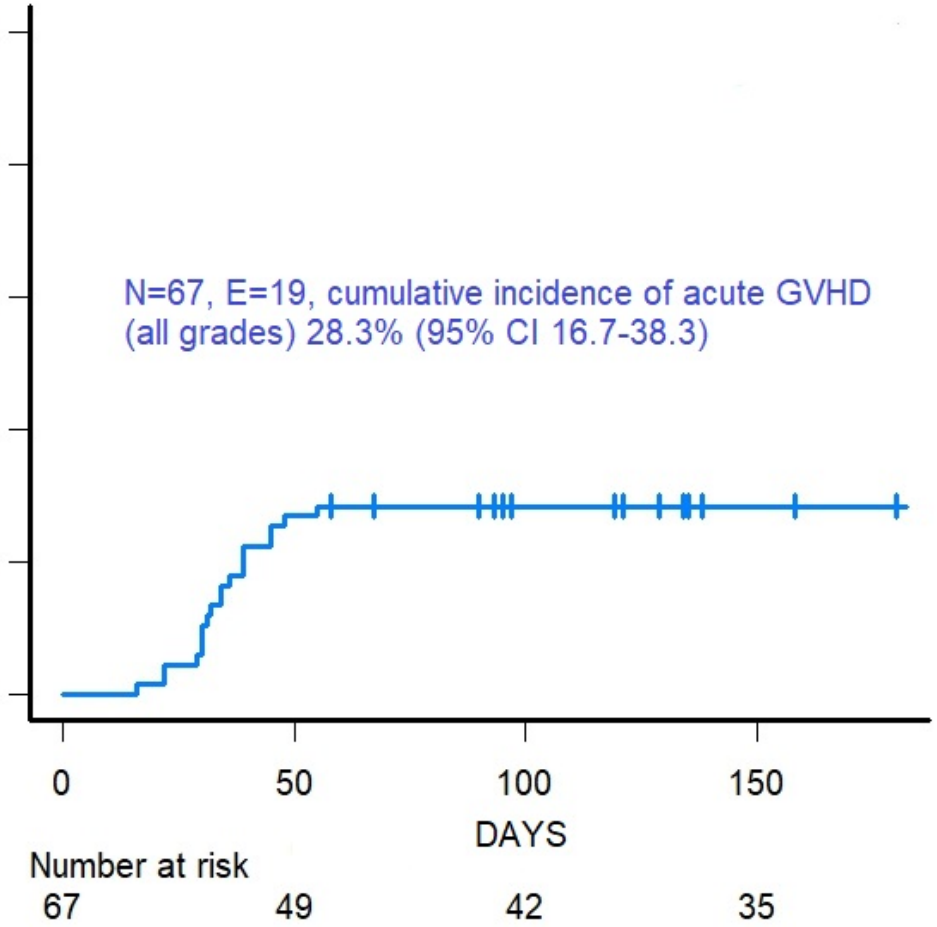
opean centers experience on
s patients enrolled in the study

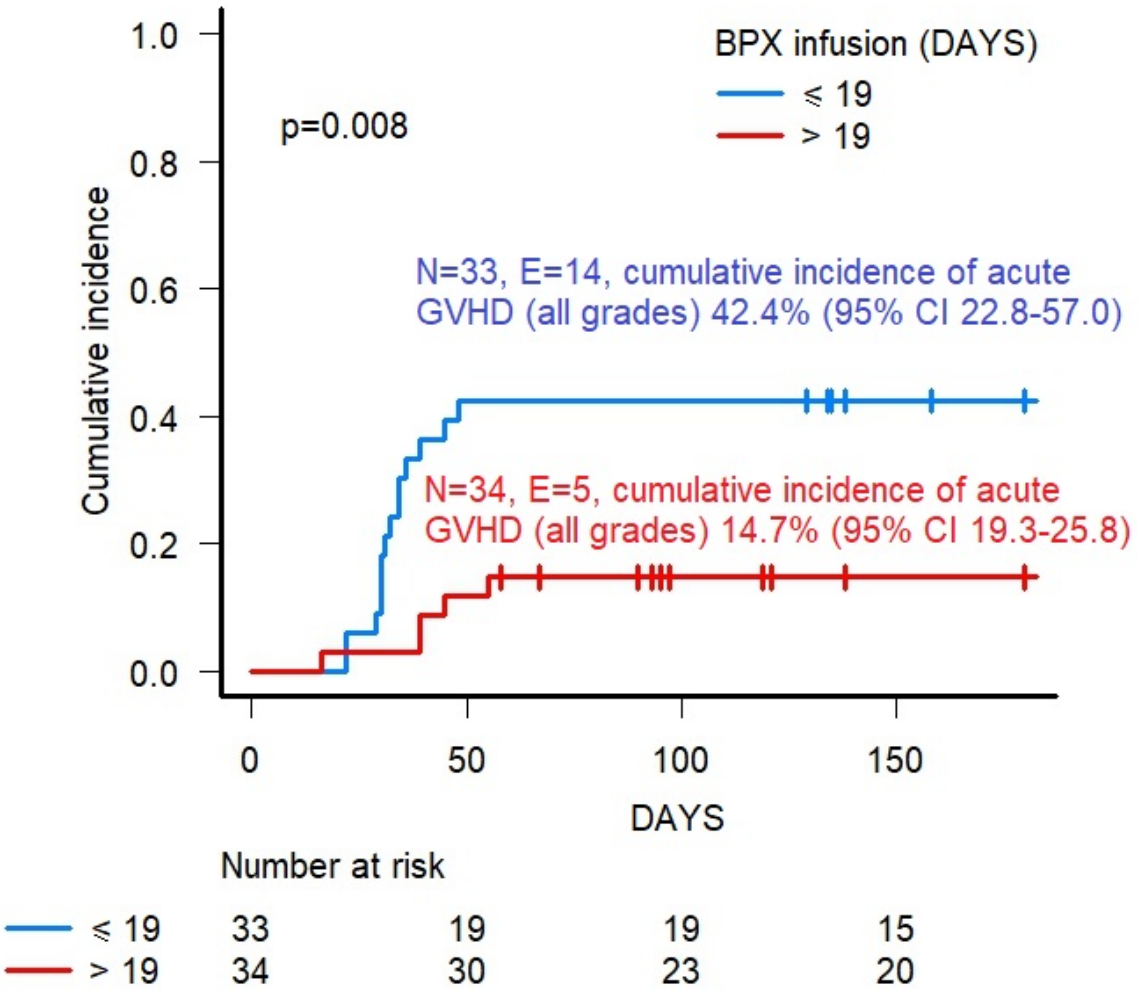
A CID patient died due to
pulmonary hemorrhage caused
by aspergillus infection, which
was already existing before
HSCT.

