

**XXVI**  
**Congresso**  
**Nazionale**  
**AIBT**  
Associazione Italiana  
di Immunogenetica  
e Biologia dei Trapianti



**Pavia, 3-5 ottobre 2019**  
**Università degli Studi di Pavia**



Fondazione IRCCS  
Policlinico San Matteo

Sistema Socio Sanitario



Regione  
Lombardia

## **Il TCSE aploidentico nel bambino mediante selezione immunomagnetica delle cellule staminali emopoietiche: una piattaforma per la terapia cellulare**

**Marco Zecca**

Oncoematologia Pediatrica

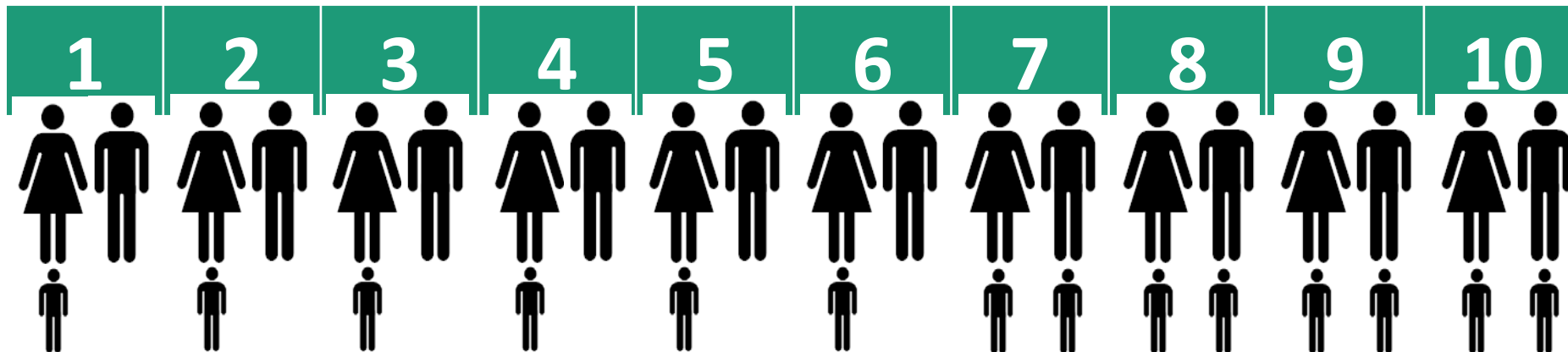
Fondazione IRCCS Policlinico San Matteo

Pavia

E-mail: [m.zecca@smatteo.pv.it](mailto:m.zecca@smatteo.pv.it)

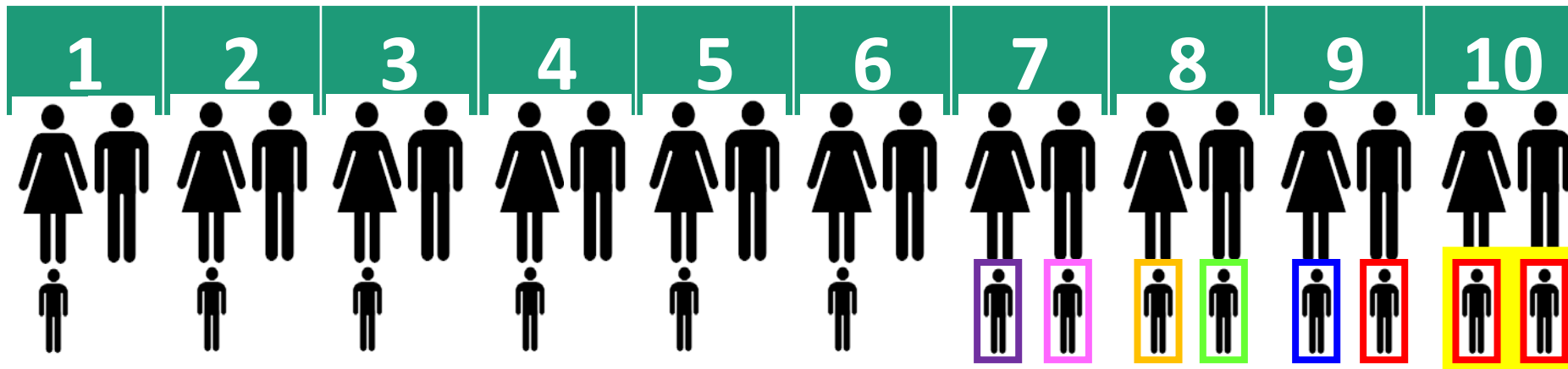
# Perché un donatore alternativo ?

Numero medio di figli per famiglia			ISTAT 2011	
	2008	2009	2010	2011
Nord	1,46	1,48	1,48	1,48
Centro	1,41	1,38	1,38	1,38
Sud	1,35	1,35	1,35	1,35
ITALIA	1,42	1,41	1,41	1,42



# Perché un donatore alternativo ?

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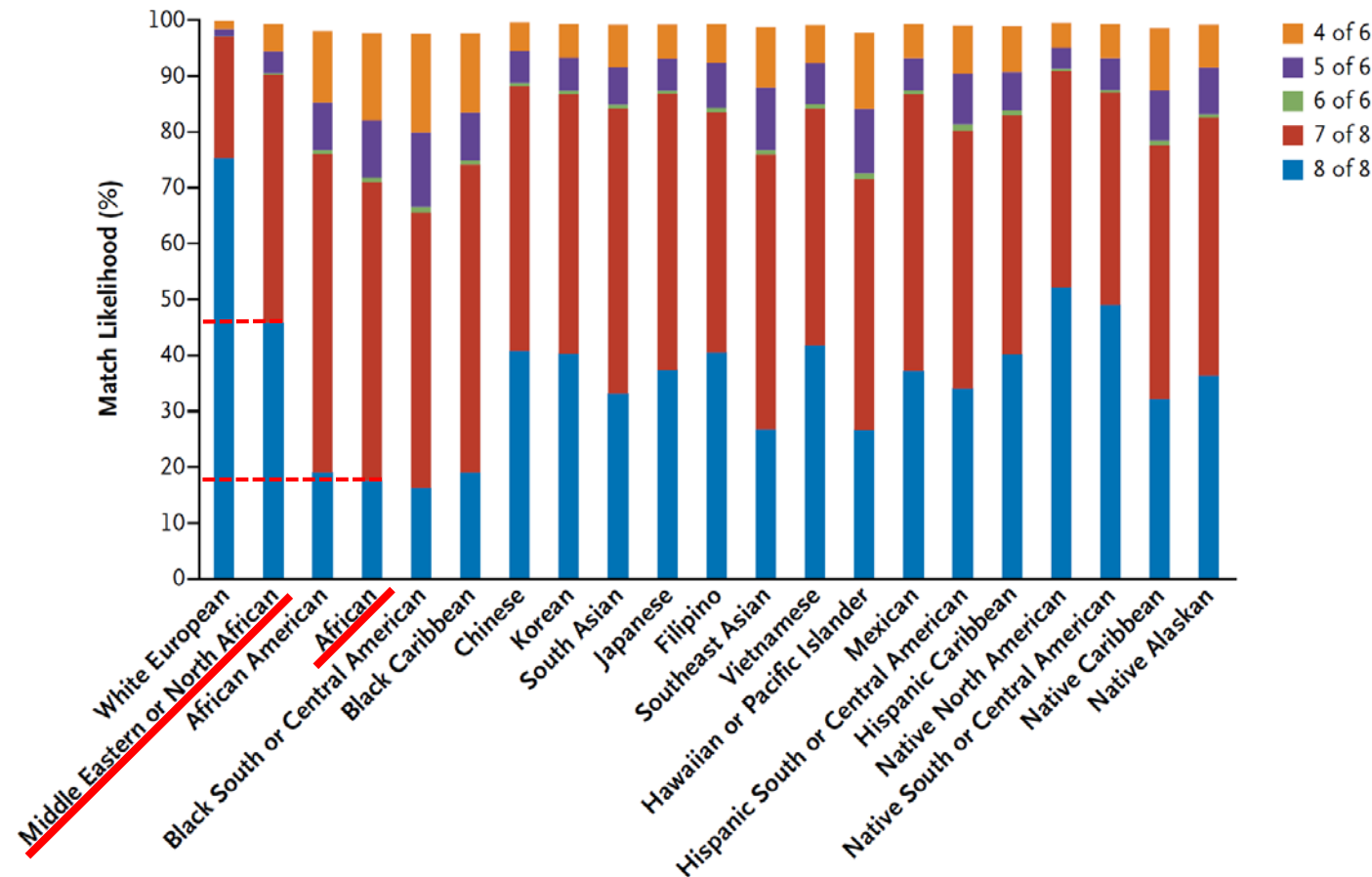


## SPECIAL ARTICLE

# HLA Match Likelihoods for Hematopoietic Stem-Cell Grafts in the U.S. Registry

Loren Gragert, B.S., B.A., Mary Eapen, M.B., B.S., Eric Williams, Ph.D., John Freeman, B.S., Stephen Spellman, M.B.S., Robert Baitty, M.P.P., Robert Hartzman, M.D., J. Douglas Rizzo, M.D., Mary Horowitz, M.D., Dennis Confer, M.D., and Martin Maiers, B.A.

**A** Patients <20 Yr of Age



## Match likelihood according to racial and ethnic group.

The likelihood of finding a match with the use of a search strategy win which an 8/8 HLA-matched donor is sought first, then a 7/8 HLA-matched donor, and thereafter a cord-blood unit with an adequate cell dose is shown.

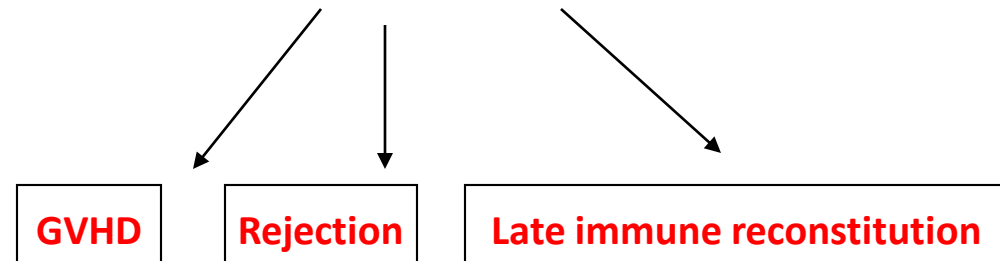
# Haploidentical transplant

## *Advantages*

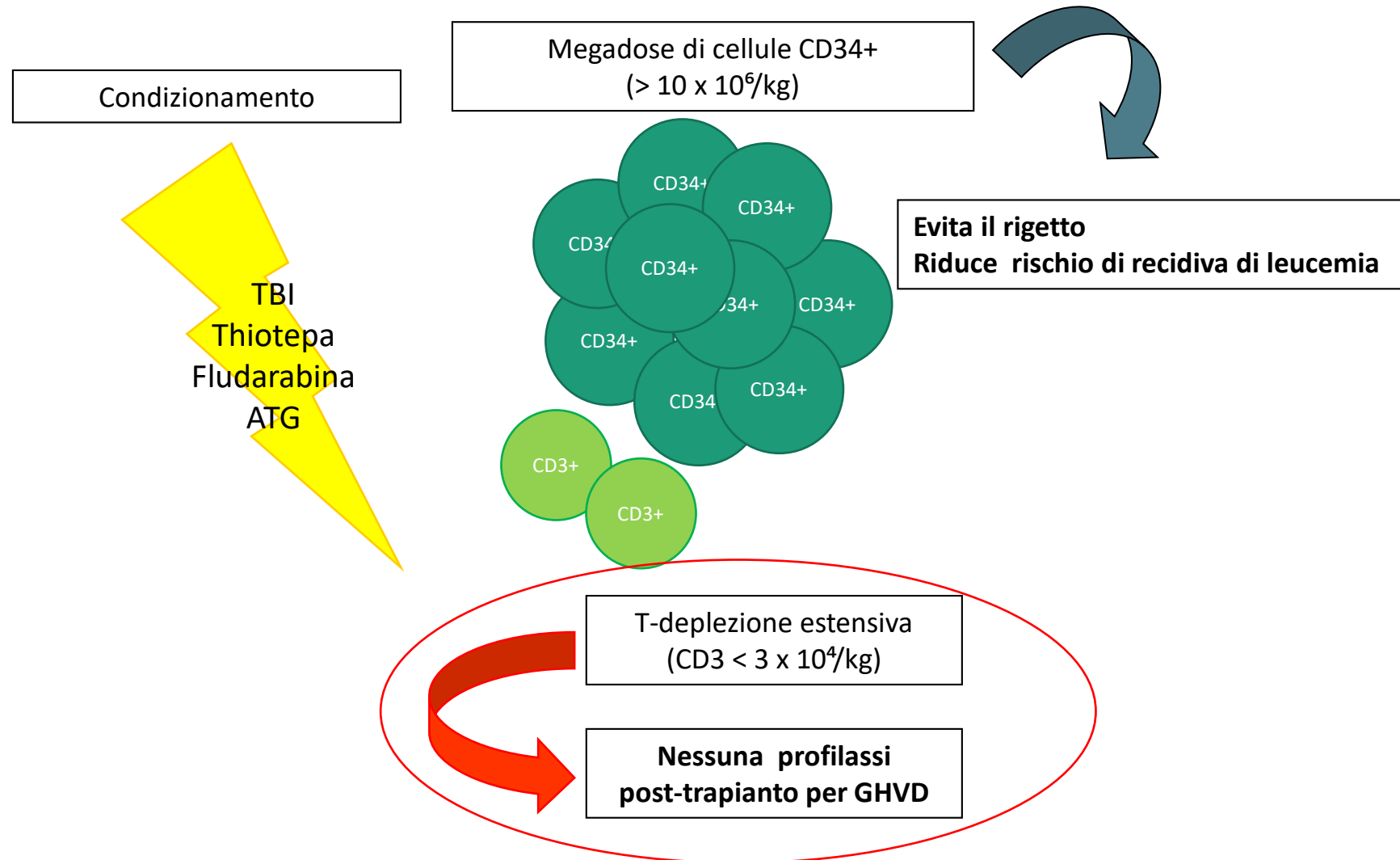
- Speed
  - (related donors easy to contact)
- Easy stem cell collection
  - (usually PBSC after 4-5 days of G-CSF administration)
- Low cost

## *Disadvantages*

- **HLA disparity**
  - (graft rich in T and B cells)



# Aplo-TCSE: come evitare il rigetto senza aumentare il rischio di GVHD



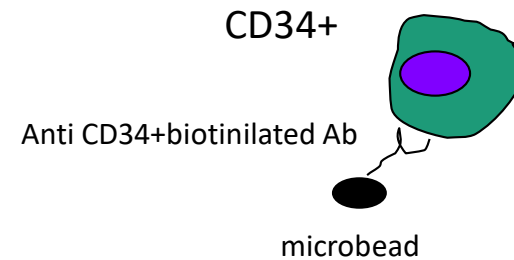


# T cell-depletion for Haplo-HSCT:

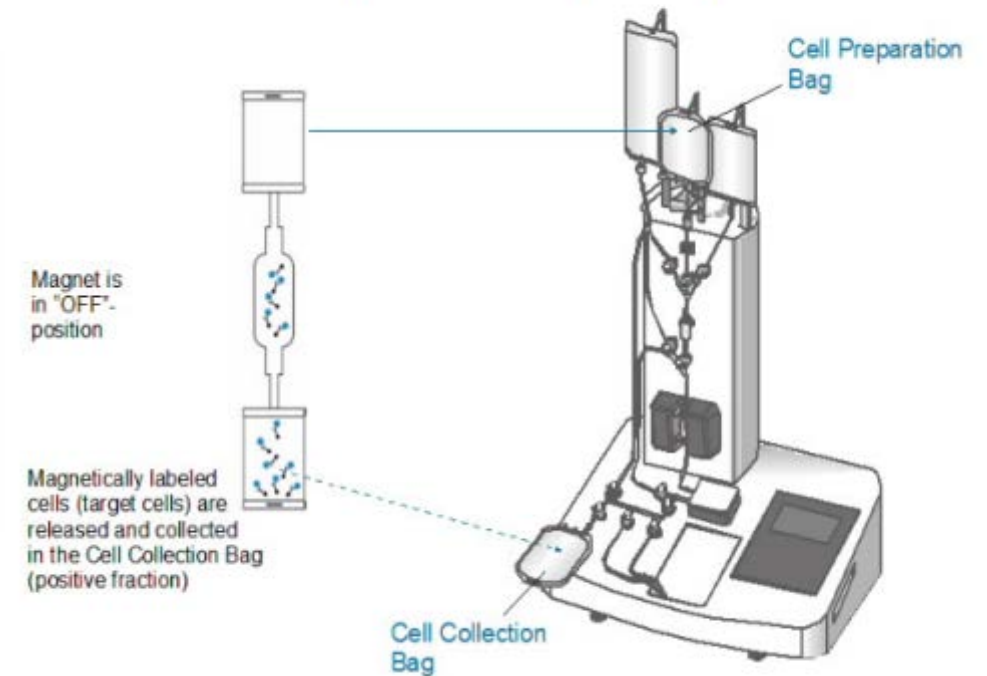
7

1995


## 1. CD34+ Selection „pure stem cells“



## Separation principle: Enrichment



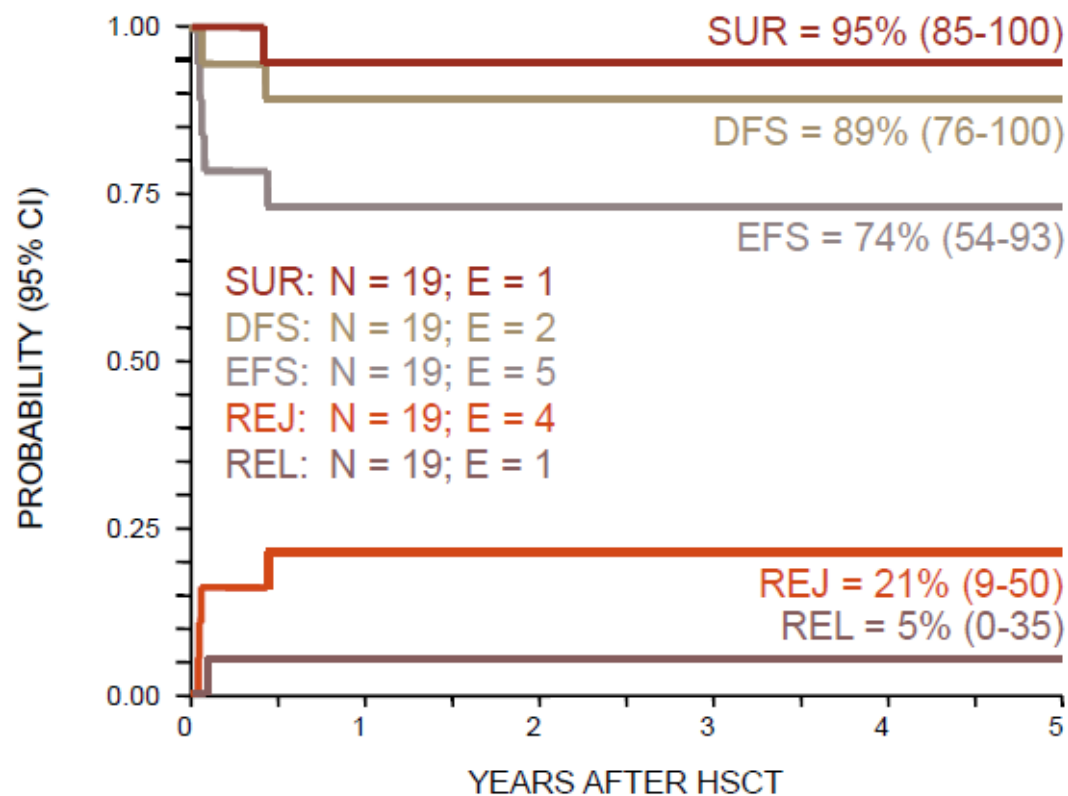
# CD34+ selection

- Depletion of T cells ( $< 1 \times 10^5/\text{kg}$ ): no GvHD
  - Higher infections
  - Rejection  prolonged lymphopenia
- 
- CD34+ megadose ( $> 10^7/\text{kg}$ ): to reduce infections and rejection. (Aversa F et al. J Clin Oncol 2005)

