3rd Cuneo City ImmunoTherapy Conference (CCITC)

Immunotherapy in Hematological Malignancies 2023

CUNEO
May 18-20, 2023
Spazio incontri Fondazione CRC

Organized by Prof. Massimo Massaia, SC Ematologia AO S.Croce e Carle, Cuneo, Italy
and Centro Interdipartimentale di Ricerca in Biologia Molecolare (CIRBM), Torino, Italy

DICHIARAZIONE

Relatore: ANDREA VELARDI

Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

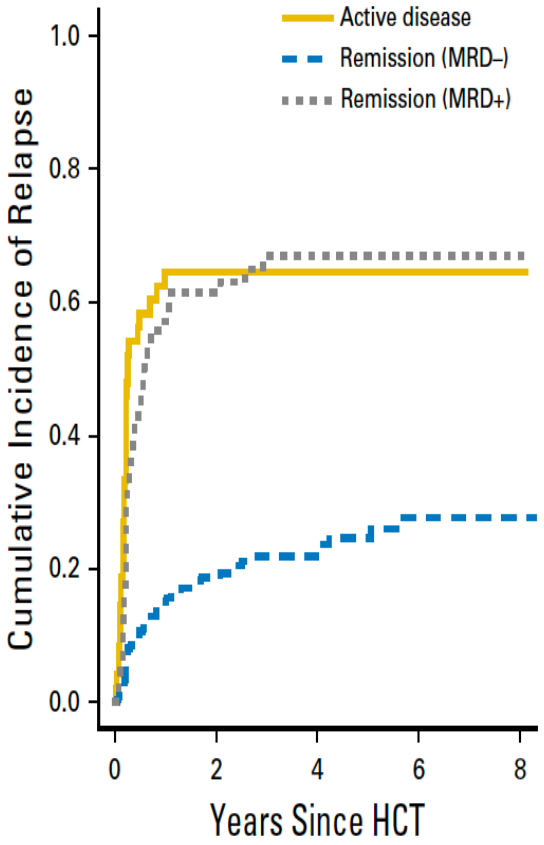
- Posizione di dipendente in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Consulenza ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Partecipazione ad Advisory Board **(NIENTE DA DICHIARARE)**
- Titolarità di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario **(NIENTE DA DICHIARARE)**
- Altro

How adoptively transferred components of the donor immune system improve outcome of HLA haploidentical transplant for acute leukemia

Andrea Velardi

Perugia Univ. Transplant Program

Disease relapse is the major cause of transplant failure in acute leukemia patients



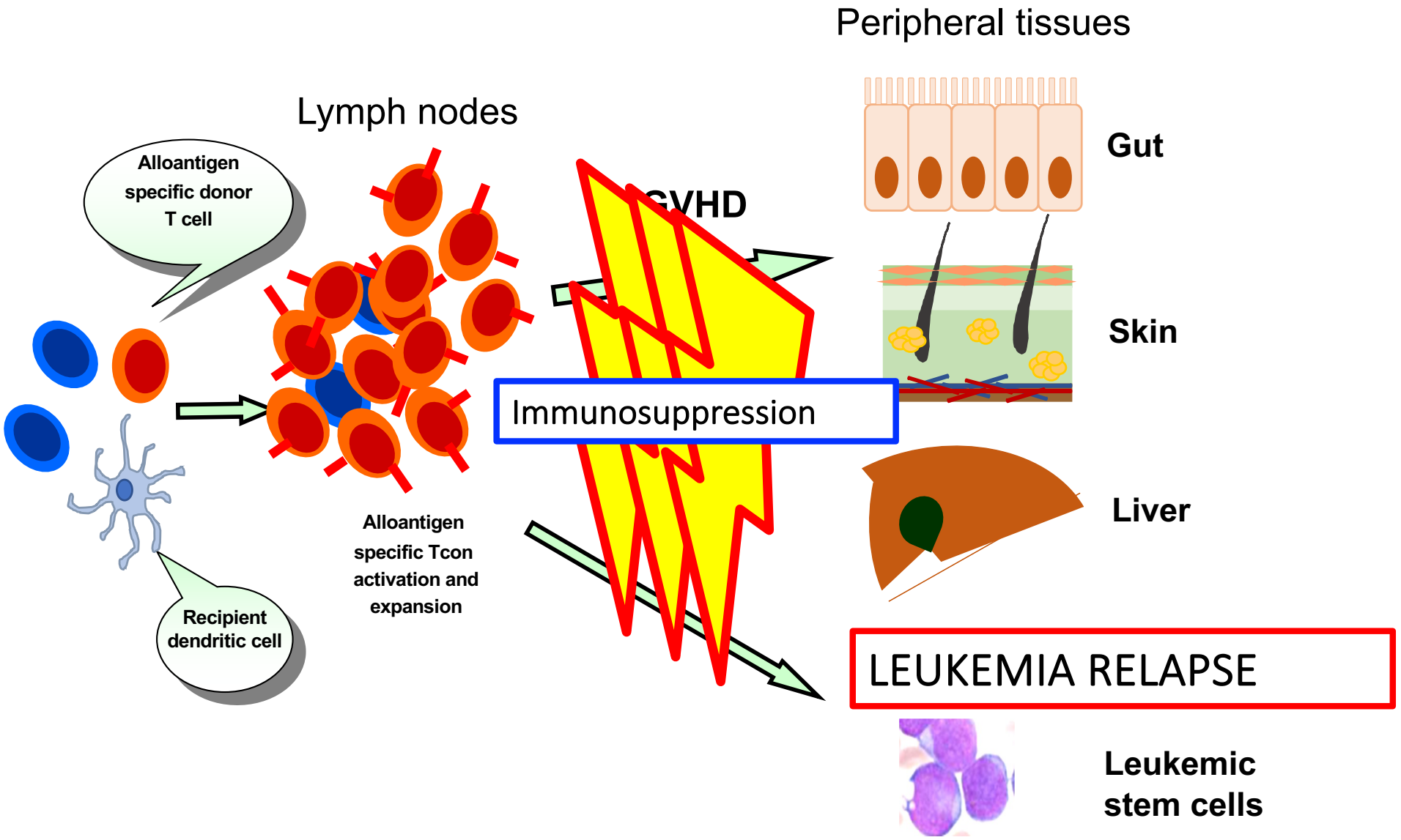
Araki et al. *JCO 2016*

	Donor	Relapse (%)	DFS (%)
Gupta et al *Blood 2010*	MSD	37	42
CR1 AML with unfav. cytogen.	MUD	40	34
Bashey et al *J Clin Oncol 2013*	MSD	34	52
	MUD	34	53
Lorentino et al *EBMT, Leukemia 2020*	MUD **PT-Cy**	28	56
Ciurea et al. *Blood 2015*	HAPLO **After RIC**	58	46
	After MA condition.	44	45
Piemontese et al. *EBMT, J Hem Onc 2019*	HAPLO **Mixed**	32	

Whatever the transplantation strategy and whoever the donor, all these diverse forms of HSCTs do not have a strong enough anti-leukemic effect.

Hypothesis:

post-transplant pharmacologic immune suppression that is required to help prevent/treat GvHD may reduce or abrogate the GvL effect



T cell depleted HLA-haploidentical HSCT
does not require post transplant pharmacologic immune suppression

A platform that allowed for the discovery of biology that helped **separate GvL from GvHD**

It unleashed safe anti-leukemia immunity, i.e., NK cell alloreactivity

It allowed for post-transplant immunotherapies, i.e., Treg/Tcon adoptive transfer

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]*

Editorial

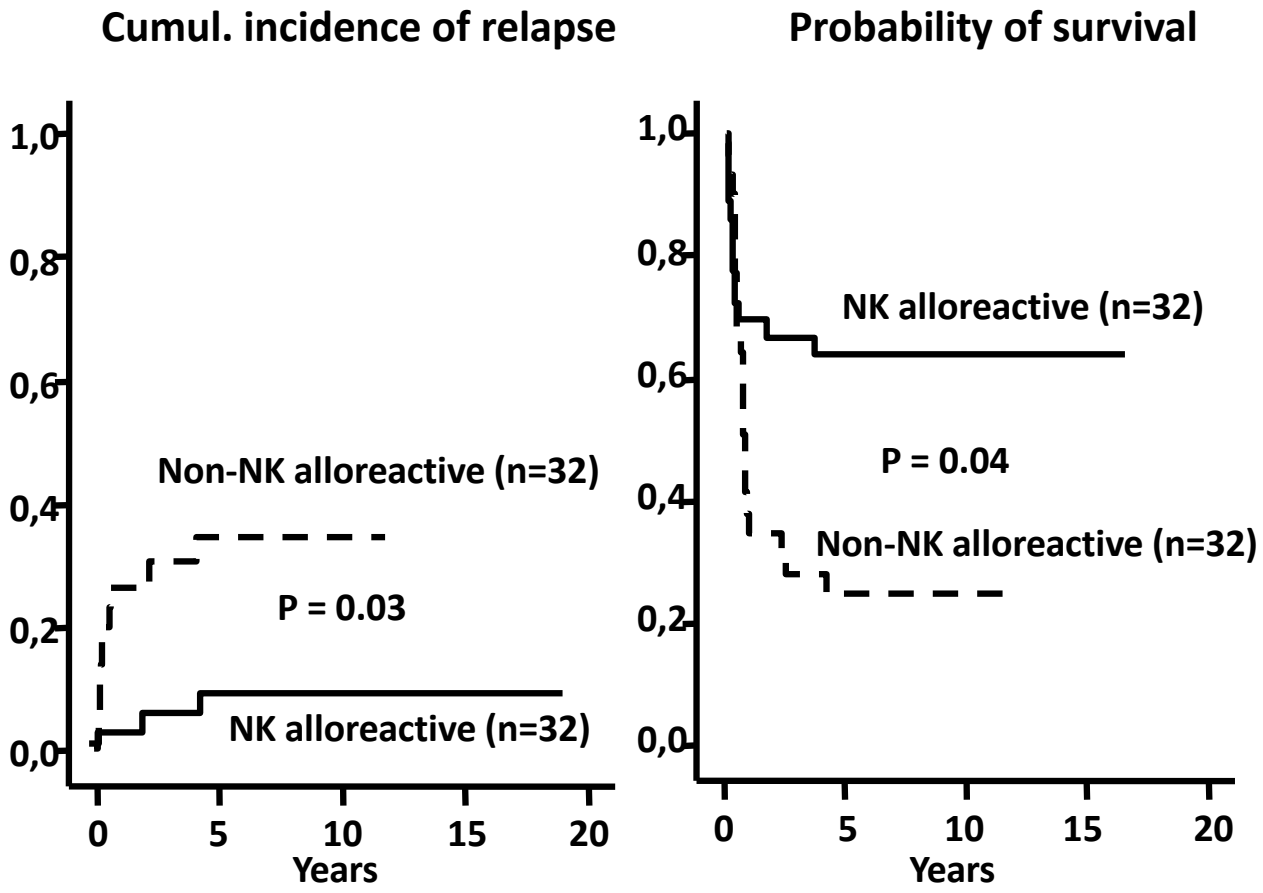
A Perfect Mismatch

Klass Kärre (Karolinska Institutet)