A WAS patient died due to
leukoencephalopathy of
unknown origin.

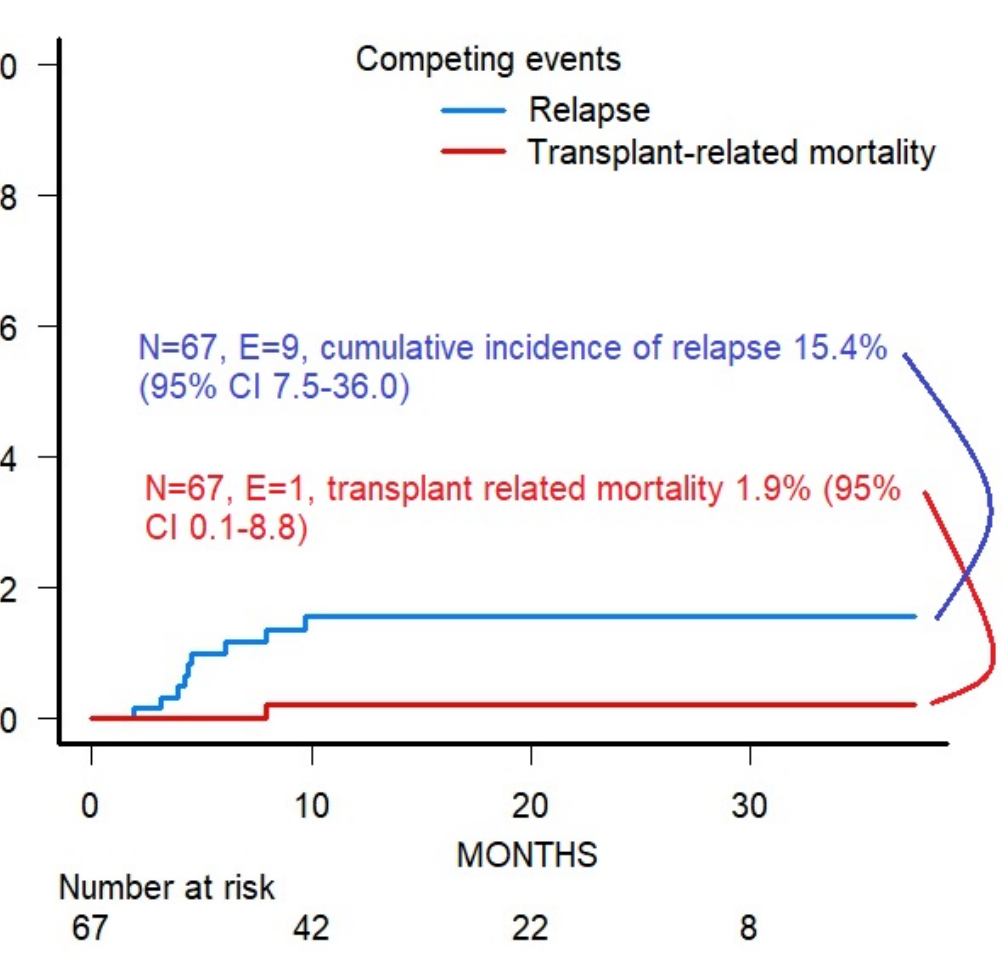


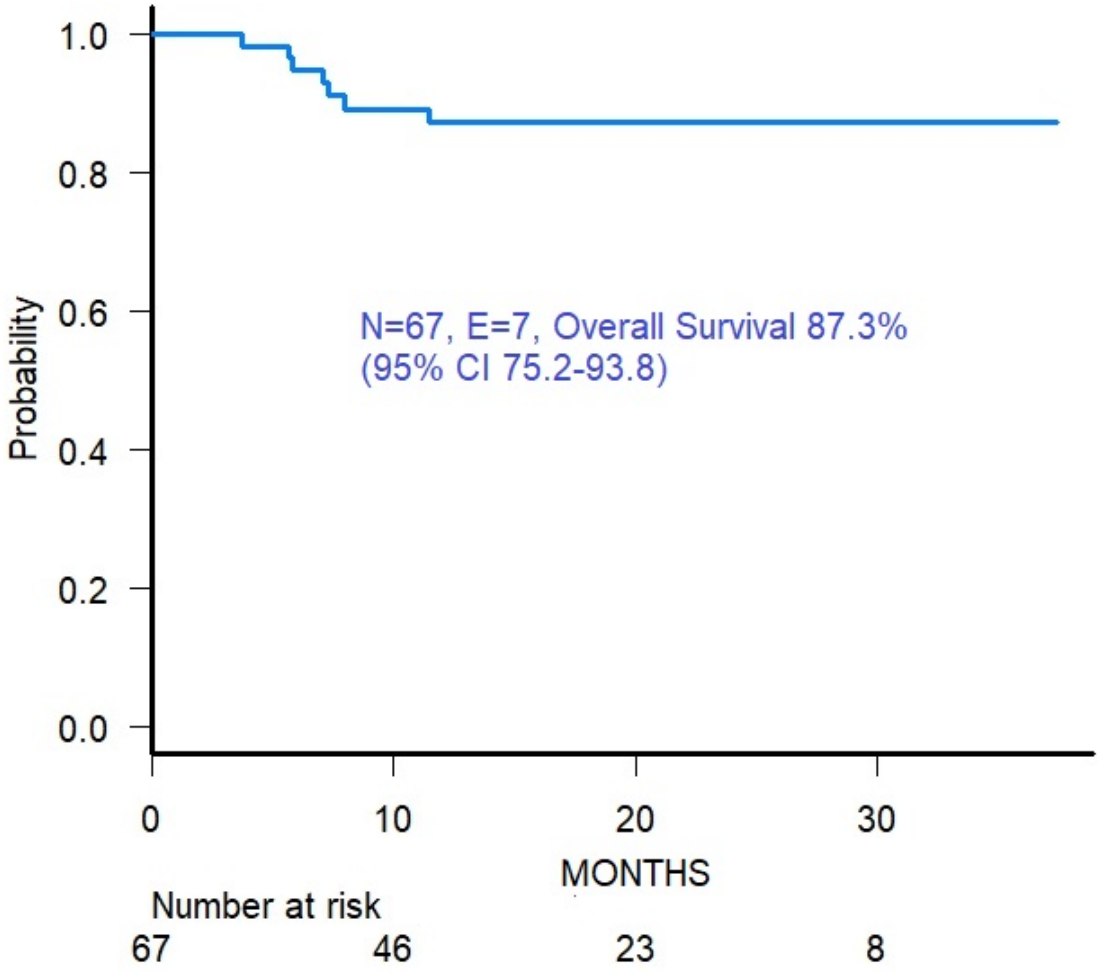
# Acute leukemias: acute GVHD



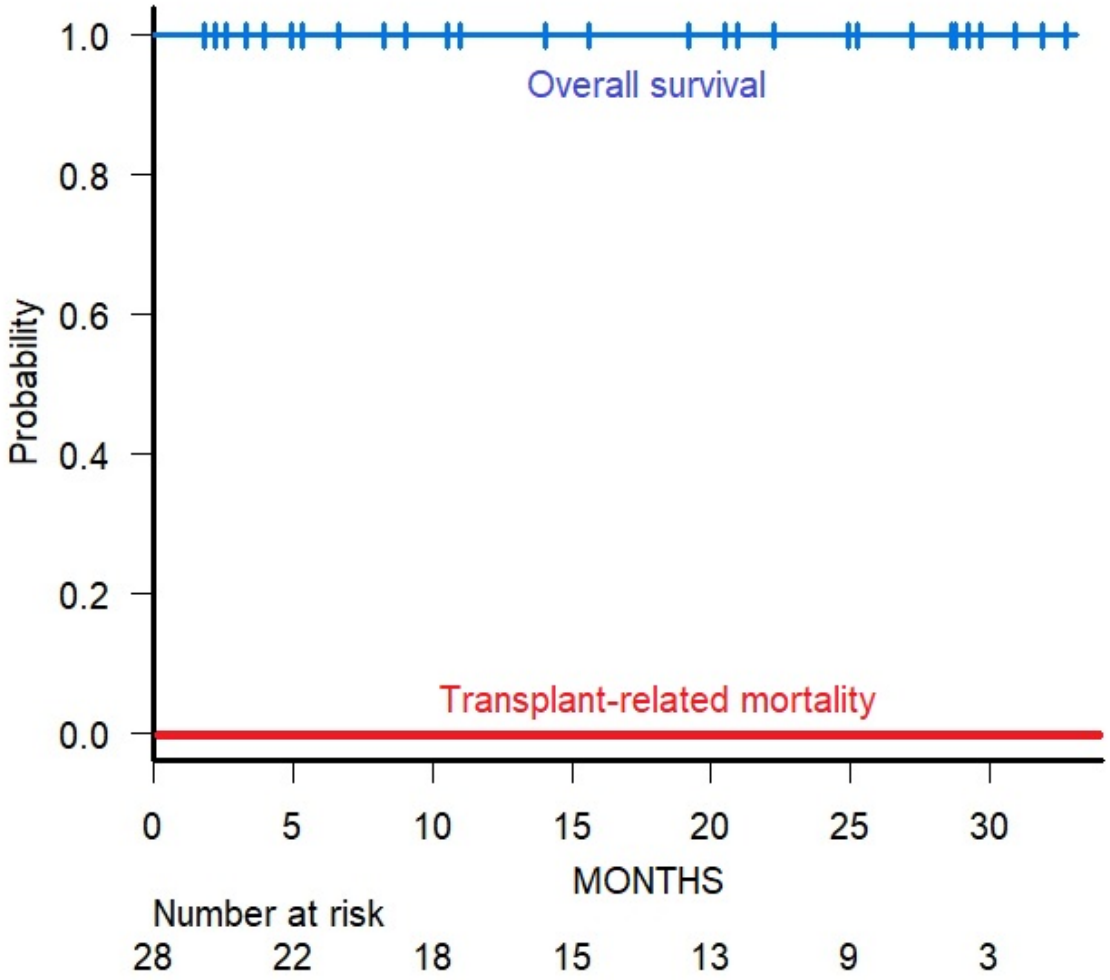



# Acute leukemias: Overall survival

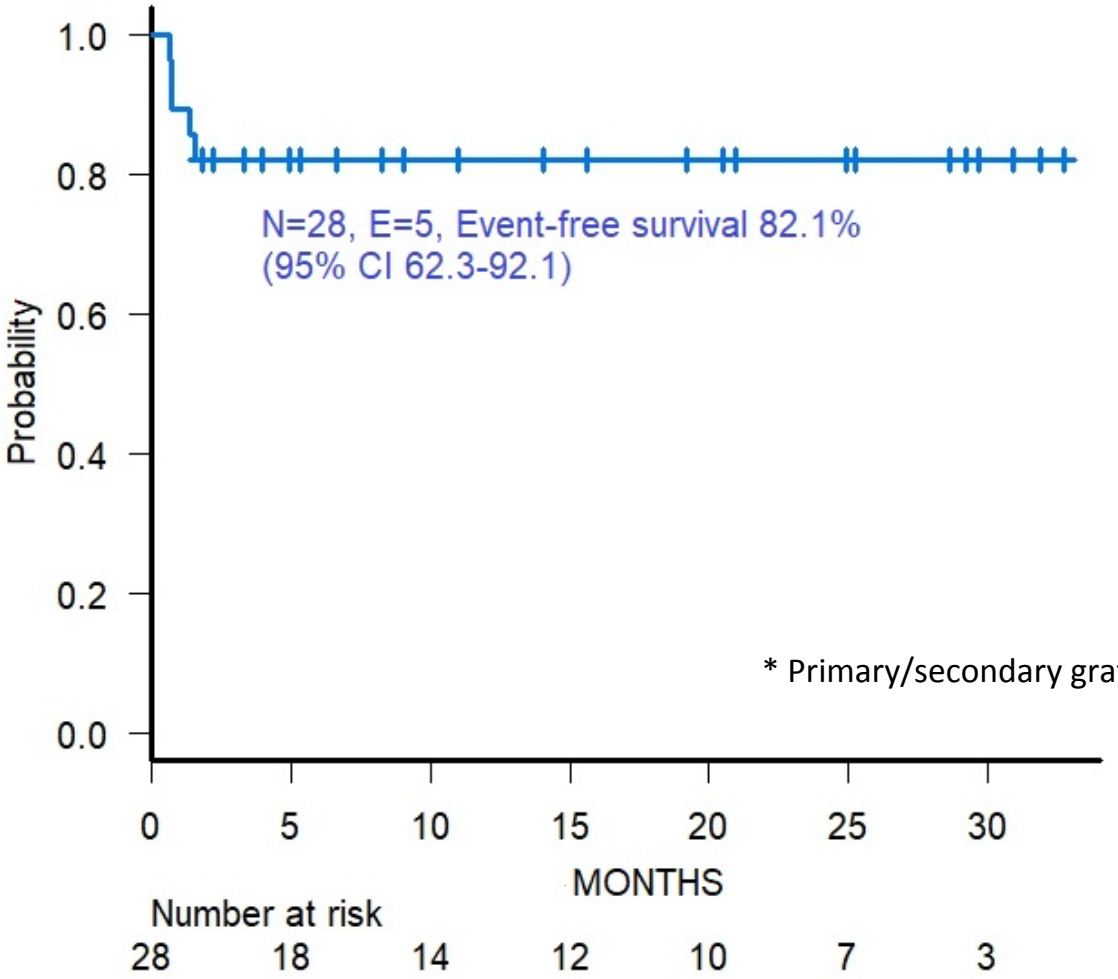