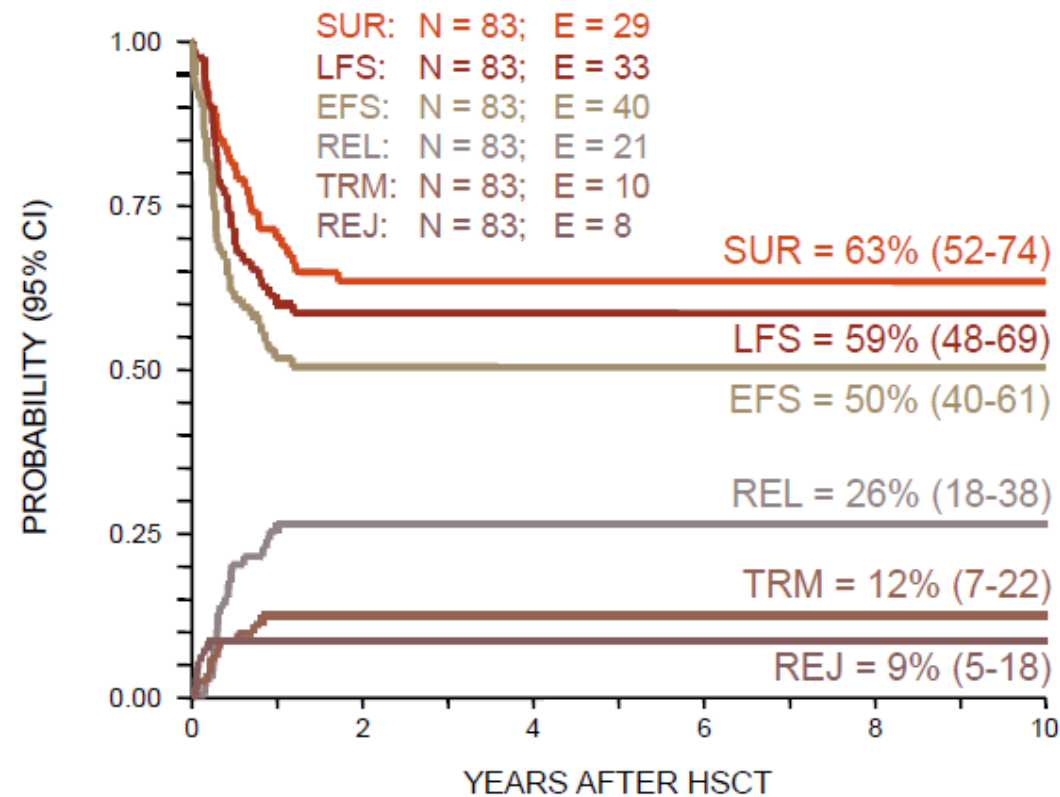


# Haplo-TCSE with positive CD34+ selection: results

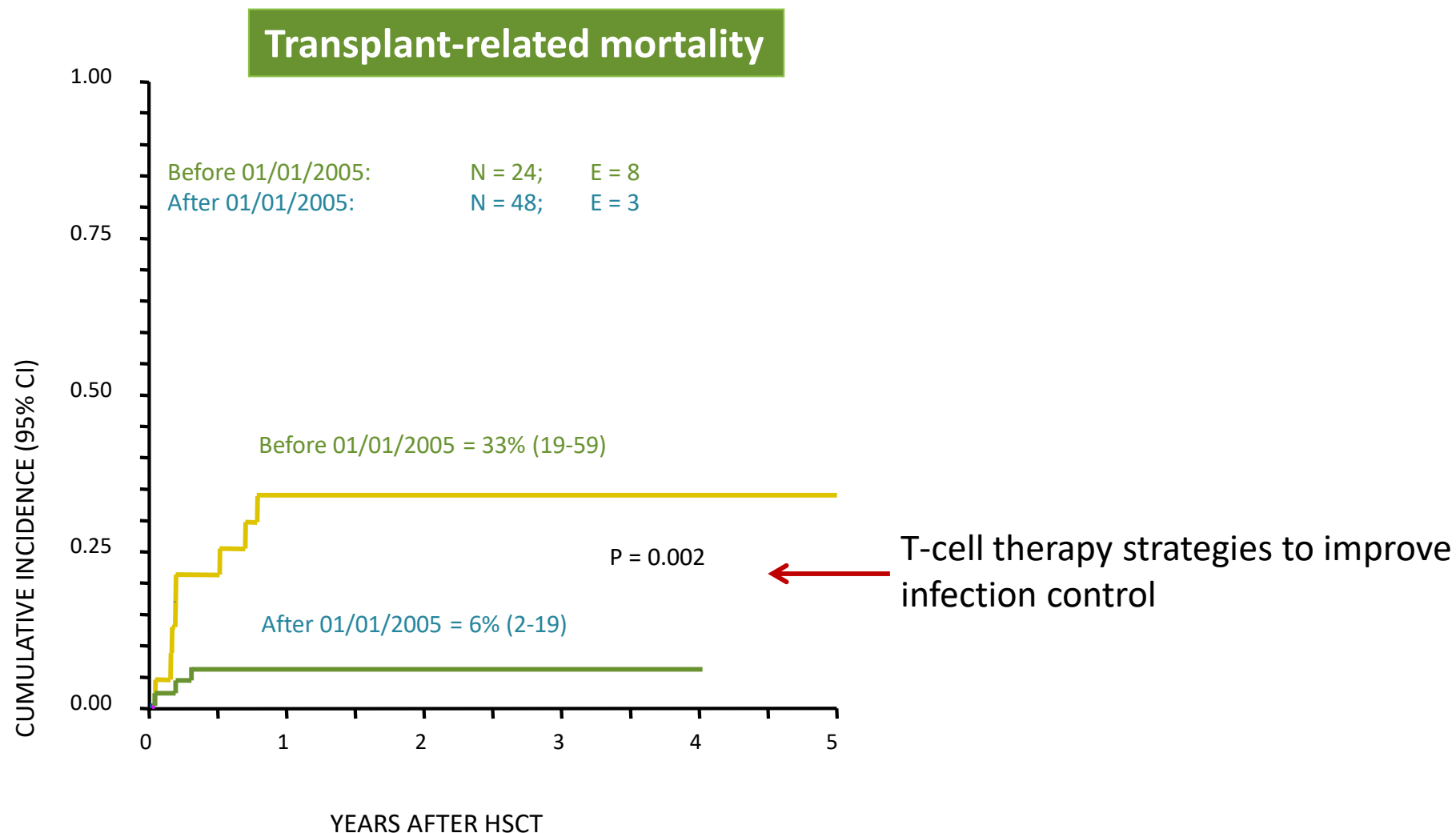
## Non malignant diseases



## Malignant diseases: outcome



# Haplo-TCSE with positive CD34+ selection: results



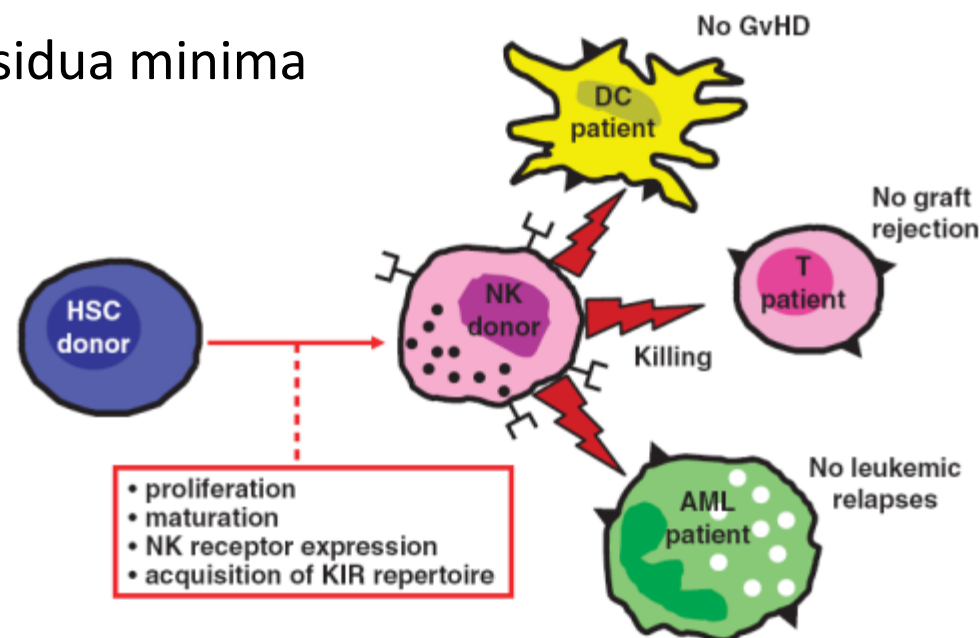
The others matter!

# Ricostituzione immunologica dopo TCSE

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Nel contesto del trapianto parzialmente compatibile T-depleto, i linfociti **NK alloreattivi** verso il ricevente possono giocare un ruolo chiave

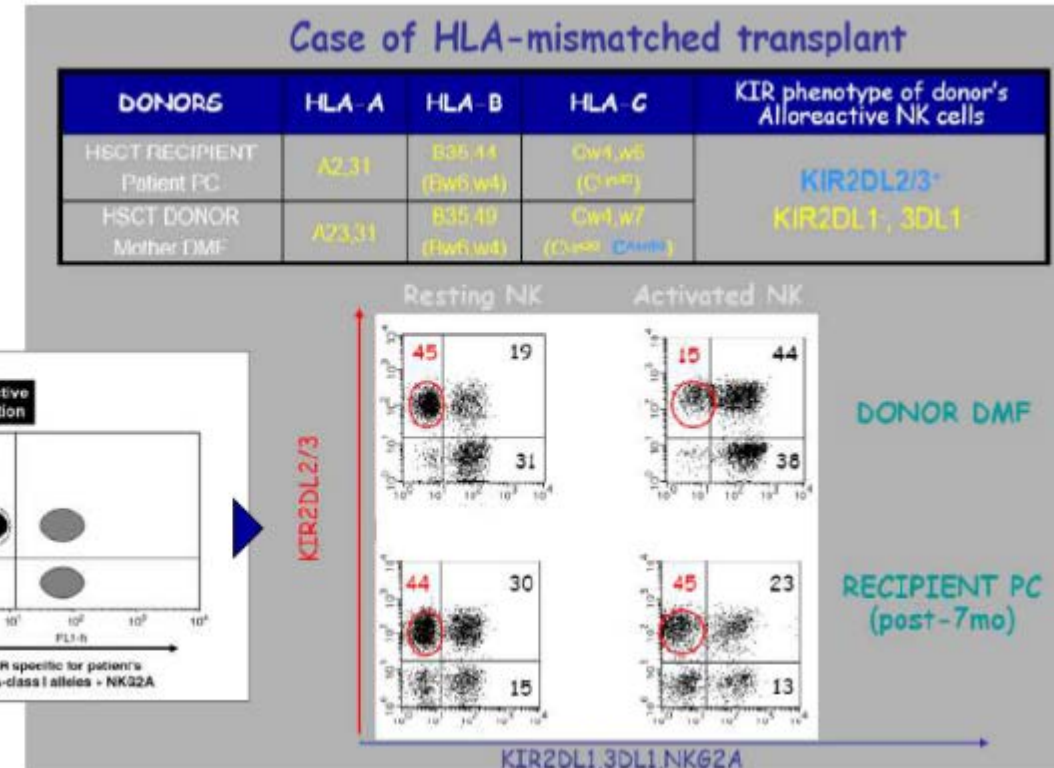
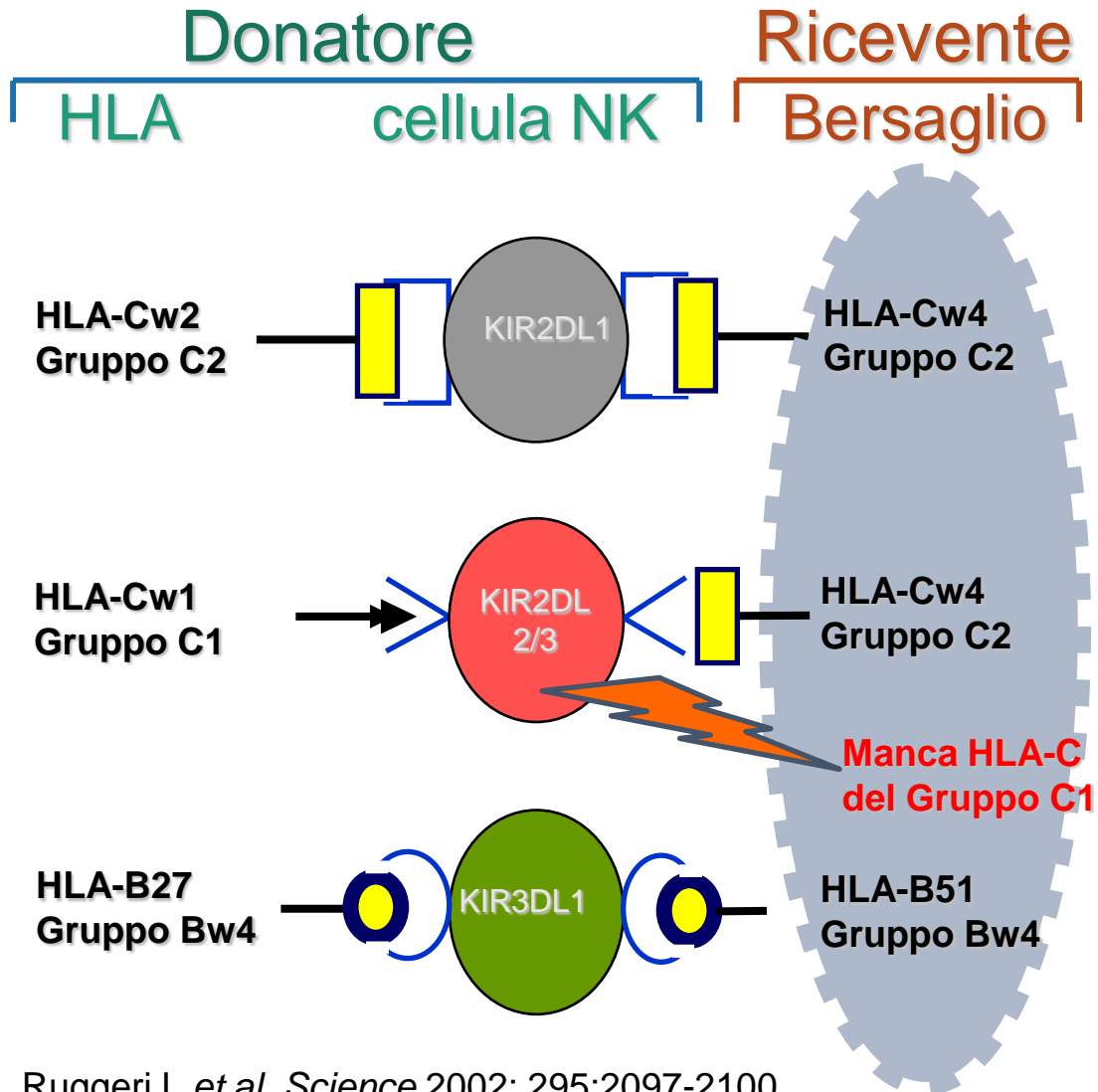
- nella prevenzione del rigetto
- nella prevenzione della GVHD
- nell'eradicazione della malattia residua minima