NK cell alloreactivity (~ 50% of haplo transplants)

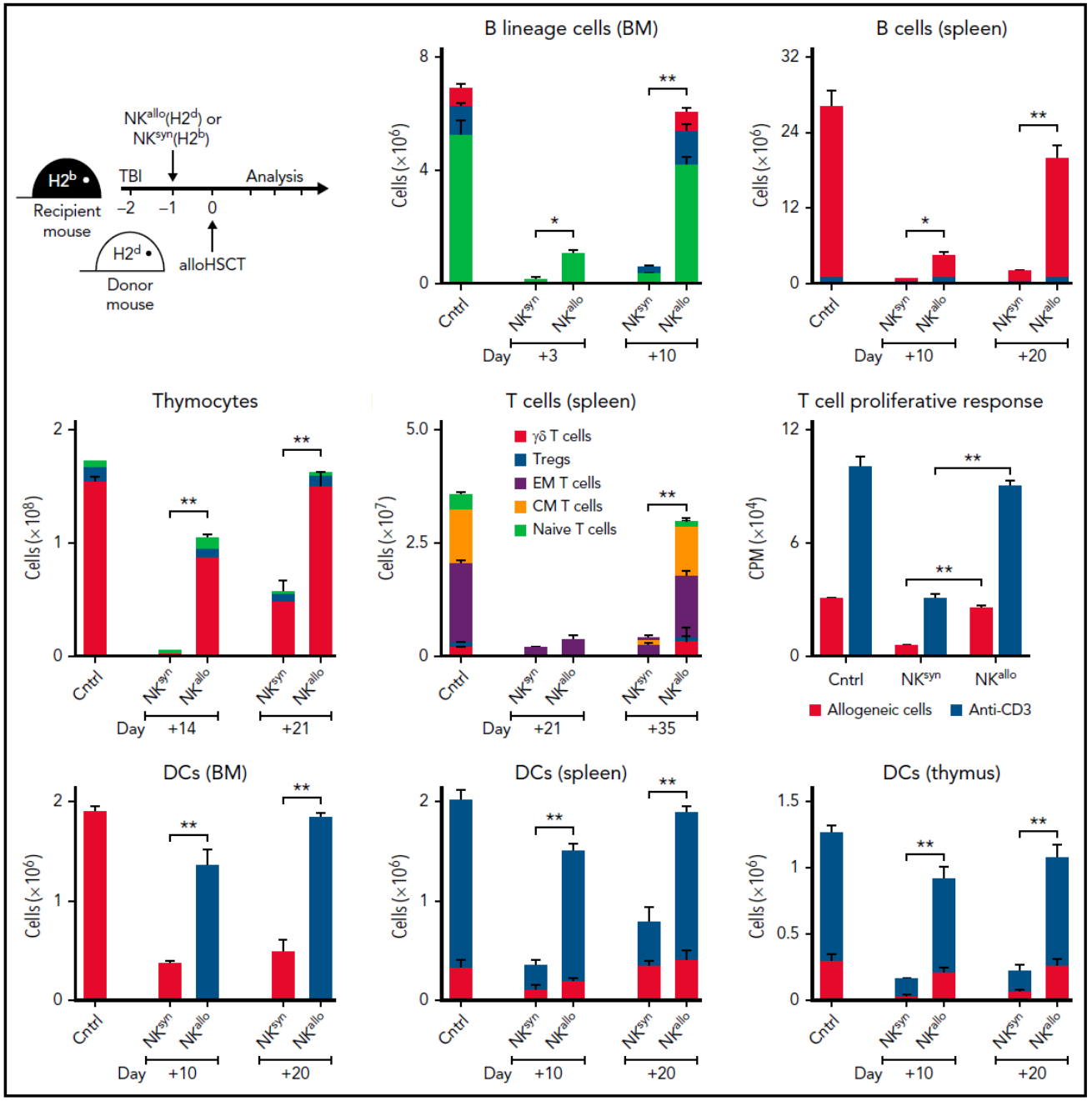
Absence of post-transplant immune suppression unleashes NK cell killing of leukemia with no attack on tissues

Relapse and survival of AML patients transplanted in any remission



Ruggeri Blood 1999; Science 2002; Blood 2007; Stern Blood 2008; Mancusi Blood 2015 and Blood 2017; updated

Unexpected fate of DONOR immune cells that develop in NK conditioned mice after BMT



December 1, 2022

This Week in Blood:

Editorial:
Donor NK cells facilitate thymopoiesis in allo-BMT
Edmund K Waller
Emory Univ

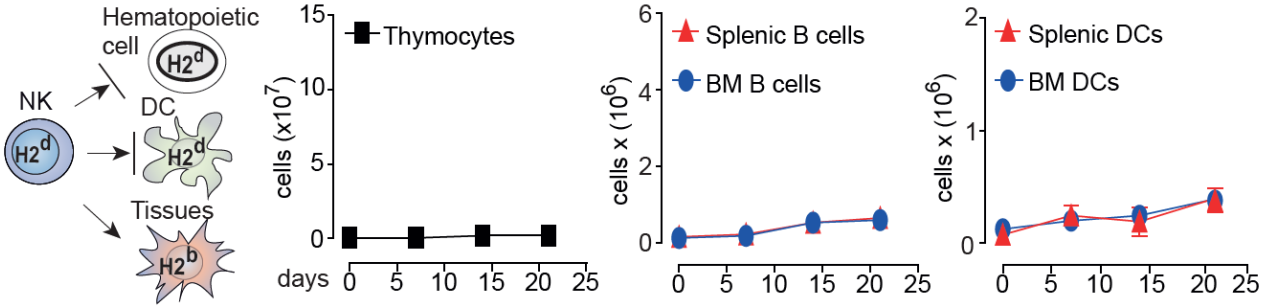
blood® Regular Article

IMMUNOBIOLOGY AND IMMUNOTHERAPY

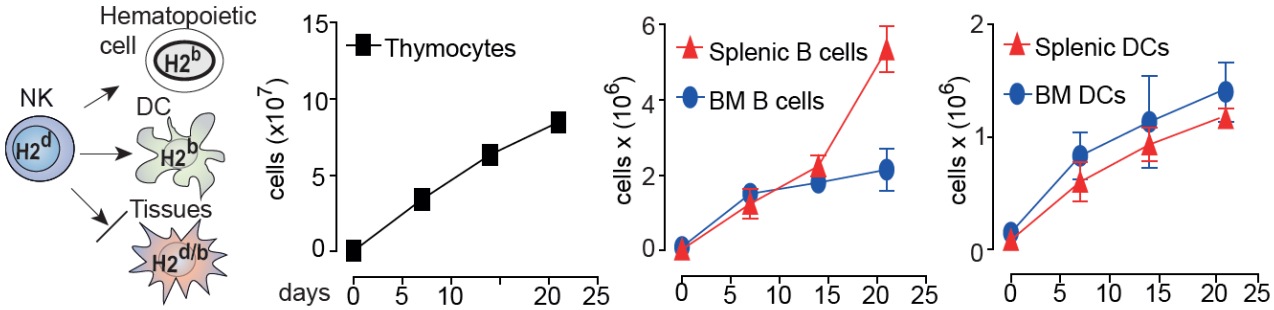
Donor natural killer cells trigger production of β-2-microglobulin to enhance post–bone marrow transplant immunity

Loredana Ruggeri,[1] Elena Urbani,[2] Davide Chiasserini,[3] Federica Susta,[3] Pier Luigi Orvietani,[3] Emanuela Burchielli,[2] Sara Ciardelli,[1] Rosaria Sola,[1] Stefano Bruscoli,[4] Antonella Cardinale,[5] Antonio Pierini,[2] Sander R. Piersma,[6] Stefano Pasquino,[7] Franco Locatelli,[5,8] Dunia Ramarli,[9] Enrico Velardi,[5] Luciano Binaglia,[2] Connie R. Jimenez,[6] Georg A. Holländer,[10-12] and Andrea Velardi[2]

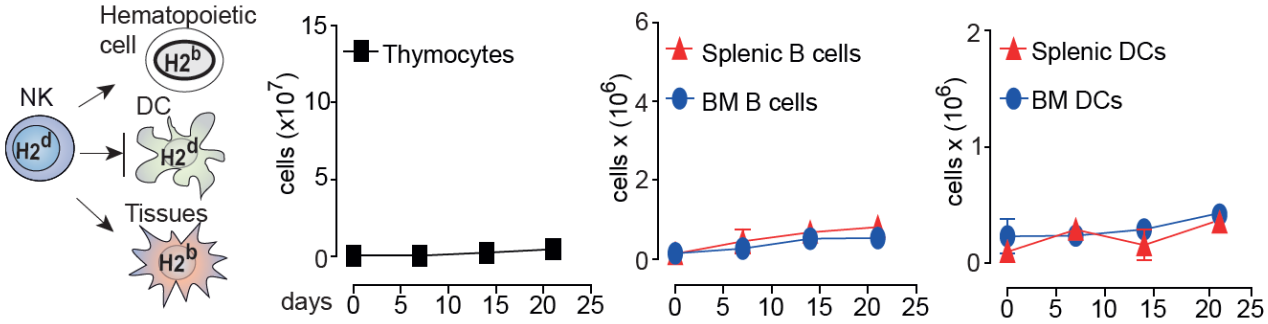
Chimera 1: NK-susceptible non-hematopoietic tissues and NK-resistant hematopoietic cells



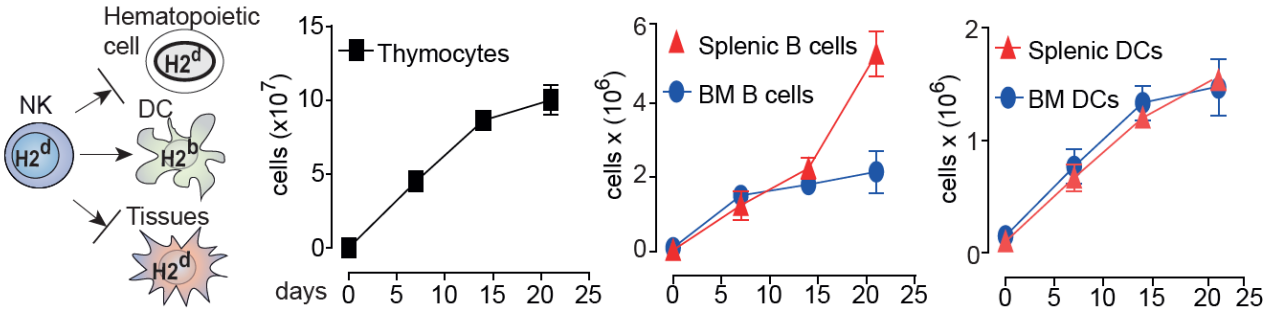
Chimera 2: NK-resistant non-hematopoietic tissues and NK-susceptible hematopoietic cells



Chimera 3: NK-resistant DCs and NK-susceptible hematopoietic cells and non-hematopoietic tissues



Chimera 4: NK-susceptible DCs and NK-resistant hematopoietic cells and non-hematopoietic tissues