# RBC disorders: Overall survival and Transplant-related mortality



# RBC disorders: Event-free survival



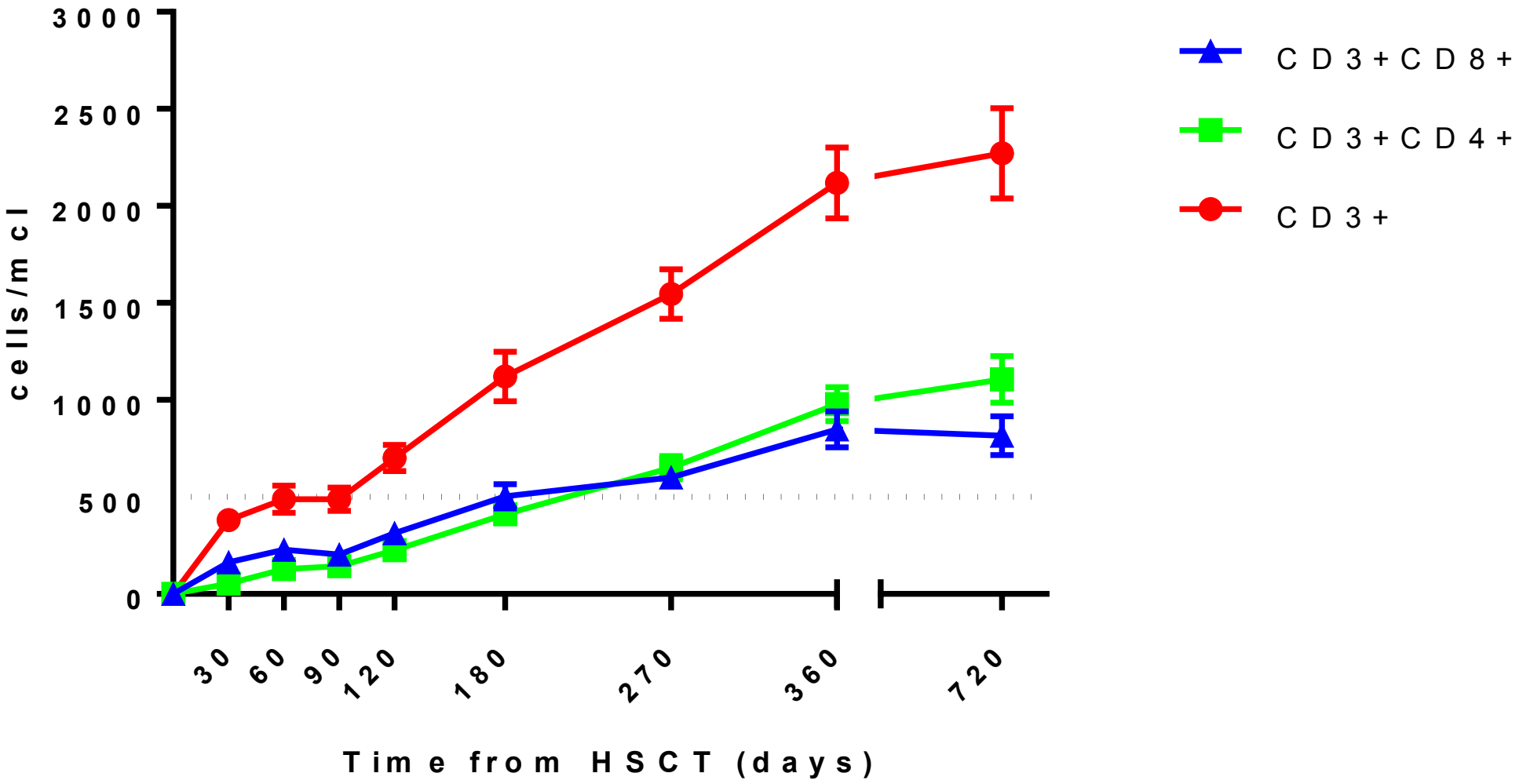

* Primary/secondary graft failure is considered as an event

# BP-004-011 Patient Demographics

Single center experience