Ruggeri et al. *Science* 2002

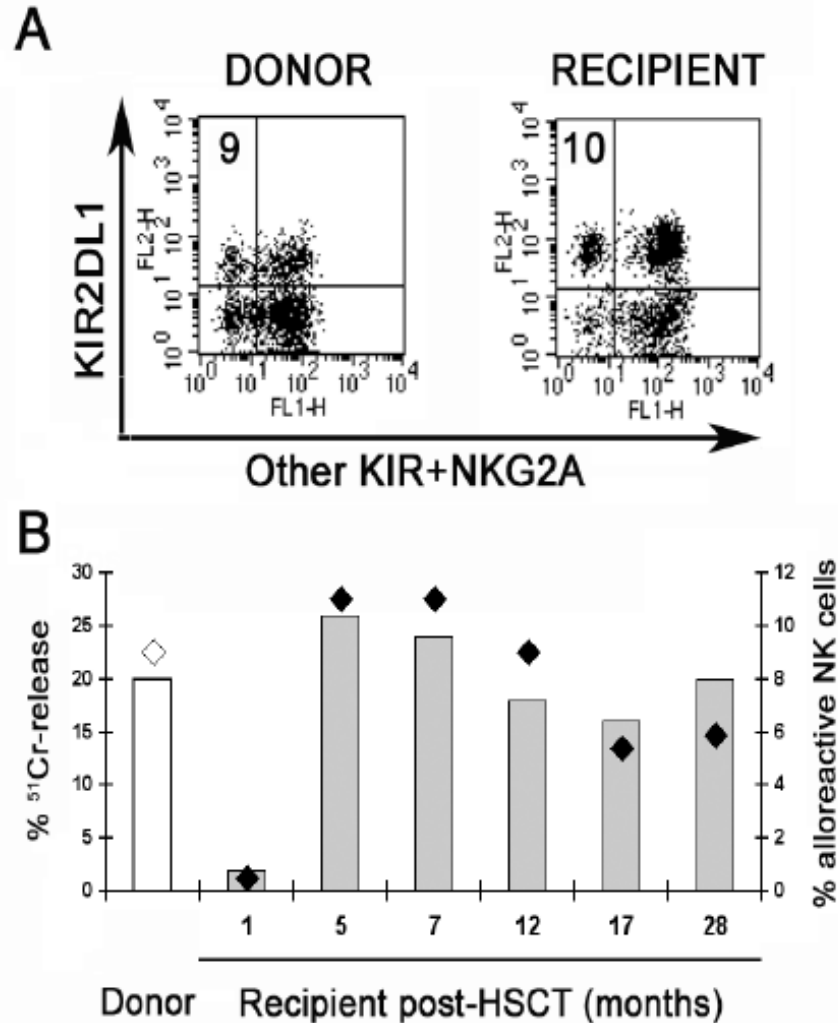
# Ricostituzione immunologica dopo TCSE

## Il trapianto aploidentico NK alloreattivo

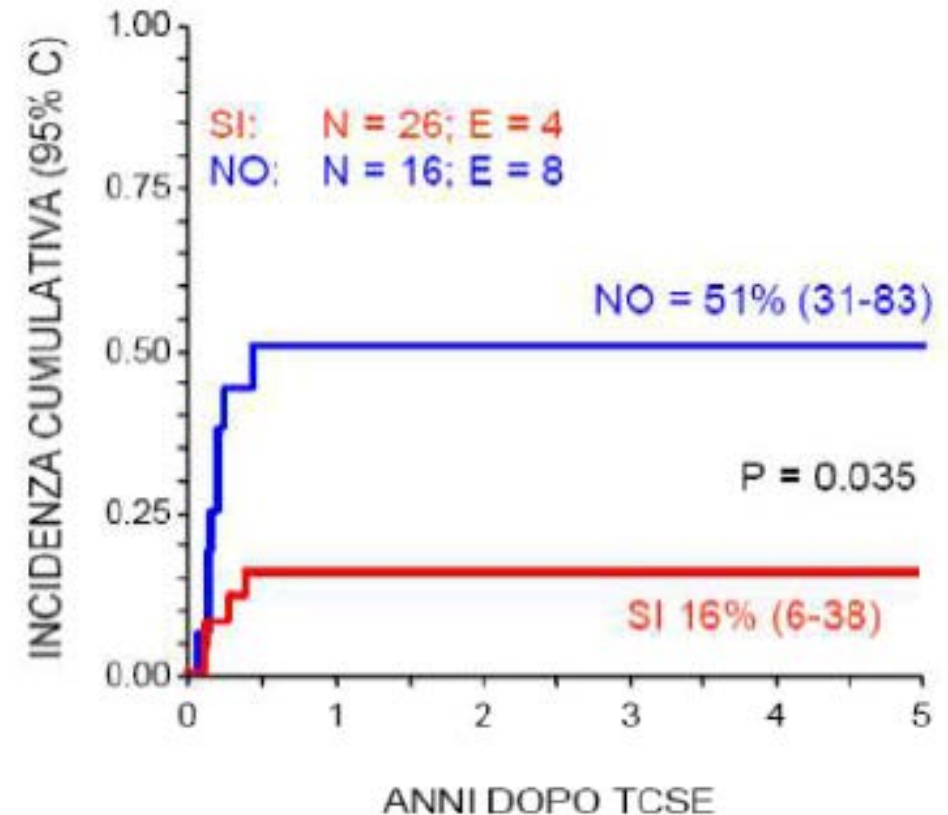


# Ricostituzione immunologica dopo TCSE

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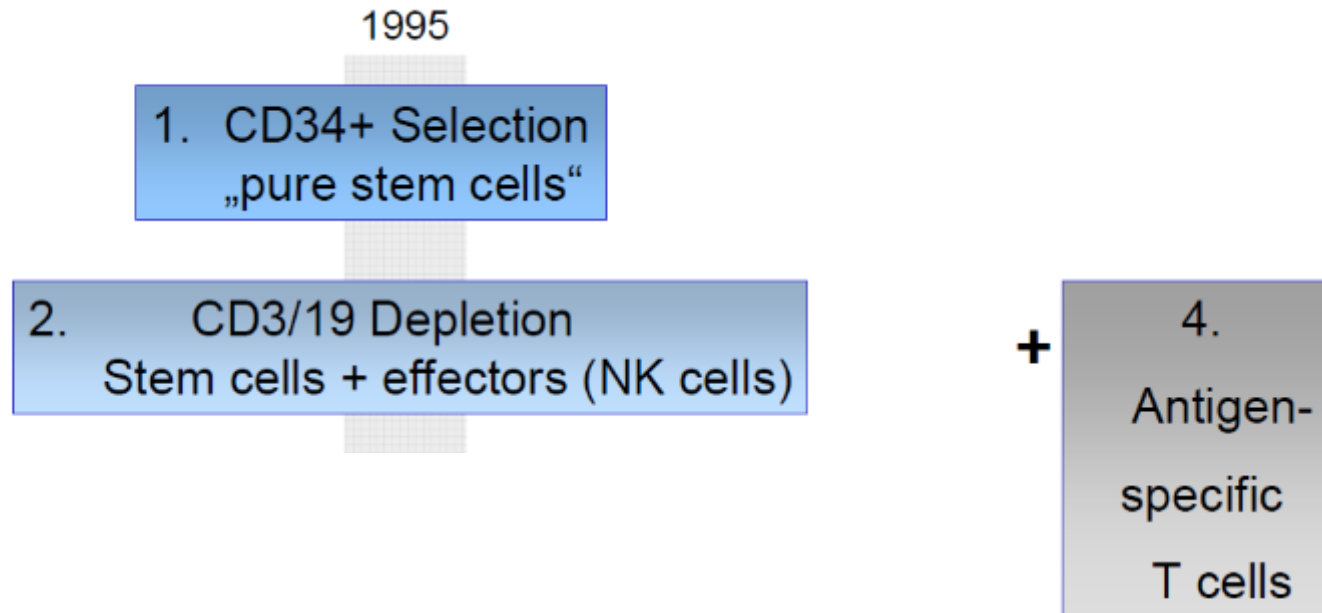


TCSE da donatore aploidentico per leucemia acuta linfoblastica:  
incidenza di ricaduta in base all'alloreattività NK



# T cell-depletion for Haplo-HSCT:

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# CD3 depletion

- Alloreactive NK cells can facilitate engraftment by elimination of residual host hematopoiesis in mouse model (Ruggeri L et al. Science 2002)
- Negative depletion strategy developed to preserve NK and accessory cells in the graft
- Reduced intensity conditioning regimens were used
- Improved clinical results in terms immune reconstitution
- High rate of aGvHD (52%) and cGvHD (28%)
- Relapse

(Chen X et al. Br J Haematol 2006, Dykes JH et al. Transfusion 2007)

# CD3/19 depletion

- T cells 10 fold higher than CD34+ ICS
- NK, Monocytes and DC content
- Avoid post transplant EBV related lymphoproliferative disease
- Less mortality
- Better immune recovery

# CD3/19 depletion in children

## Early evaluation of immune reconstitution following allogeneic CD3/CD19-depleted grafts from alternative donors in childhood acute leukemia

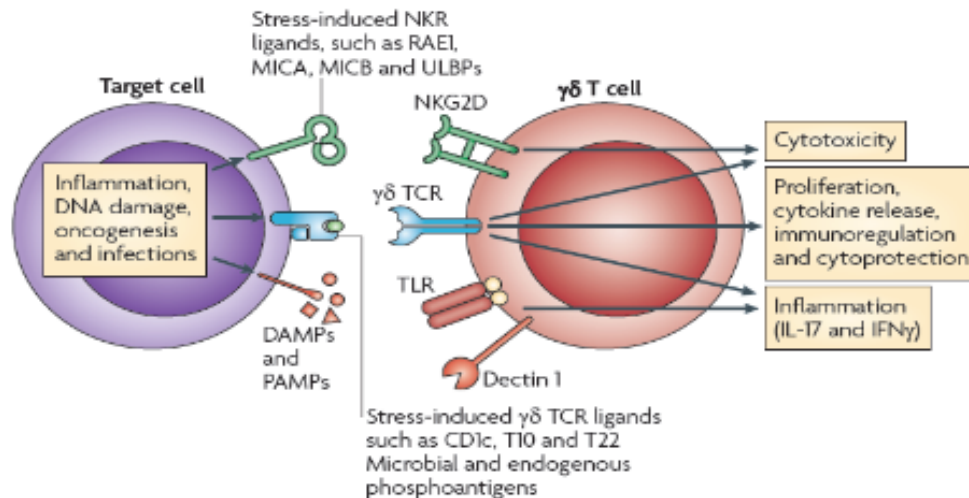
A Pérez-Martínez<sup>1</sup>, M González-Vicent<sup>1</sup>, J Valentín<sup>1</sup>, E Aleo<sup>1</sup>, A Lassaletta<sup>1</sup>, J Sevilla<sup>1</sup>, JL Vicario<sup>2</sup>, M Ramírez<sup>1</sup> and MA Díaz<sup>1</sup>

Graft engineering procedures for hematopoietic SCT (HSCT) may improve the chance of success in matched unrelated donor (MUD) and haploidentical donor transplantations. Successful donor immune reconstitution is important to mediate GVL effects in reduced-intensity conditioning (RIC) HSCT. We prospectively investigated early immune reconstitution and clinical outcome in 30 CD3/CD19-depleted MUD ( $n = 15$ ) or HP ( $n = 15$ ) HSCTs for high-risk childhood leukemia using a fludarabine-based RIC without serotherapy. The graft consisted of a mean of  $10.5 \times 10^6/\text{kg}$  CD34<sup>+</sup>,  $77 \times 10^3/\text{kg}$  CD3<sup>+</sup> and  $39 \times 10^6/\text{kg}$  CD56<sup>+</sup> cells. After transplantation, 86% of the patients engrafted. In all, 13% of patients had >grade 3 acute GVHD. Natural killer (NK) cell, DC and T-cell recovery achieved normal values within the first 60 days after transplantation. DC recovery was dominated by the DC2<sup>+</sup> subset. NK-cell phenotype was altered and cytotoxicity was lower compared with their donors. EFS was  $50 \pm 9\%$  ( $73 \pm 11\%$  for those in CR1 and  $26 \pm 11\%$  for those with advanced disease). Faster DC2<sup>+</sup> recovery was associated with better outcome, especially in the MUD setting. In summary, CD3/CD19-depleted HSCT with fludarabine-based RIC without serotherapy resulted in favorable patient survival, and rapid NK, DC and T-cell recovery.

*Bone Marrow Transplantation* (2012) **47**, 1419–1427; doi:10.1038/bmt.2012.43; published online 12 March 2012

The others matter!

# $\gamma\delta$ T cell effector functions: a blend of innate programming and acquired plasticity



**Figure 1 | Sensing of cellular stress and infection by  $\gamma\delta$  T cells.**  $\gamma\delta$  T cells can recognize separately, additively or synergistically three sets of stress-induced stimuli: MHC-related and -unrelated T cell receptor (TCR) ligands (such as the weakly polymorphic MHC class I-like human CD1c molecules and mouse T10 and T22 molecules, and microbial and endogenous phosphoantigens), various cell surface molecules (such as retinoic acid early transcript 1 (RAE1) and MHC class I polypeptide-related sequence A (MICA)) that engage the activating natural killer receptors (NKR) such as NK group 2, member D (NKG2D), and/or danger-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) recognized by pattern recognition receptors (such as Toll-like receptors (TLRs) and dectin 1). IFN $\gamma$ , interferon- $\gamma$ ; IL-17, interleukin-17; ULBP, cytomegalovirus UL16-binding protein.

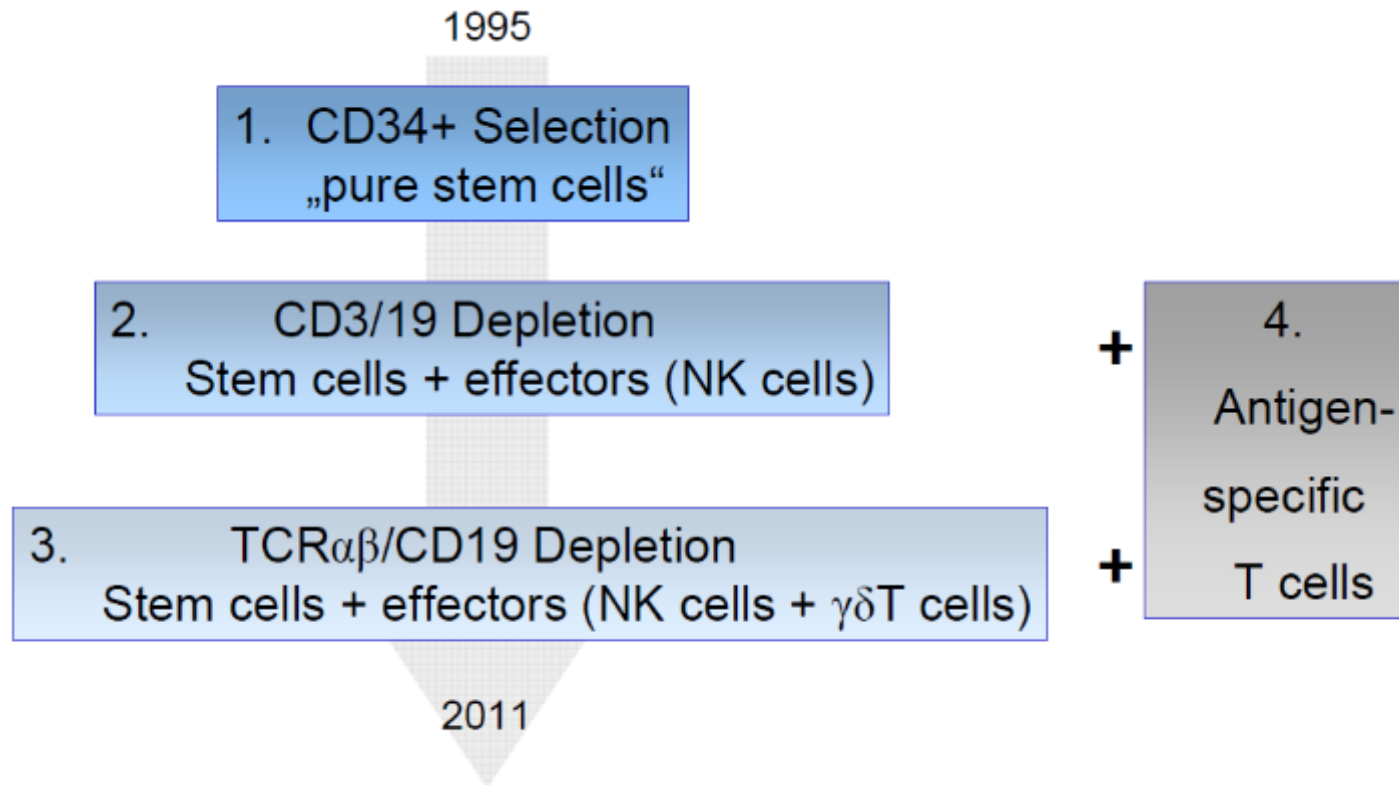
**Table 1**  
Comparison of  $\alpha\beta$  T cells and  $\gamma\delta$  T cells.

Feature	$\alpha\beta$ T cells	$\gamma\delta$ T cells
Proportion of CD3 <sup>+</sup> cells	90–99%	1–10%
TCR V gene germline repertoire	Large	Small
CD4/CD8 phenotype		
CD4 <sup>+</sup>	~60%	<1%
CD8 <sup>+</sup>	~30%	~30%
CD4 <sup>+</sup> CD8 <sup>+</sup>	<1%	<1%
CD4 <sup>+</sup> CD8 <sup>+</sup>	<1%	~60%
MHC restriction	CD4 <sup>+</sup> : MHC class II CD8 <sup>+</sup> : MHC class I	No MHC restriction
Ligands	Peptide + MHC	Phospholipid antigen

Adapted by D. Kabelitz et al. (1999), *Springer Seminars in Immunopathology* 21: 55, p. 36.

