Alloreactivity of NK cells (and beyond..)

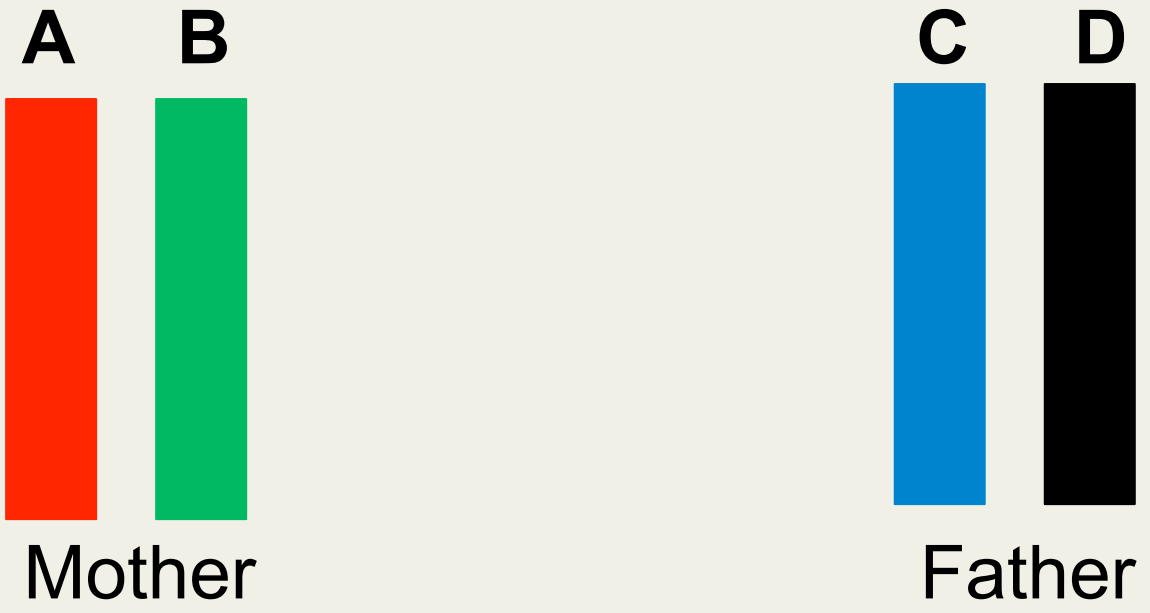
Andrea Velardi
Transplantation Programme, Univ. of Perugia

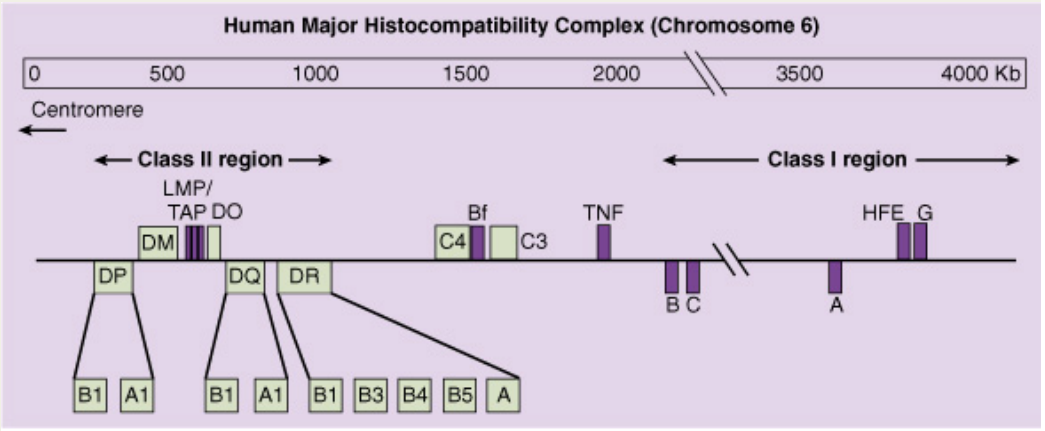
The beginning of ex-vivo manipulation for safe haplo BMT..

Reisner Y, Kapoor N, Kirkpatrick D, Pollack MS, Cunningham-Rundles S, Dupont B, Hodes MZ, Good RA, O'Reilly RJ

Transplantation for severe combined immunodeficiency with HLA-A,B,D,DR incompatible parental marrow cells fractionated by soybean agglutinin and sheep red blood cells. *Blood* 1983, 61:341-8.

One HLA haplotype-mismatched ("haplo-identical") donor

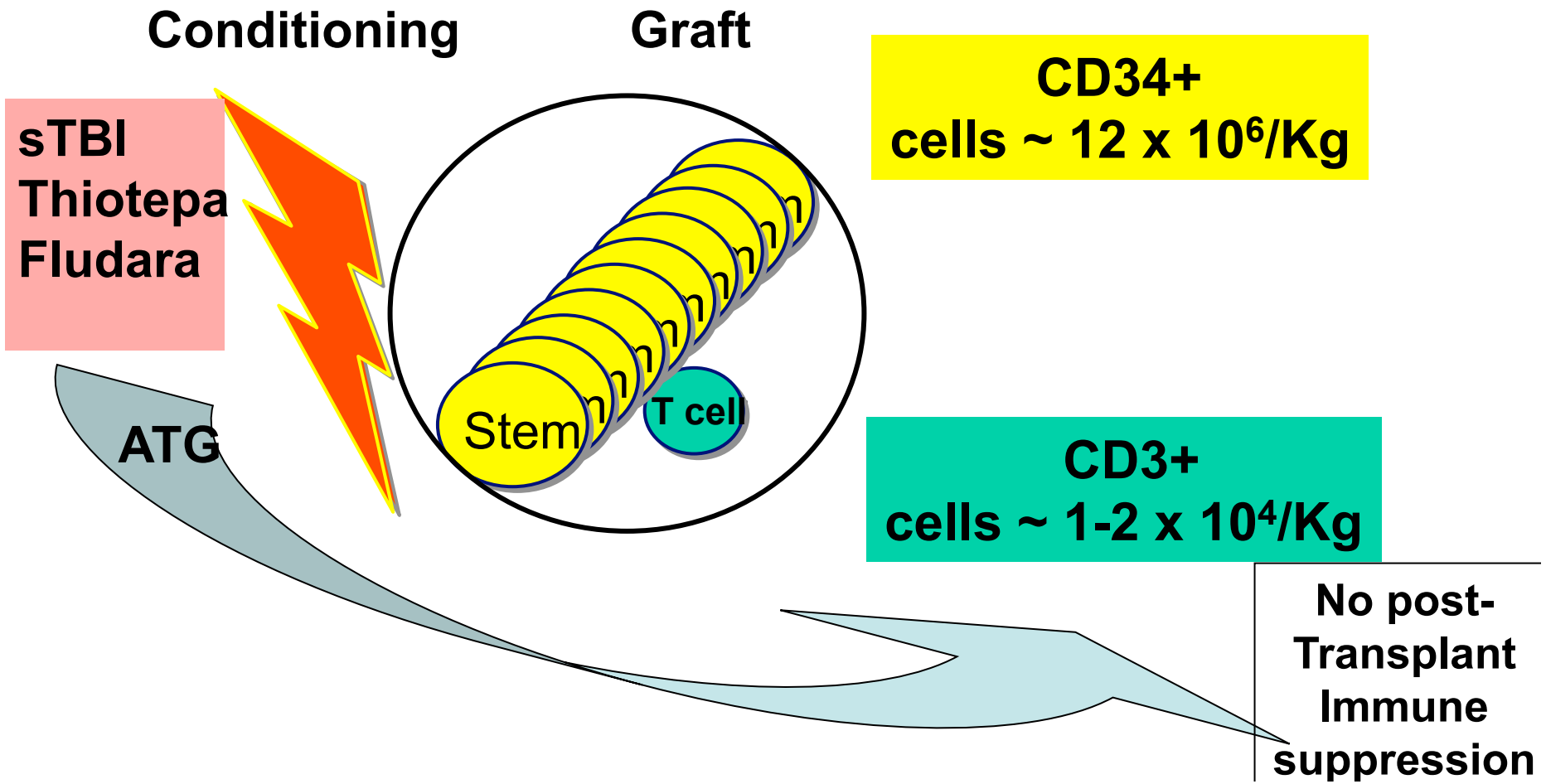




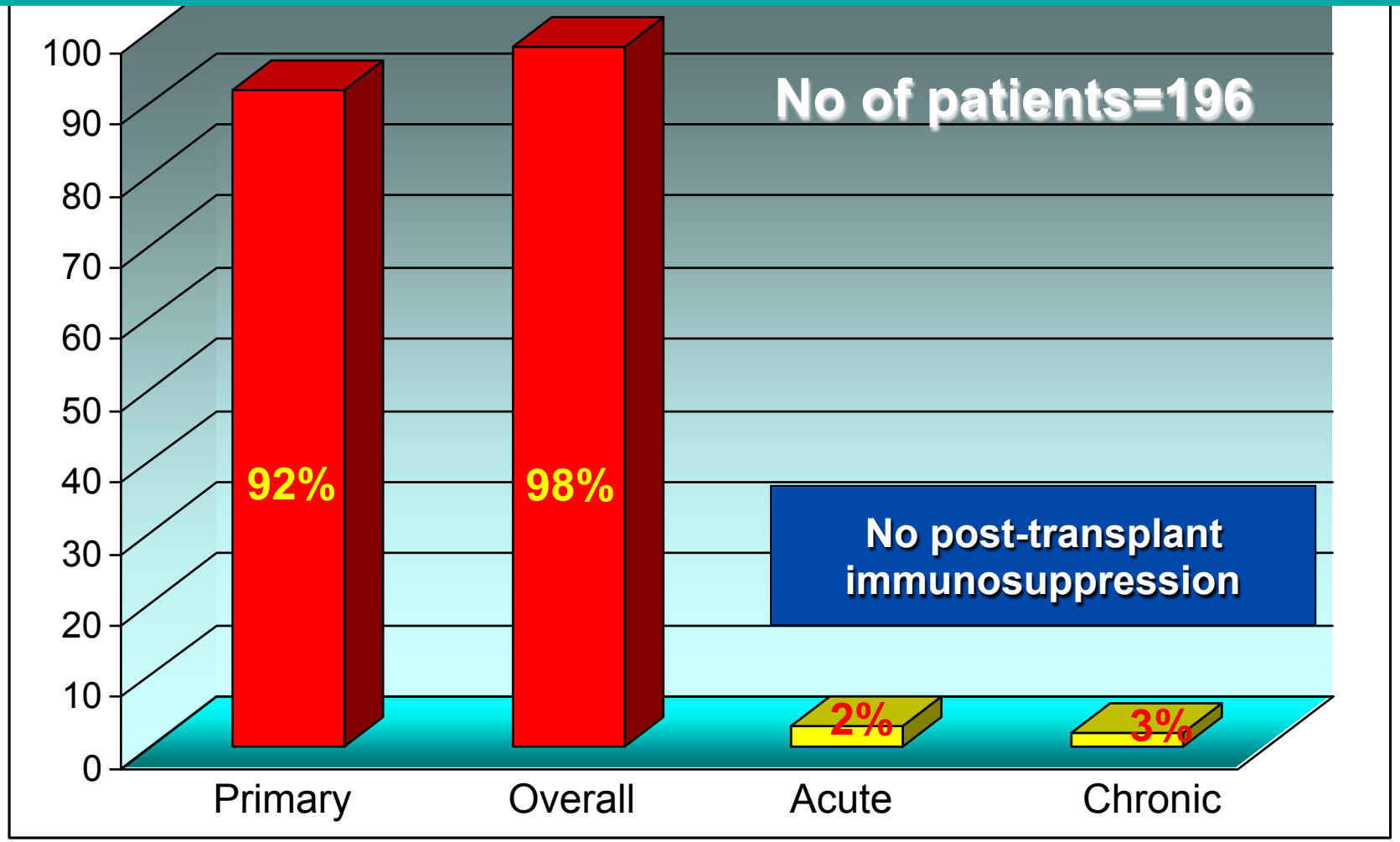
Fully mismatched HLA haplotype

Clinical traslation of the “megadose” stem cell concept

In mice: Reisner and coll: Nat Med 1995,
In leukemia: Aversa et al, Blood 1994; NEJM 1998; JCO 2005



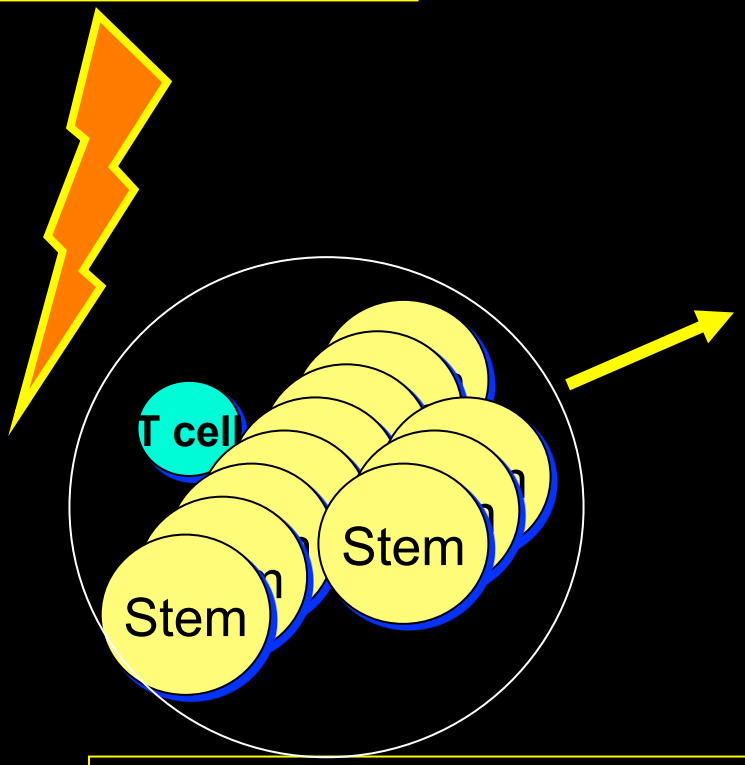
Engraftment GvHD

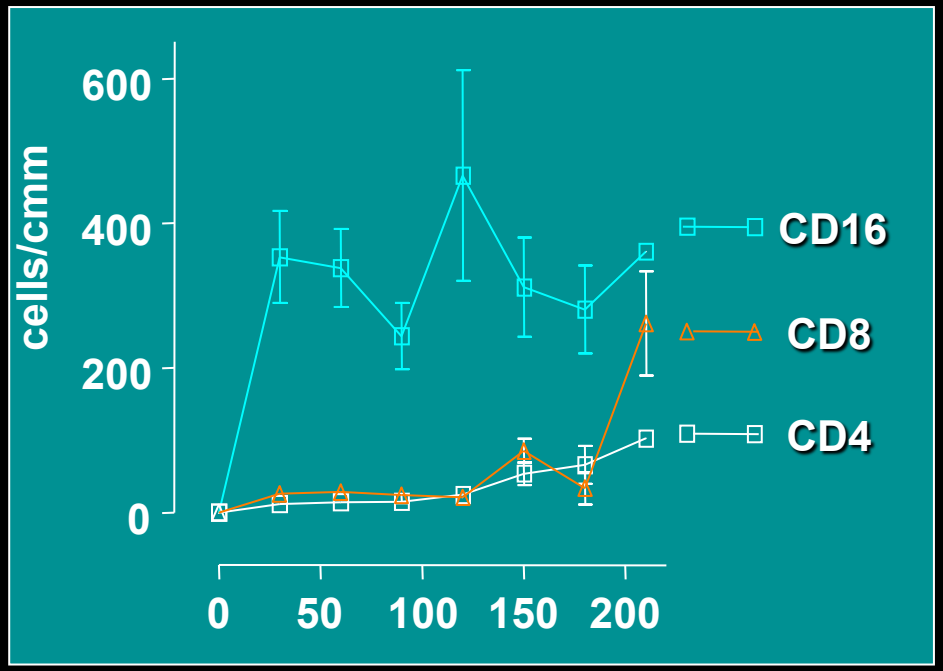


Aversa F. et al., N Engl J Med 1998;339:1186-1193
Aversa F. et al., J Cln Oncol 2005;23:3447-3454