on all patients follow-up > 90 days enrolled in the study

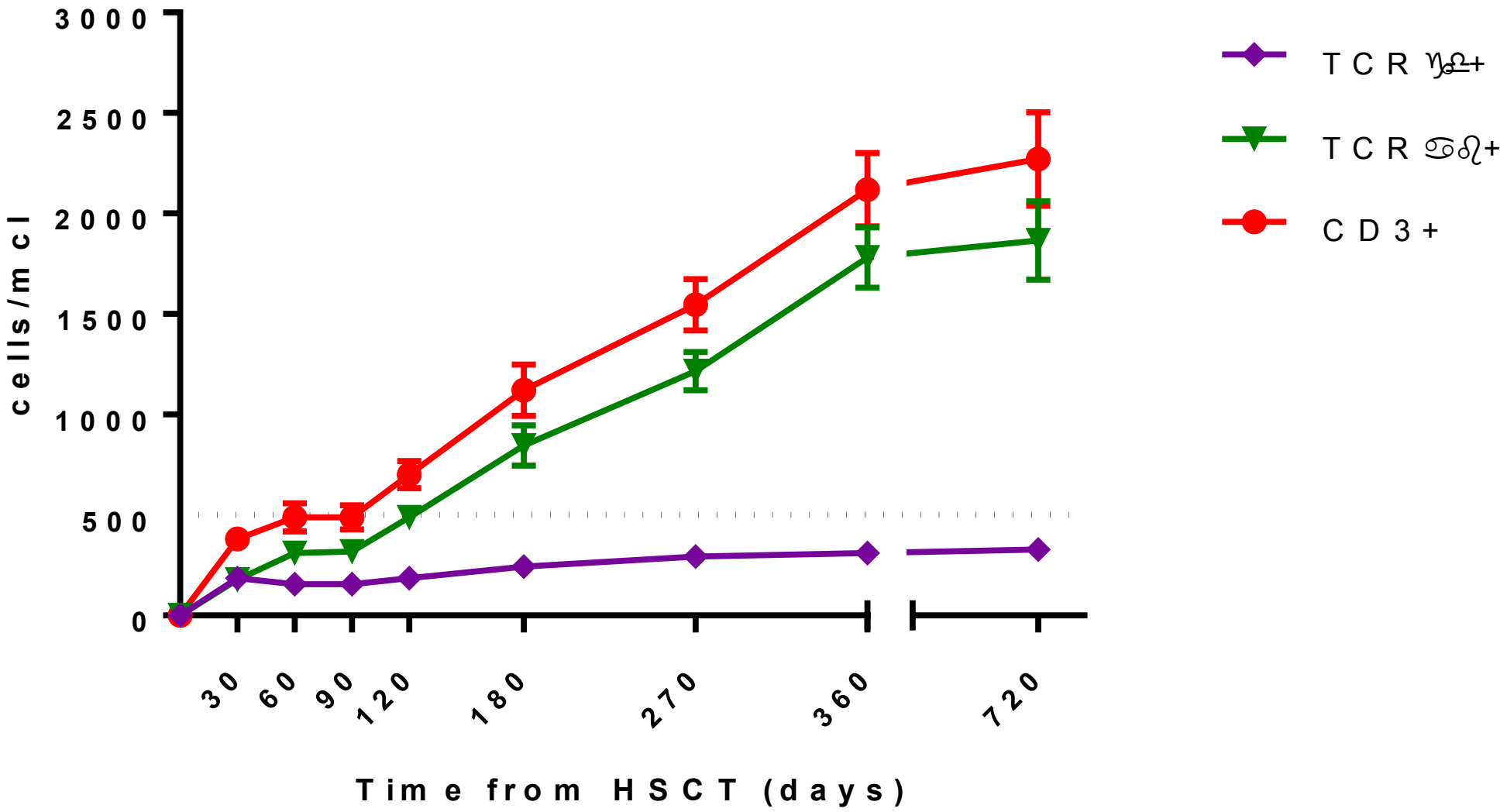
| | N | % (or range) |
|---|---|---|
| Patients | 112 | |
| Gender | | |
| M/F | 63 /49 | 56% / 44% |
| Median Age (yrs) | 5.6 | (0.3-17.9) |
| Conditioning Regimen | | |
| Busulfan-based | 46 | 41% |
| TBI-based | 44 | 40% |
| Treosulfan-based | 18 | 16% |
| Other | 4 | 3% |
| CMV status | | |
| Rec- Don- | 3 | 5.5% |
| Rec+ Don- | 6 | 11% |
| BPX-infusion (days) | | |
| median (range) | 17 | 10-82 |
| Median Follow-up (months) (surviving patients) | 17.5 | (3-35.6) |