# T cell-depletion for Haplo-HSCT:

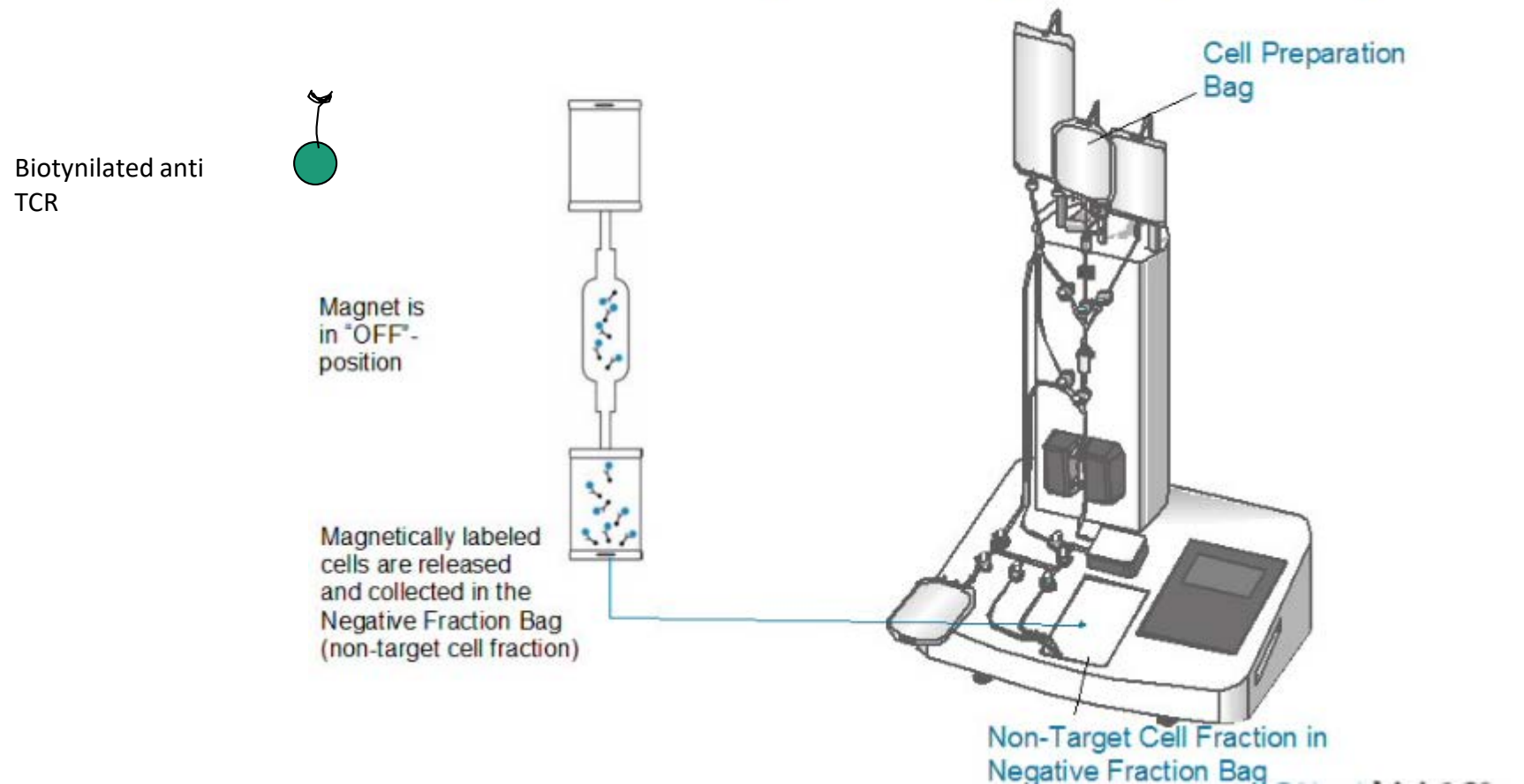
21



# TCR $\alpha$ - $\beta$ CD19 ICS

22

## Separation principle: Depletion

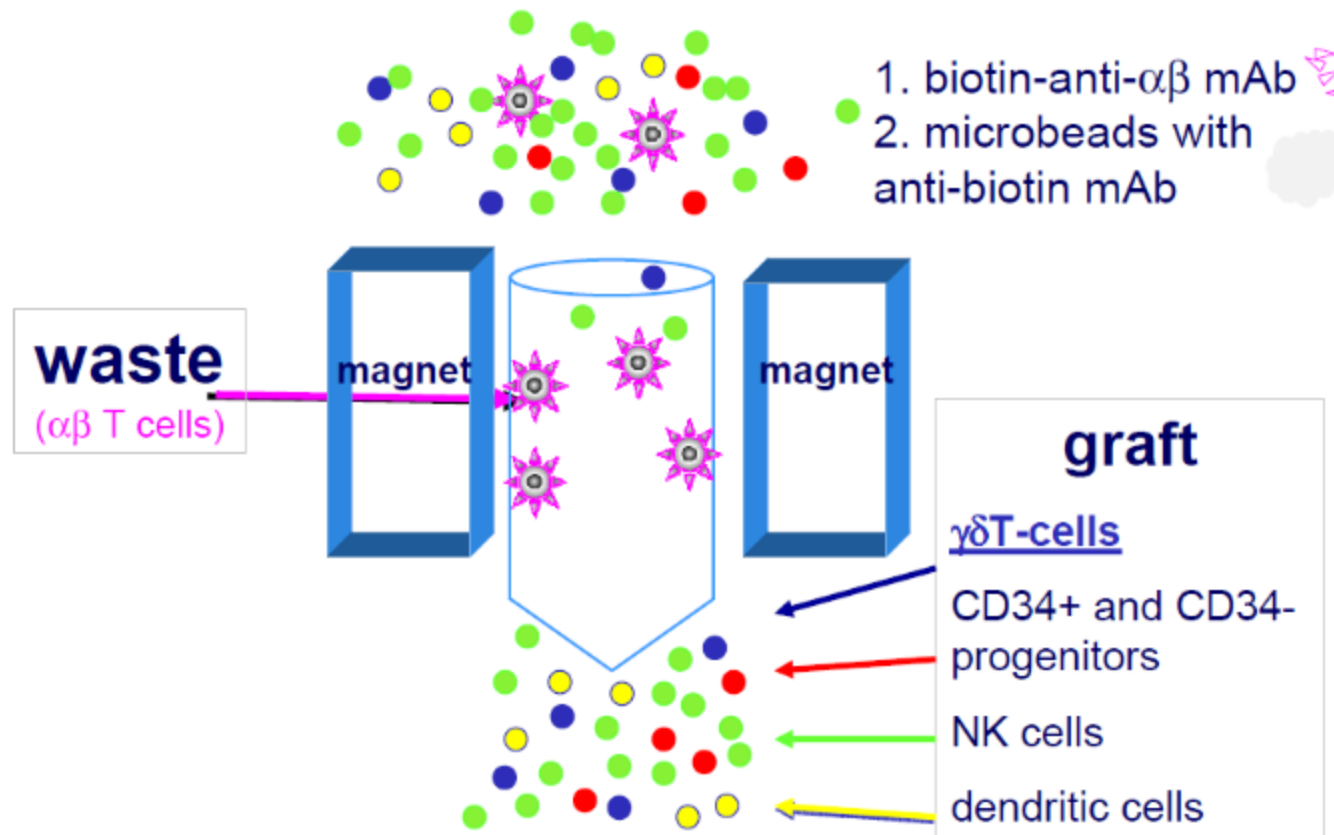


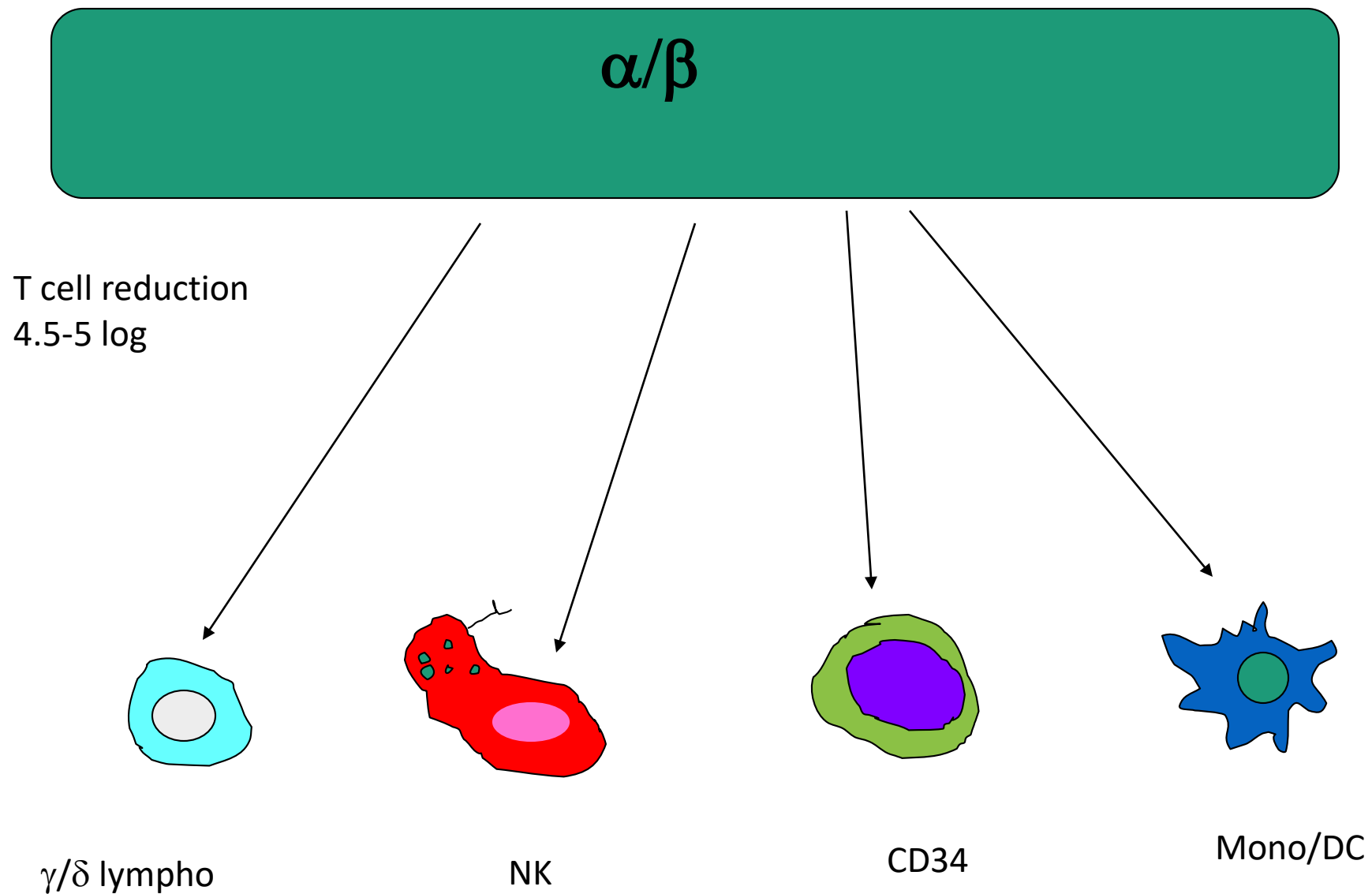


# TCR $\alpha/\beta$ + T / CD19+ B cell-depleted Haplo-HSCT: Negative selection method

## Strategy for depletion of $\alpha\beta$ + T-cells

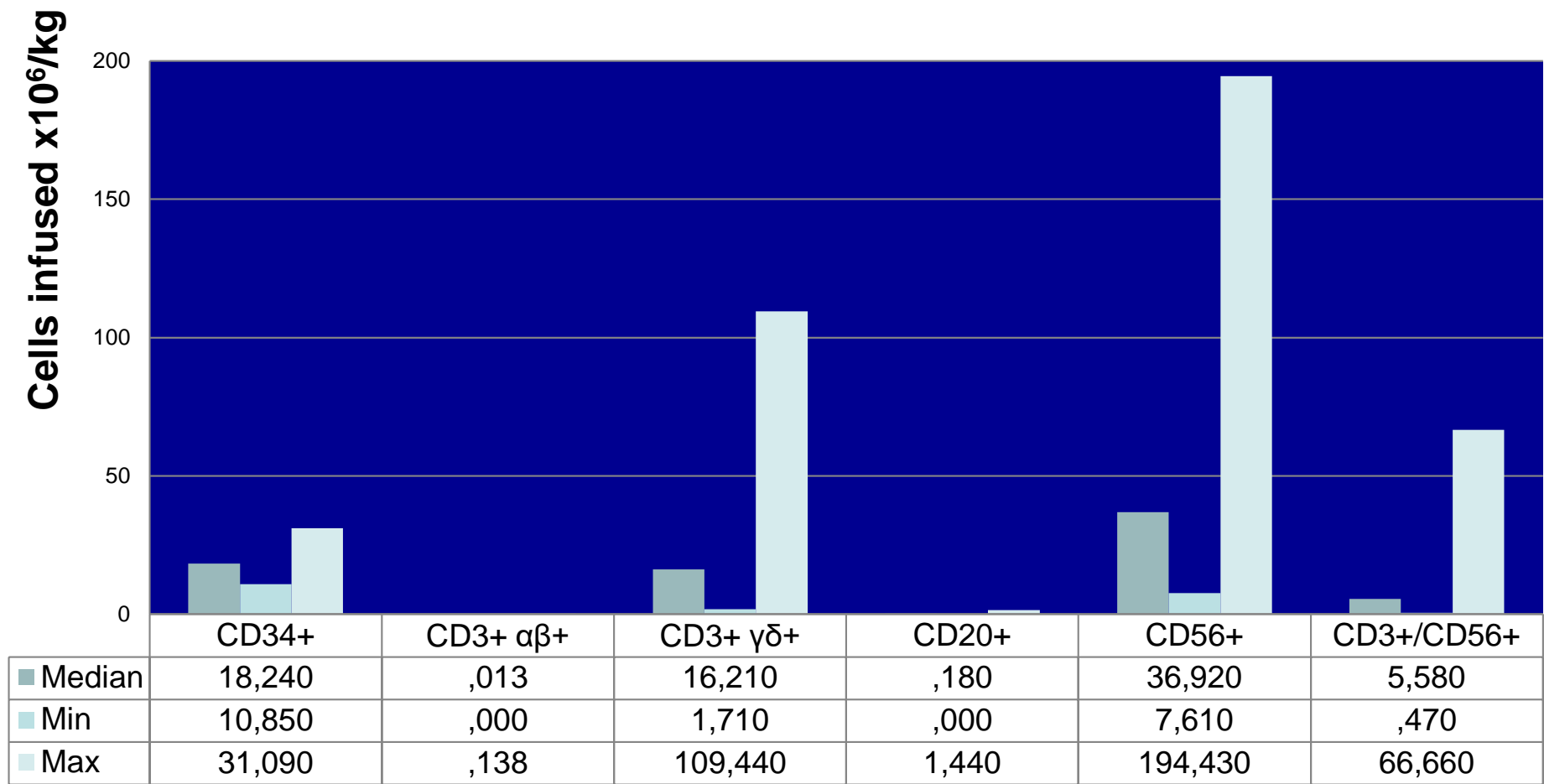
Chaleff S. et al.: A large scale method for the selective Depletion of  $\alpha/\beta$  T-lymphocytes from PBSC for allogeneic Transplantation. Cytotherapy, 2007





Two cell sources:

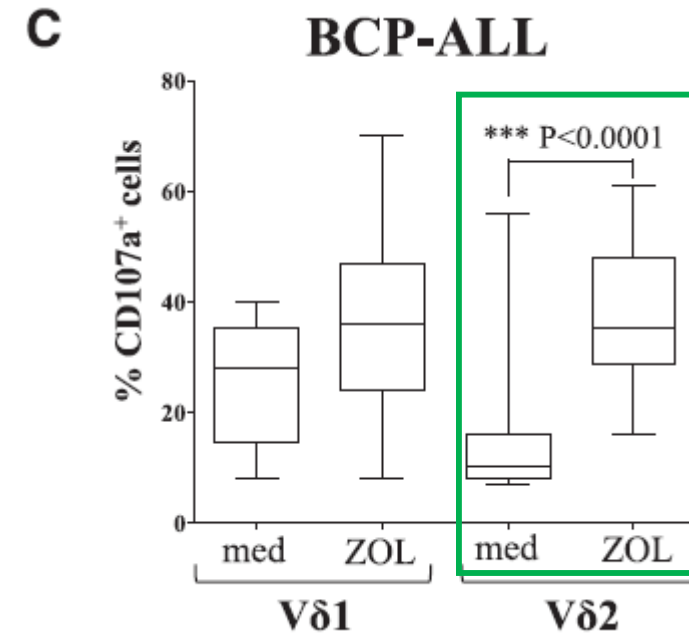
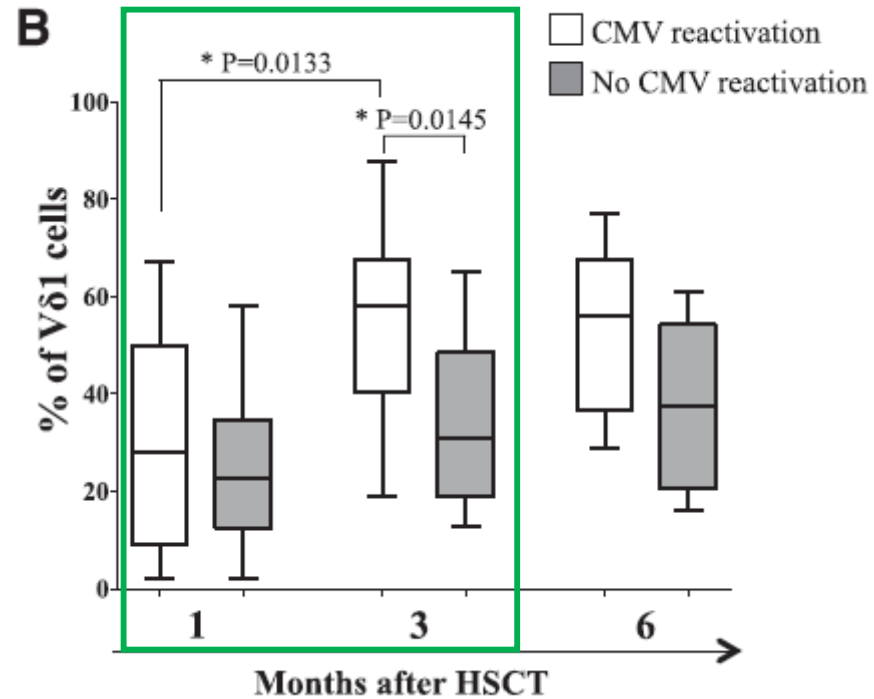
cells contained in the graft at time of transplantation  
cells derived from CD34+ infused



# Clinical experience

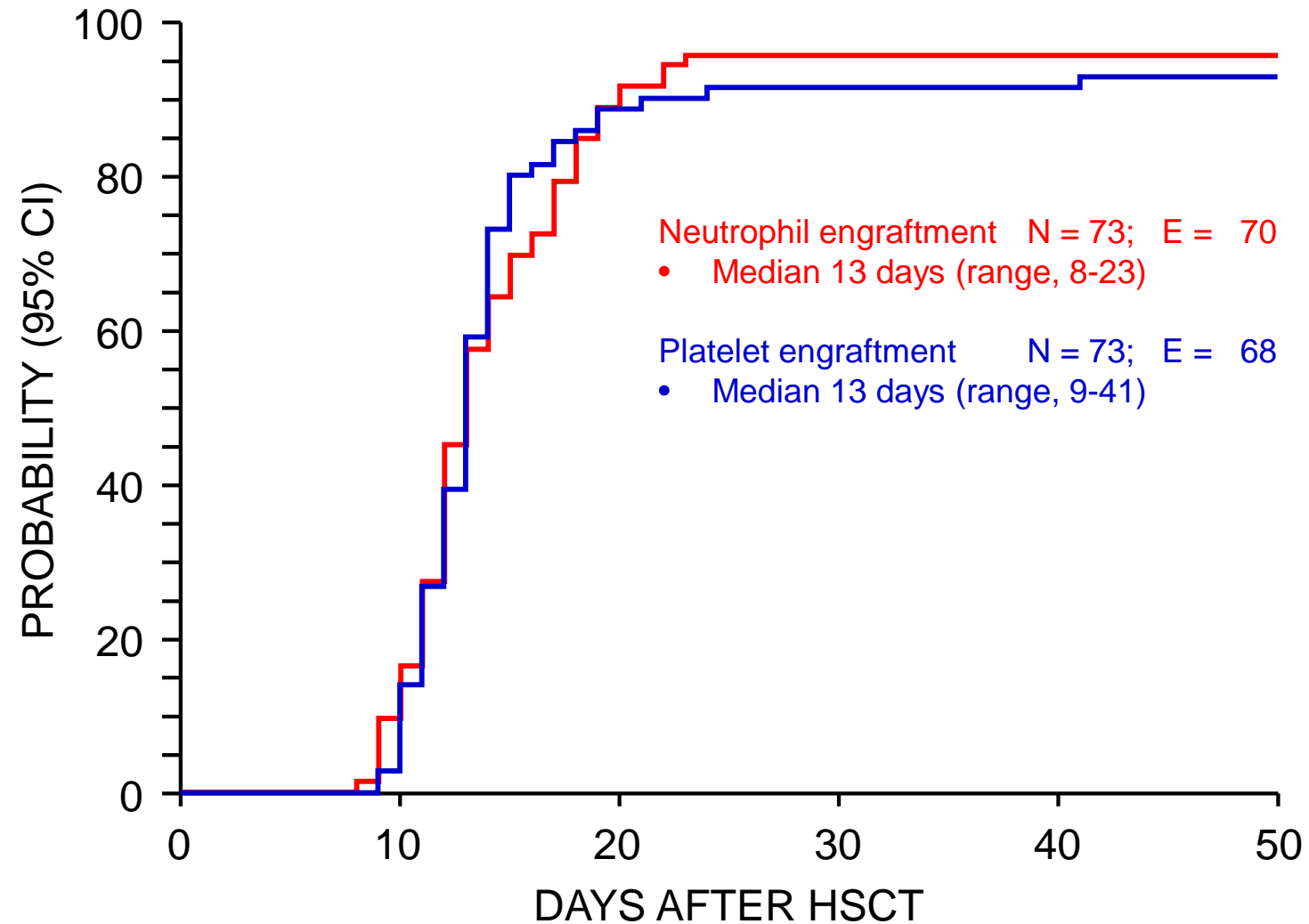
- Handgretinger et al. 2011. Robust engraftment and rapid immune reconst in children with high risk leukemia.
- Bertaina et al. Blood 2014. 23 children with non malignant diseases: low aGvHD (skin grade I-II), no cGvHD, no engraftment in 4/23. At a median follow up of 18 months, 21 pts alive.
- In the italian cohorts: no post transplant GvHD profilaxis