Post-transplant immune reconstitution

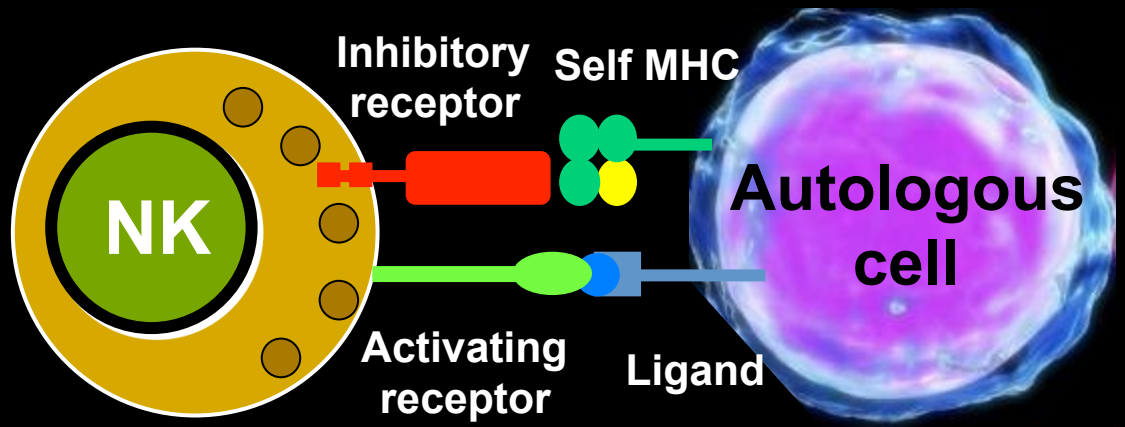
High-intensity conditioning



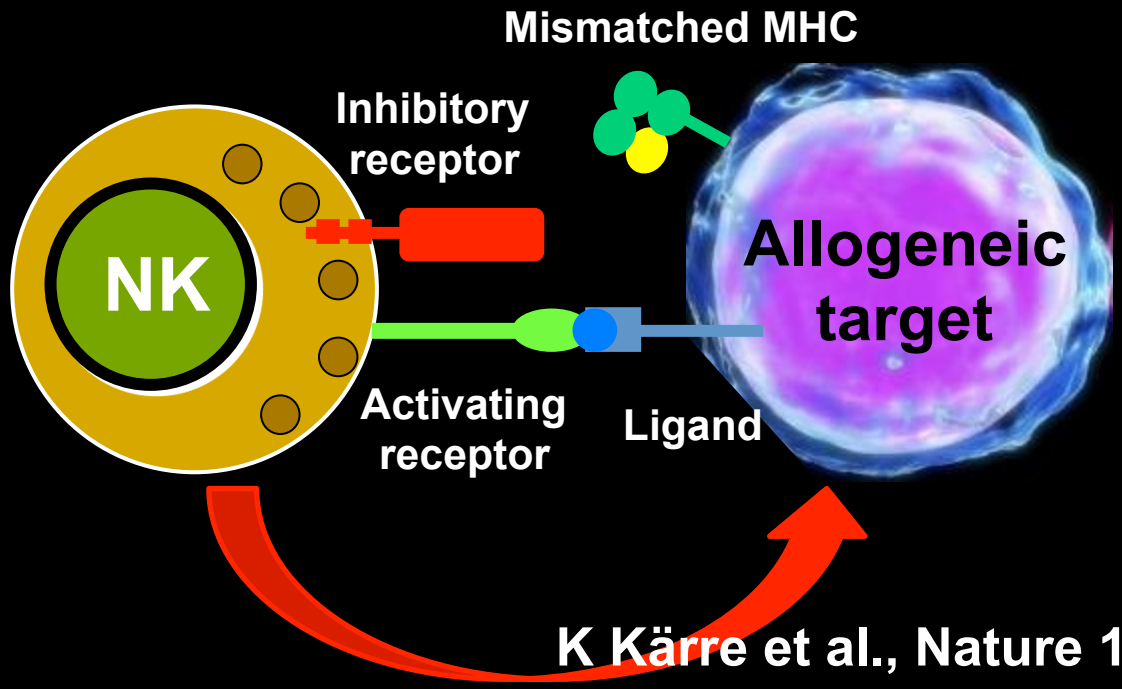


Mega-dose stem cells from “allo NK” donor
No post-transplant immune suppression

“Missing self” recognition and alloreactivity



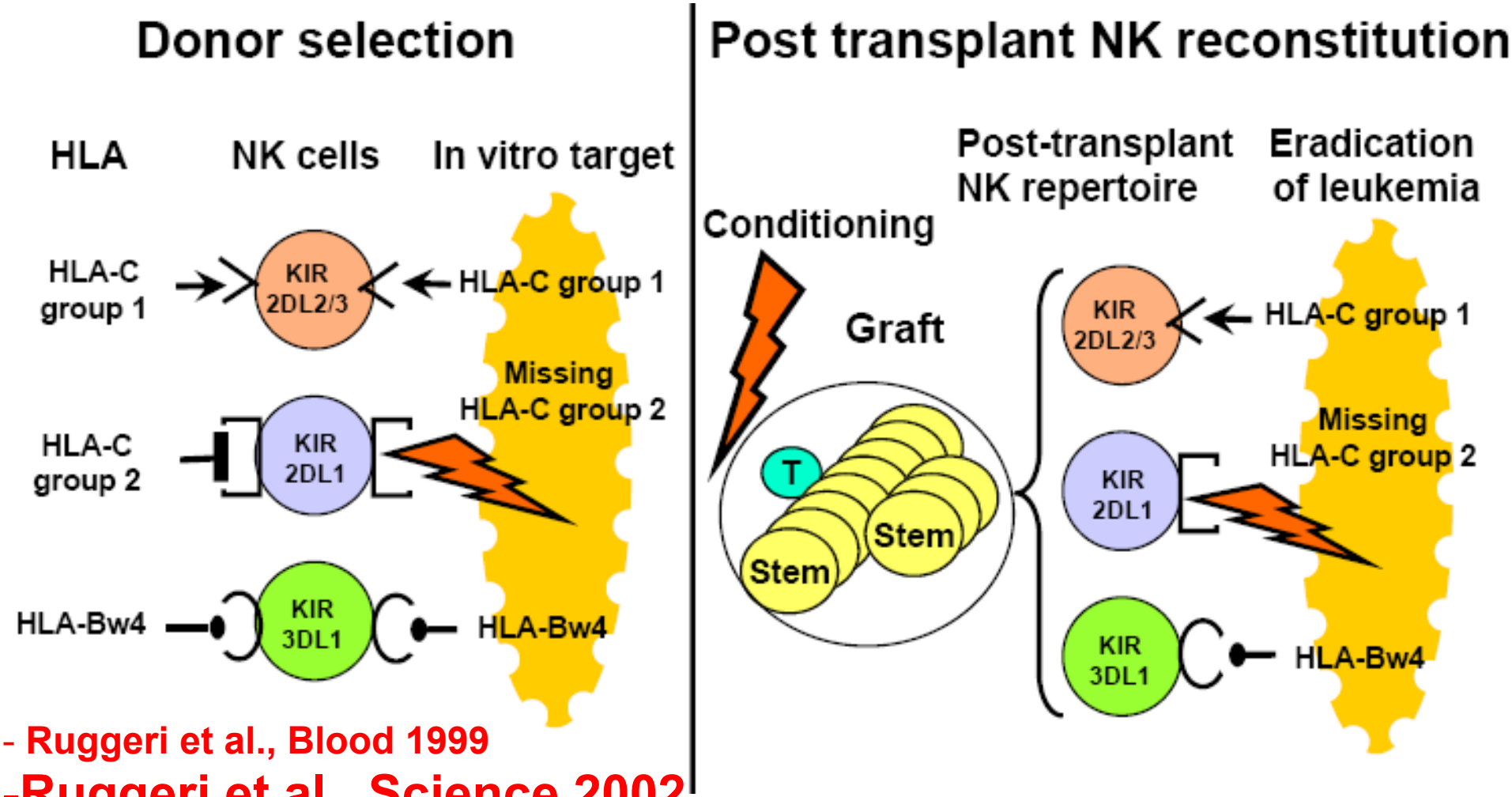
Protection



Lysis

K Kärre et al., Nature 1986
E Ciccone…A Moretta, JEM 1992
M Colonna…J Strominger, Science 1993

Donor versus recipient NK cell alloreactivity



- Ruggeri et al., Blood 1999

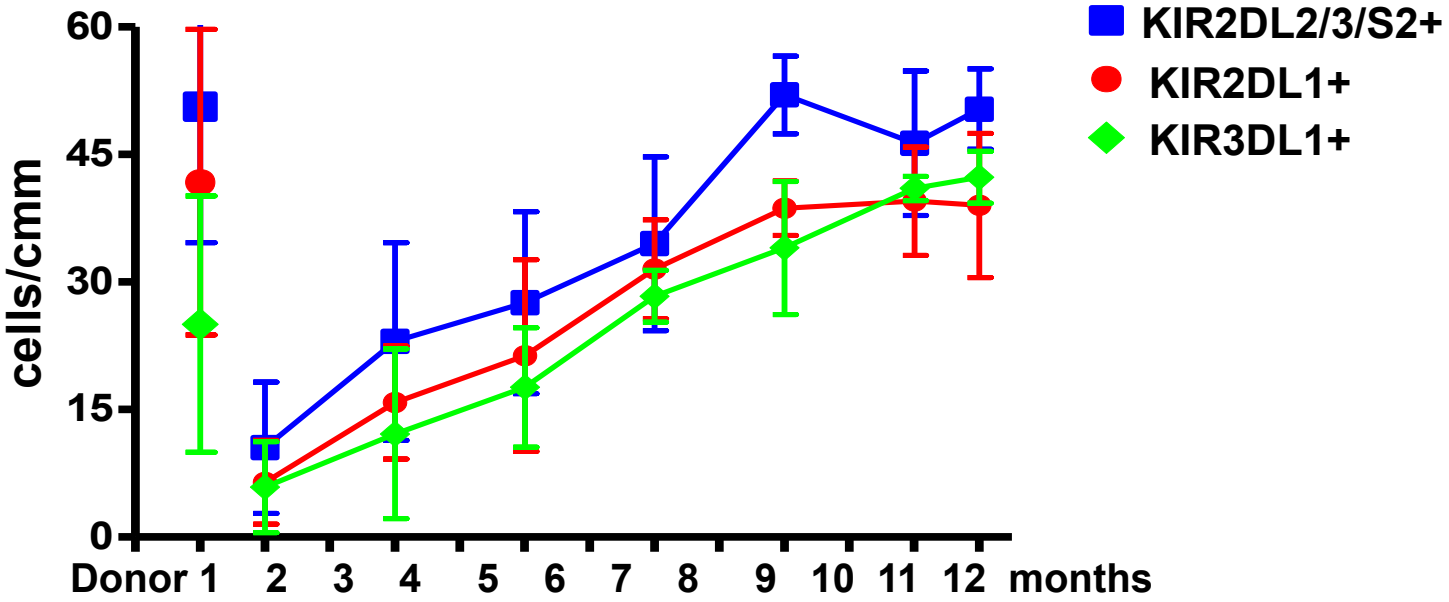
-Ruggeri et al., Science 2002

- Ruggeri et al., Blood 2007
- Stern et al., Blood 2008
- Mancusi et al., Blood 2015

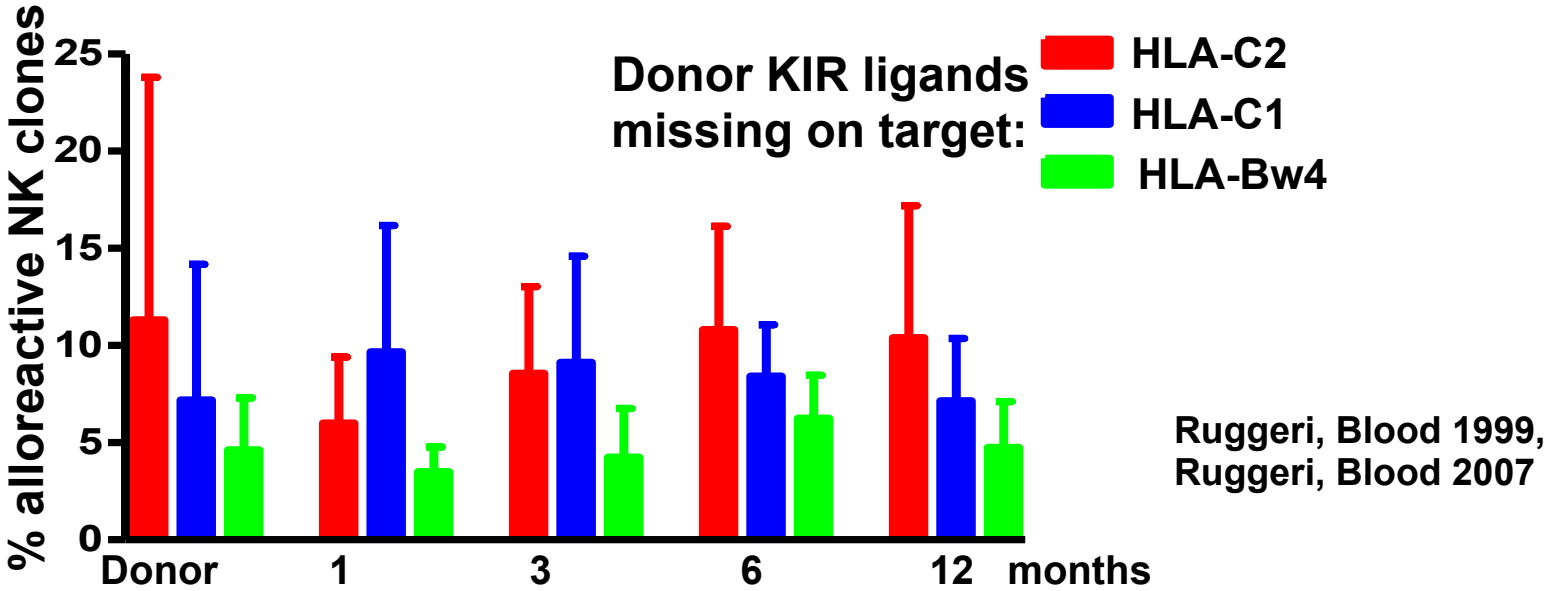
- EBMT Van Bekkum Award, Montreax 2002
- AICF's Prize for Excellence in Clinical Medicine, New York, 2003