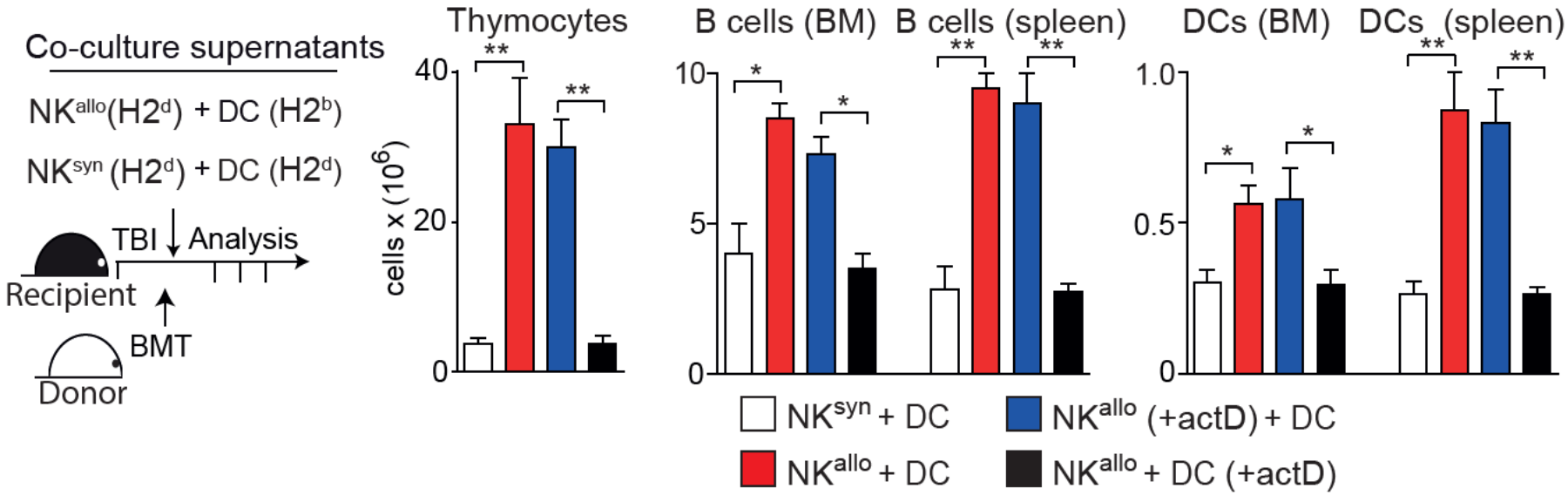


What triggers NK cells?

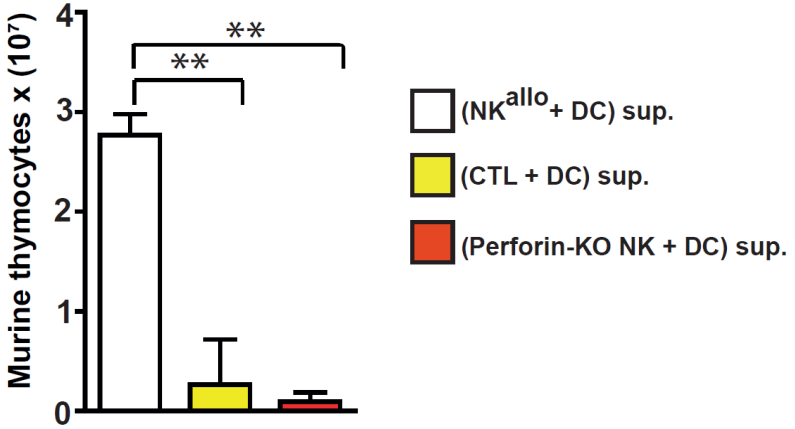
Donor alloreactive NK cells are specifically triggered by recipient DCs to promote accelerated post-BMT immune reconstitution

When attacked by alloreactive NK cells, DCs synthesize and release an “immune rebuilding factor”

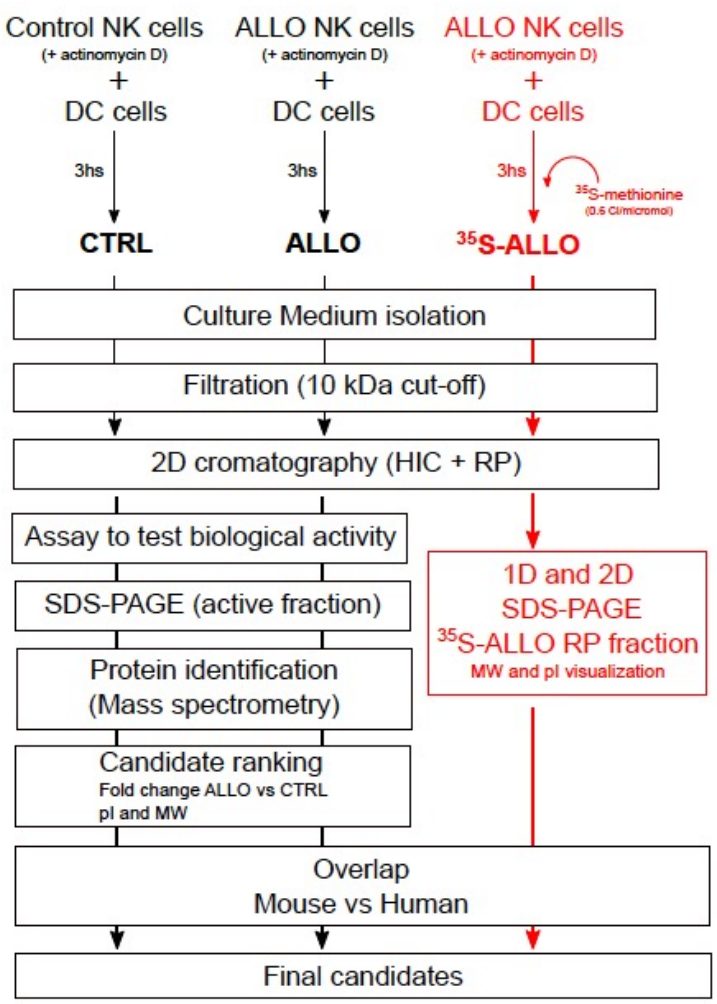
1) Also **supernatants** of alloreactive NK/DC co-cultures promote accelerated immune rebuilding
2) Supernatants in which **DNA transcription is blocked in DCs,** but not in NK cells, fail to accelerate post-BMT immune reconstitution.

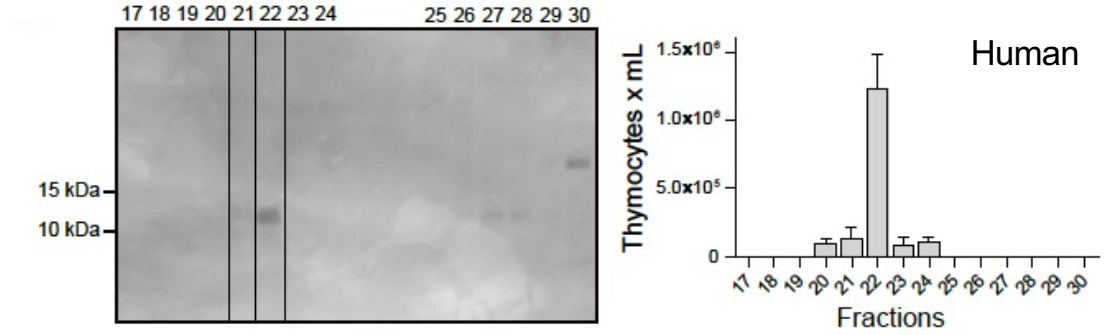


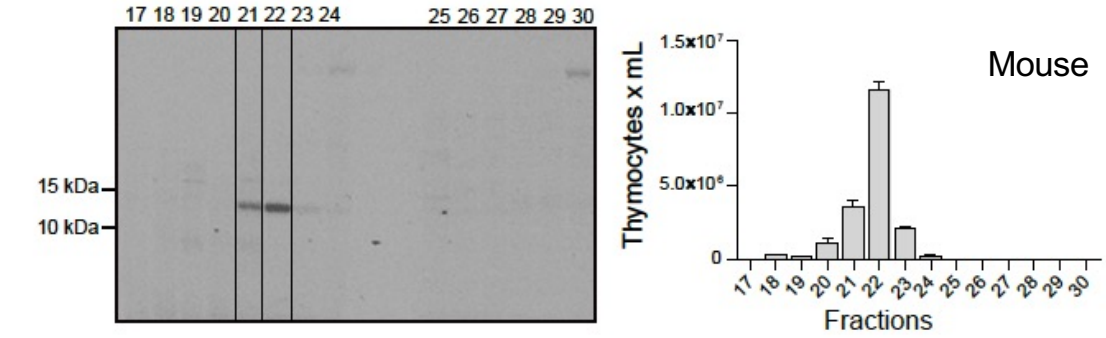
3) Perforin-KO NK cells to not promote immune rebuilding:
Killing of DCs allows release of the “immune rebuilding factor”

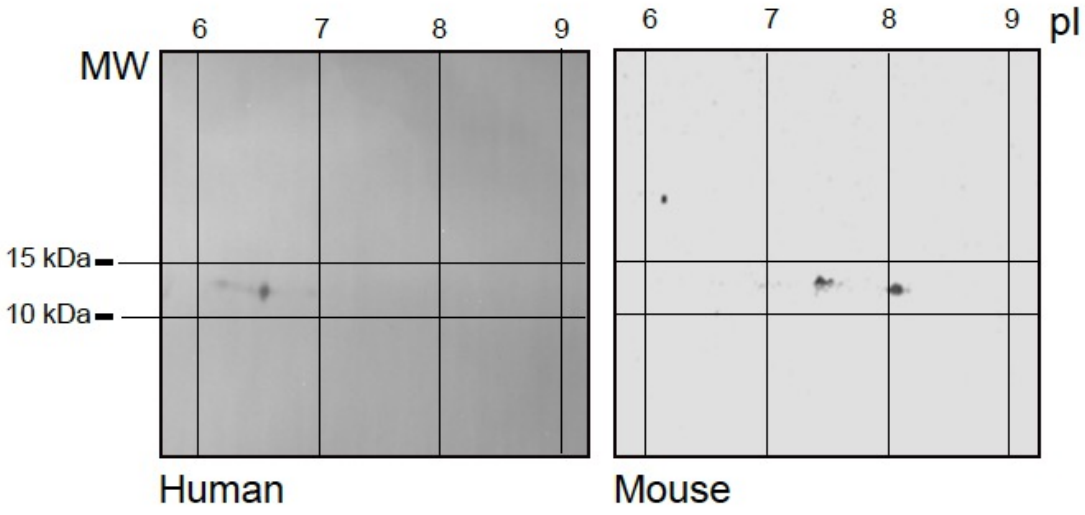


Chromatography fractionation of alloreactive NK/DC co-culture supernatants identified a protein with molecular weight and iso-electric points of B2M