# $\gamma\delta$ T cells - role in haplo-HSCT: control of infection and leukemia



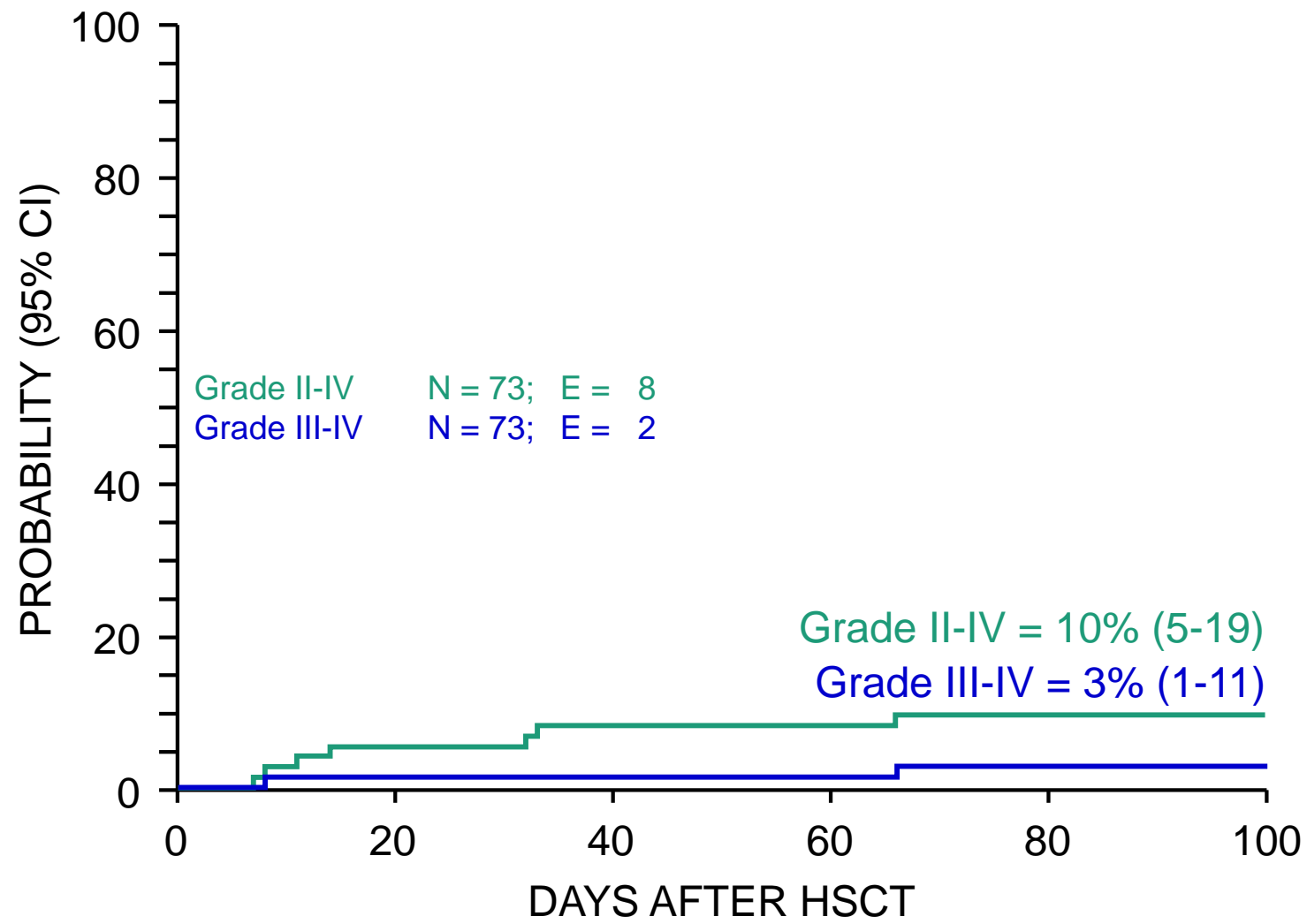
**Results on TCR $\alpha\beta$  CD19 negative ICS performed  
in pediatric pts at Policlinico S.Matteo (2013-2019)**

# Neutrophil and platelet engraftment



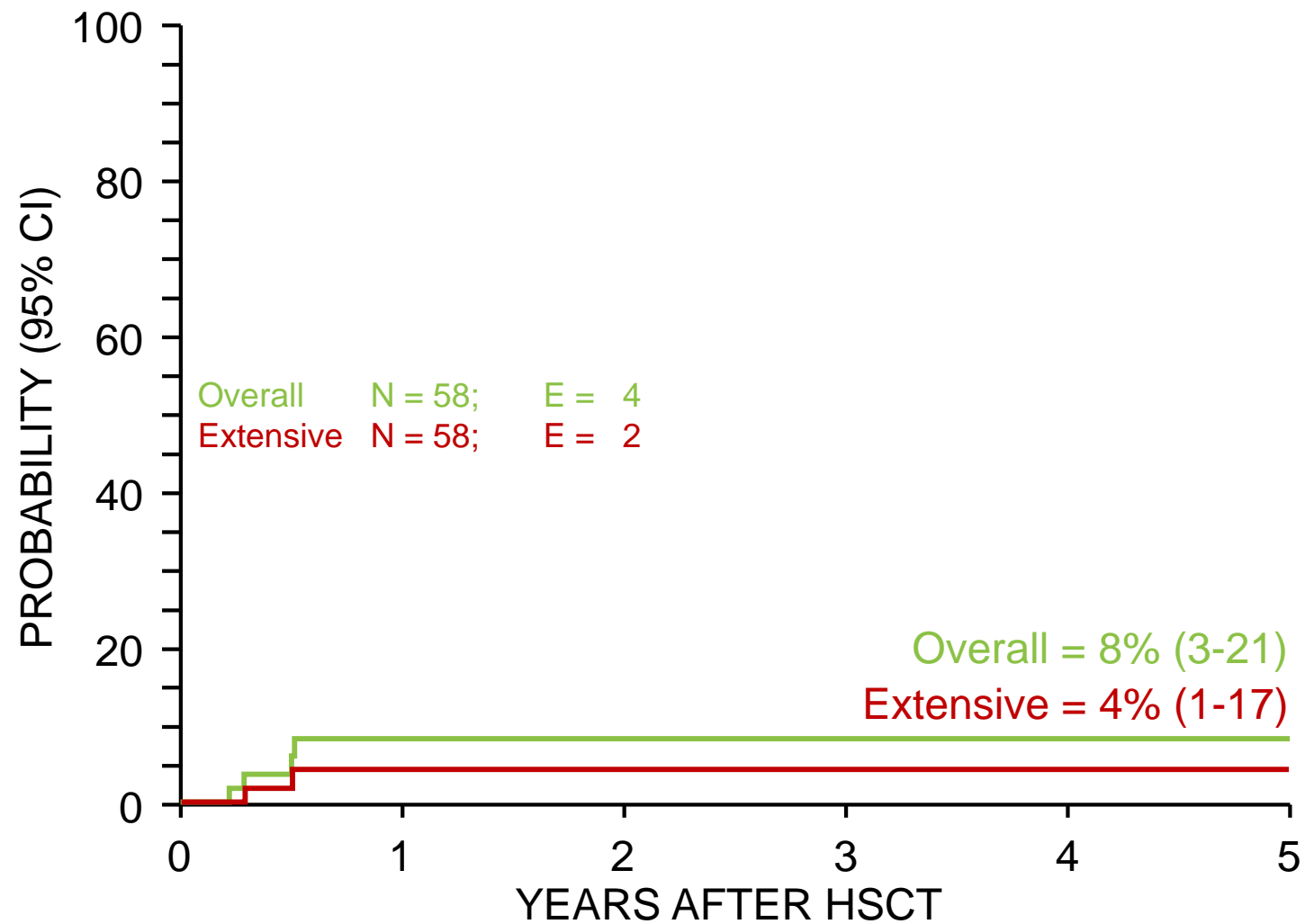


# Acute GVHD

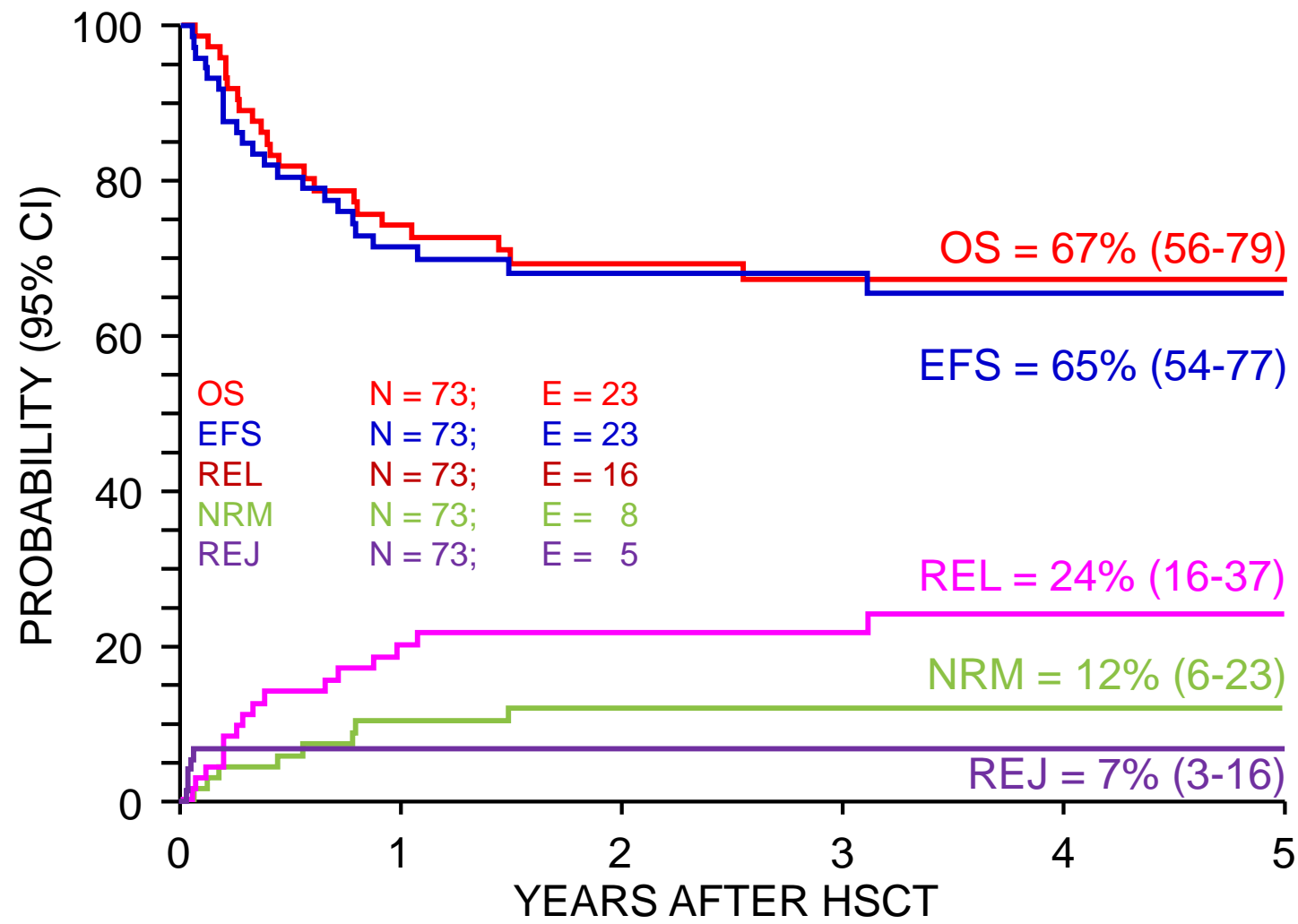


# Chronic GVHD

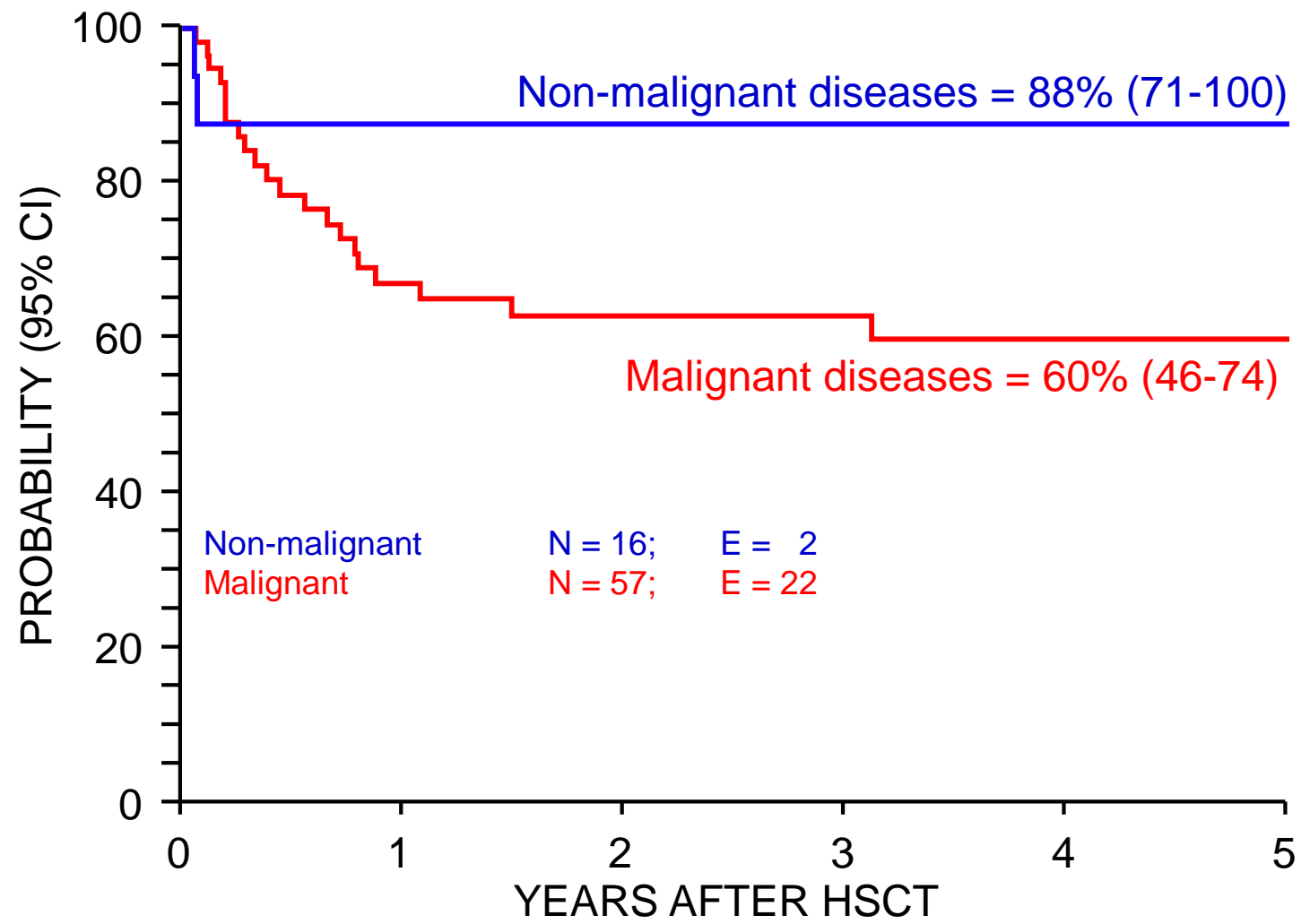
31



Overall survival, event-free survival,  
non-relapse mortality, rejection and relapse



# Event-free survival by diagnosis





## HAPLOIDENTICAL T-CELL DEPLETED HSCT IN CHILDREN: COST ANALYSIS COMPARING 2 DIFFERENT T-CELL DEPLETION TECHNIQUES

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Technique	Nr. procedures	Total costs (Euro)	Mean $\pm$ SD (Euro)	Median (Euro)	Range (Euro)
Positive selection	14	216.964	15.497 $\pm$ 5.650	13.095	6.026 – 24.107
Negative selection	13	230.509	17.731 $\pm$ 6.580	18.080	13.095 – 31.175

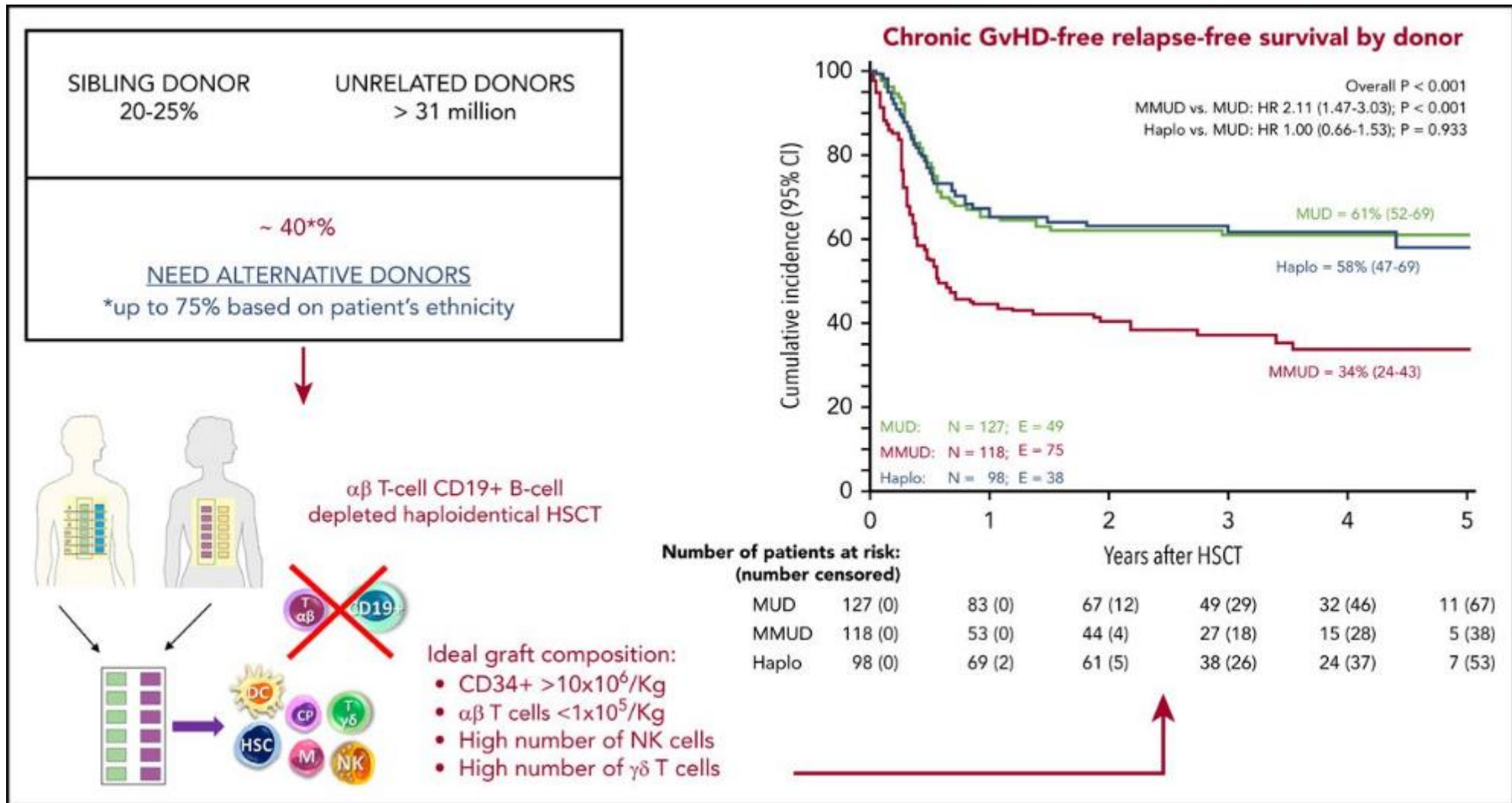
Elements considered for the cost analysis:

cost of the T cell depletion procedure

cost of hospitalization after HSCT  
length of hospital stay

Total cost of hospitalization			
Technique	Nr. HSCT	Median	(Range)
Positive selection	12	82.080	36.936 – 217.512
Negative selection	13	39.672	23.256 – 109.440