Post-transplant regeneration of donor vs recipient alloreactive NK repertoires

Phenotype of potentially alloreactive NK subsets

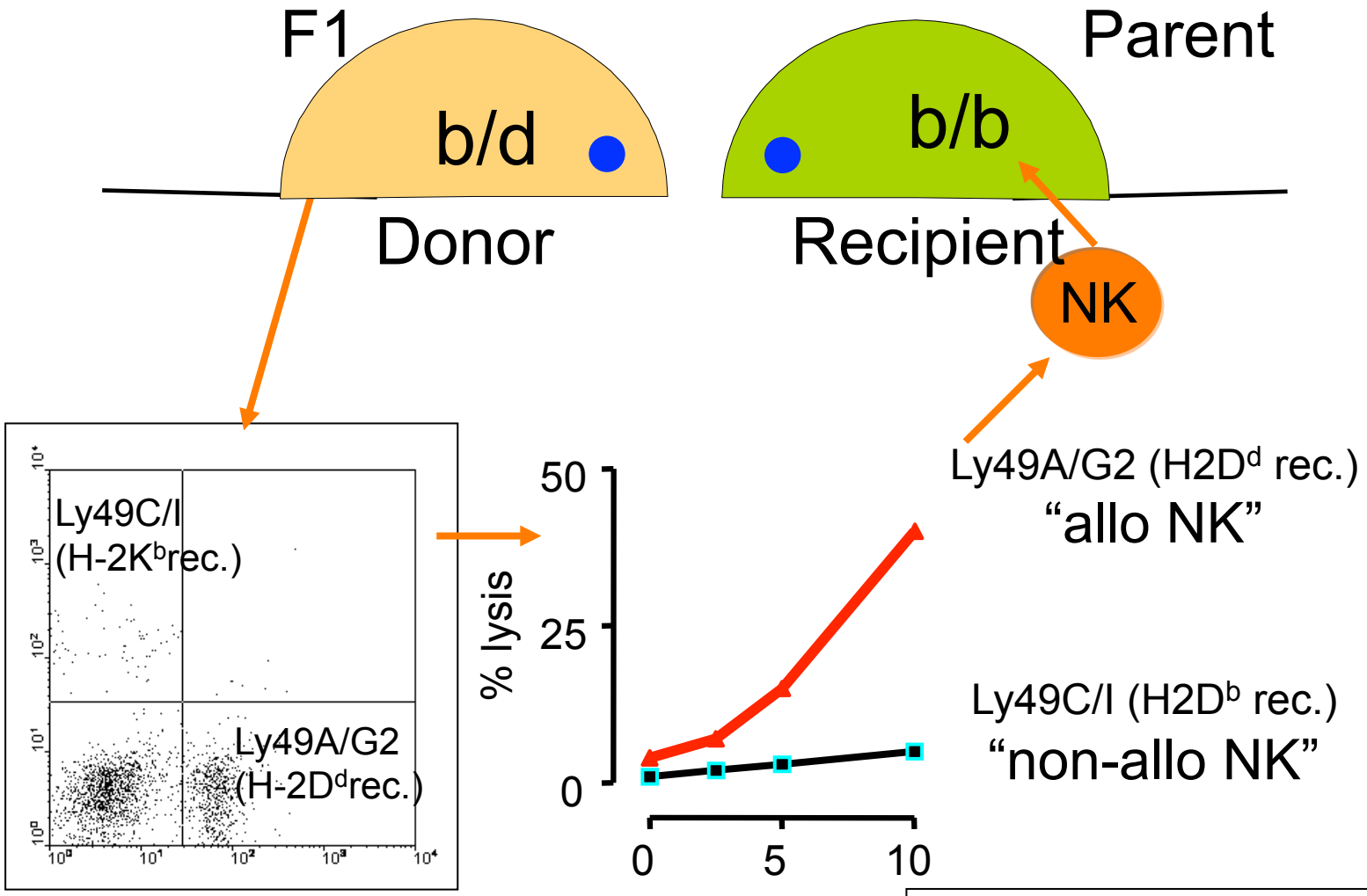


Frequencies of alloreactive NK clones

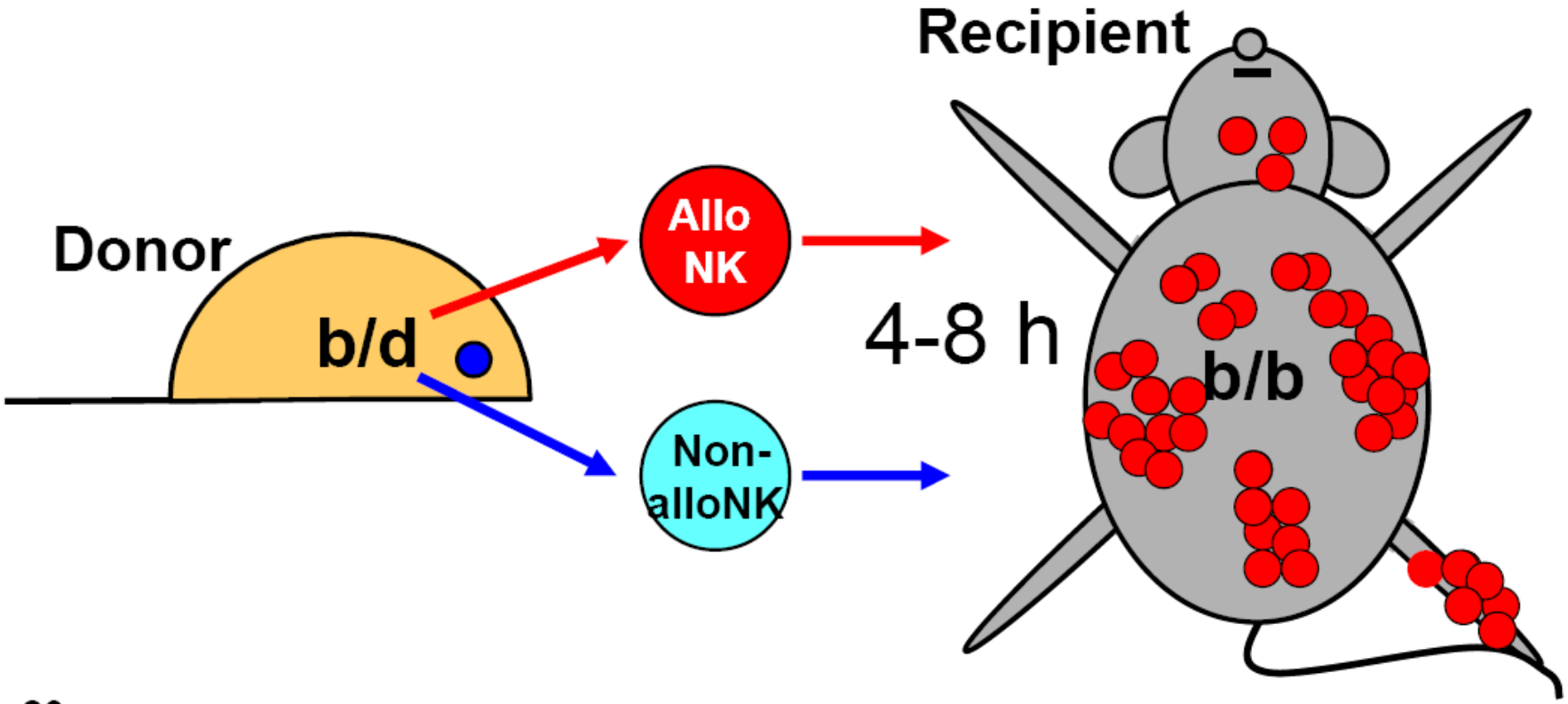


Ruggeri, Blood 1999,
Ruggeri, Blood 2007

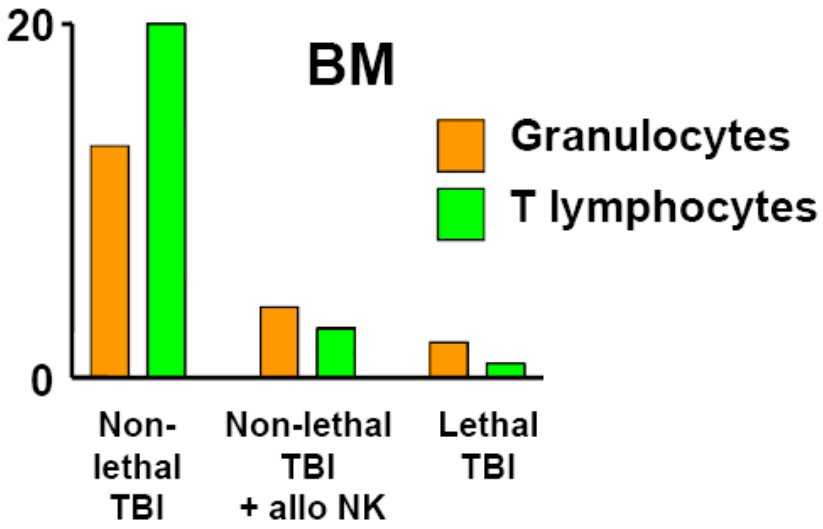
A mouse model of donor-vs-recipient NK cell alloreactivity in transplantation

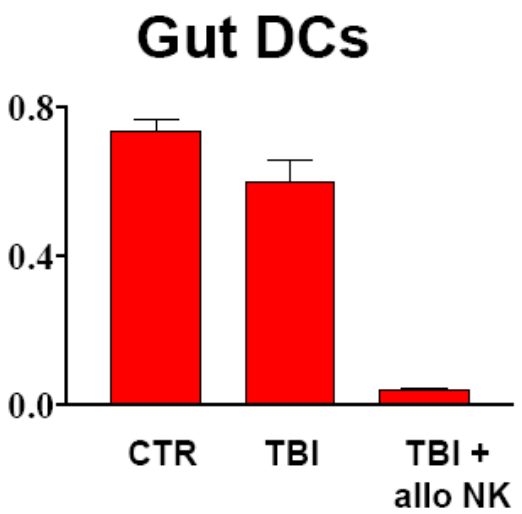


Ruggeri et al. Science 2002



No GVHD

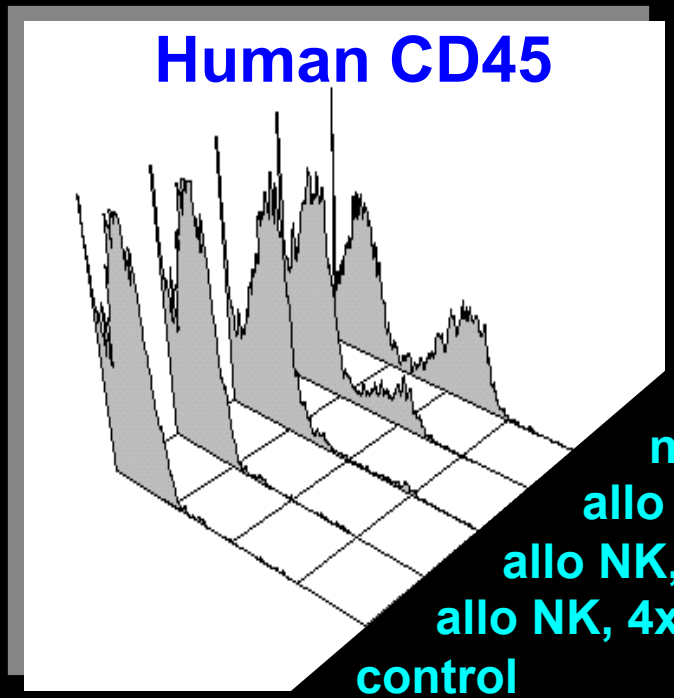


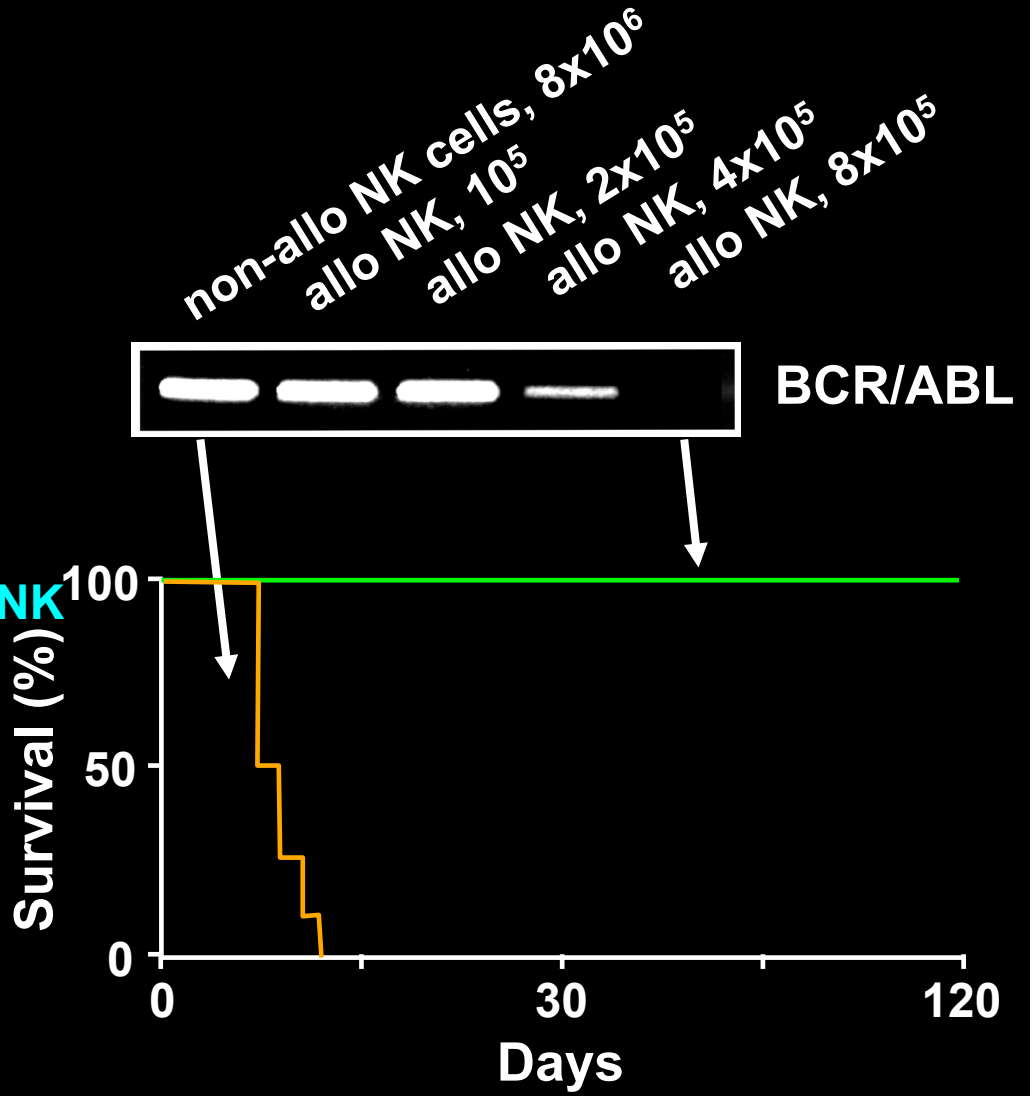


Ruggeri et al. Science 2002

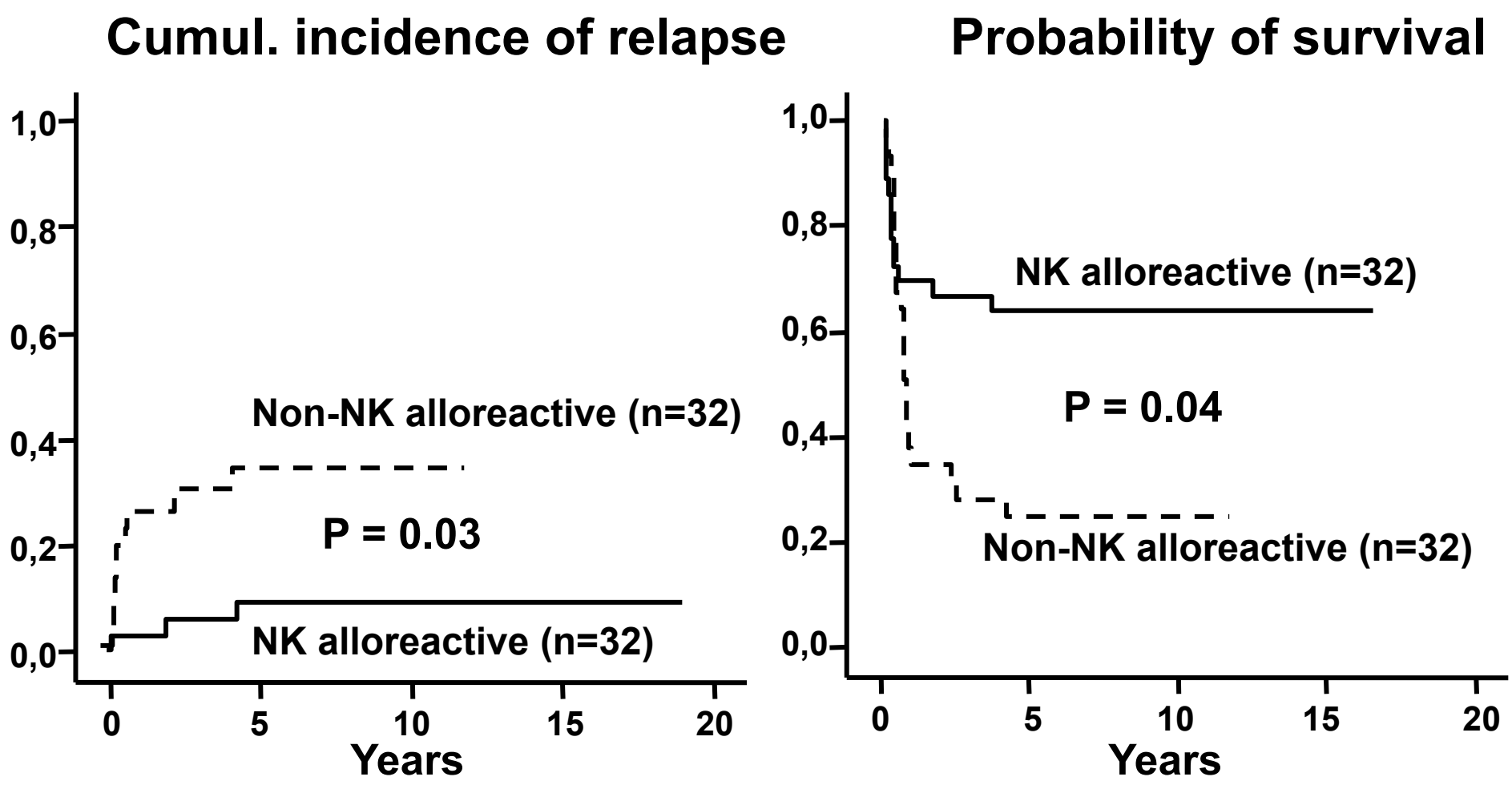
Clearance of human leukemia by human alloreactive NK clones in SCID mice

CML blastic crisis in NOD-SCID mice

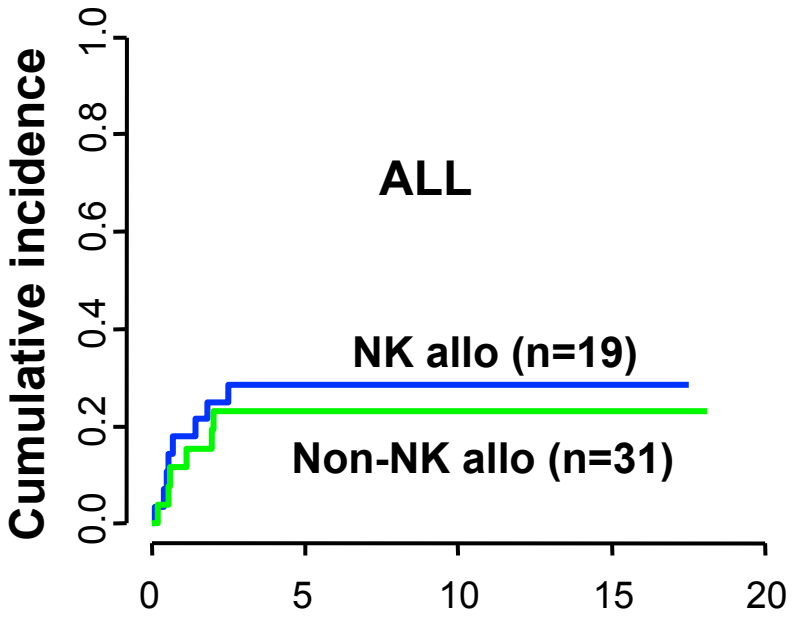




Relapse and survival of AML patients transplanted in any remission

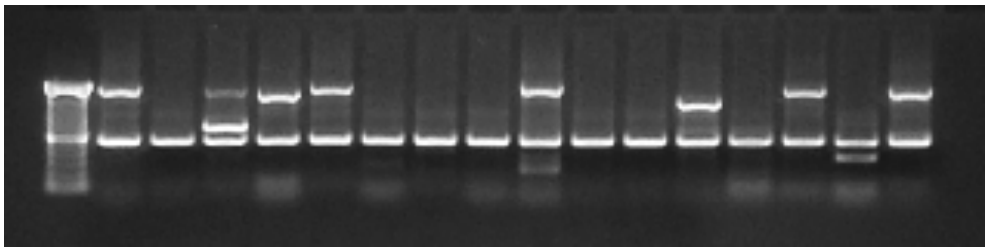


Ruggeri Blood 1999; Science 2002; Blood 2007; Stern Blood 2008; Mancusi Blood 2016