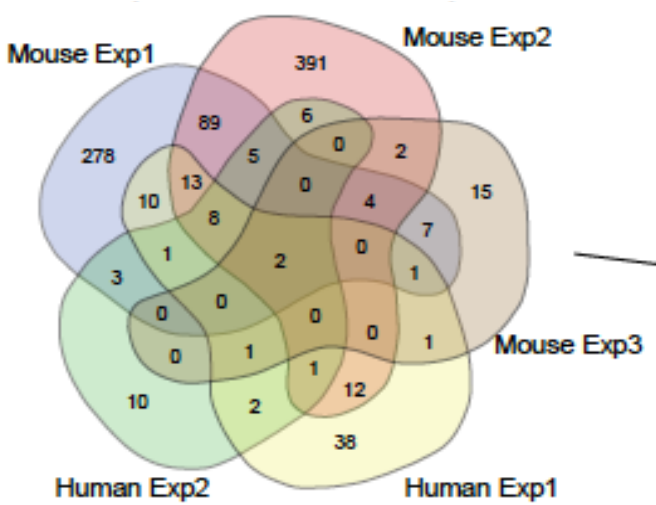


High-sensitivity nano liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis identified amino acid sequences specific of B2M (Proteomics Lab, VU University, Amsterdam)

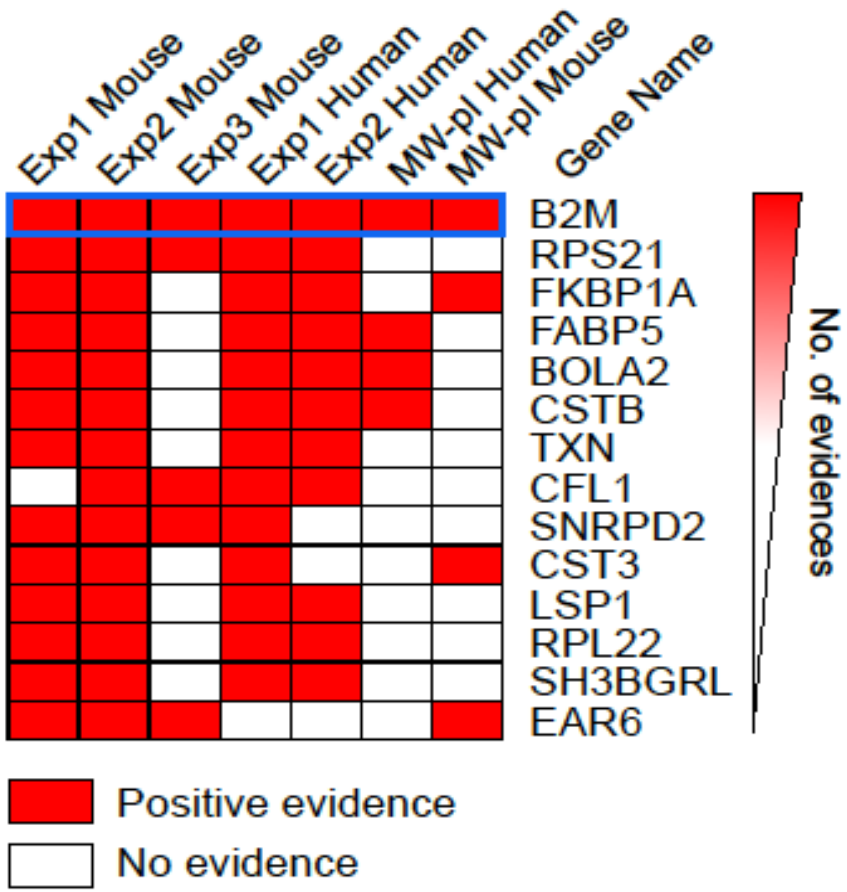
Ranking Criteria:

1) ≥1,5 fold increase in protein content
2) in all mouse and human experiments

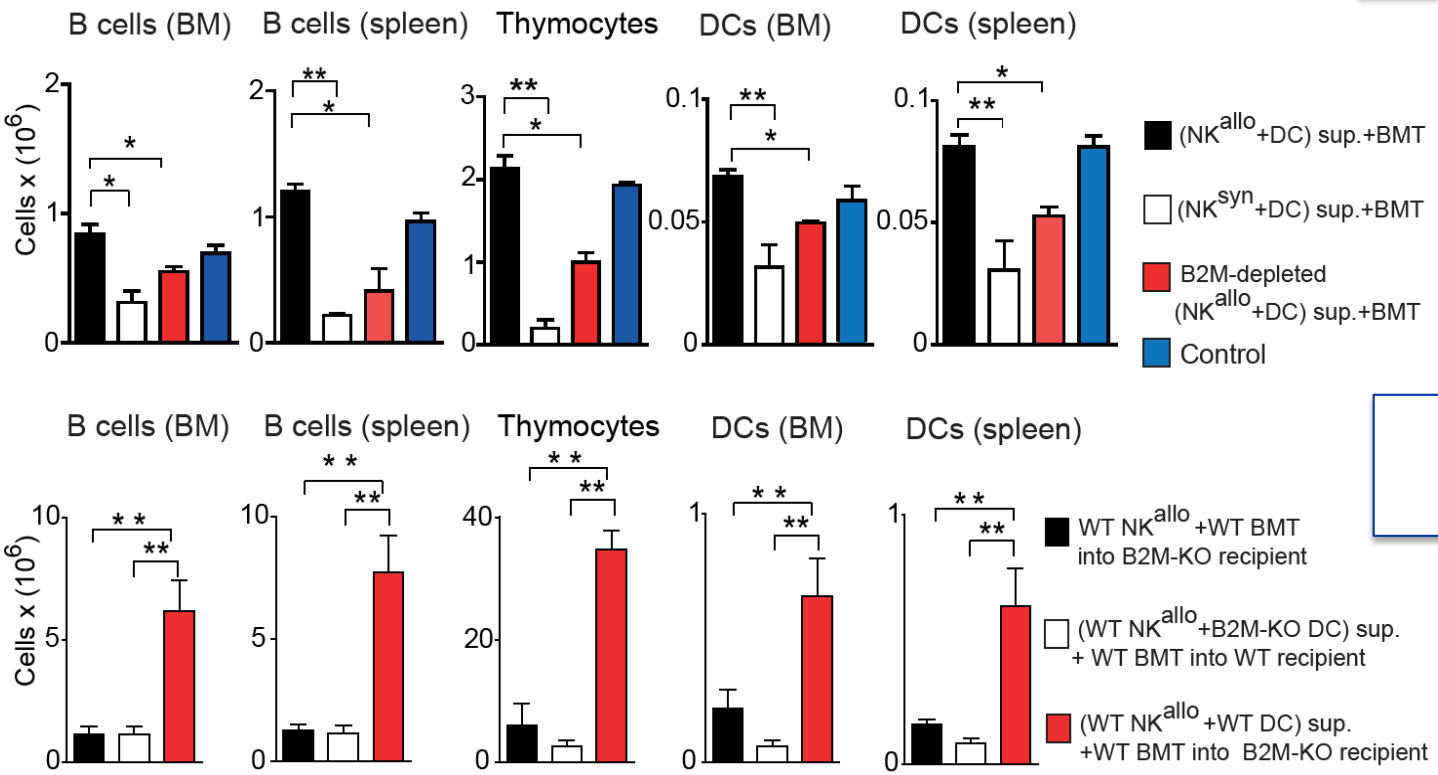
Only one, out of 853 proteins detected, i.e., B2M, fulfilled the 4 ranking criteria



3) Molecular weight specific of B2M
4) Iso-electric points specific of B2M



Functional and genetic evidence that B2M is the “immune rebuilding factor”



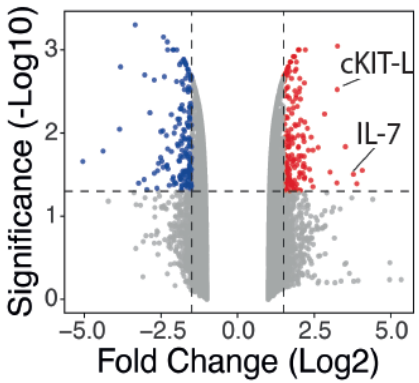
Anti-B2M antibody depletion of NK/DC co-culture supernatants (partially) abrogates the immune rebuilding effect

- B2M-KO mice conditioned with wild-type allo-NK cells are unable to undergo accelerated immune reconstitution
- and even wild-type mice infused with supernatants from co-cultures of wild-type allo-NK but B2M-KO DCs are unable to undergo accelerated immune reconstitution.

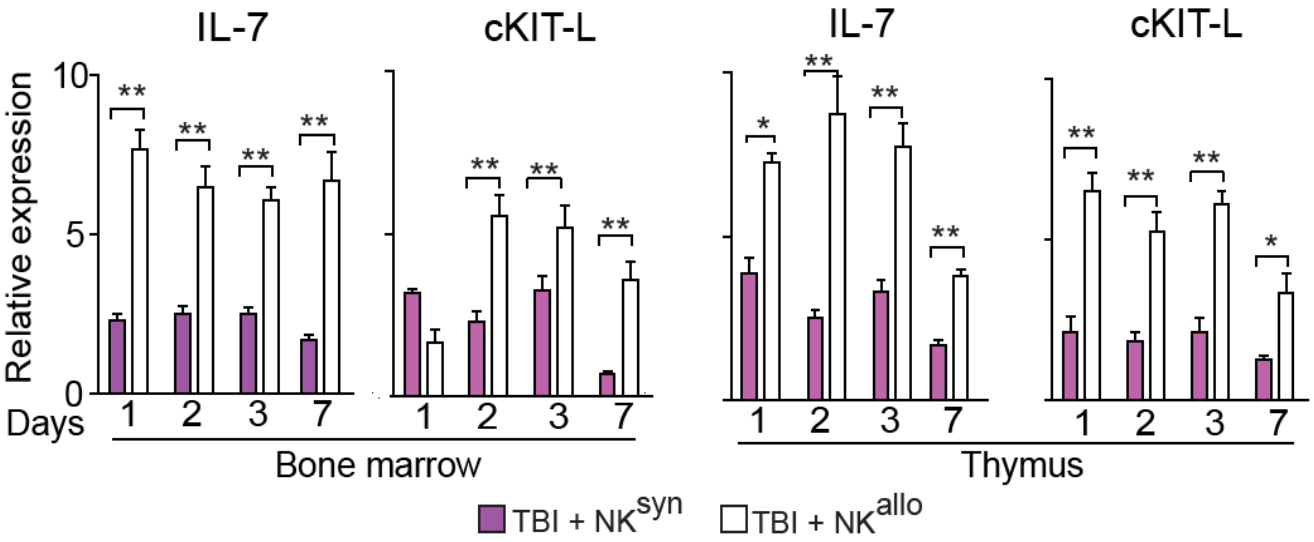
“Cure” of B2M-KO mice by infusing supernatants from co-cultures of wild-type allo-NK and wild-type DCs.