# Immune reconstitution – CD3+



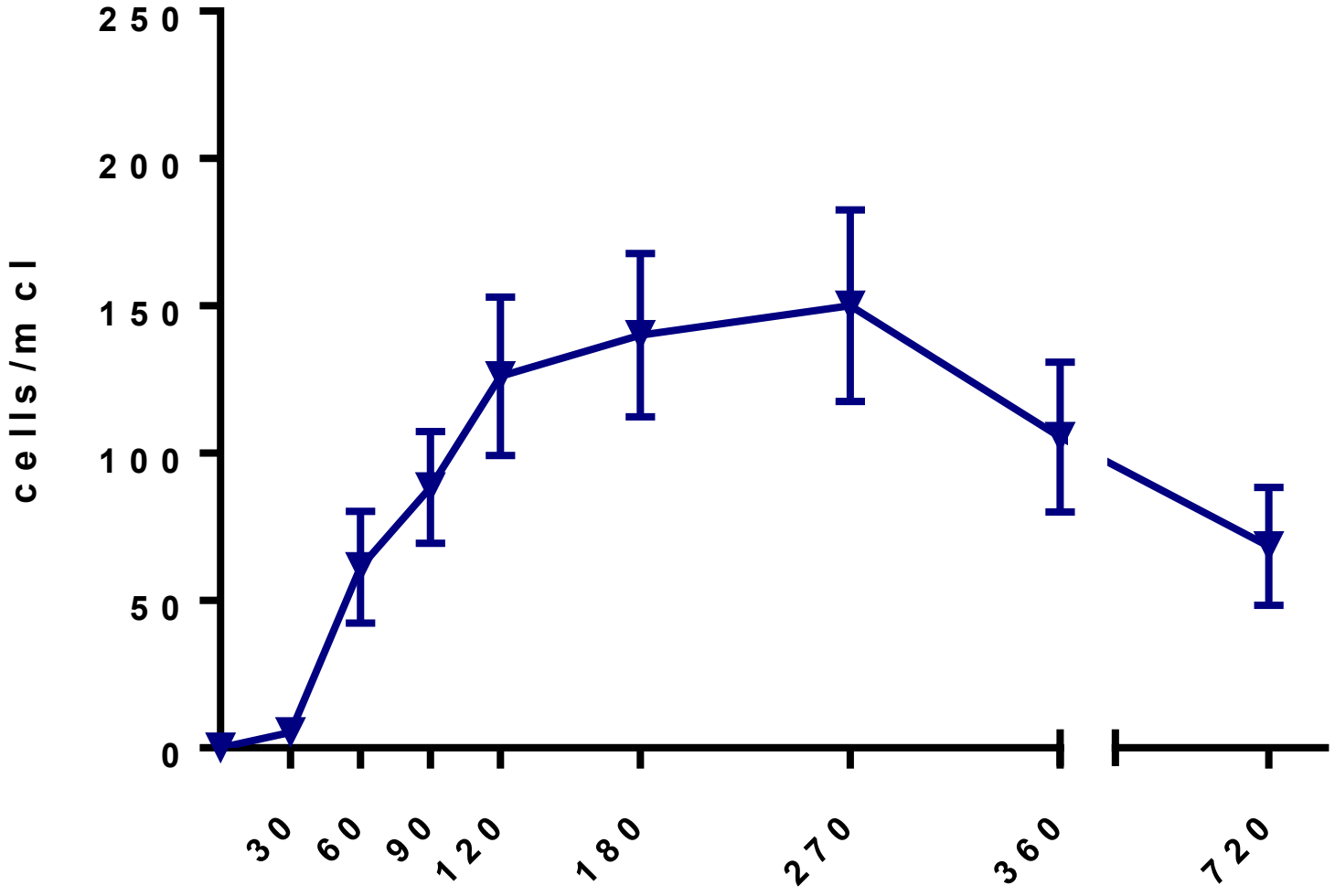

| Number at risk | 112 | 112 | 112 | 102 | 96 | 71 | 67 | 23 |
|---|---|---|---|---|---|---|---|---|