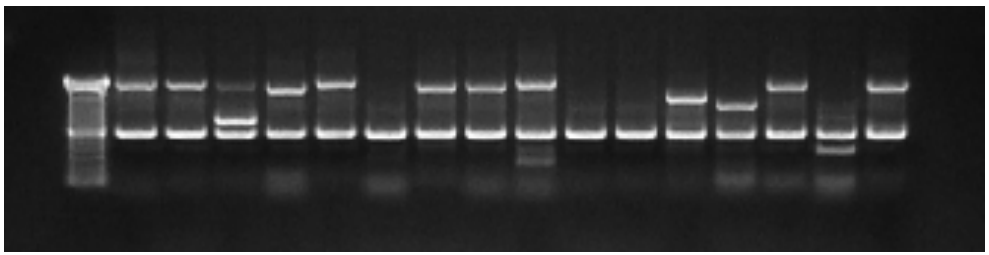

Cumulative incidence
0.0
0.2
0.4
0.6
0.8
1.0
ALL
NK allo (n=19)
Non-NK allo (n=31)
0
5
10
15
20

Killer cell Ig-like receptor (KIR) haplotypes

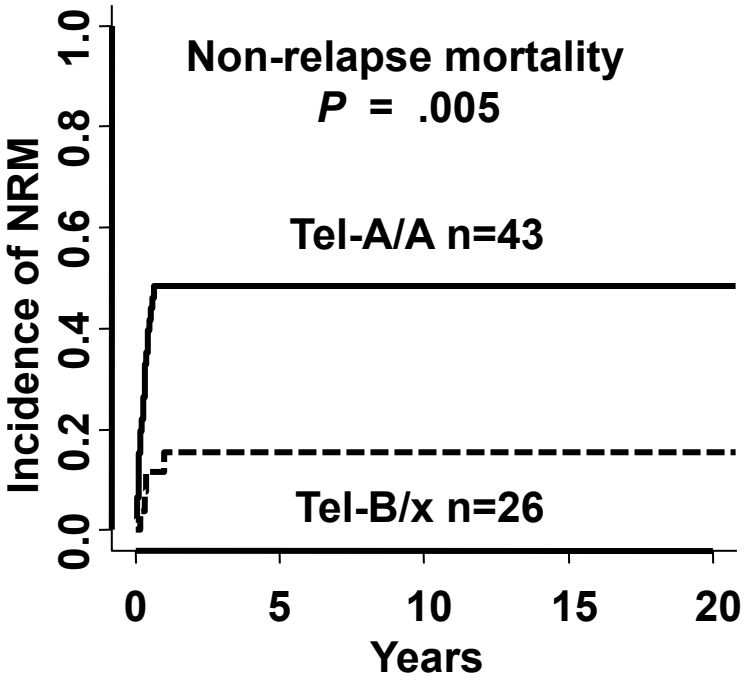
Group A haplotype KIR genes
"AA" (~25% of individuals)

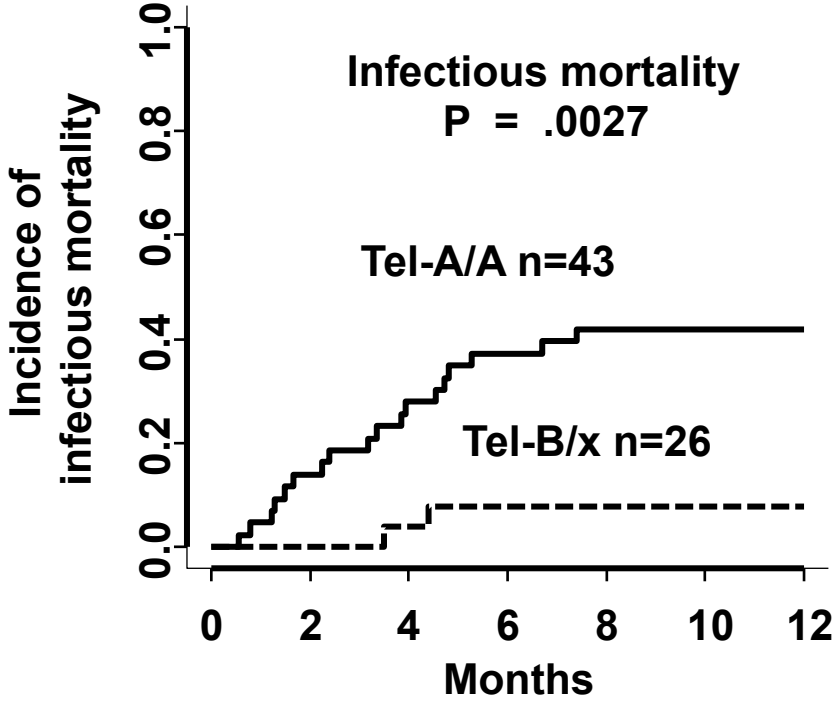


Group B haplotype KIR genes
"BX" (~75% of individuals)



Transplantation from NK alloreactive donors with concomitant activating KIRs reduces non-relapse (infectious) mortality





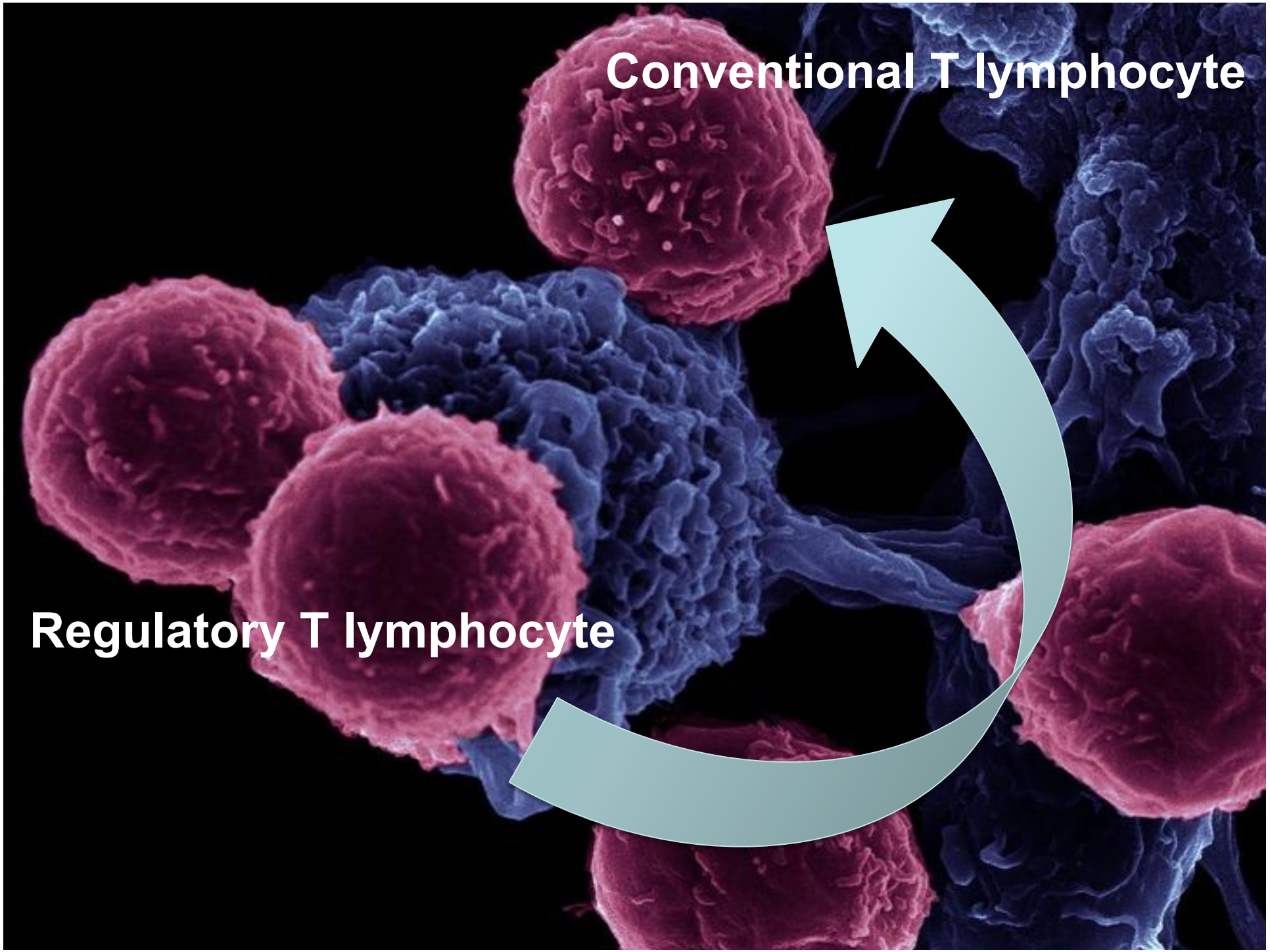
Tel-B/x versus Tel-A/A:HR: 0.23; 95% CI: 0.08-0.66; *P* = .007

Tel-B/x versus Tel-A/A: HR: 0.13; 95% CI: 0.03-0.58; *P* = .007

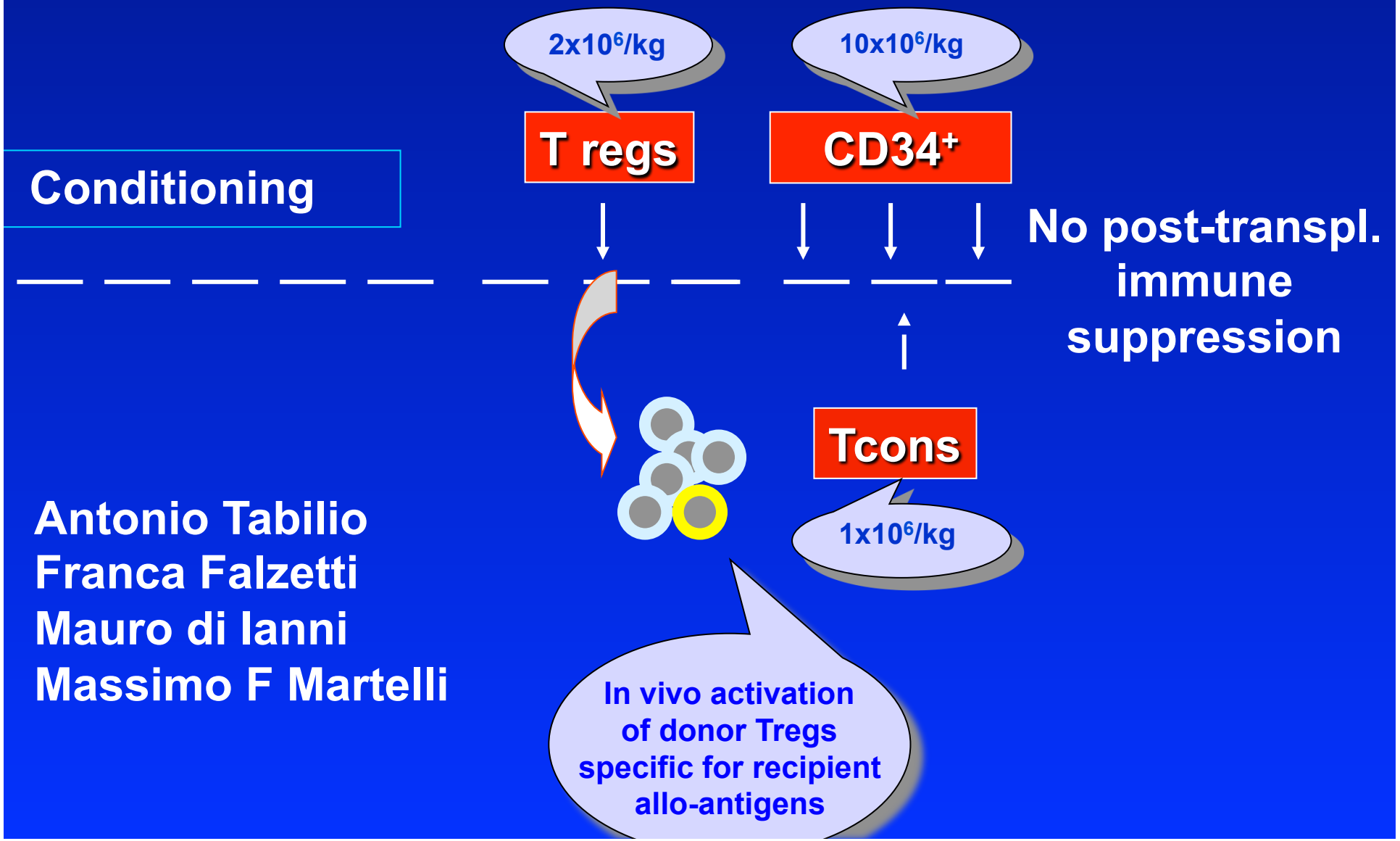
Mancusi et al., Blood 2015

Limitations of allo NK cell immunotherapy:

1) KIR ligand mismatches present in 50% of transplants
2) Not effective in (B cell precursor) ALL

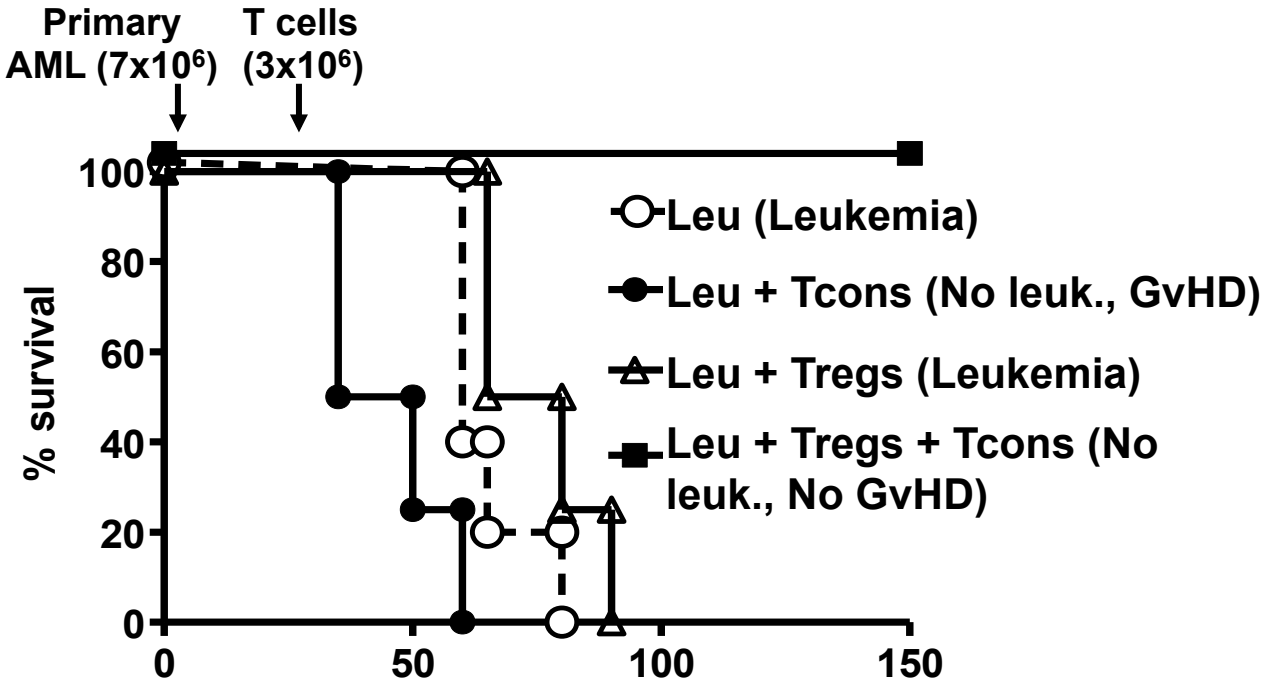

Conventional T lymphocyte
Regulatory T lymphocyte

Improving on NK cell-mediated GvL: Treg and Tcon adoptive immunotherapy?