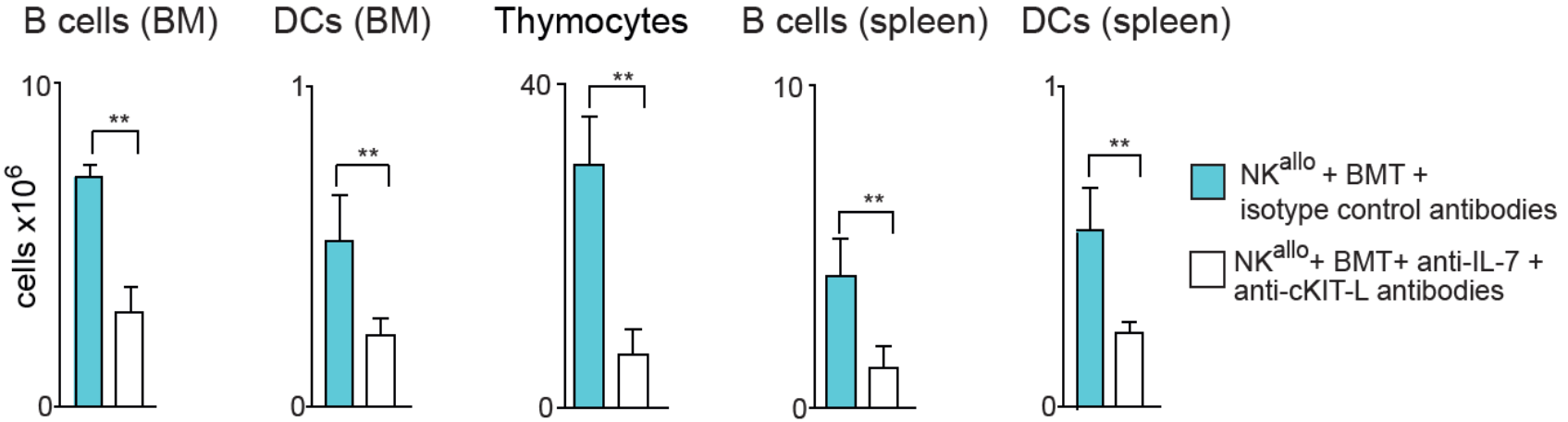
B2M triggers production of two master regulators of lymphocyte development, such as c-KIT ligand and IL-7

RNA-seq analysis

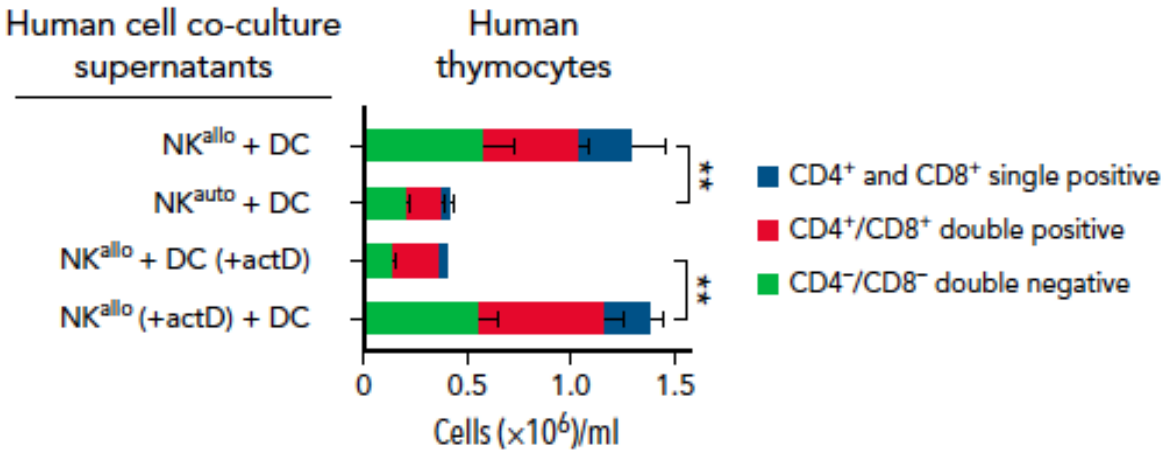


mRNA quantification by qPCR in NK-conditioned mice

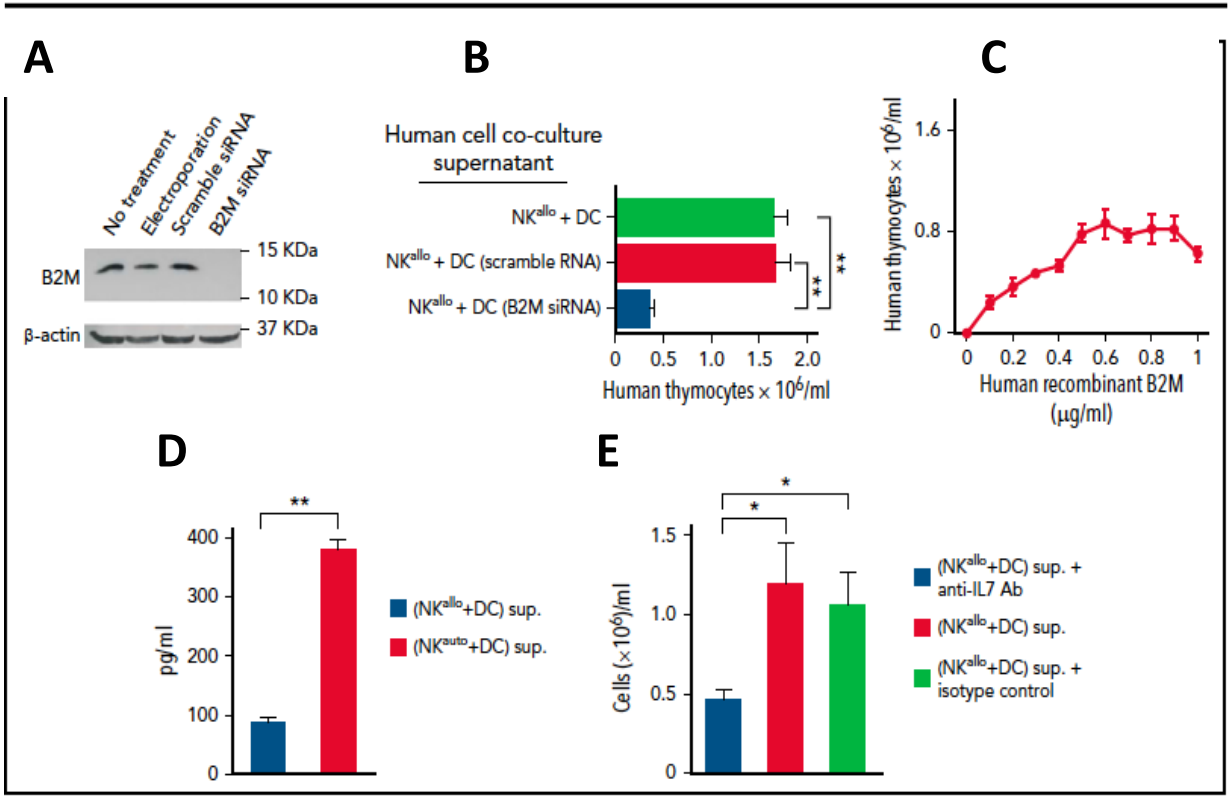




The human counterpart and its exact same cell dynamics



- In a thymocyte/TEC culture system, addition of human allo-NK/DC supernatants increases thymocyte counts.
- DNA transcription blockade in DCs, but not in NK cells, prevents the increase in human thymocyte counts.



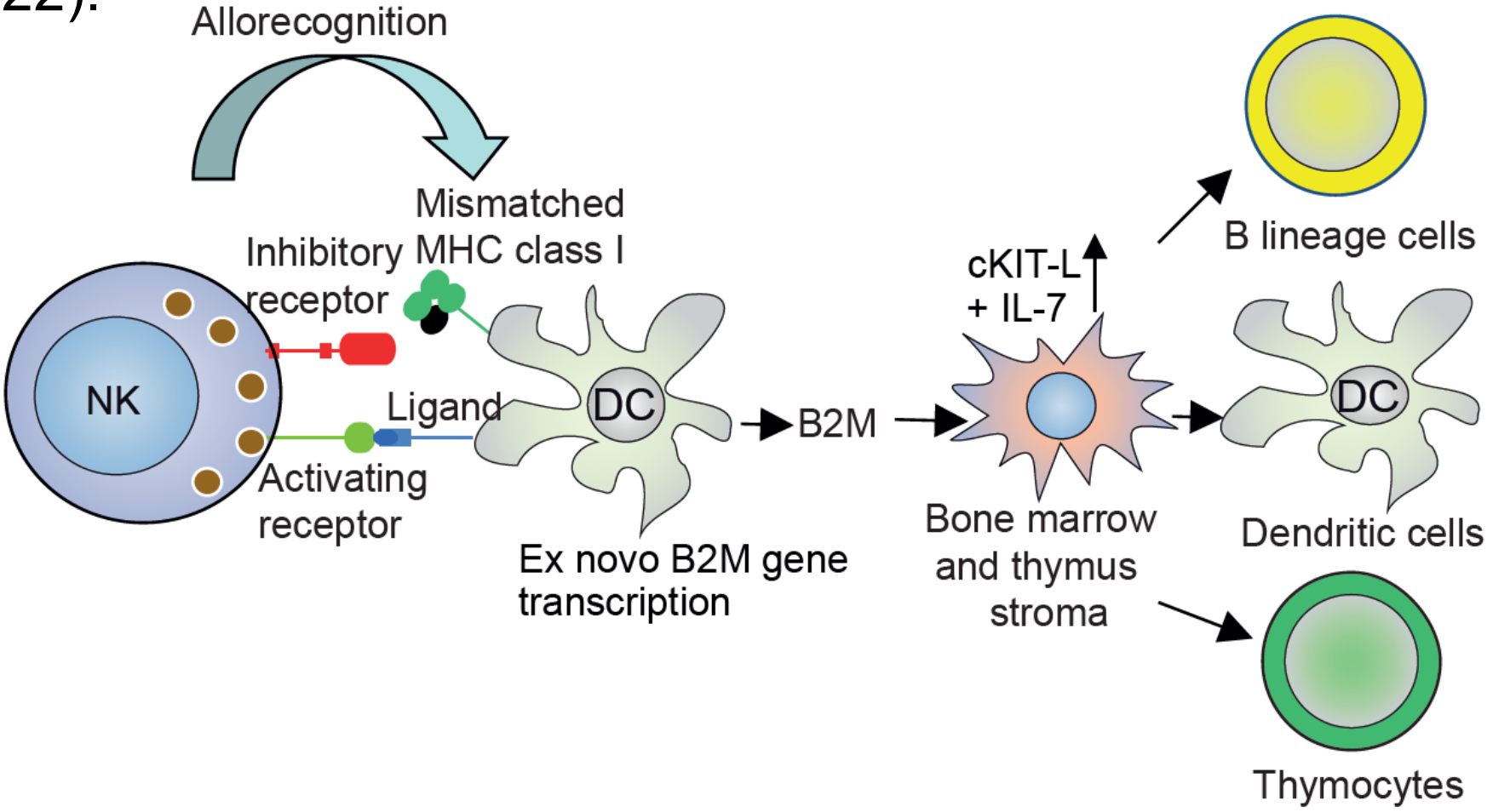
A + B: Silencing the B2M gene in DCs prevents the increase in thymocyte cellularity.

