Is PTCy the standard GvHD prophylaxis for haplo-HSCT in 2023? NO

Mauro Di Ianni

Nothing to Disclose

T-cell replete HSCT: GvHD and relapse

		Relapse (%)	DFS (%)	GvHD %(grade II-IV)
Luznik et al. *BBMT 2008*	NMA/PTCY Haplo	51	42	34
Bashey et al *J Clin Oncol 2013*	MA/PTCy -Haplo	33	60	33
McCurdy et al. *BBMT 2015*	MA/PTCy -Haplo	**21 LR** **48 IR** **67 HR**		

Ex vivo T cell depletion

- ***non-selective***
  - all T-cells are removed
    - SBA/ E_N
    - HSC specific monoclonal antibodies in conjunction with magnetic beads: positive selection of CD34+ cells

- ***Selective***
  - **only a given T-cell subset is targeted**
    - **Removal of αβ T-cells**
    - Removal of naive T-cells (CD45RA T-cell subset)
    - Tregs selection

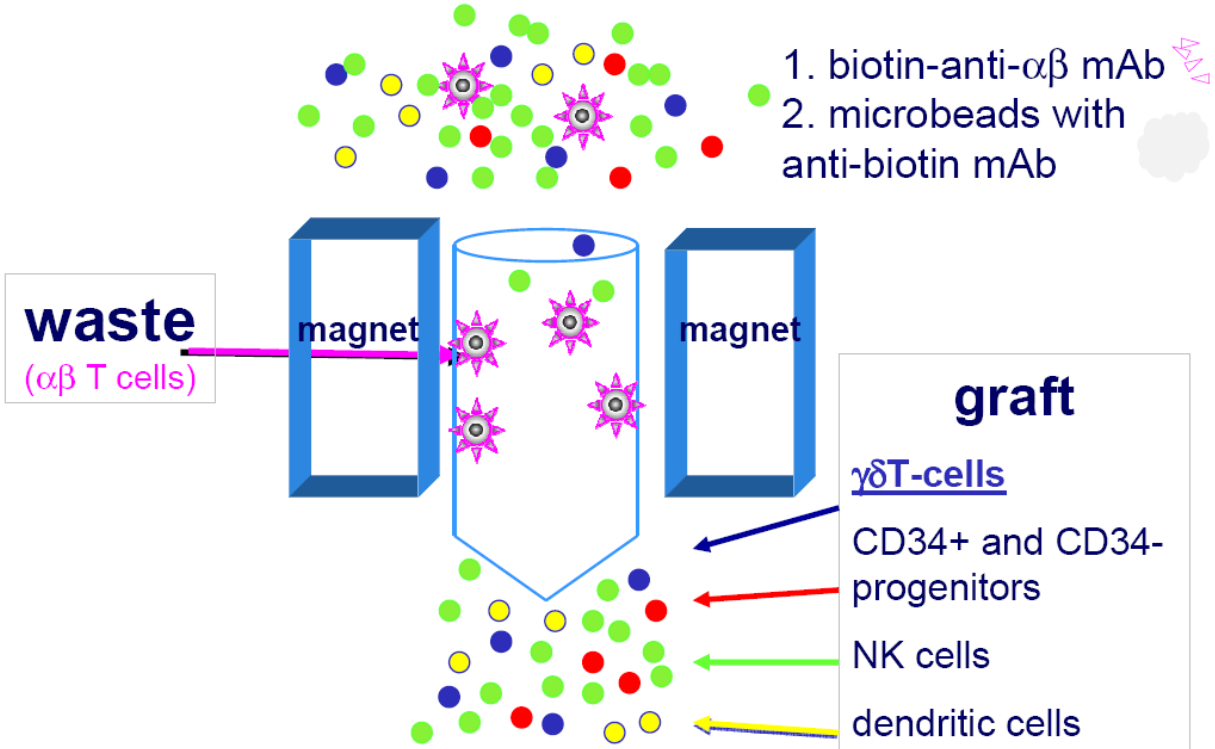
Rationale

4. Rationale

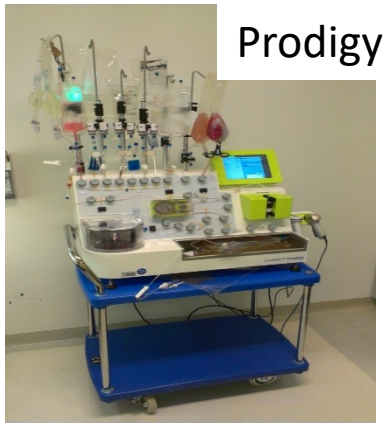
- Efficient TCRα/β$^+$ cell depletion
  → Potentially reducing the risk of GvHD
  → Reducing the need for strong pharmaceutical immunosuppression
    → A basis for enhanced immune reconstitution and GvL effects
- Maintenance of stem cells and facilitating cells, such as NK cells and TCRγ/δ$^+$ T cells
  → Facilitate engraftment
  → Drive immune reconstitution
  → Exert GvL effects
  → Reduce risk for infections