Antonio Tabilio
Franca Falzetti
Mauro di Ianni
Massimo F Martelli

Clearance of human leukemia w/o GvHD in immunodeficient (NSG) mice given peripheral blood human T regs + T cons



Ruggeri L., unpublished

Clinical scale immunomagnetic selection of human peripheral blood CD4+/CD25+/Fox P3+ regulatory T cells

1st step:
Depletion of
CD8+/CD19+cells

2nd step:
Selection of
CD25+ cells

Cell processing

Franca Falzetti

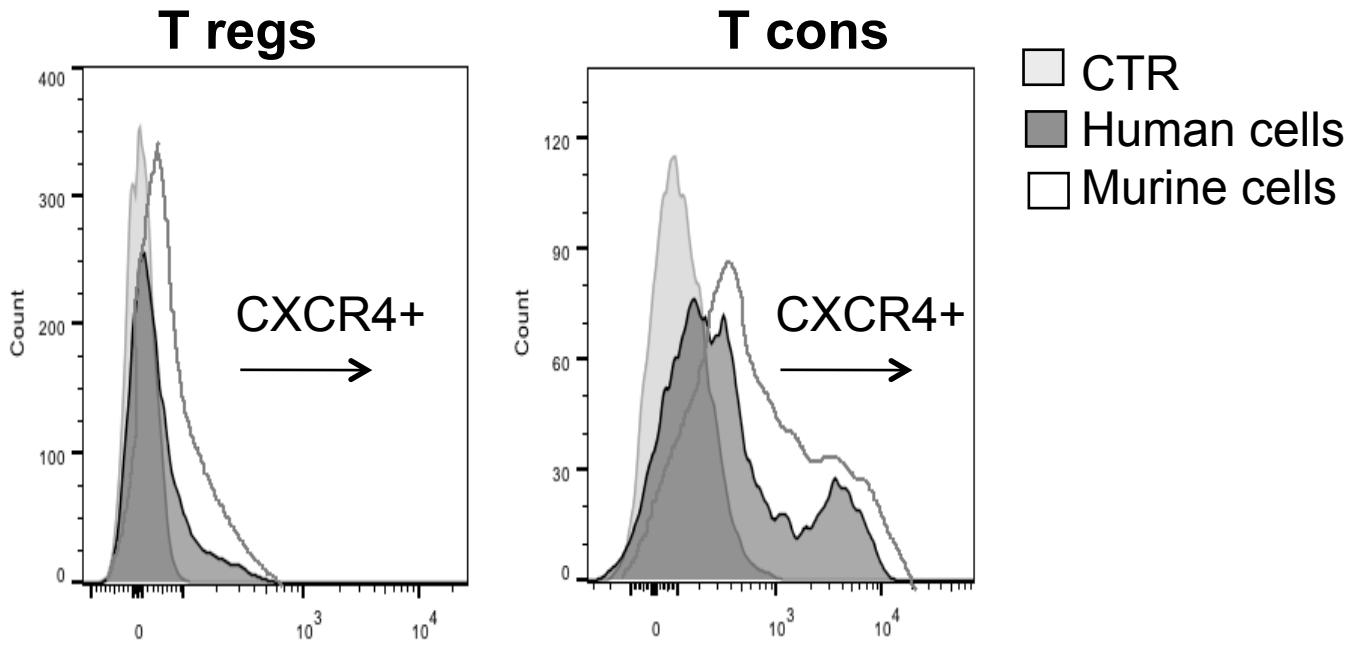
Roberta Iacucci

Tiziana Zei

Final product

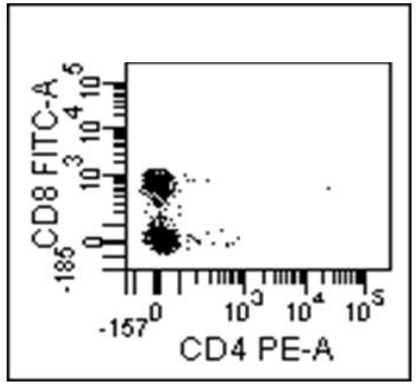
- Cells ($x10^9$) = 280 (202- 390)
- CD4/CD25+ = 92% (90-97%)
- Fox P3+ cells = 90%

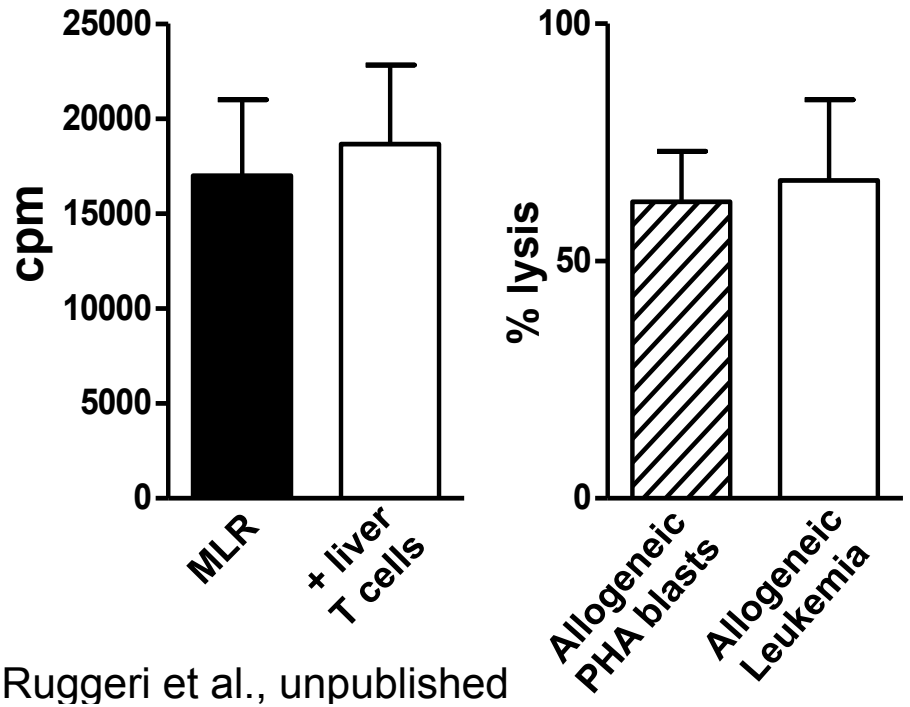
Unlike T cons, human peripheral blood T regs (and mouse spleen T regs) are largely negative for CXCR4 bone marrow homing receptor



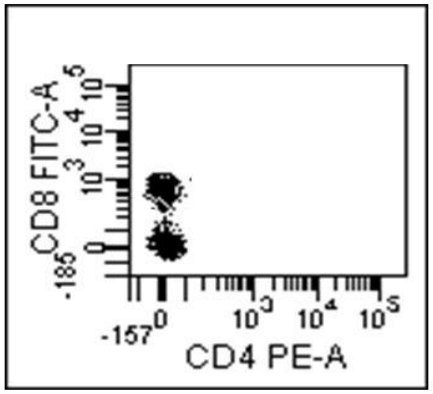
Immunodeficient mice + human T cons (clear leukemia but die of GvHD)

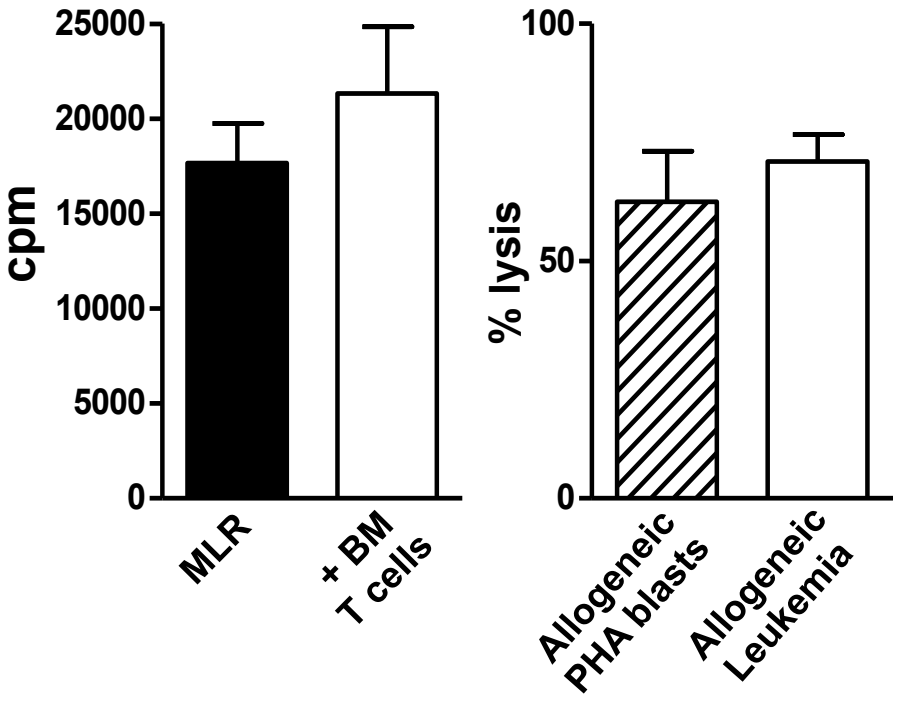
Liver and Gut





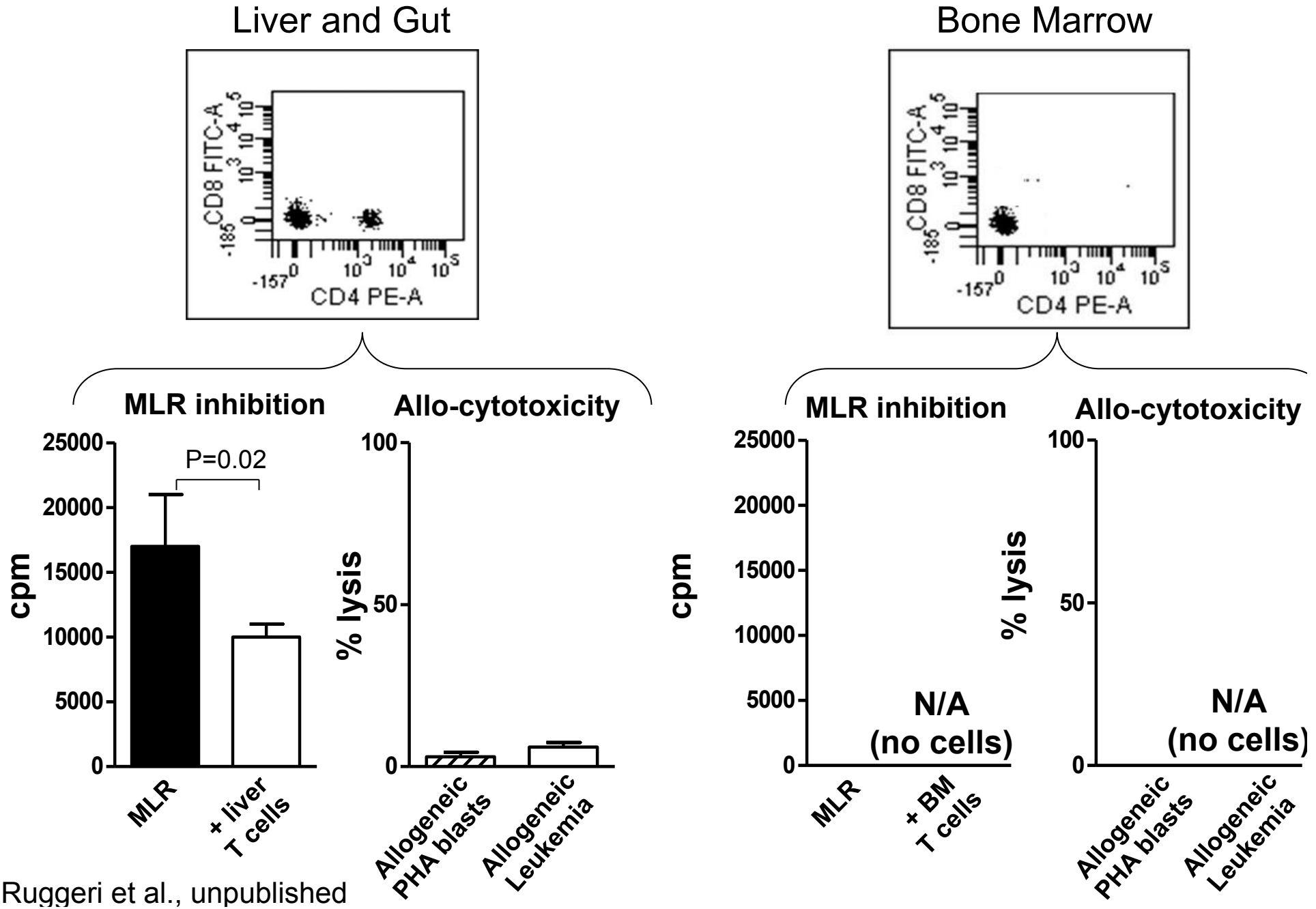
Bone Marrow





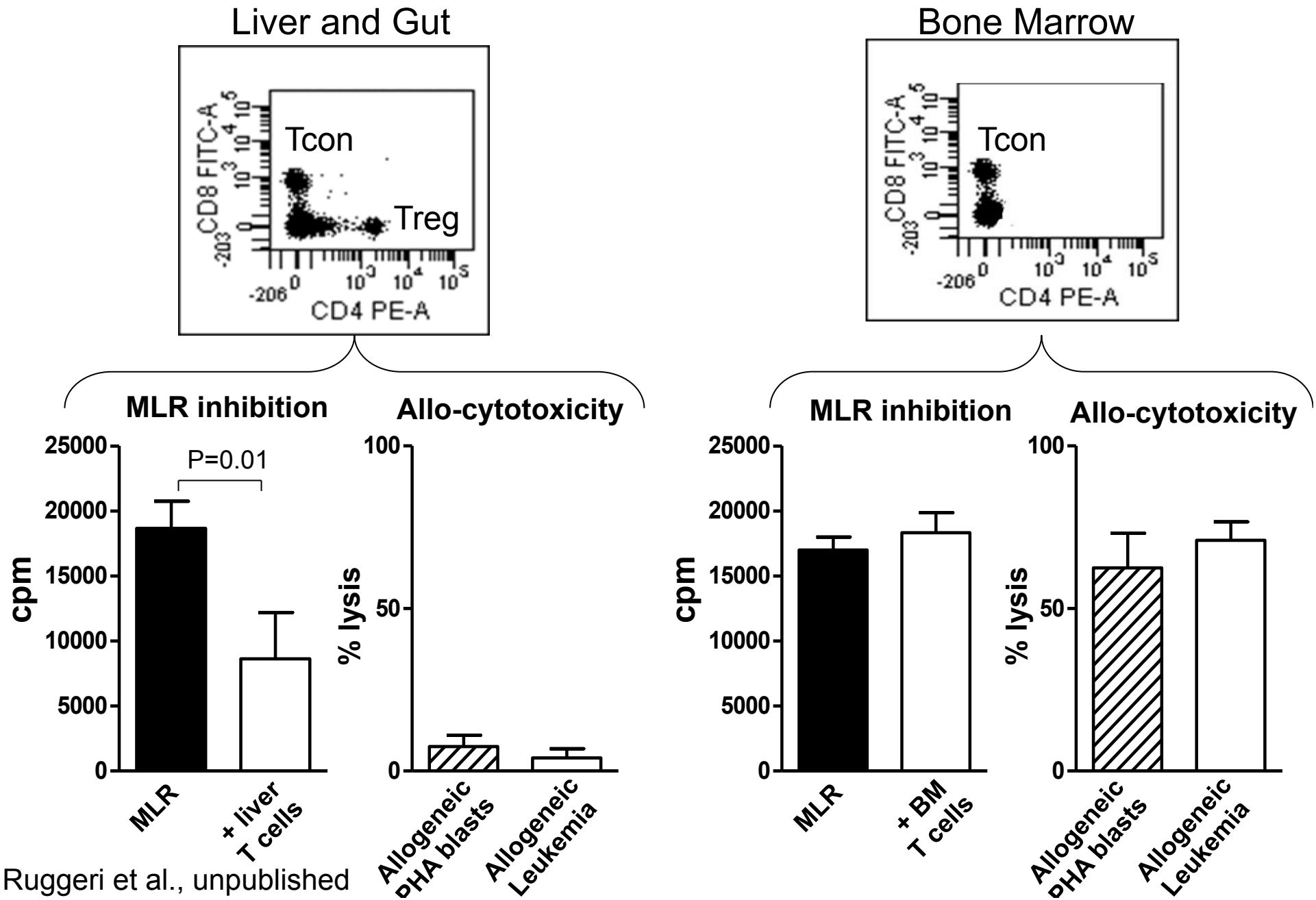
Ruggeri et al., unpublished

Immunodeficient mice + human Tregs (die of leukemia, w/o GvHD)



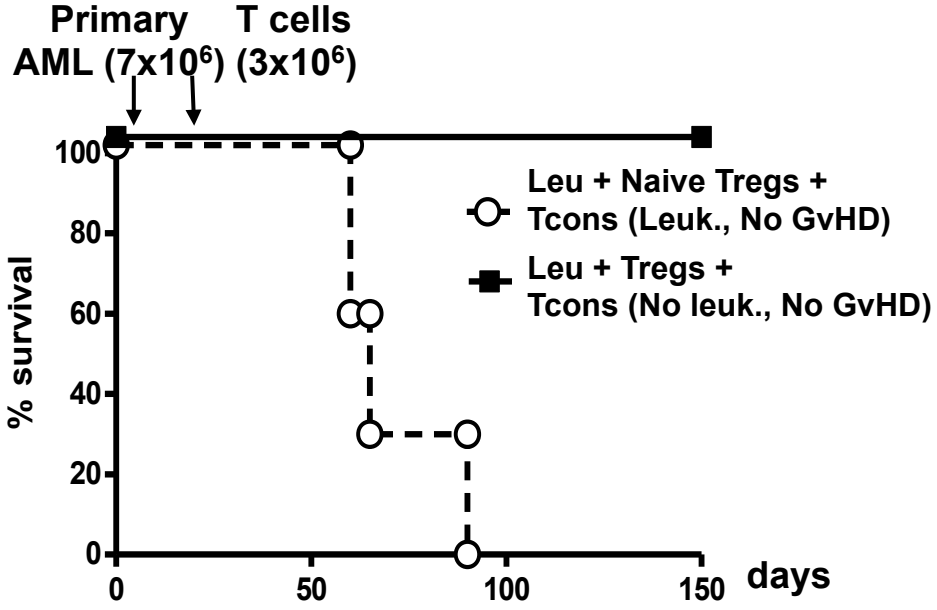
Ruggeri et al., unpublished

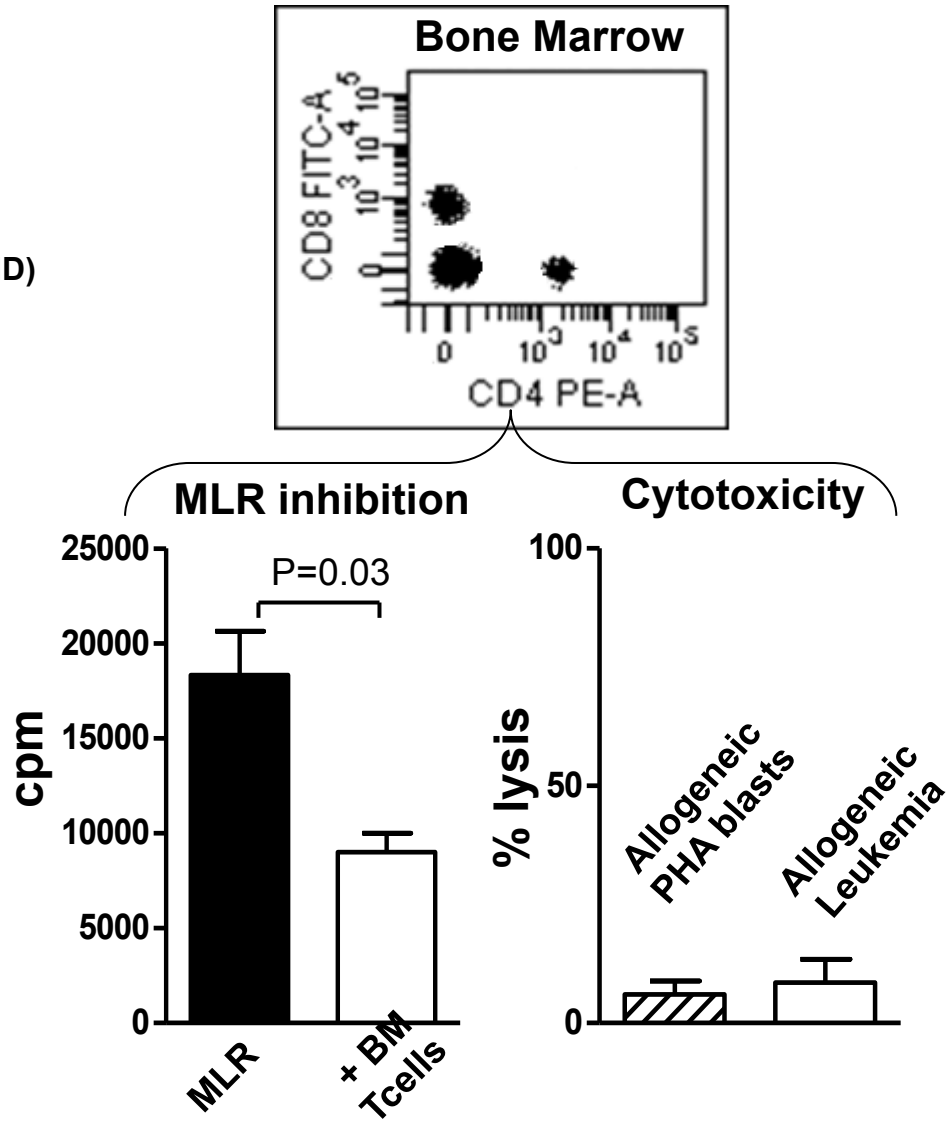
Immunodeficient mice given human Tcons and Tregs clear leukemia w/o GvHD