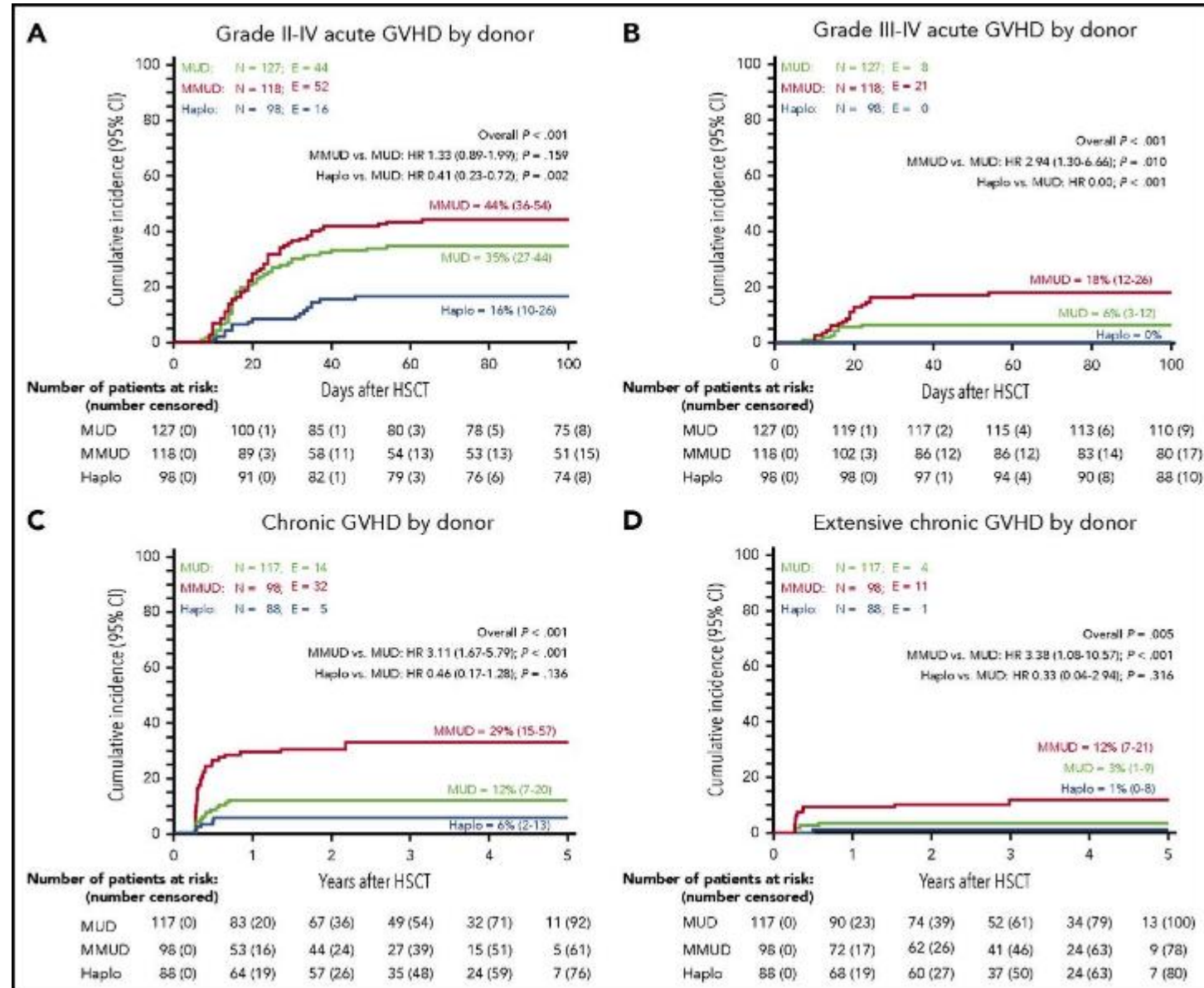
**Conclusions:** Even though the cost of graft manipulation using negative  $\alpha\beta^+$  T cell and CD19+ B cell selection was higher than the cost of a classical CD34+ cell positive selection, the shorter duration of hospitalization after HSCT counterbalanced the higher cost of graft manipulation. Negative  $\alpha\beta^+$  T cell and CD19+ B cell selection could be cost effective as compared to other graft manipulation techniques in the context of haploidentical stem cell transplantation in children.



Bertaina A, Zecca M. et al. Blood 2018;132:2594-2607

# Cumulative incidence of acute and chronic GVHD in $\alpha\beta$ haplo-HSCT, MUD-HSCT, and MMUD-HSCT recipients.

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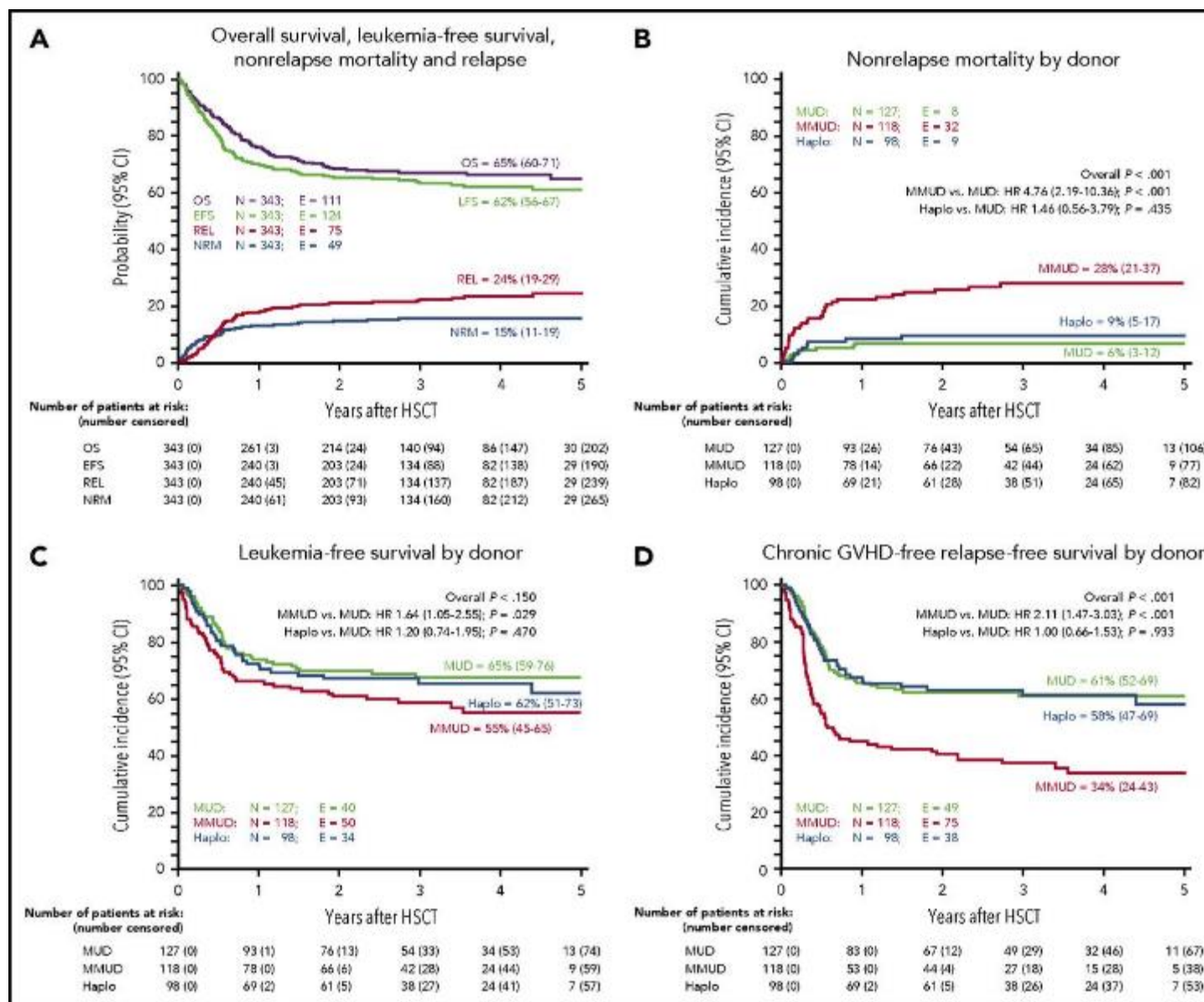


Bertaina A, Zecca M. et al. Blood 2018;132:2594-2607

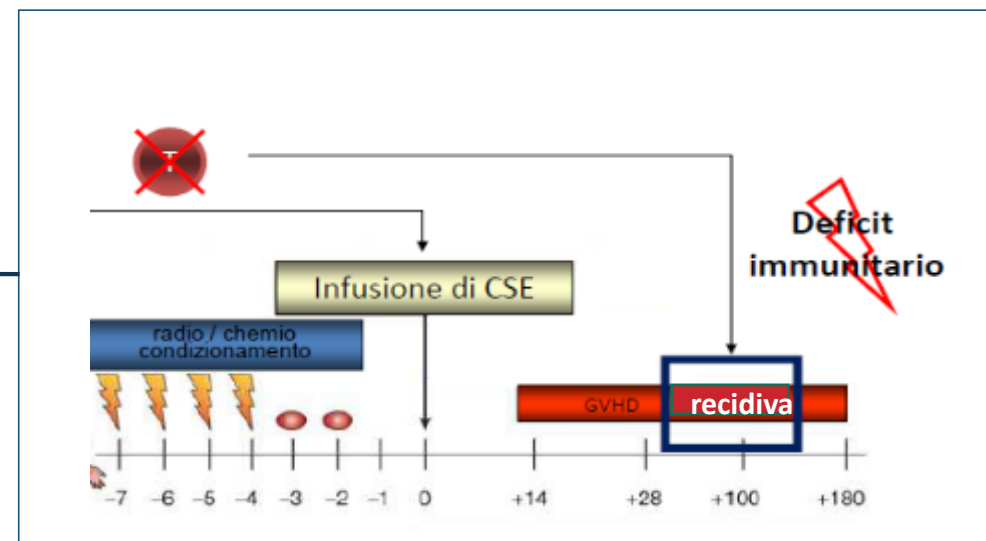
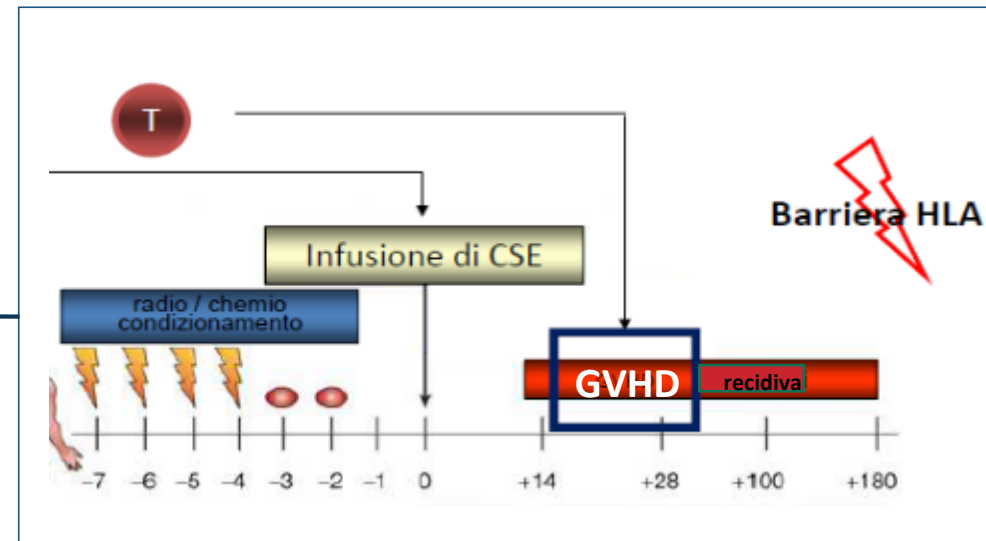
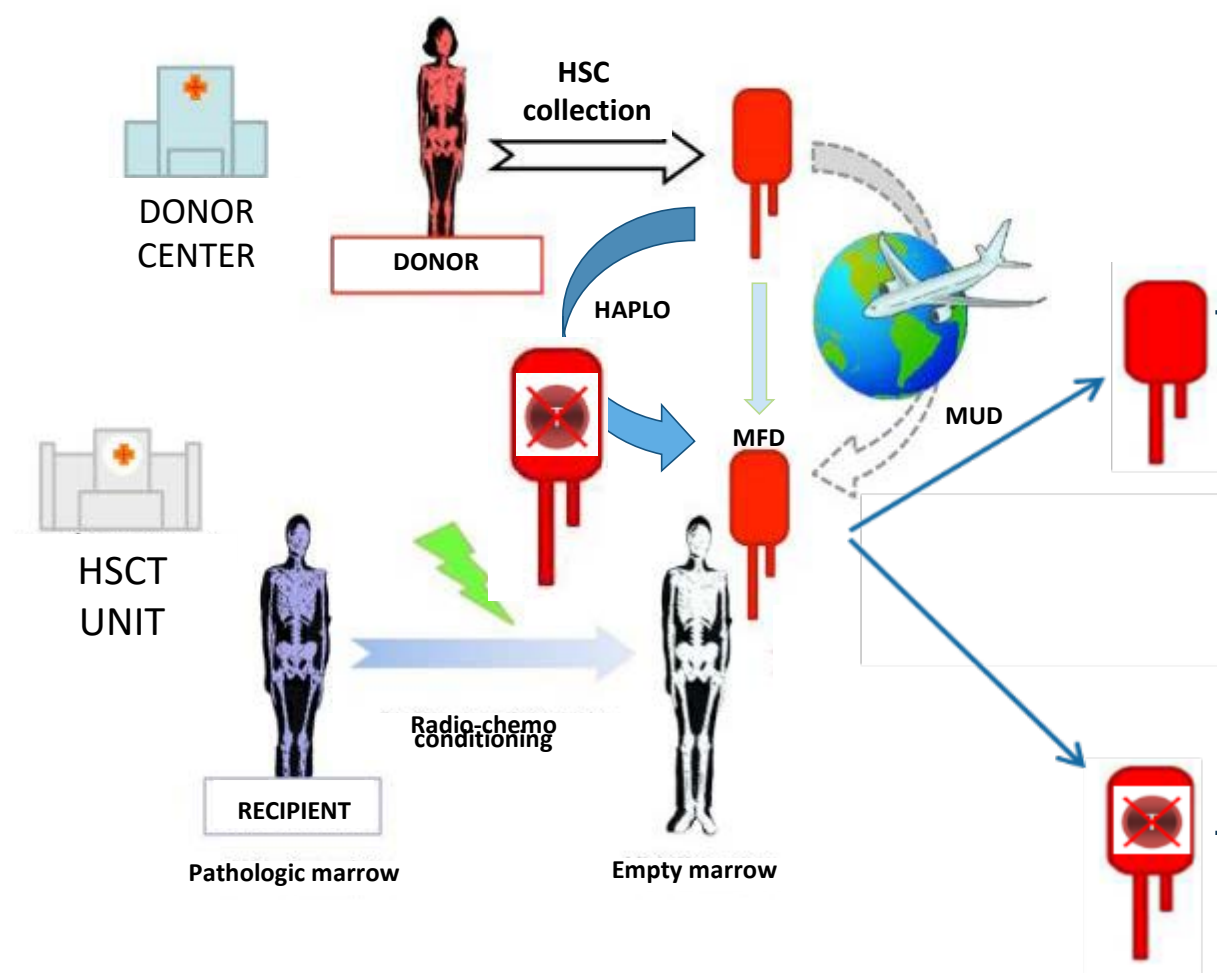


# Probability of OS, LFS, NRM, relapse incidence (REL), and chronic GVHD-free, relapse-free survival (GFRS).

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Bertaina A, Zecca M. et al. Blood 2018;132:2594-2607



## T-cell based:

### DLI

- unmanipulated
- insertion of suicide gene to block flare in case of GVHD
- physical selection of specific subpopulations (i.e. CD45RA)
- induction of tolerance
- depletion of alloreactive populations

### cultured CIKs

### cultured minor HLA antigen-specific T cells

### cultured leukemia antigen-specific T cells

- leukemia whole blasts
- leukemia-associated antigens: i.e. peptides derived from NPM1mut, WT1

### gene-modified T cells targeting leukemia antigens

- insertion of natural T cell receptor
- insertion of chimeric antigen receptor

## NK-cell based:

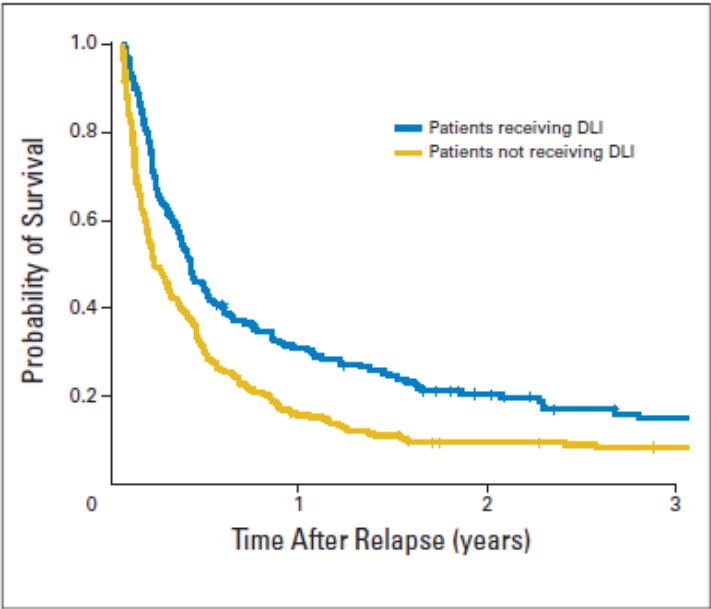
### selected NK cells

### selected and activated NK cells

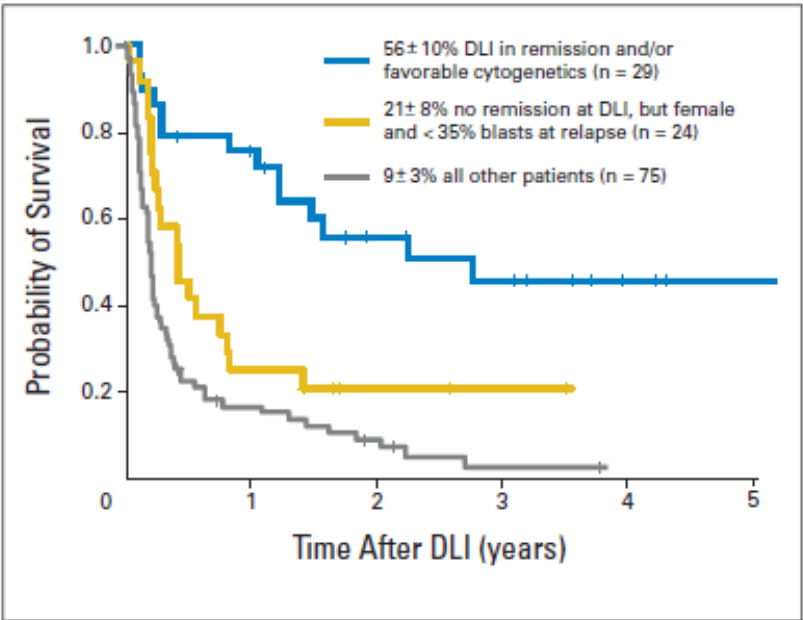
### gene-modified NK cells targeting leukemia antigens

- insertion of chimeric antigen receptor

Donor lymphocyte infusions (DLI) is the simplest way to boost the GvL effect



**Fig 1.** Unadjusted survival of patients with first hematological relapse of acute myeloid leukemia after allogeneic hematopoietic stem-cell transplantation