C: Human recombinant 14.0 kDa B2M precursor increases thymocyte counts

D + E: In a thymocyte/TEC culture system, allo-NK/DC co-culture supernatants promote IL-7 production by TECs which in turn supports thymocyte cellularity

Conclusion 1

In addition to eradicating high-risk myeloid leukemia and improving leukemia-free survival (2002), donor alloreactive NK cells (in pre-clinical mouse models and in human in vitro systems) trigger recipient DCs to synthesize and release beta-2-microglobulin that boosts post bone marrow transplant immunity (2022).



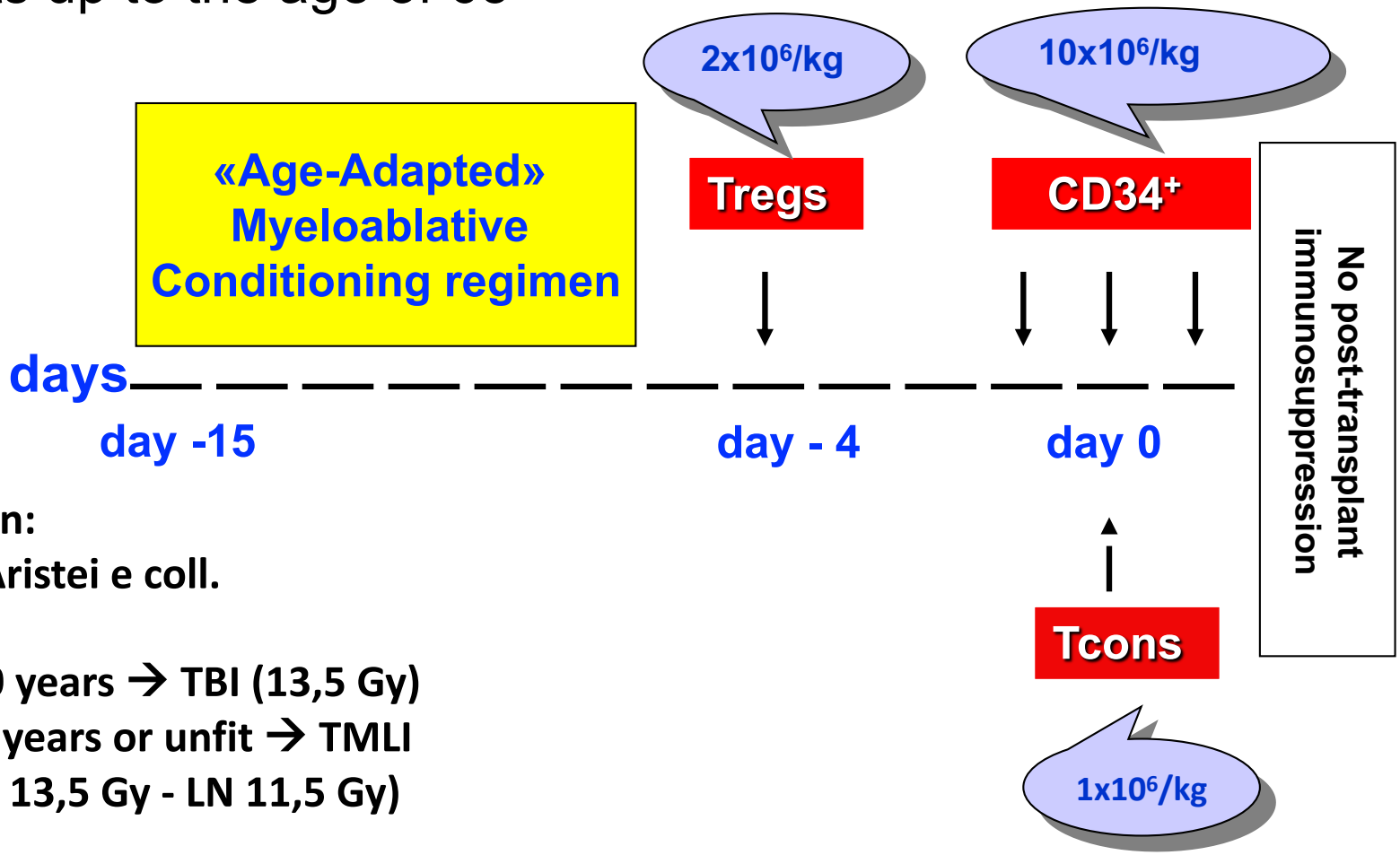
Limitations of T cell depletion + allo NK cell immunotherapy:

1) HLA class I allele (KIR ligand) mismatches that allow donor versus recipient NK cell alloreactivity are present in only up to 50% of transplants

1) NK cells are not effective in (B cell precursor) ALL

3) Infections (due to T cell depletion) result in 35-40% TRM

A myeloablative, irradiation-based, age-adaptable conditioning regimen followed by adoptive immunotherapy with regulatory and conventional T cells for haploidentical transplant in acute leukemia patients up to the age of 65

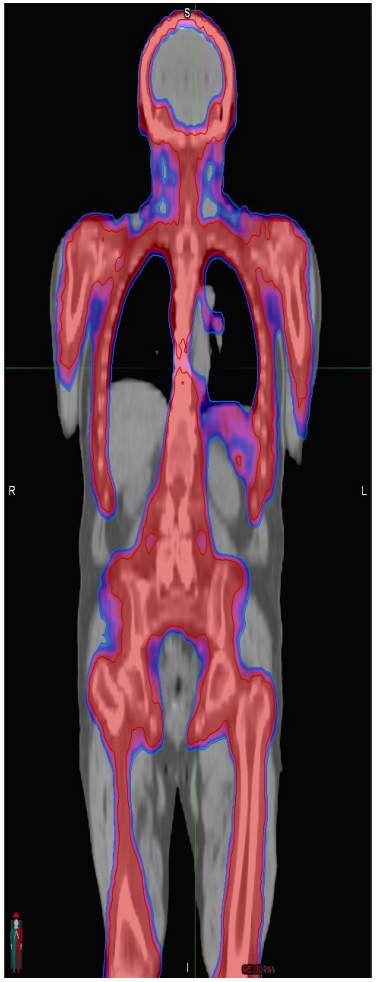


Irradiation:
Cynthia Aristei e coll.

- up to 50 years → TBI (13,5 Gy)
-50 to 65 years or unfit → TMLI
(marrow 13,5 Gy - LN 11,5 Gy)
+
Thiotepa (3,75mg/kg/day for 2 days)
Fludarabine (30 mg/m2/day for 5 days)
Cyclophosphamide (10-15 mg/kg/day for 2 days)

Pierini et al., Blood Advances 2021

Key achievements of TMLI technology in HSCT



- **Reduced toxicity to organs that are not site of disease**

  → **Unfit and older patients can SAFELY receive myeloablative HSCT**

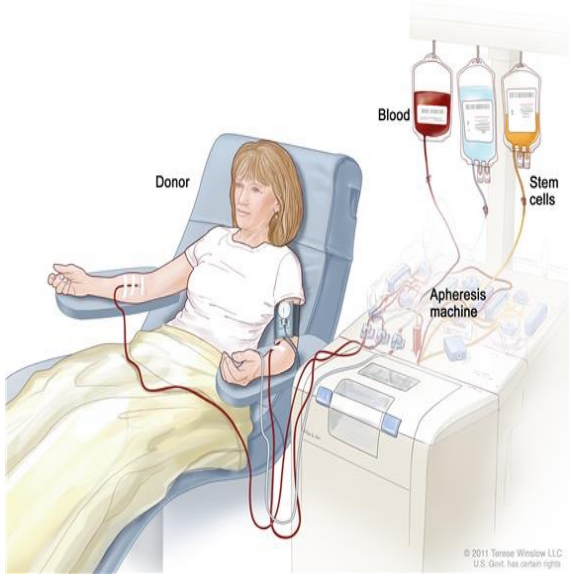
- **Possibility to boost areas of disease during conditioning**

  → **Increase antileukemic activity of the conditioning regimen**

City of Hope, seminal work by
Professor Jeffrey Wong
Professor Susanta Hui

Perugia Radiation Oncology
Professor Cynthia Aristei
Dr. Simonetta Saldi

Selection of peripheral blood CD4+/CD25+ regulatory T Cells



Fully automated immunomagnetic selection by commercially available kits and device

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

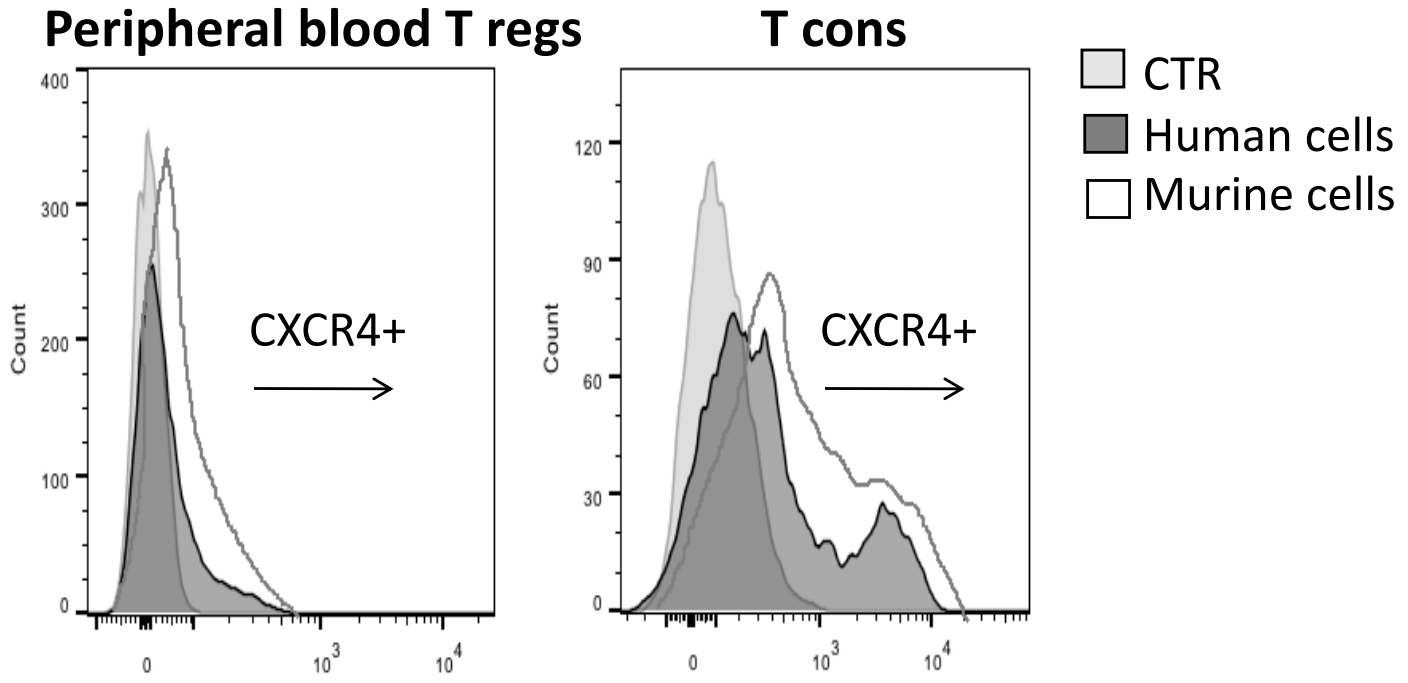
2nd step:
Selection of CD25+ cells

«Treg»