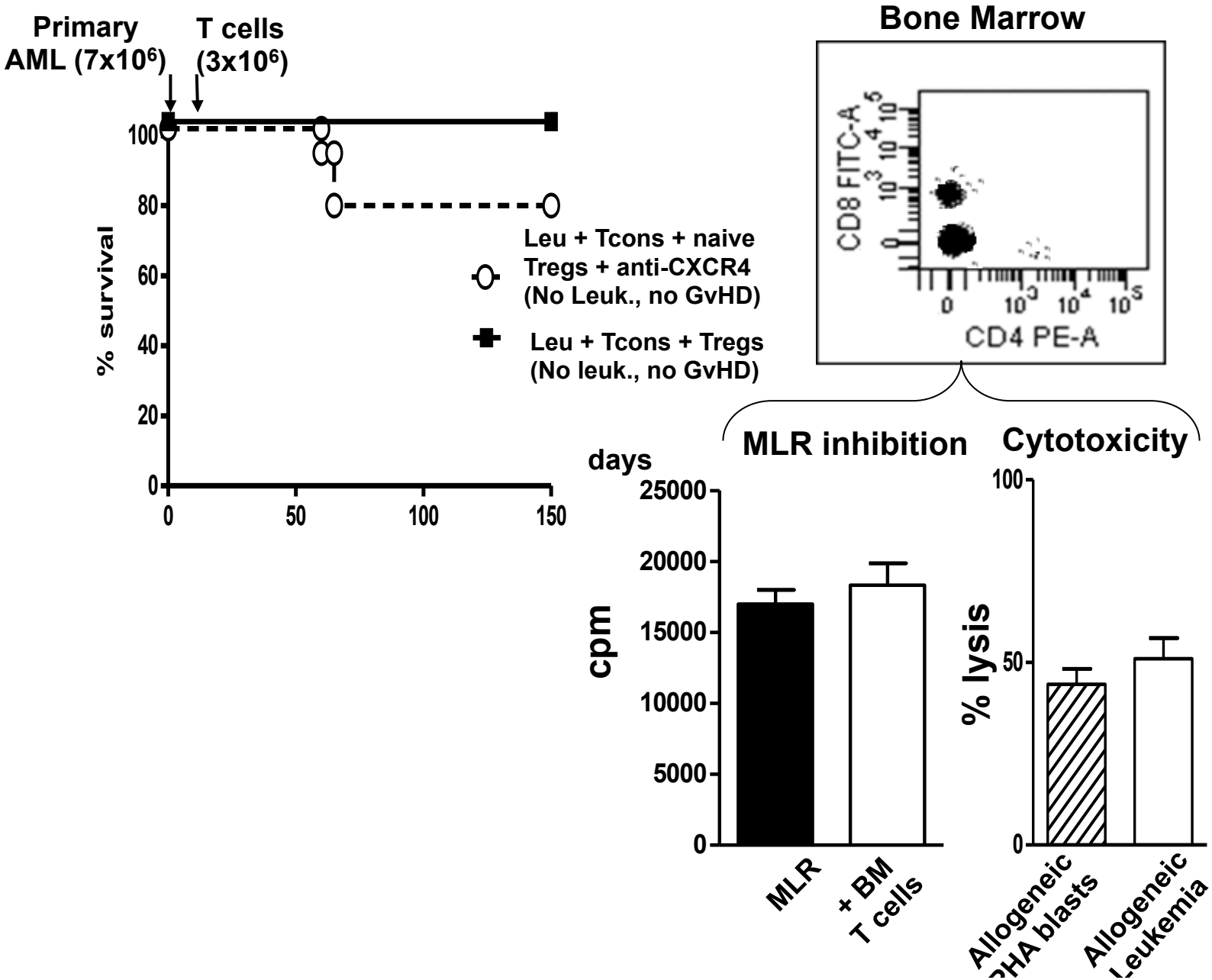
Ruggeri et al., unpublished

A control experiment: mice given Tcons + **naive** (CD45RA+) T regs die of leukemia. Unlike memory Tregs, naive Tregs possess CXCR4 bone marrow homing receptor. They home to the bone marrow and prevent conventional T cells from exerting thier GvL effect





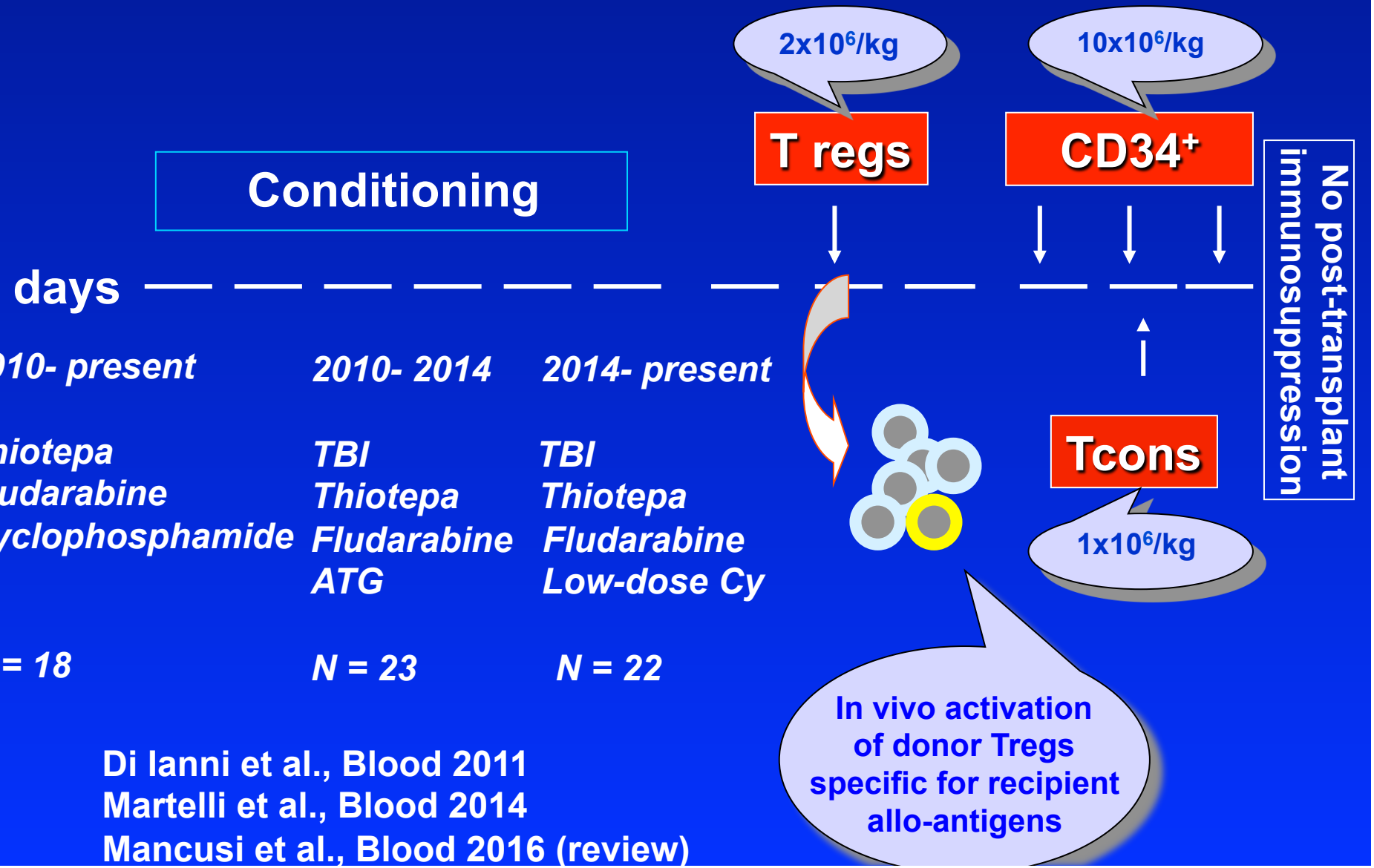
Key role of CXCR4: anti-CXCR4 antibody treatment prevents naive Tregs from entering the bone marrow and reinstates the GvL effect mediated by T cons



Hypothesis:

In clinical trials, immunotherapy with Treg/Tcon may be able to eradicate leukemia without causing GvHD because human peripheral blood Tregs are largely CXCR4-negative. They may migrate to the periphery where they may block T cons and prevent GvHD but may not home to the bone marrow. Consequently, in the bone marrow, T cons may be free to exert allo-antigen recognition and leukemia killing

Treg and Tcon adoptive immunotherapy in haplo HSCT for high-risk acute leukemia patients



Di Ianni et al., Blood 2011
Martelli et al., Blood 2014
Mancusi et al., Blood 2016 (review)

1ST Clinical Trial

Di Ianni et al. Blood 2011

TBI
Thiotepa
Fludarabine
Cyclophosphamide

GVHD prevention:
Low aGvHD rates
Very Low cGvHD rates

High TRM:
High toxicity and infection rates

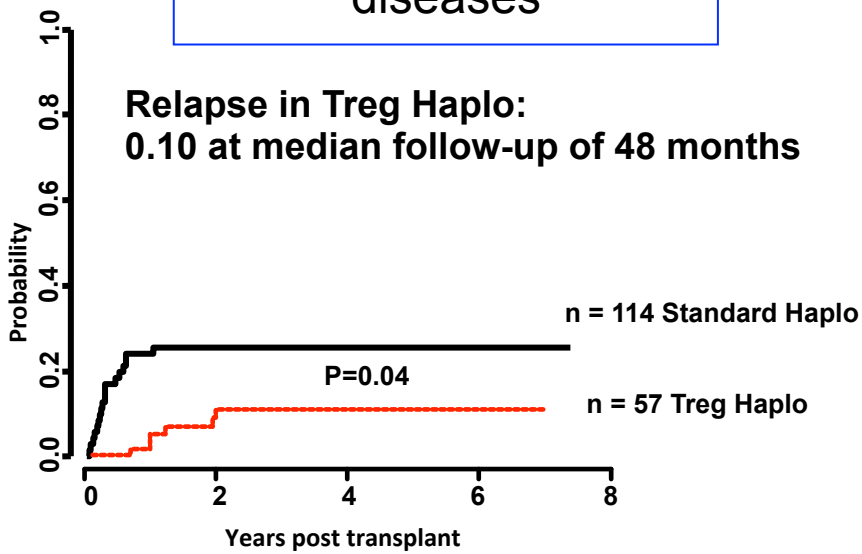
2nd Clinical Trial

Martelli et al. Blood 2014

TBI
Thiotepa
Fludarabine
ATG

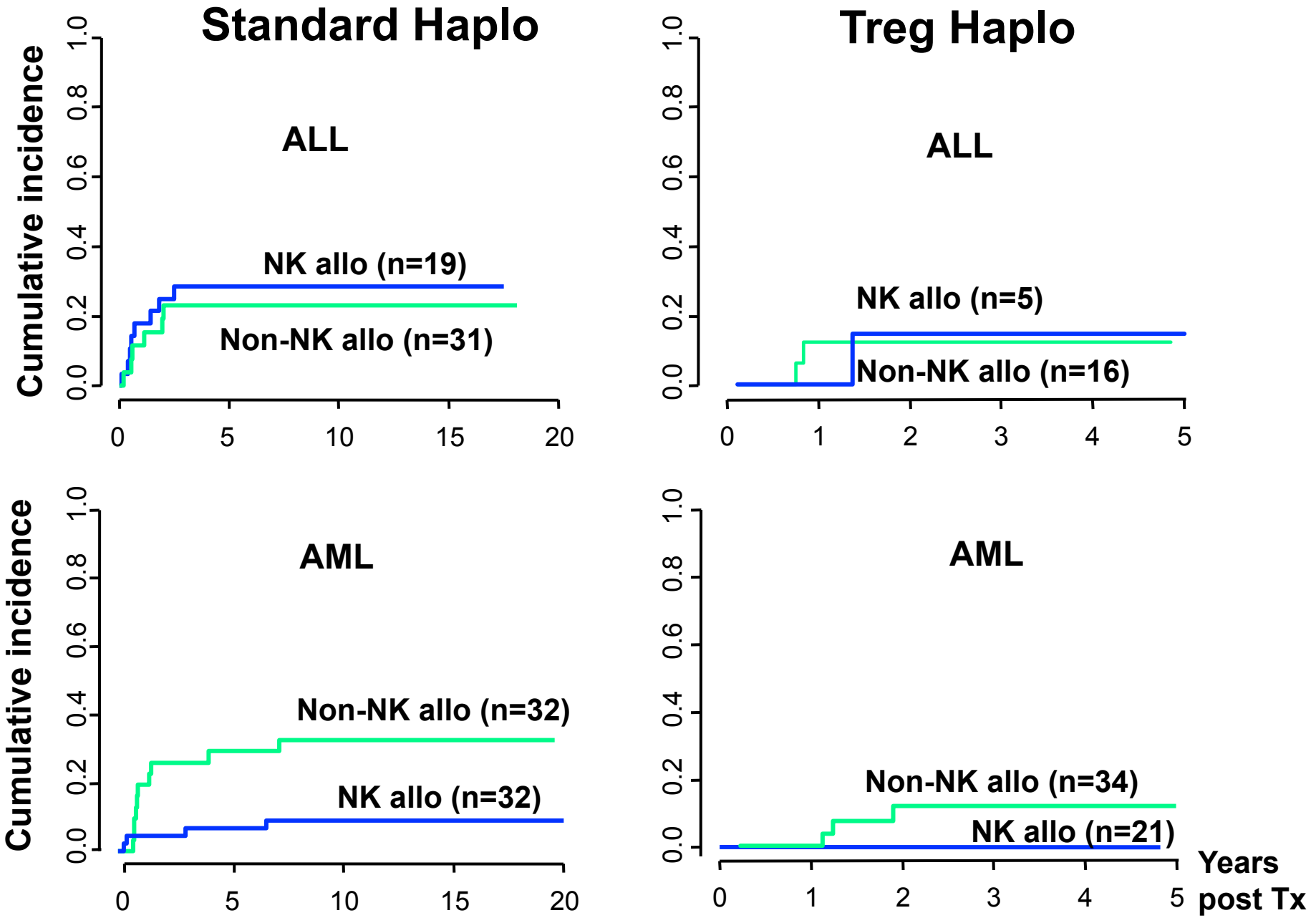
GvL effect:
Low Relapse rates despite high-risk diseases