Strategy for depletion of αβ+ T-cells

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



CliniMACS



Prodigy

Efficient TCRα/β+ cell depletion

→ Potentially reducing the risk of GvHD

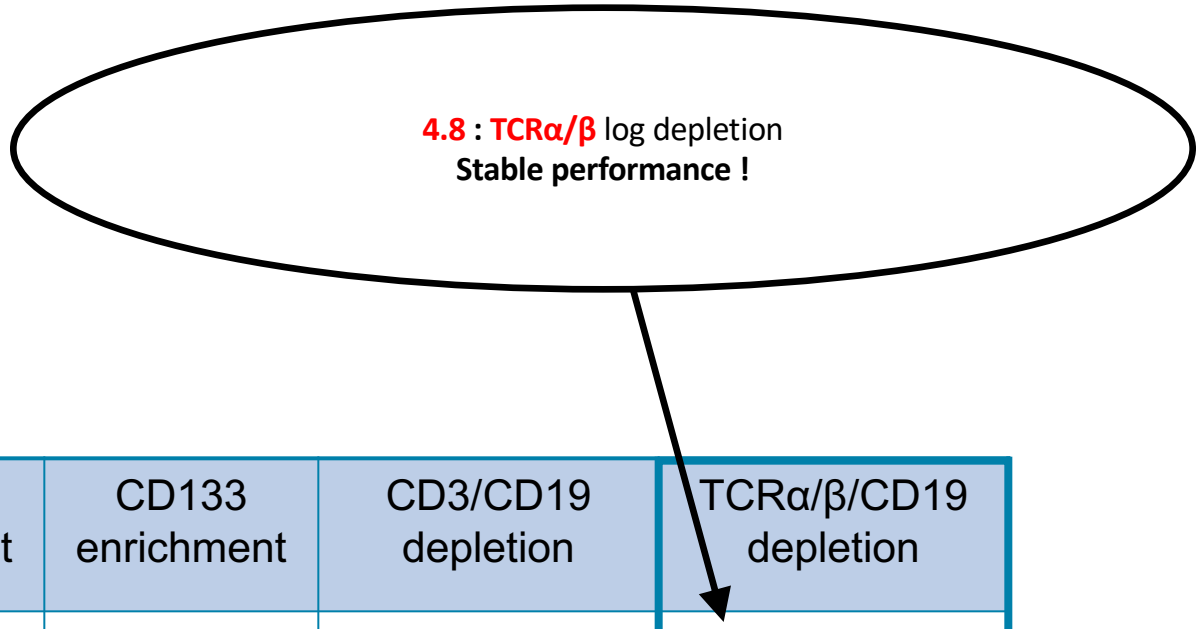
Maintenance of stem cells and facilitating cells (TCRγδ T cells, NK cells)

→ might facilitate engraftment,

→ exerts a GvL effect and reduces the risk for infections.

Performance

3. High log depletion



	CD34 enrichment	CD133 enrichment	CD3/CD19 depletion	TCRα/β/CD19 depletion
Median T cell log depl.	**4.6 - 5.1***	**3.8 - 4.2***	**3.0 - 4.1***	**4.8 ± 0.3**** (range: 4.3 – 5.1)
Median B cell log depl.	**3.2 - 3.7***	**3.1***	**2.2 - 3.7***	**3.5 ± 0.1***** (range: 3.4 – 3.6)