**Table 4.** Multivariate Analysis of Risk Factors for Survival Among Patients Receiving DLI for Treatment of Hematological Relapse After HSCT for AML

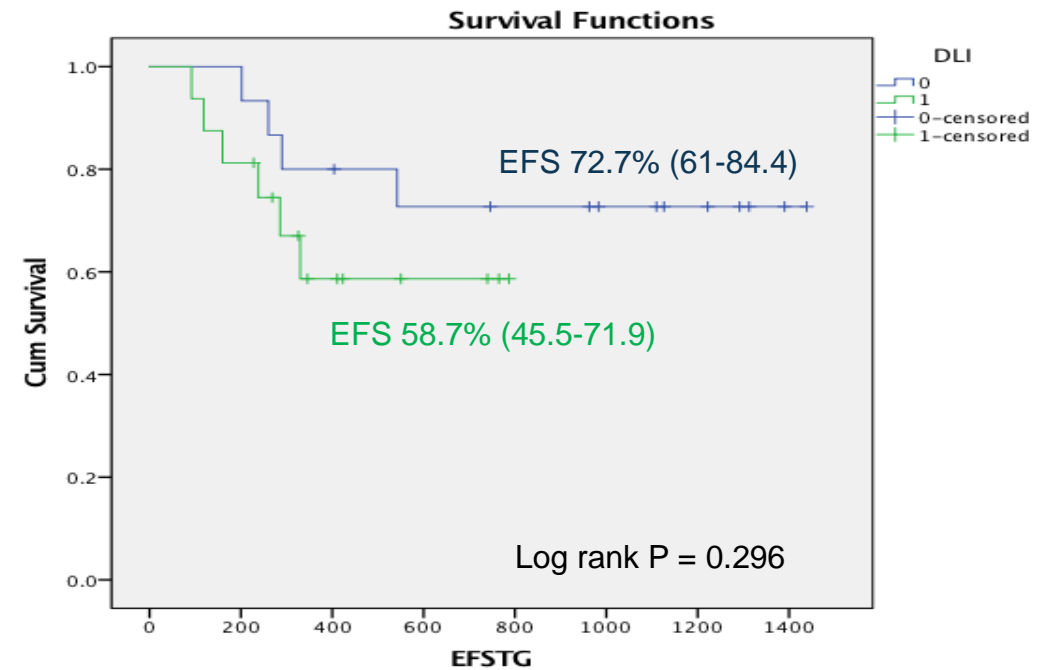
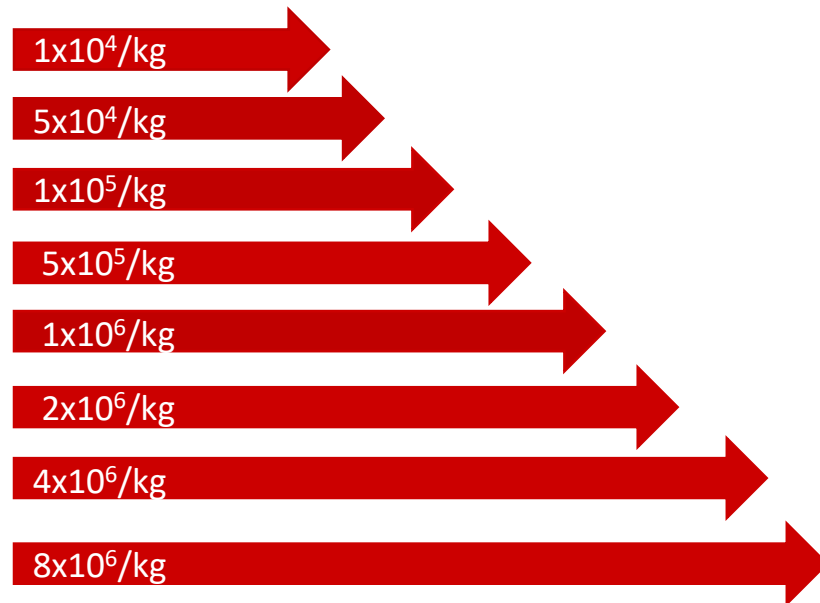
Prognostic Factor	P	Relative Risk for OS	95% CI
% blast at relapse (BM), > 35%	.006	0.56	0.38 to 0.85
Female v male	.02	1.6	1.07 to 2.4
Cytogenetics (favorable v other)	.004	5.6	1.76 to 1.8
Disease stage at DLI (remission v no remission)	< .0001	5.8	2.5 to 13.7

Abbreviations: DLI, donor lymphocyte infusion; HSCT, hematopoietic stem-cell transplantation; AML, acute myeloid leukemia; OS, overall survival; BM, bone marrow.

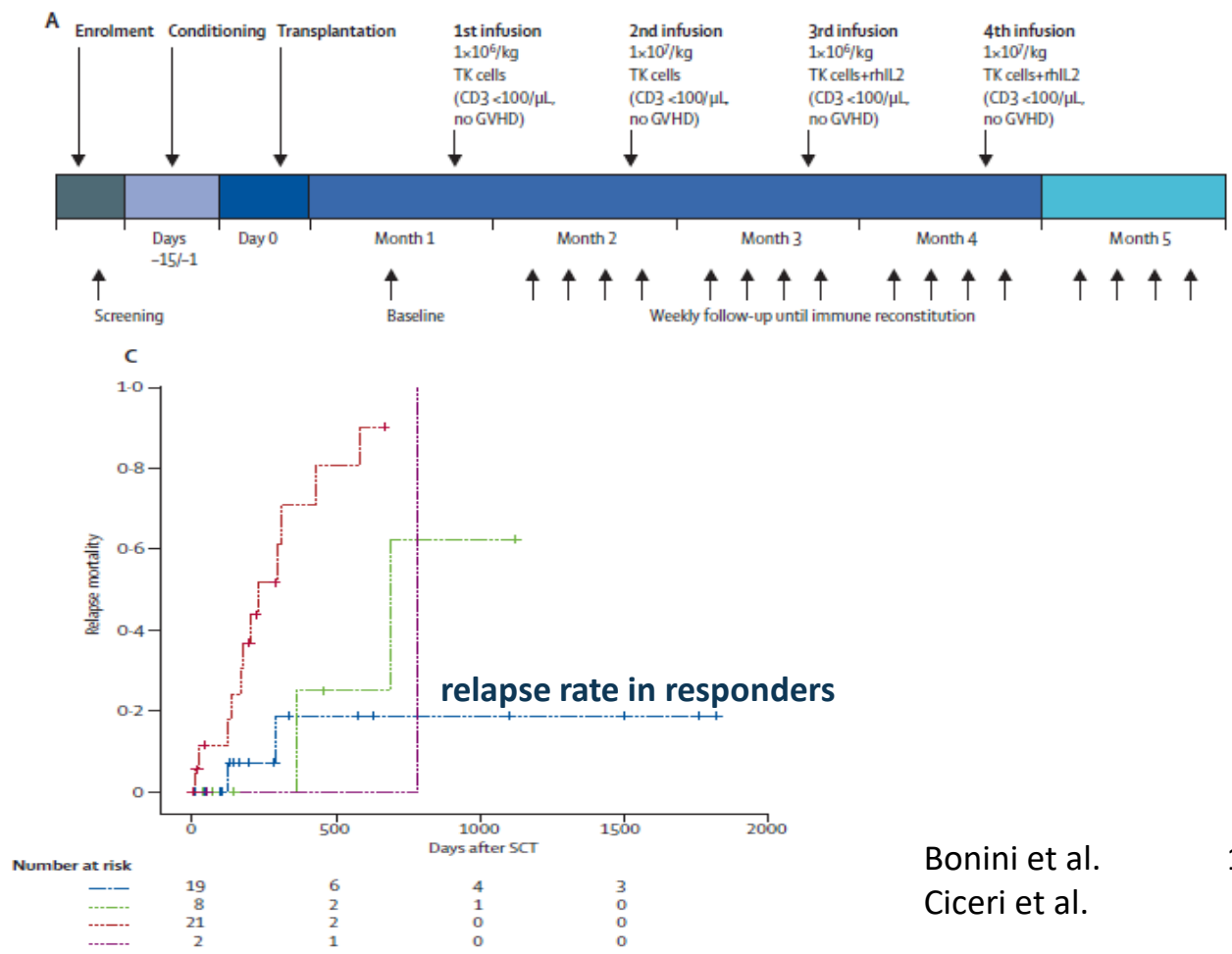
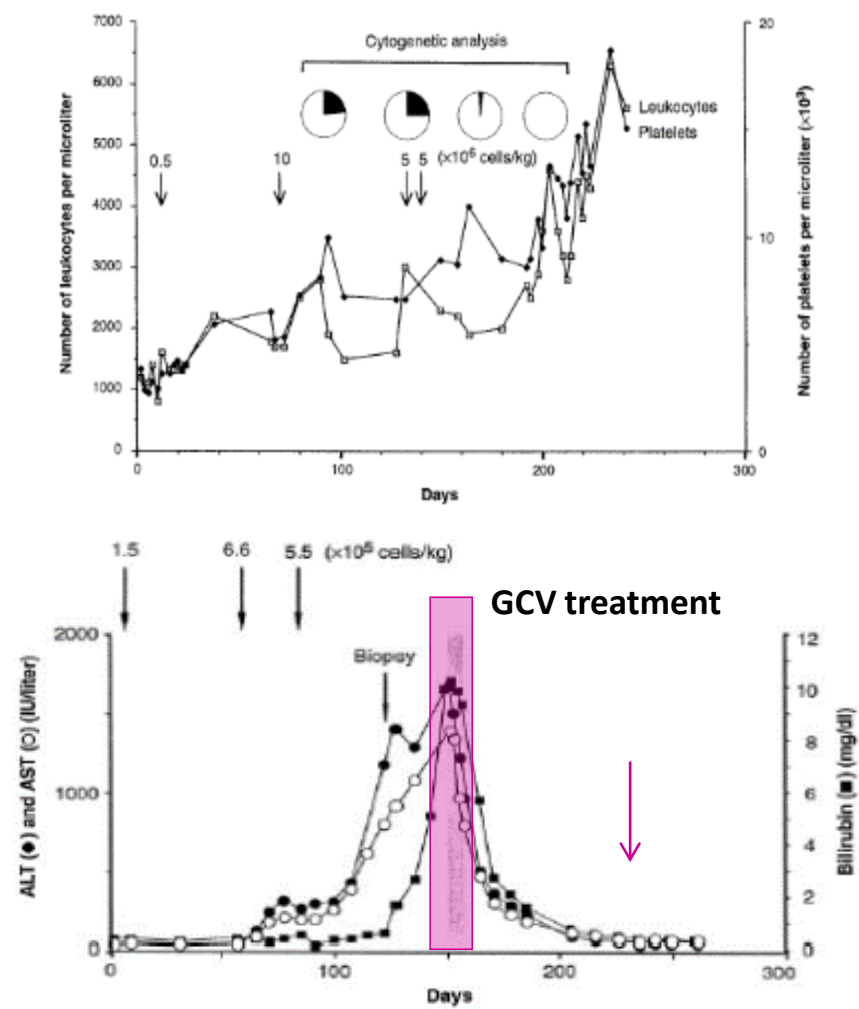
$\alpha\beta$ 

We employed DLI in haplo-HSCT recipients with **high relapse risk** (vs standard risk)

DLI schedule: escalating T cell dose every 3 weeks



Insertion of HSV-TK gene in lymphocytes:  
ability to control cell expansion by treatment with ganciclovir in case of GVHD

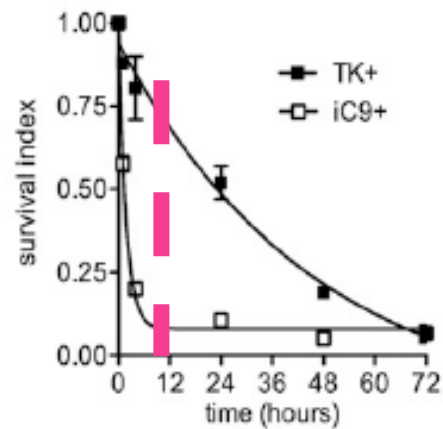
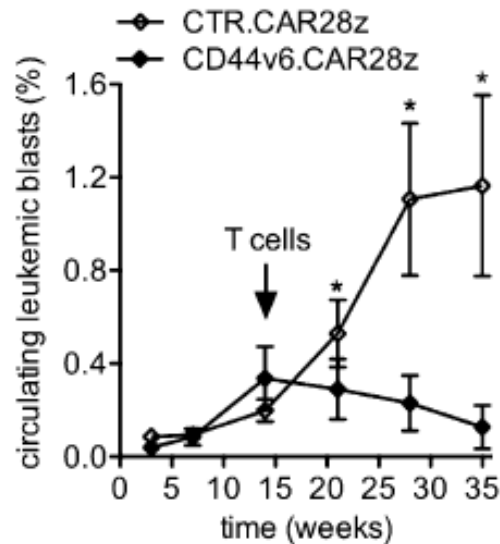


Bonini et al. 1997  
Ciceri et al. 09

## Insertion of HSV-TK gene in lymphocytes:

kinetics of cell control through ganciclovir exposure in case of GVHD are slow, and GVHD may still develop  
iC9 suicide gene allows for a faster reduction of alloreactive populations

CD44v6 CAR  
for AML/MM



$\alpha\beta$  T-cell and B-cell depleted  
Haplo-HSCT  
+  
BPX-501<sup>1</sup>  
(NO post-HSCT GvHD prophylaxis)

Rimiducid for patients  
who develop GvHD or  
are refractory to SOC  
treatment



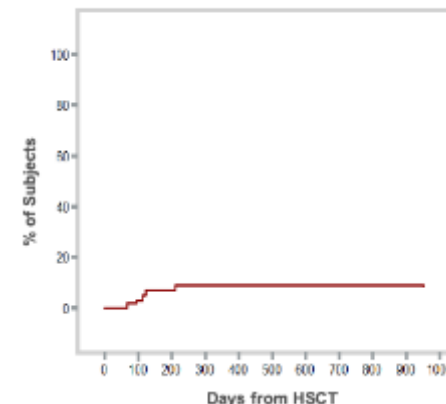
**Phase I: 3+3 design (no MTD reached)**

2.5x10<sup>5</sup>, 5x10<sup>5</sup>, 1x10<sup>6</sup> BPX-501 T-cells/kg (no DLTs observed)

**Phase II:**

1x10<sup>6</sup> BPX-501 T-cells/kg (chosen for further evaluation)

Low TRM incidence of 8.7%

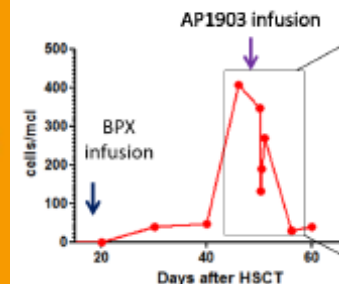


**Grade II-IV:**  
8.9% (95% CI, 1.5-16.4)

**Grade III-IV:**  
1.8% (95% CI, 0.0-5.3)

Cases of acute GvHD within 100 days included:

- Grade II (n=4)
  - Stage 3 skin (n=3)
  - Stage 1 upper GI (n=1)
- Grade III (n=1)
  - Stage 3 liver



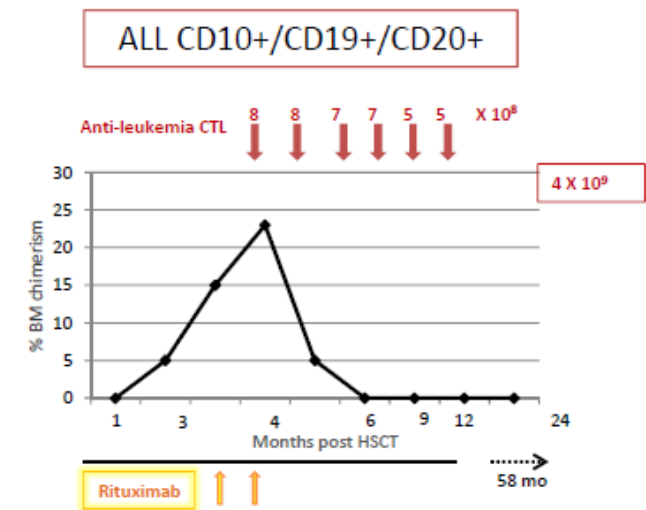
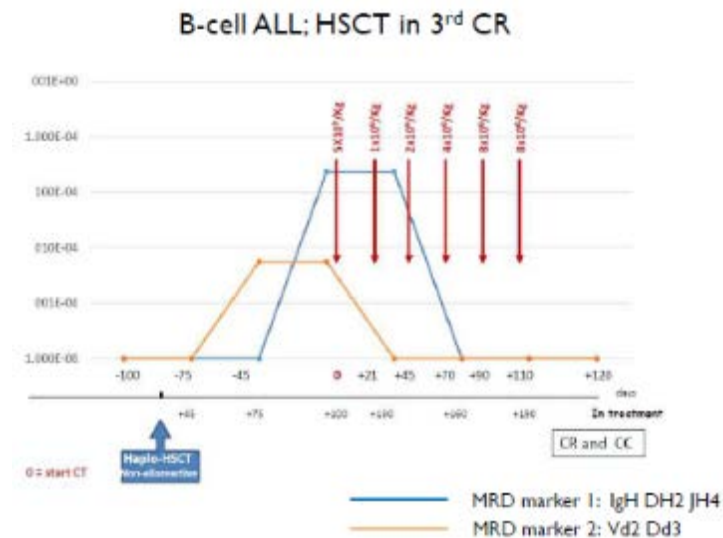
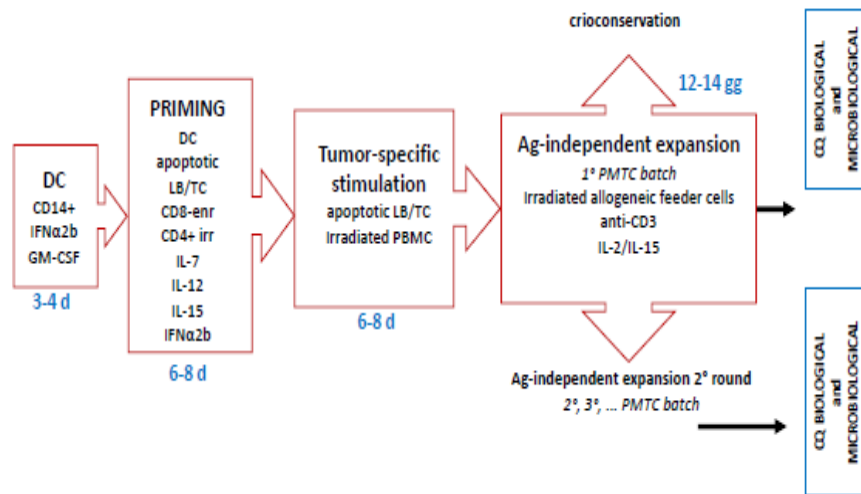


Our center has optimized a protocol to expand anti-leukemia CTLs by stimulating donor lymphocytes with apoptotic whole leukemic blasts

The approach has two advantages:

Knowledge of leukemia-associated antigen not needed

It works with any HLA specificity



Montagna et al.