Relapse in Treg Haplo:
0.10 at median follow-up of 48 months



Martelli et al. Blood 2014, updated

Clinical GvL effect



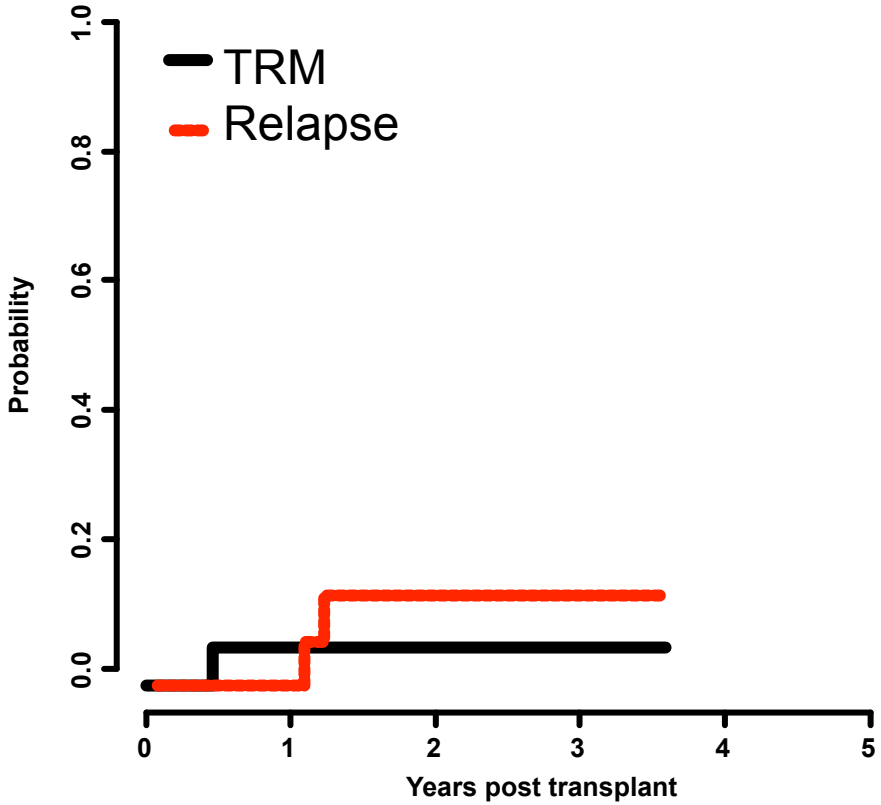
Ongoing Trial

TBI/HF-TBI
Thiotepa
Fludarabine
Low-dose Cyclophosphamide

21 patients transplanted to date:
- Median age: 30 yrs
- High risk leukemias:
  15 AML, 6 ALL
- All patients in 1ST - 3RD CR

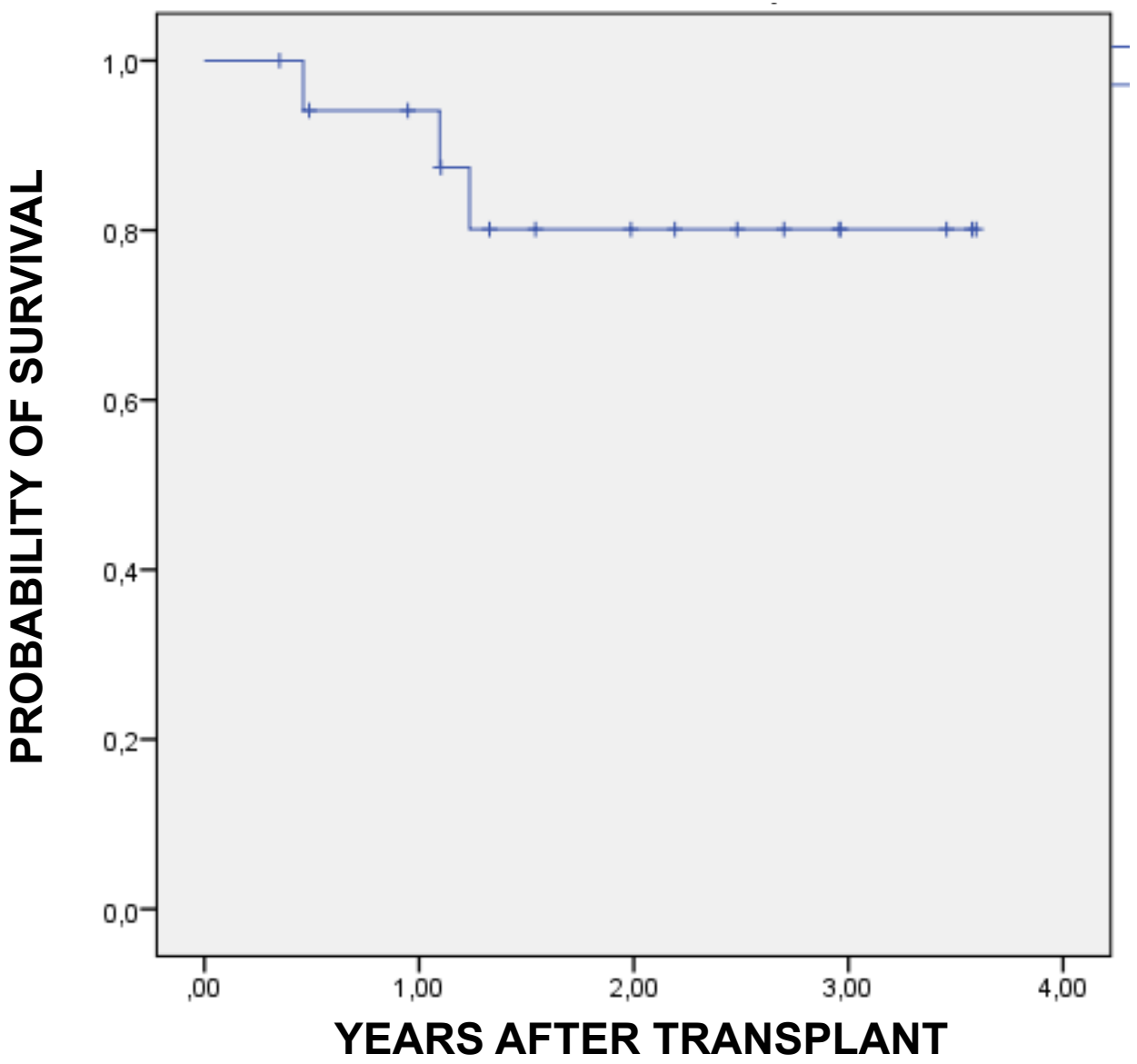
OUTCOMES:
- Rejection: 1/21
- **Relapse: 2/20**
- **aGvHD: 2/20 (off therapy)**
- **cGvHD: 1/20**
- **TRM: 1/20**

Cumulative Incidences

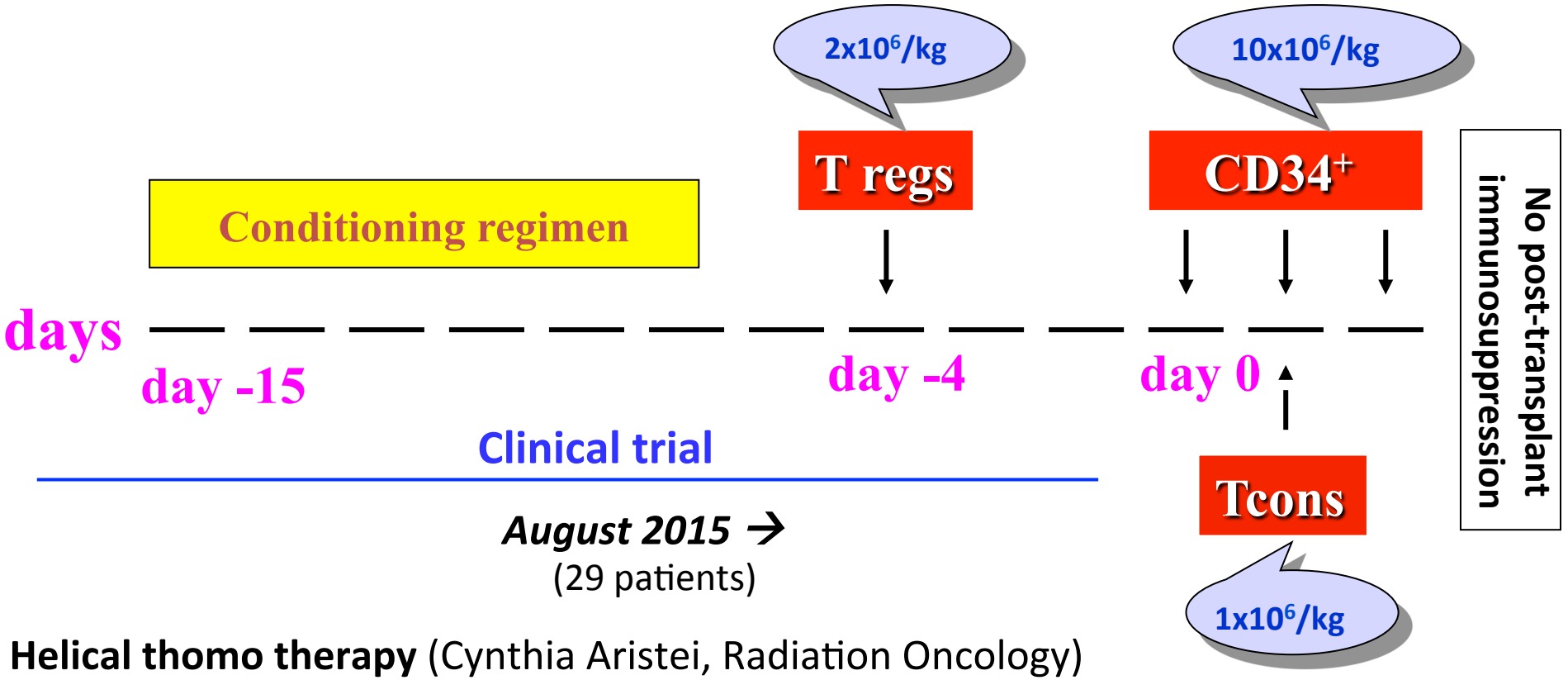


cGvHD/Leukemia-free Survival

Median follow-up = 1.5 yrs



Total lymphoid + total marrow irradiation:
Haplo-HSCT with Treg and Tcon immunotherapy
made feasible for elderly and/or unfit patients with high risk AL

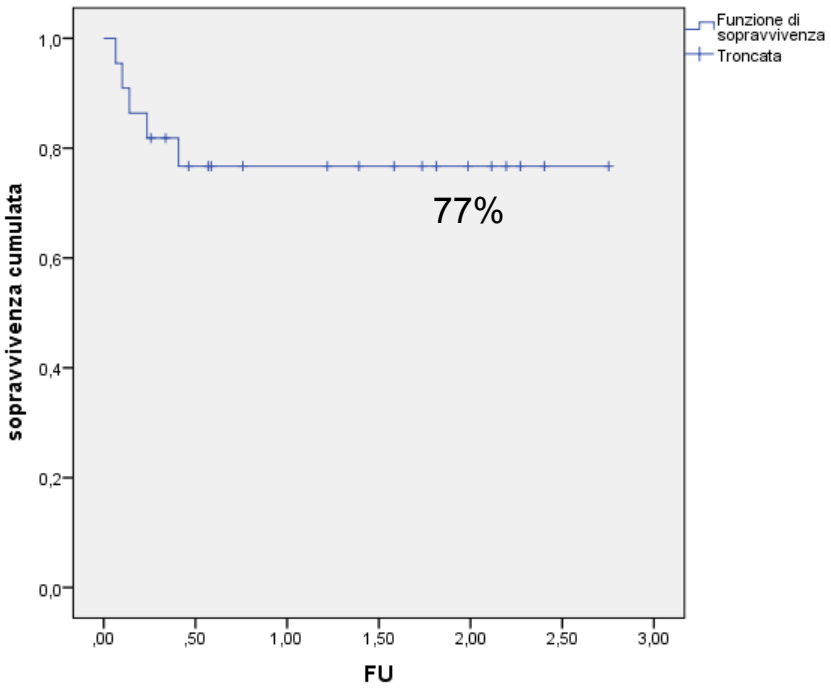


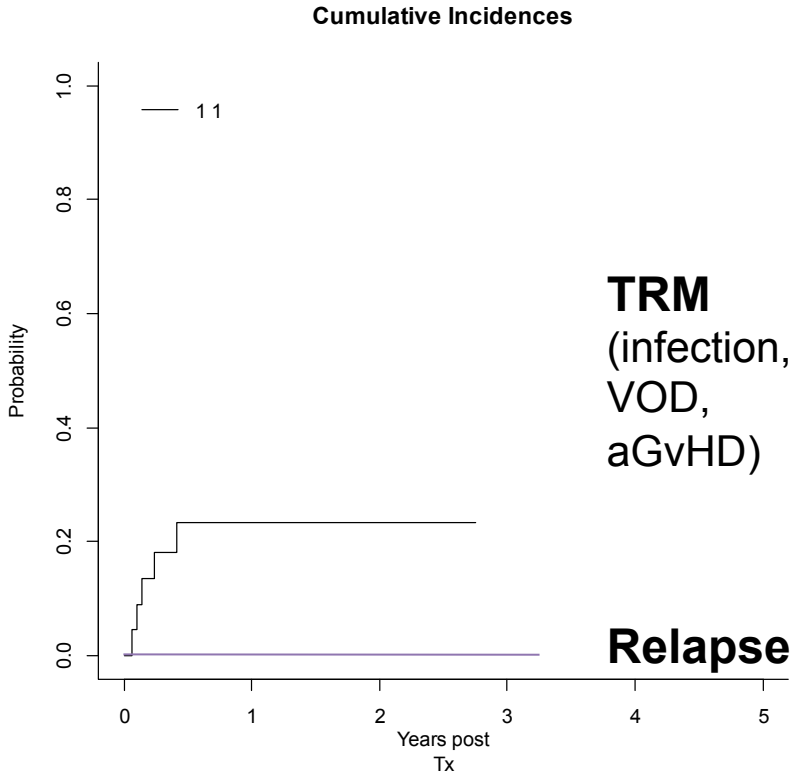
August 2015 →
(29 patients)

Helical thomo therapy (Cynthia Aristei, Radiation Oncology)
Total Lymphoid Irradiation (11,7 Gy in 9 fraction)
Total Marrow Irradiation (13,5 Gy in 9 fraction)

Thiotepa (5mg/kg per day for 2 days)
Fludarabine (30 mg/m2 per day for 5 days)
Cyclophosphamide (15 mg/kg per day for 2 days)

cGvHD/Leukemia-free Survival





TRM (infection, VOD, aGvHD)

Relapse

T-cell replete HSCT: Relapse and DFS in high-risk AL patients

		Relapse (%)	DFS
Gupta et al *Blood 2010* **CR1 AML with unfav cytogen**	MSD	**37**	42
	MUD	**40**	34
Bashey et al *J Clin Oncol 2013*	MSD	**34**	52
	MUD	**34**	53
	HAPLO T-replete cyclo post HSCT	**33**	60
Scaradavou et al *Blood 2013*	Double UCB	**36**	32
	Single UCB	**32**	32
Di Bartolomeo et al *Blood. 2013*	HAPLO T-replete GCSF primed BM	**26 (28)** ✦	44 (30) ✦

Conclusions

Ex vivo-manipulated (T cell-depleted) HLA haplo-identical HSCT (with no post-transplant immune suppression):

1. **An opportunity to exploit the megadose stem cell engraftment effect to allow transplantation across HLA barriers w/o GvHD**
2. **An opportunity to uncover Graft vs Leukaemia Natural Killer cell alloreactivity**
3. **An opportunity to transfer Graft vs Leukemia conventional T cells w/o GvHD ("Treg/Tcon haplo trial")**
4. **An opportunity to uncover the "mother donor effect".**

Transplantation Program

Adults Unit

Alessandra Carotti
Adelmo Terenzi
Rita Felicini
Antonio Pierini
Lucia Amico
Luca De Carolis
Genni Casarola
Sara Tricarico
Samuele Bagnasco
Samanta Bonato
Matteo Paradiso

Graft processing

Franca Falzetti
Roberta Iacucci
Tiziana Zei

Data management

Mara Merluzzi

Transplantation Immunology

Loredana Ruggeri
Antonella Mancusi
Antonio Pierini
Elena Urbani
Sara Ciardelli
Sara Piccinelli
Maria Speranza Massei

Pediatrics Unit

Maurizio Caniglia

University of L'Aquila

Mauro Di Ianni

University of Parma

Franco Aversa

Professor Emeritus Univ. of Perugia

Massimo F Martelli

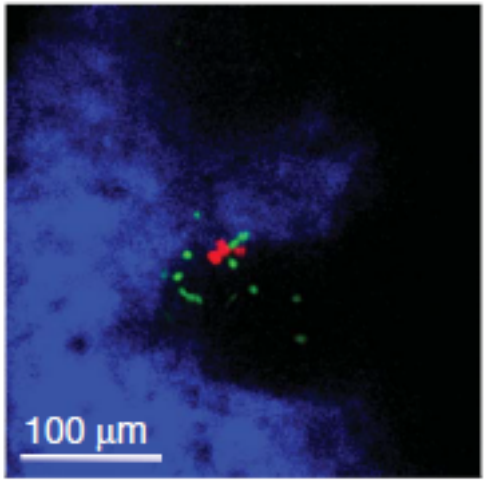
AIRC

The Leukemia & Lymphoma Society
Fighting Blood Cancers

Regulatory T cell functions

- Treg infusion prevents GVHD in animal models and clinical trials *(Edinger et al, Nat Med 2003; Di Ianni et al, Blood 2011; Brunstein et al, Blood 2011; Martelli et al, Blood 2014)*

- Treg protect allogeneic hematopoietic stem cells from in vivo killing *(Fujisaki et al, Nature 2011)*