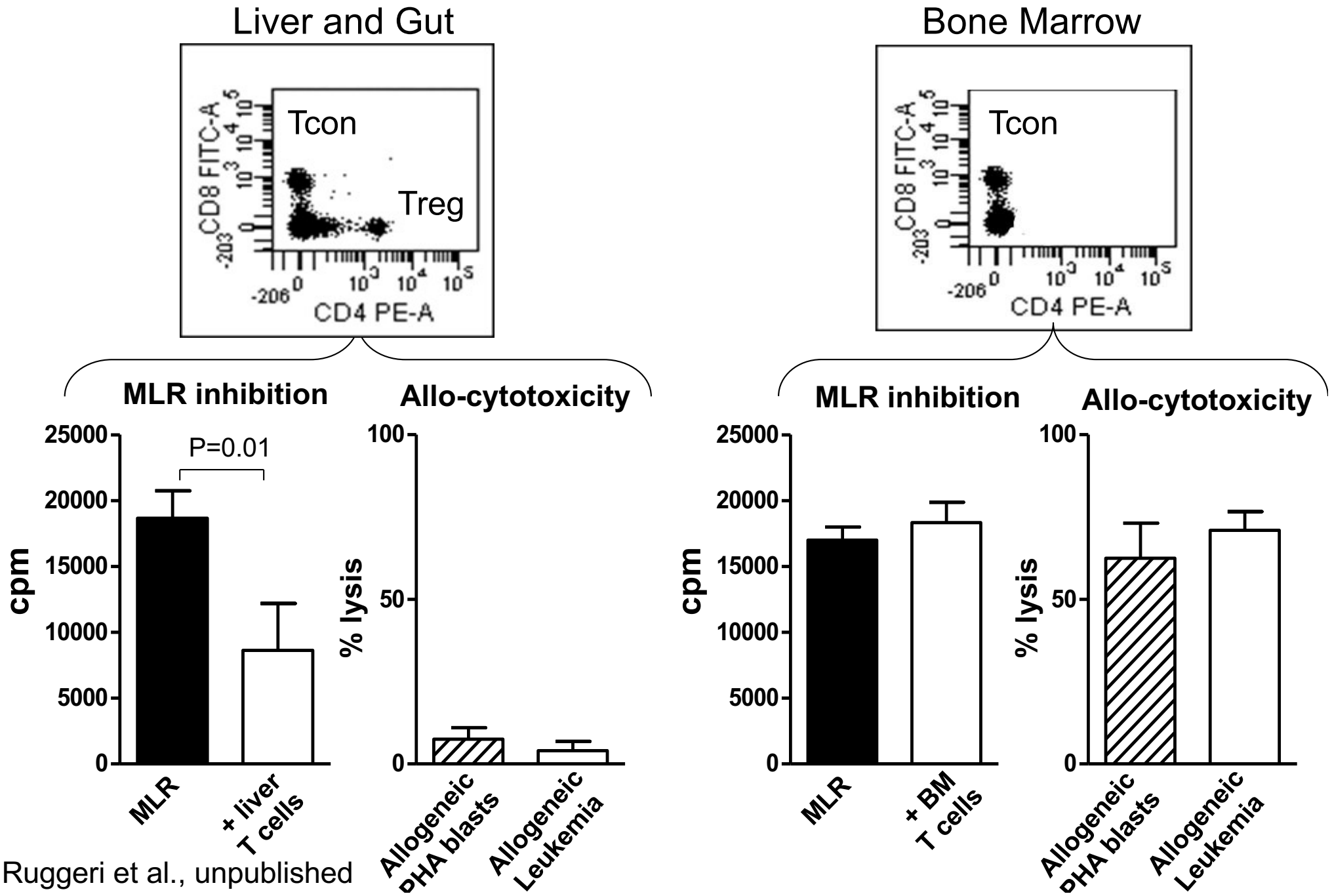
Final product

- **Cells ($x10^9$) = 280 (202- 390)**
- **CD4/CD25+ = 92% (90-97%)**
- **FOXP3+ cells = up to 90%**

Unlike T cons, human peripheral blood T regs are largely negative for CXCR4 bone marrow homing receptor

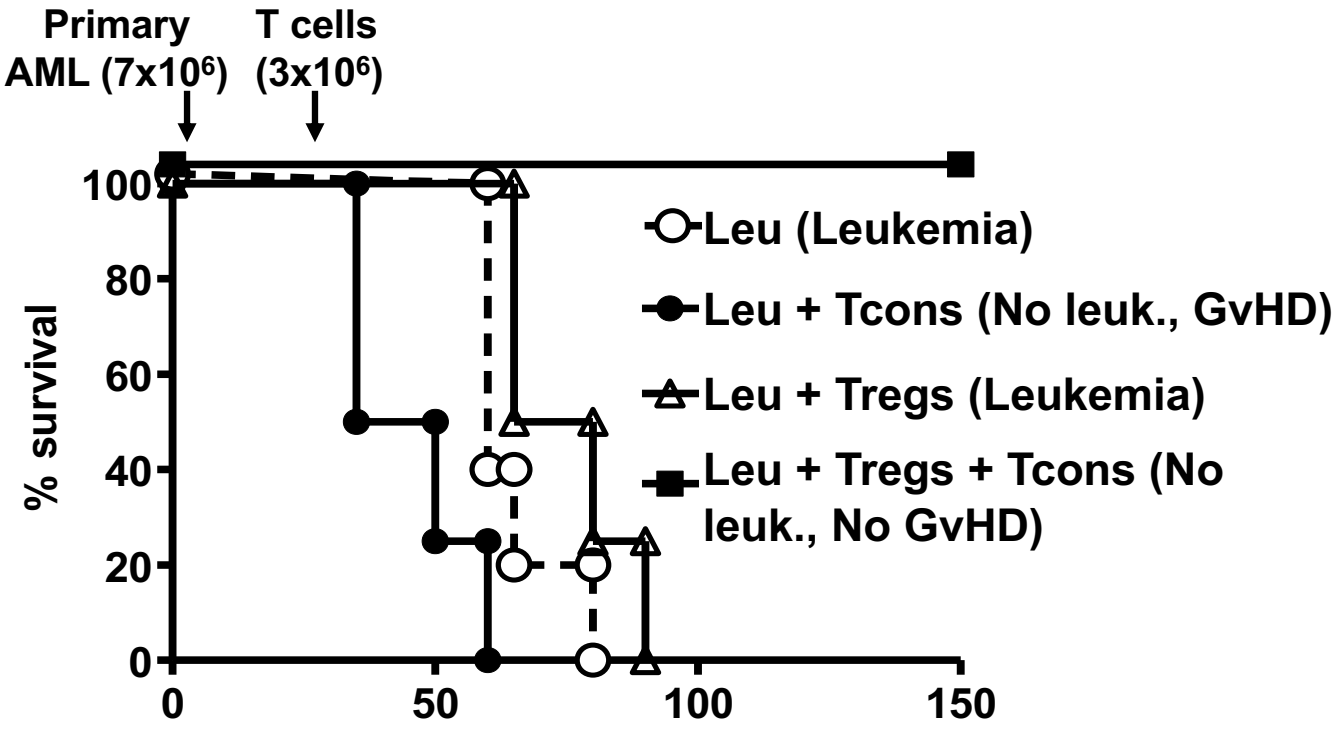


Differential homing and functional consequences of Treg/Tcon immunotherapy in immunodeficient mice



Ruggeri et al., unpublished

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



Ruggeri L., unpublished

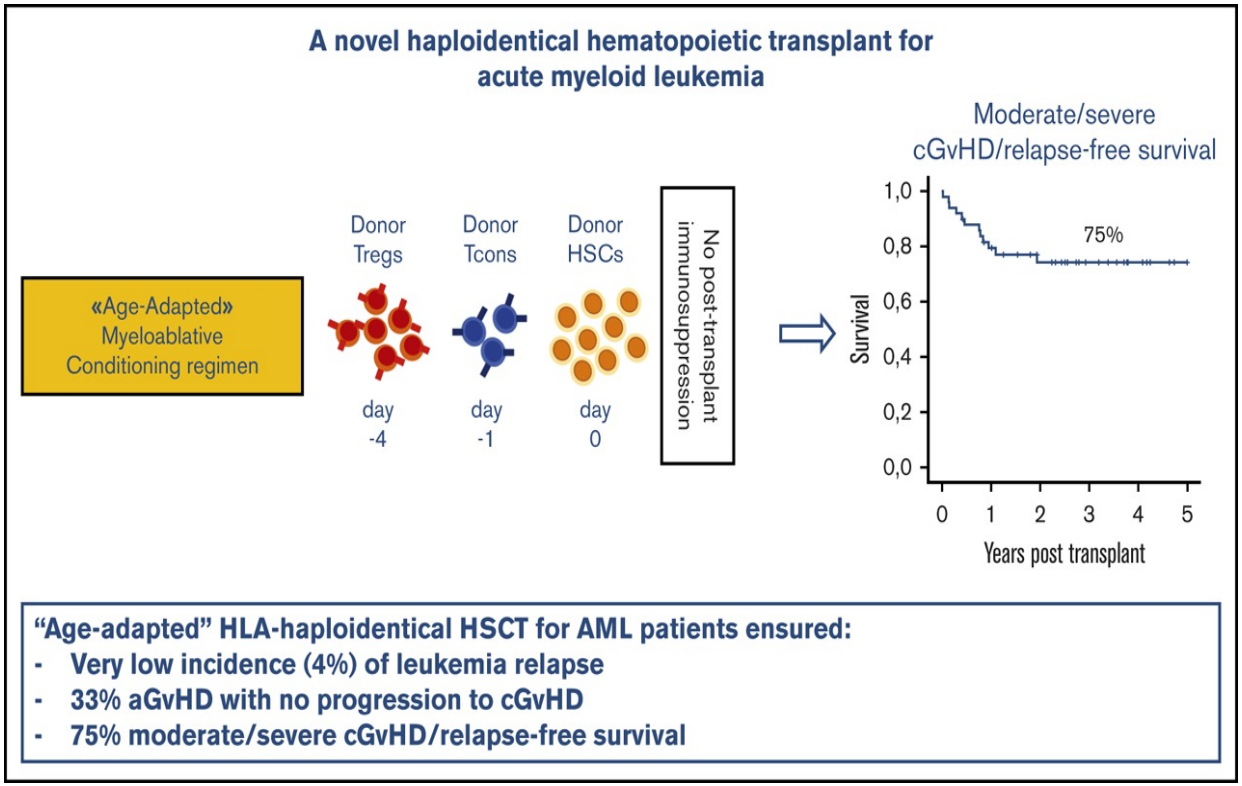
REGULAR ARTICLE

blood advances

Haploidentical age-adapted myeloablative transplant and regulatory and effector T cells for acute myeloid leukemia