2001;

2008



We still have a clinical need: expanding CTLs for patients lacking cryopreserved tumor cells  
Identification of suitable leukemia targets for cellular therapy

We have recently succeeded in expanding  $p^{190}$  BCR/ABL-specific CTLs by stimulation with peptides derived from the BCR/ABL fusion region

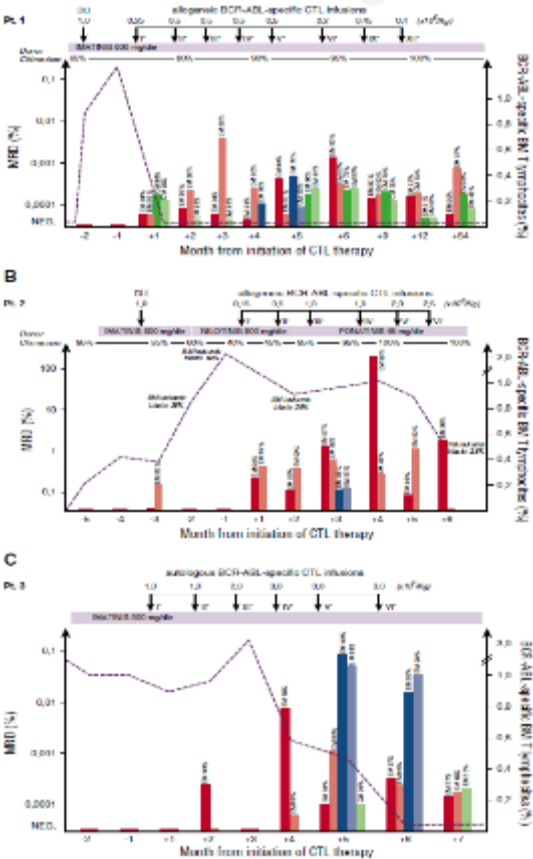


Issue Highlights

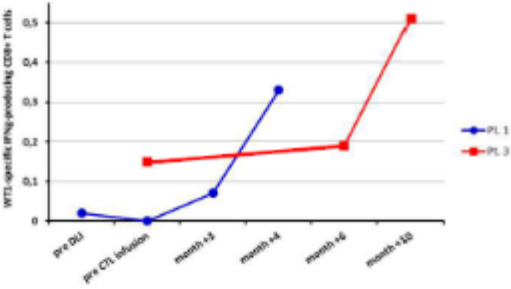
BCR-ABL-specific T-cell therapy in  $Ph^{+}$  ALL patients on tyrosine-kinase inhibitors  
Blood 2017 129:582-586;

INSIDE *BLOOD* COMMENTARIES  
Clinical Trials and Observations

Fall of the mutants: T cells targeting BCR-ABL

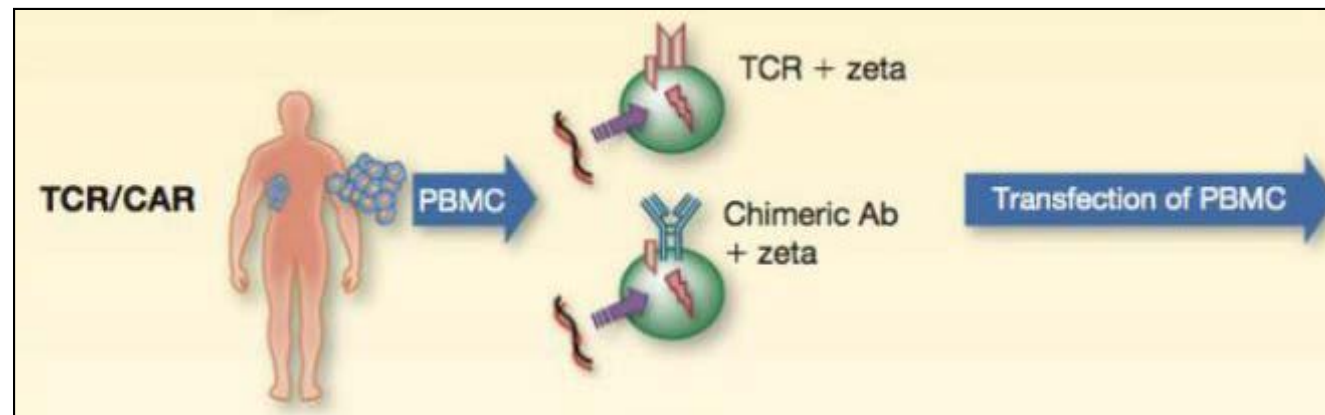


Infused  $p^{190}$  BCR/ABL-specific CTLs induce epitope spreading: emergence of WT1-specific T cells



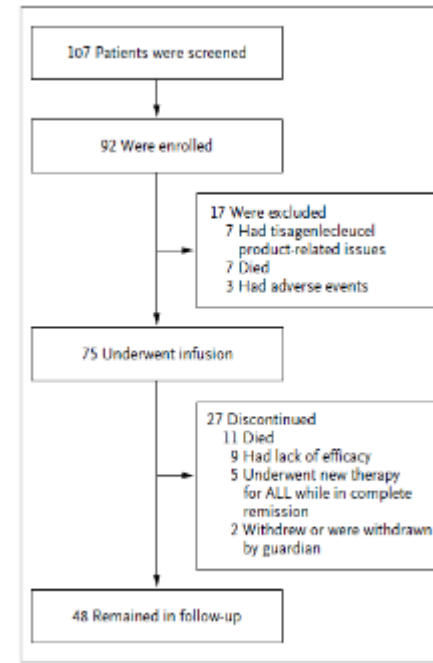
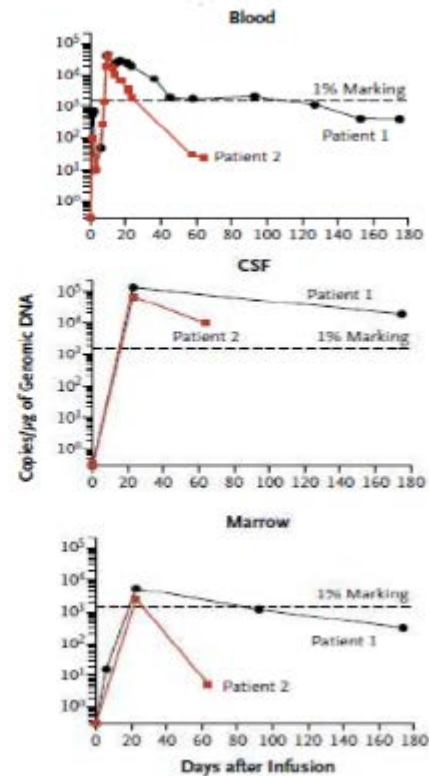
It is feasible to graft specificities for antigens expressed on tumor cells through genetic manipulation:

- insertion of natural TA-specific TCR
- insertion of chimeric antigen receptors



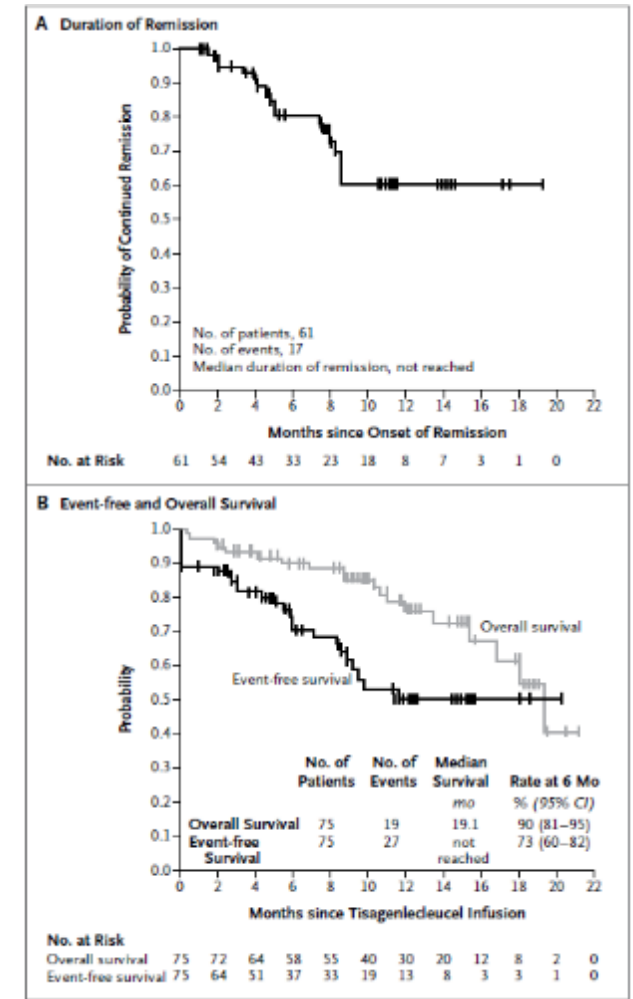
CAR-T cells employed in the clinical trials so far have been of recipient origin

Patient and Tissue	Dominant Clone Reads	Tumor Clone Frequency %
<b>Patient 1</b>		
<b>Blood</b>		
Day -1	185	97.88
Day 23	0	0
Day 87	0	0
Day 180	0	0
<b>Marrow</b>		
Day -1	59,774	99.97
Day 23	33	89.19
Day 87	10	100.00
Day 180	0	0
<b>Patient 2</b>		
<b>Blood</b>		
Day -1	30,425	79.71
Day 23	18	19.60
<b>Marrow</b>		
Day -1	50,887	74.43
Day 23	946	66.90
Day 60	363,736	68.90

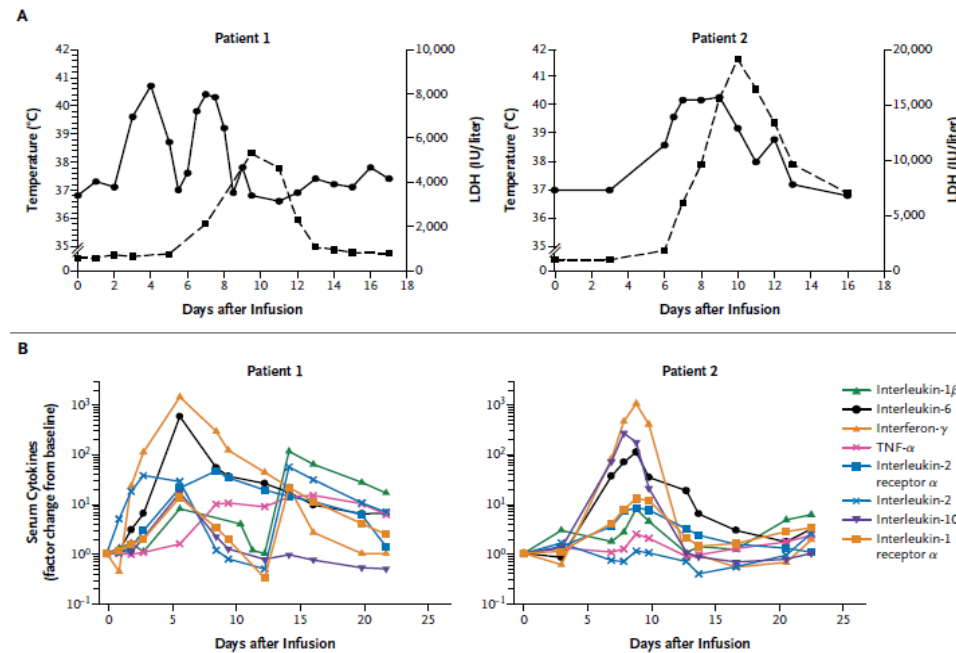


Grupp et al. N Engl J Med 2013

Maude et al. N Engl J Med 2018



Massive T cell expansion leading to cytokine storm  
 On target toxicity (hypogammaglobulinemia)  
 On target but out of organ toxicity (presence of Ag on normal tissues)



**Table 3. Adverse Events of Special Interest within 8 Weeks after Infusion, Regardless of Relationship to Tisagenlecleucel.\***

Type of Event	Any Grade (N=75)	Grade 3 (N=75)	Grade 4 (N=75)
	<i>number of patients (percent)</i>		
Any adverse event of special interest	67 (89)	26 (35)	30 (40)
Cytokine release syndrome	58 (77)	16 (21)	19 (25)
Neurologic event	30 (40)	10 (13)	0
Infection	32 (43)	16 (21)	2 (3)
Febrile neutropenia	26 (35)	24 (32)	2 (3)
Cytopenia not resolved by day 28	28 (37)	12 (16)	12 (16)
Tumor lysis syndrome	3 (4)	3 (4)	0

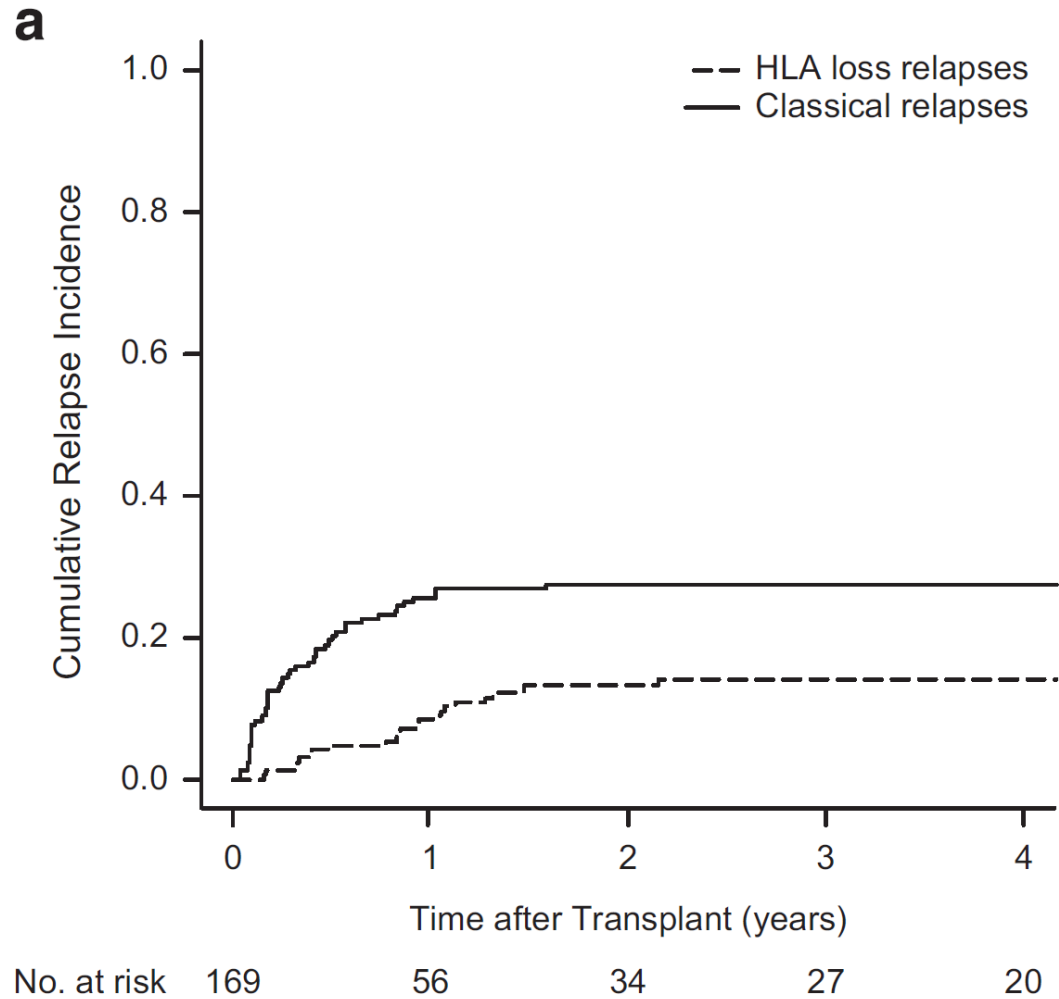
Morgan et al. Mol Ther 2010  
 Brentjens et al. Mol Ther 2010  
 Grupp et al. N Engl J Med 2013  
 Lee et al. Lancet 2015

Maude et al. N Engl J Med 2018

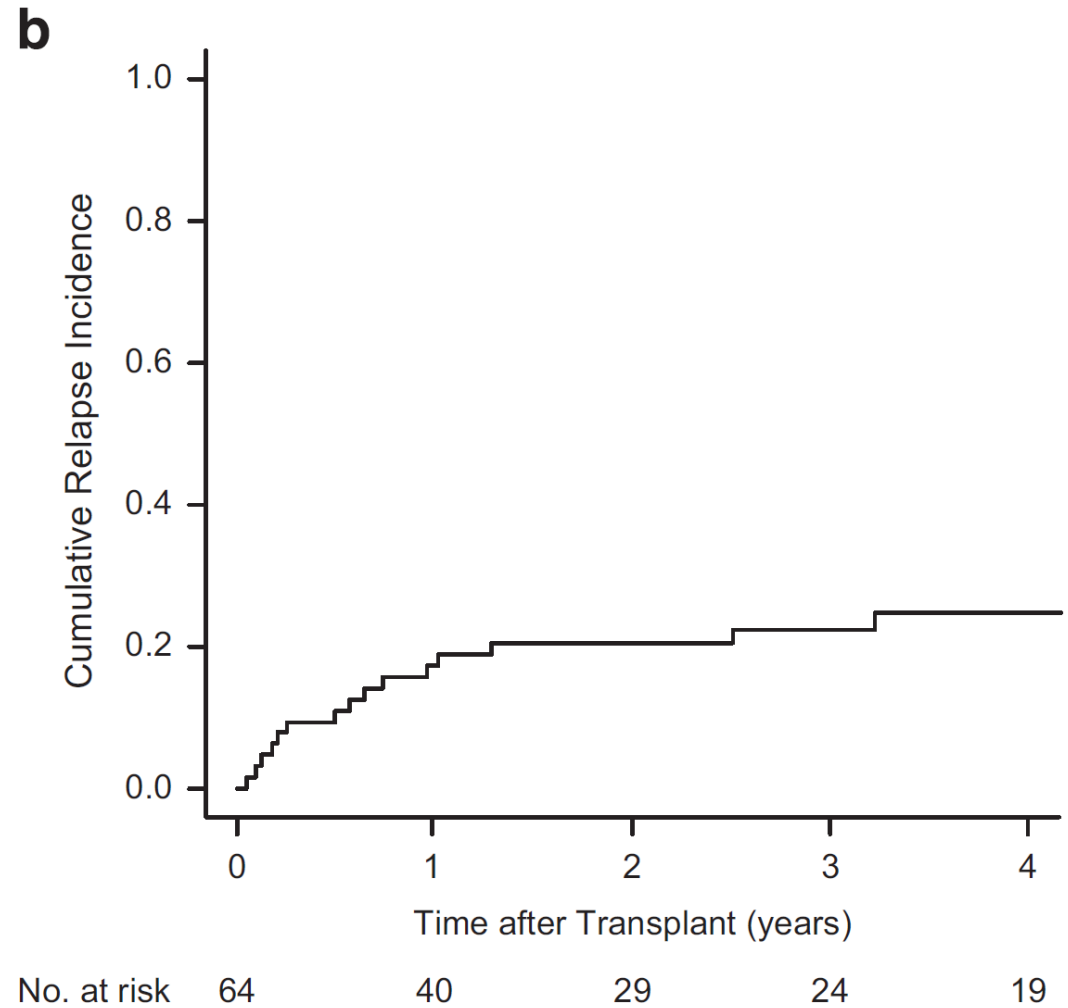
# Recidiva con perdita dell'aplotipo HLA non condiviso

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## TCSE aploidentico



## TCSE da MUD mismatched



**Servizio di Immunoematologia e medicina  
Trasfusionale,**

**Unità di aferesi e Laboratorio di  
manipolazione cellulare e citofluorimetria**

Direttore: Cesare Perotti

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