# Immune reconstitution – TCRαβ+ and TCRγδ+



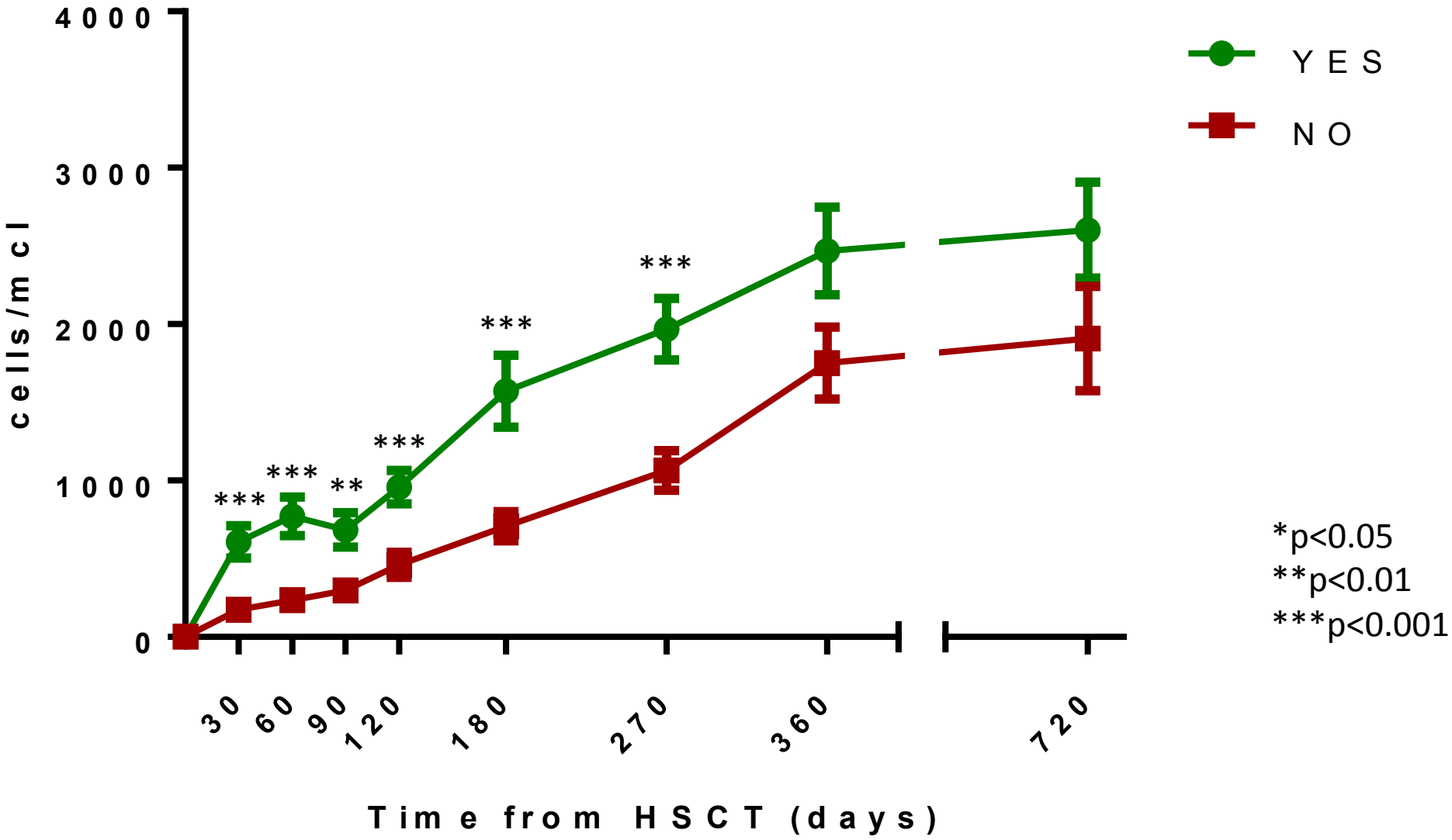

| Number at risk | 112 | 112 | 112 | 102 | 96 | 71 | 67 | 23 |
|---|---|---|---|---|---|---|---|---|