Antonio Pierini,[1,*] Loredana Ruggeri,[1,*] Alessandra Carotti,[1] Franca Falzetti,[1] Simonetta Saldi,[2] Adelmo Terenzi,[1] Claudio Zucchetti,[3] Gianluca Ingrosso,[2] Tiziana Zei,[1] Roberta Iacucci Ostini,[1] Sara Piccinelli,[1] Samanta Bonato,[1] Sara Tricarico,[1] Antonella Mancusi,[1] Sara Ciardelli,[1] Roberto Limongello,[1] Mara Merluzzi,[1] Mauro Di Ianni,[4] Rita Tognellini,[1] Olivia Minelli,[1] Cristina Mecucci,[1] Maria Paola Martelli,[1] Brunangelo Falini,[1] Massimo Fabrizio Martelli,[1] Cynthia Aristei,[2] and Andrea Velardi[1]

[1]Division of Hematology and Clinical Immunology, Department of Medicine, University of Perugia, Perugia, Italy; [2]Department of Surgical and Biomedical Science, University of Perugia and Perugia General Hospital, Perugia, Italy; [3]Section of Medical Physics, Perugia General Hospital, Perugia, Italy; and [4]Department of Medicine and Aging Sciences, University of Chieti-Pescara, Pescara, Italy



Main Eligibility Criteria:

- **Diagnosis of AML** with indication to transplant
- Absence of an HLA-matched family donor.
- Availability of a donor family member with a full haplotype mismatch HLA with the patient
- Age >18 and <65 years
- ECOG ≤ 2

Endpoints:

PRIMARY:

- 2 years probability of Chronic Graft versus Host Disease – Relapse Free Survival (CRFS)

SECONDARY:

- Engraftment
- Cumulative incidences of aGvHD, cGvHD, NRM, Relapse

Demographics:

	TBI-based conditioning regimen	TMLI-based conditioning regimen	TOTAL
Number of patients	19	31	50
Sex (M/F)	8/11	18/13	26/24
Median Age (Range)	33 (20-50)	56 (38-65)	53 (20-65)
Genetic risk stratification at diagnosis			
Favorable Risk	2 (11%)	3 (10%)	5 (10%)
Intermediate Risk	8 (42%)	14 (45%)	22 (44%)
Adverse Risk	9 (47%)	11 (35%)	20 (40%)
Missing Informations	0 (0%)	3 (10%)	3 (6%)
Other risk factors			
Secondary AML	5 (26%)	11 (35%)	16 (32%)
PIF	5 (26%)	12 (39%)	17 (34%)

Demographics:

	TBI-based conditioning regimen	TMLI-based conditioning regimen	TOTAL
Disease Status at HSCT			
1st CR,MRD^{NEG}	8 (42%)	9 (29%)	17 (34%)
1st CR,MRD^{POS}	7 (37%)	10 (32%)	17 (34%)
≧ 2nd CR, MRD^{NEG}	0 (0%)	0 (0%)	0 (0%)
≧ 2nd CR, MRD^{POS}	1 (5%)	7 (23%)	8 (16%)
AD	3 (16%)	5 (16%)	8 (16%)
DRI			
Low	2 (11%)	2 (6%)	4 (8%)
Intermediate	6 (32%)	14 (45%)	20 (40%)
High	10 (53%)	8 (39%)	18 (36%)
Very High	1 (5%)	4 (13%)	5 (10%)
Missing Informations	0 (0%)	3 (10%)	3 (6%)

33 (66%) patients **MRD positive** or with **active disease** at the time of transplant

Engraftment

- All patients achieved full donor type engraftment:
  - Neutrophil engraftment: 13 days
    (range: 8-23 days)
  - Platelet recovery: 17 days
    (range: 14-72 days)

- Full Donor Chimerism was sustained in all patients starting from 1 month after transplant

Pierini et al. Blood Advances 2021

Toxicity

- Most conditioning regimen-related AEs were mild:
  - No patient developed more than grade 3 oral and intestinal mucositis
- TMLI ensured lower mucosal toxicity to older patients

TBI-treated patients

	GRADE II	GRADE III	GRADE IV	GRADE V
ORAL MUCOSITIS	13 (68%)	6 (32%)		
CNS				
HEPATIC	1 (5%)		4 (16%)	
GASTRIC	18 (95%)	1 (5%)		
INTESTINAL	11 (58%)	8 (42%)		
RENAL				
PULMUNARY	6 (32%)	1 (5%)		
BLADDER		1 (5%)		
CARDIAC	6 (32%)	1 (5%)		

TMLI-treated patients

	GRADE II	GRADE III	GRADE IV	GRADE V
ORAL MUCOSITIS	25 (81%)	6 (19%)		
CNS	2 (6%)	2 (6%)		1 (3%)
HEPATIC	3 (10%)	1 (3%)	2 (6%)	
GASTRIC	29 (94%)	2 (6%)		
INTESTINAL	21 (68%)	10 (32%)		
RENAL				
PULMUNARY	1 (35%)			
BLADDER	2 (6%)			
CARDIAC	3 (10%)			

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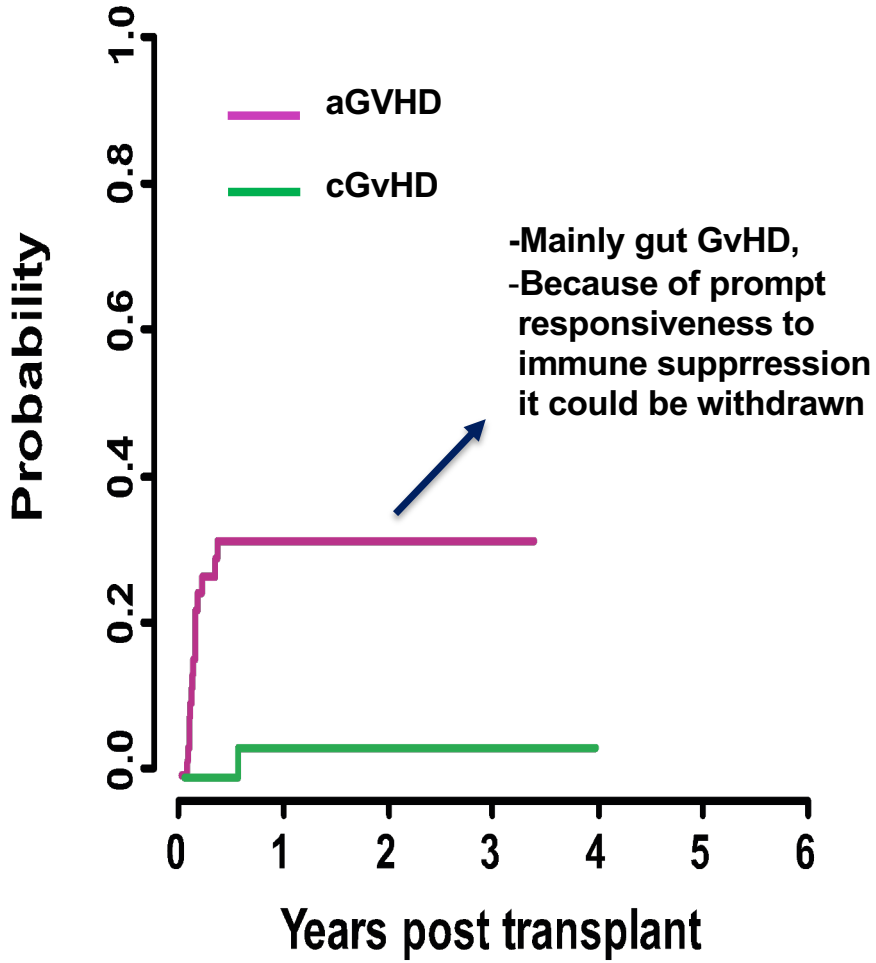
GvHD

Grade ≥2 acute GvHD: 15 pts (CI=33%)

- 12/15 alive and off-immunosuppressive therapy

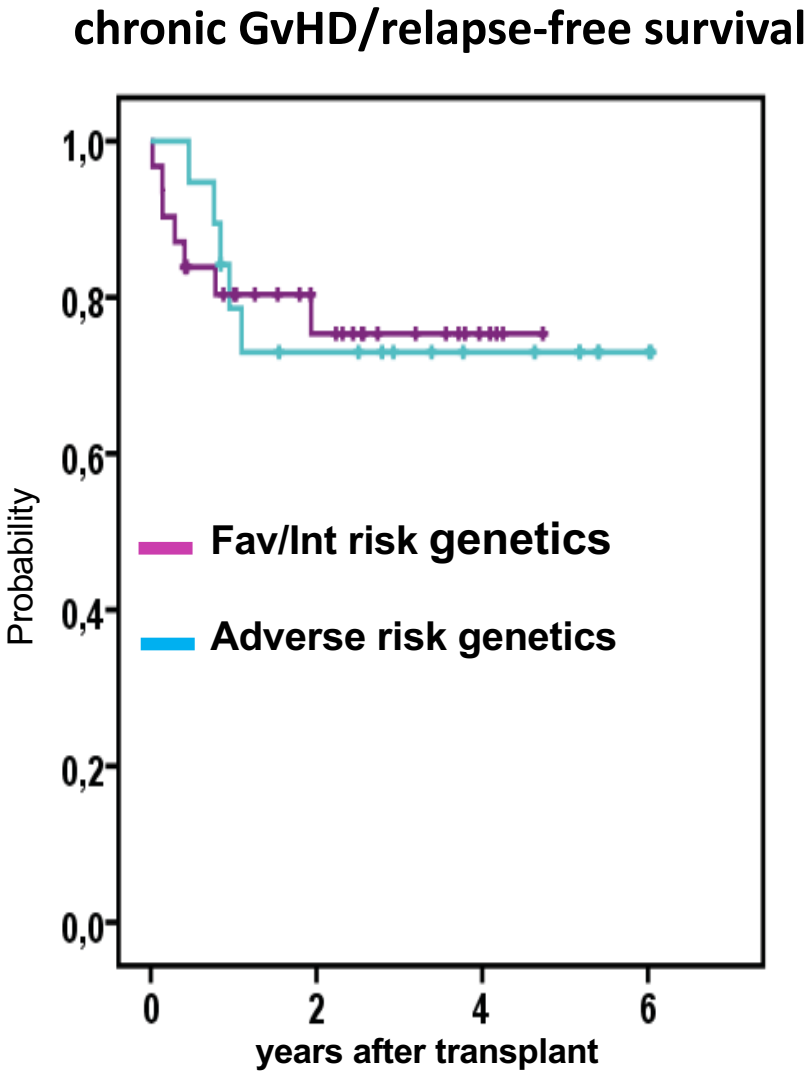
Moderate/Severe chronic GvHD: 1 pt (CI=3%)

- The patient died because of pnuemonia during treatment



No impact of Adverse Genetics

Adverse genetics at diagnosis (including monosomal and/or complex karyotype) had **no impact on chronic GvHD/relapse-free survival**



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Conclusion 2

An age-adaptable, TBI/TMLI-based conditioning regimen combined with Treg/Tcon immunotherapy allowed for “unprecedented” >70% cGvHD/Leukemia free survival in haploidentical transplants for AML patients up to the age of 65, regardless of adverse cytogenetics.

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