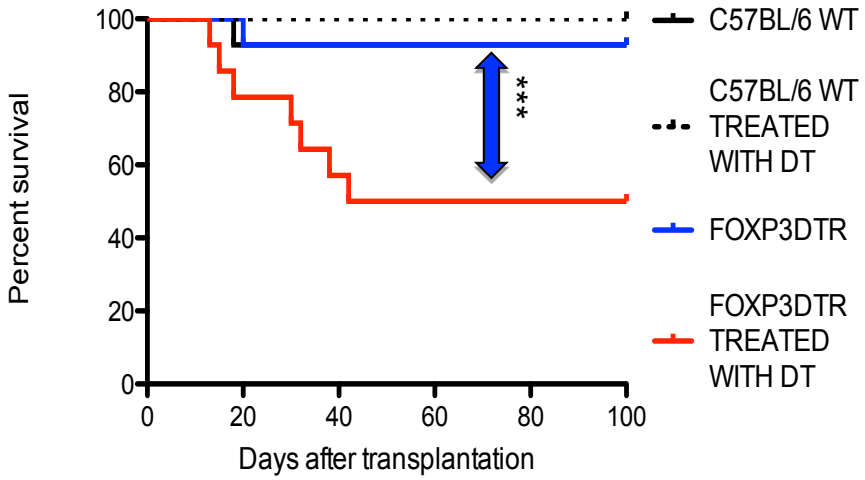


Red = HSCs
Green = Tregs

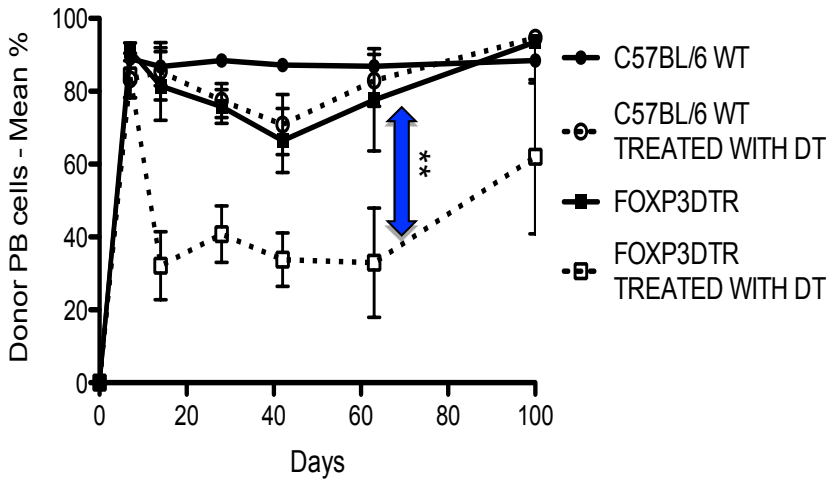
(Fujisaki et al, Nature 2011)

Treg depletion causes rejection of allogeneic T depleted BMT

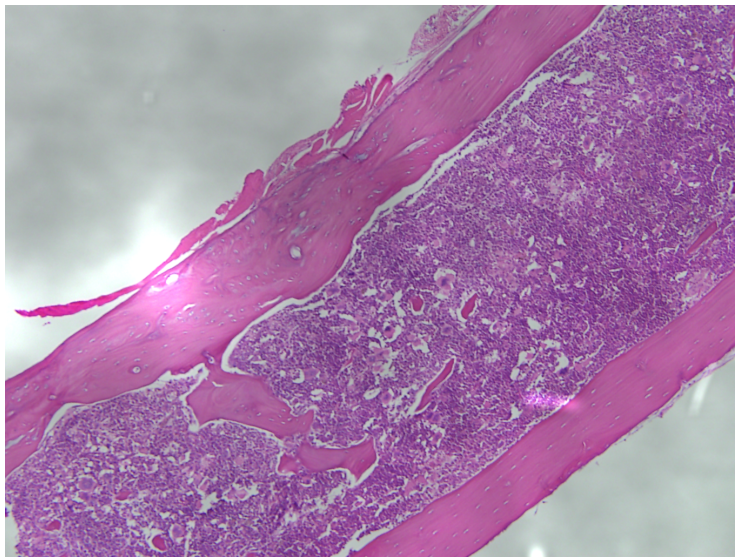
Mouse Survival



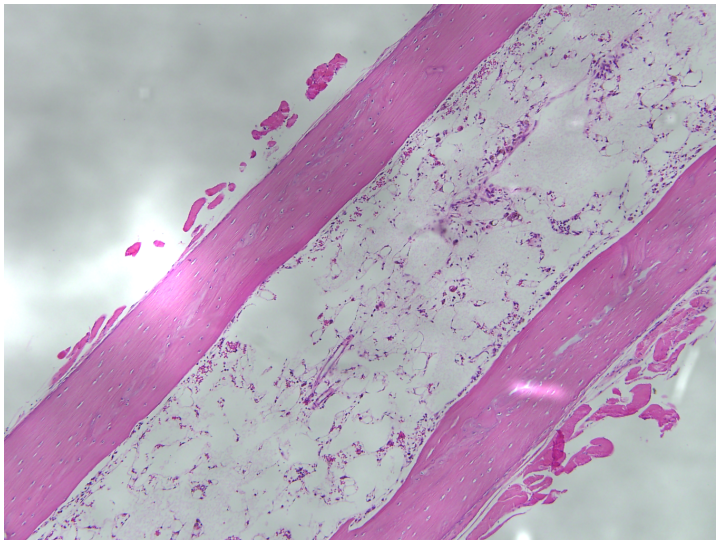
Peripheral Blood Chimerism



T depleted BM



T depleted BM + Treg Depletion

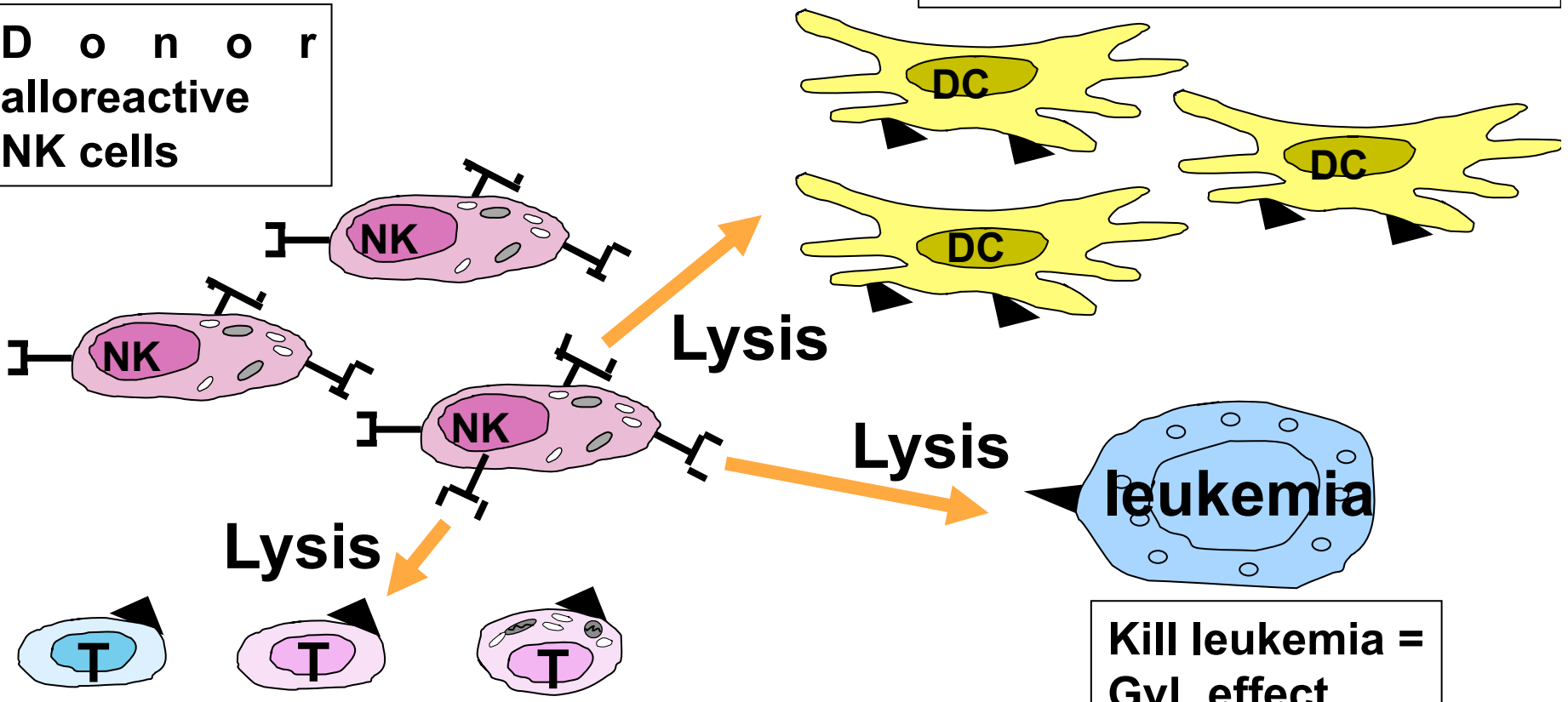


Pierini et al. *Nature Communications 2017*

Allo NK-based conditioning in murine transplant models: Ablation of recipient targets

Kill recipient APCs = protect from GvHD. T-replete mismatched BMT with 40 times the lethal dose of allogeneic T cells

D o n o r alloreactive NK cells



Kill leukemia = GvL effect

Kill recipient T cells = improve engraftment. Reduced-intensity BMT

Ruggeri et al. Science 2002

Issue Highlights:
NK Cells: Heroes in Bone Marrow Transplants

Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants

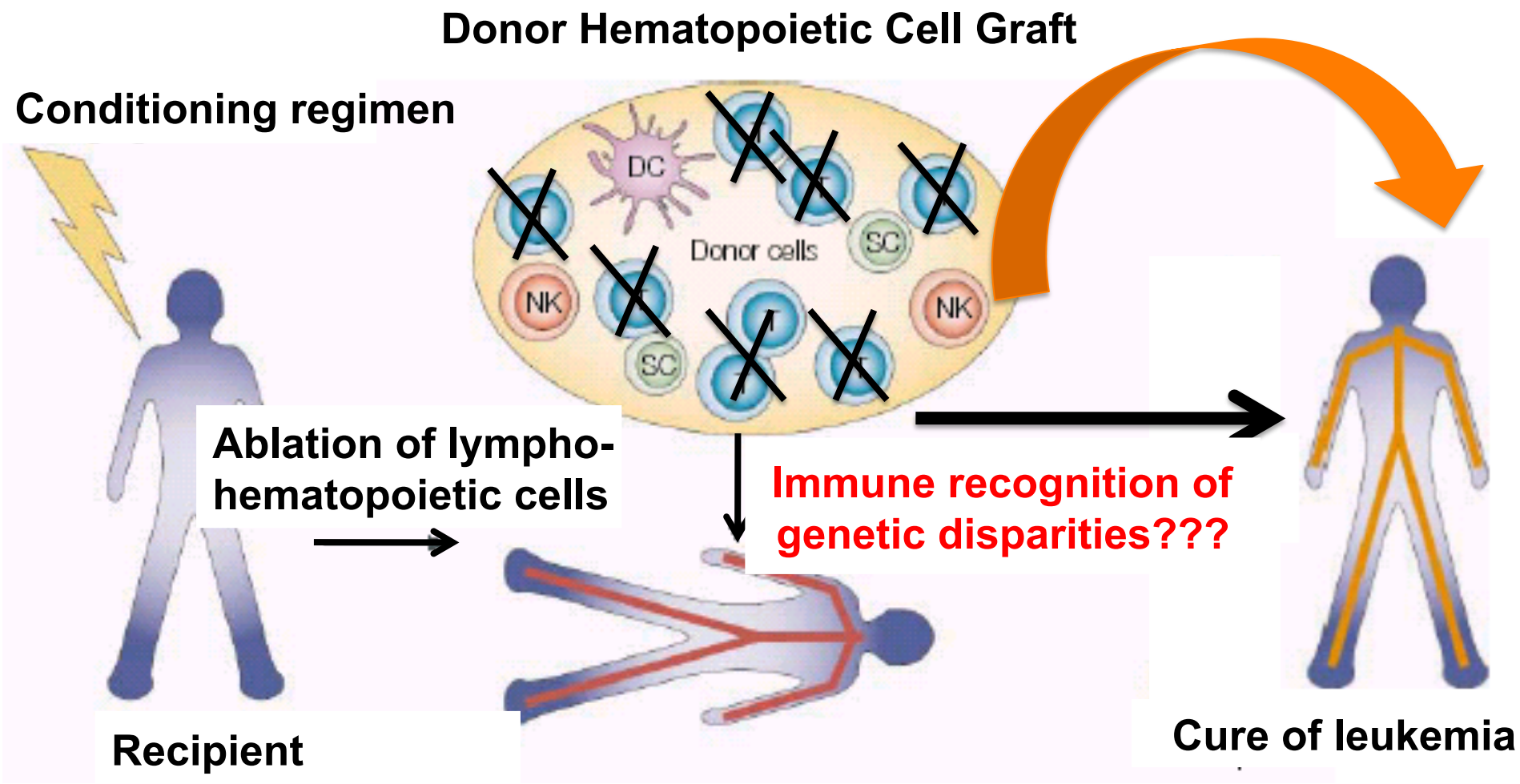
Loredana Ruggeri,[1] Marusca Capanni,[1] Elena Urbani,[1] Katia Perruccio,[1] Warren D. Shlomchik,[2] Antonella Tosti,[1] Sabrina Posati,[1] Daniela Rogaia,[1] Francesco Frassoni,[3] Franco Aversa,[1] Massimo F. Martelli,[1] Andrea Velardi[1]*

>2000 citations

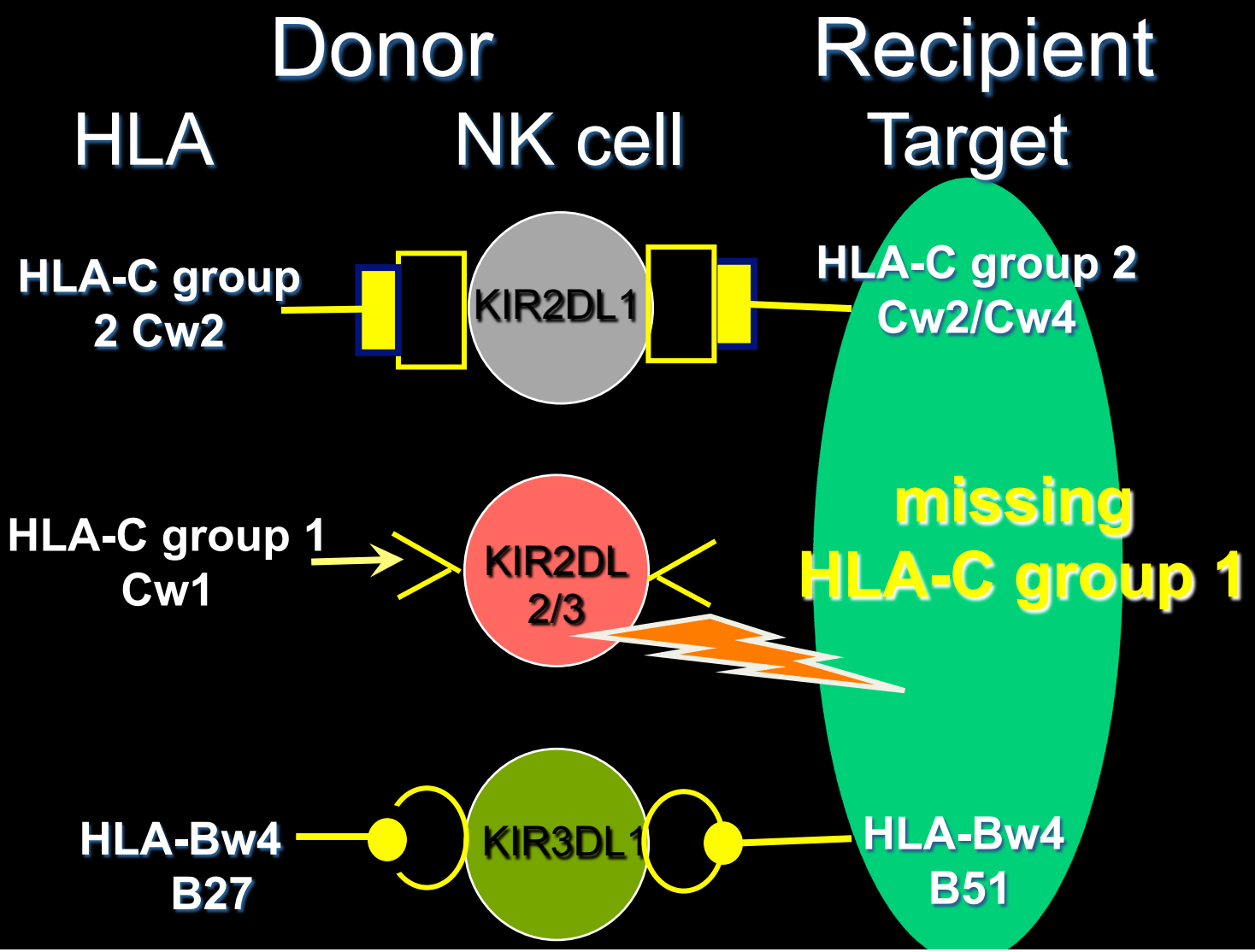
Editorial

A Perfect Mismatch

Klass Kärre (Karolinska Institutet)


Donor Hematopoietic Cell Graft
Conditioning regimen
DC
Donor cells
SC
NK
NK
SC
Ablation of lympho-hematopoietic cells
Immune recognition of genetic disparities???
Recipient
Cure of leukemia

Inhibitory KIR recognition of self HLA class I educates NK cells to become fully functional and alloreactive



THE JOURNAL OF IMMUNOLOGY

Determinants of Antileukemia Effects of Allogeneic NK Cells

Wing Leung, Rekha Iyengar, Victoria Turner, Peter Lang, Peter Bader, Paul Conn, Dietrich Niethammer and Rupert Handgretinger

J Immunol 2004; 172:644-650; ;
http://www.jimmunol.org/content/172/1/644

T cell depleted megadose stem cell transplant