# Immune reconstitution – BPX-501 cells (CD3+CD19+)

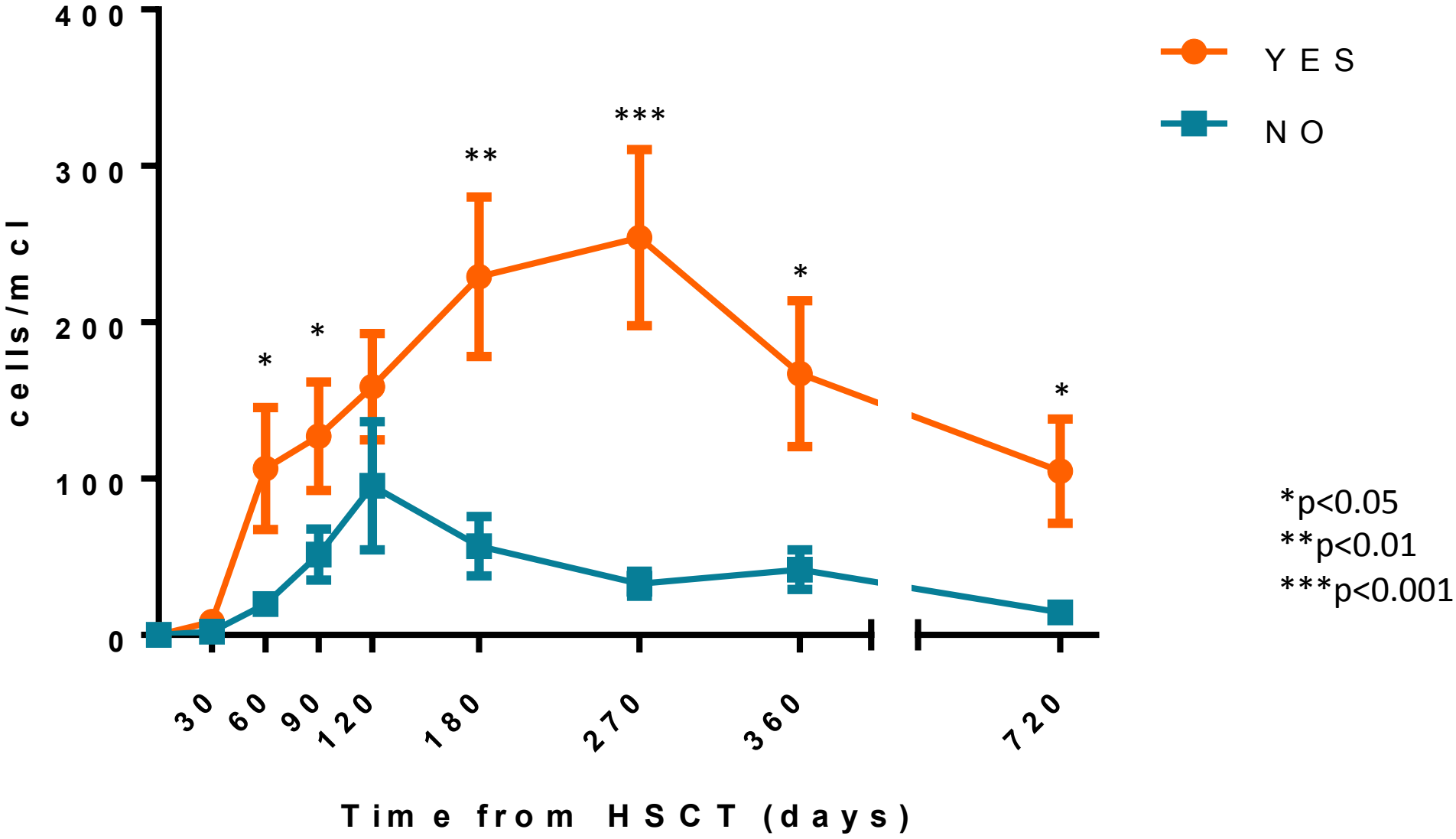



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number at risk | 112 | 112 | 112 | 101 | 95 | 70 | 67 | 23 |

# Immune reconstitution – CD3+ by CMV reactivation



# Immune reconstitution – BPX-501 cells by CMV reactivation



# CD3+ – Multivariate analysis

tor associated with an accelerated immune
onstitution in univariate analysis:
CMV reactivation
No TBI in the conditioning regimen
Age < 2 years
PID diagnosis

ctor associated with an accelerated
mune reconstitution in multivariate
alysis:
CMV reactivation
Age < 2 years

| Dependent Y | cd3 |
|---|---|

**Least squares multiple regression**

| | |
|---|---|
| Method | Backward |
| Enter variable if P< | 0,05 |
| Remove variable if P> | 0,1 |

| | |
|---|---|
| Sample size | 75 |
| Coefficient of determination $R^2$ | 0,3524 |
| $R^2$-adjusted | 0,3344 |
| Multiple correlation coefficient | 0,5937 |
| Residual standard deviation | 842,9211 |

**Regression Equation**

| Independent variables | Coefficient | Std. Error | $r_{partial}$ | t | P |
|---|---|---|---|---|---|
| (Constant) | 2316,5778 | | | | |
| Age | -1277,7684 | 255,5921 | -0,5076 | -4,999 | <0,0001 |
| CMV | 522,5381 | 199,1974 | 0,2954 | 2,623 | 0,0106 |

| Variables not included in the model |
|---|
| Diagnosis |
| TBI |

**Analysis of Variance**

| Source | DF | Sum of Squares | Mean Square |
|---|---|---|---|
| Regression | 2 | 27840354,448 | 13920177,224 |
| Residual | 72 | 51157154,932 | 710516,041 |

| | |
|---|---|
| F-ratio | 19,592 |
| Significance level | P<0,0001 |

## nclusions

TCRαβ/CD19 depleted haplo-HSCT: ideal platform for approaches of po
ransplant immunotherapies

TCRγδ cells can be exploited to improve GvL effect

TCRαβ/CD19 depleted haplo-HSCT and addback of iCaspase9 transduc
lonor T-cells (BPX-501) result into an improved outcome (as compared
istorical controls) for patients affected by acute leukemia, PIDs and r
lood cell disorders;

Rimiducid effectively treated uncontrollable aGVHD due to BPX-501 T cells;

Our results indicate that BPX-501 cells infused after αβ haplo-HSCT expand
*ivo* and persist over time, improving adaptive immunity recovery;

CMV reactivation is the main driver of BPX-501 cell expansion, this findi
uggesting that BPX-501 cells cooperate to the viral clearance;

Multimodal approaches

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