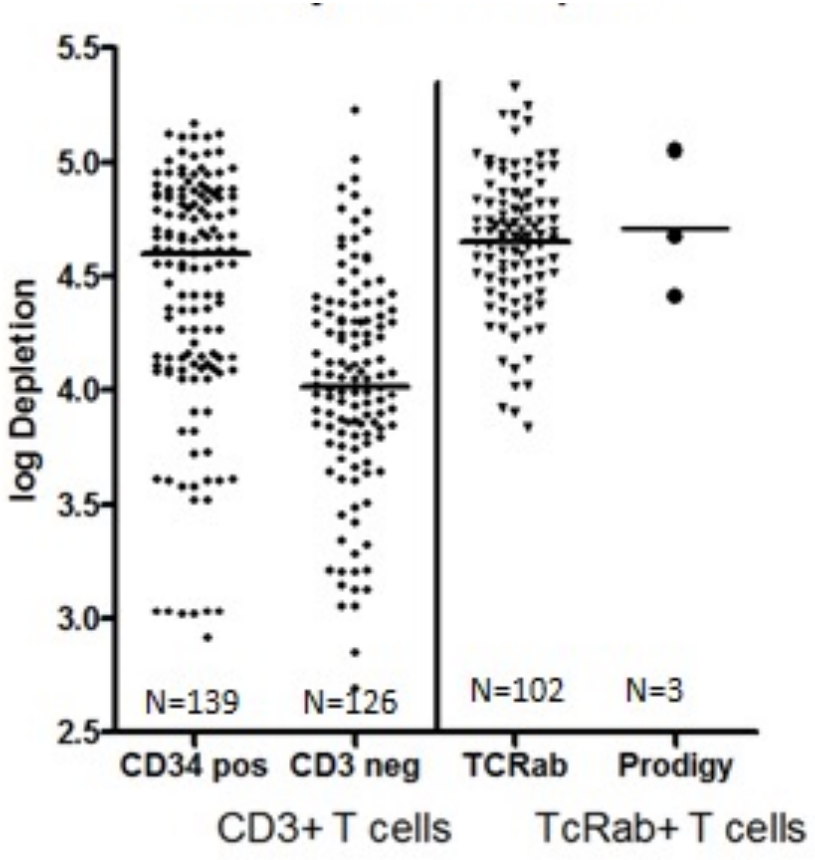
*** Median depletion results from different publications**

**** TCRα/β log depl.; n=6; In-house project 2; (mean ± SD)**

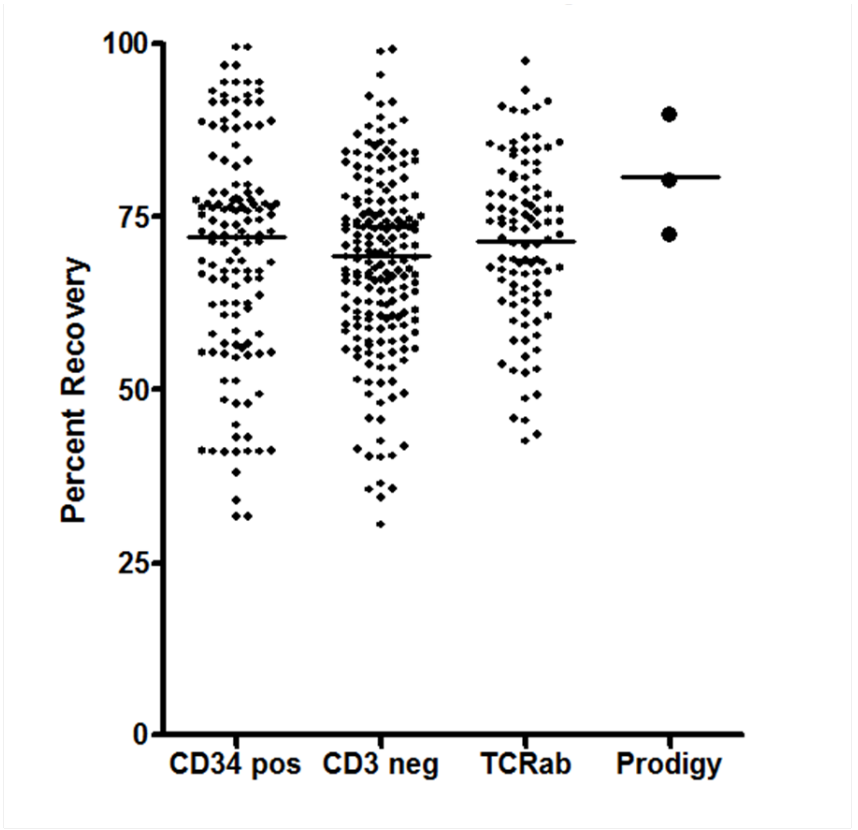
***** Combined TCRα/β/CD19 depl.; n=3; In-house project 2; (mean ± SD)**

TcRαβ-Depletion

Log Depletion



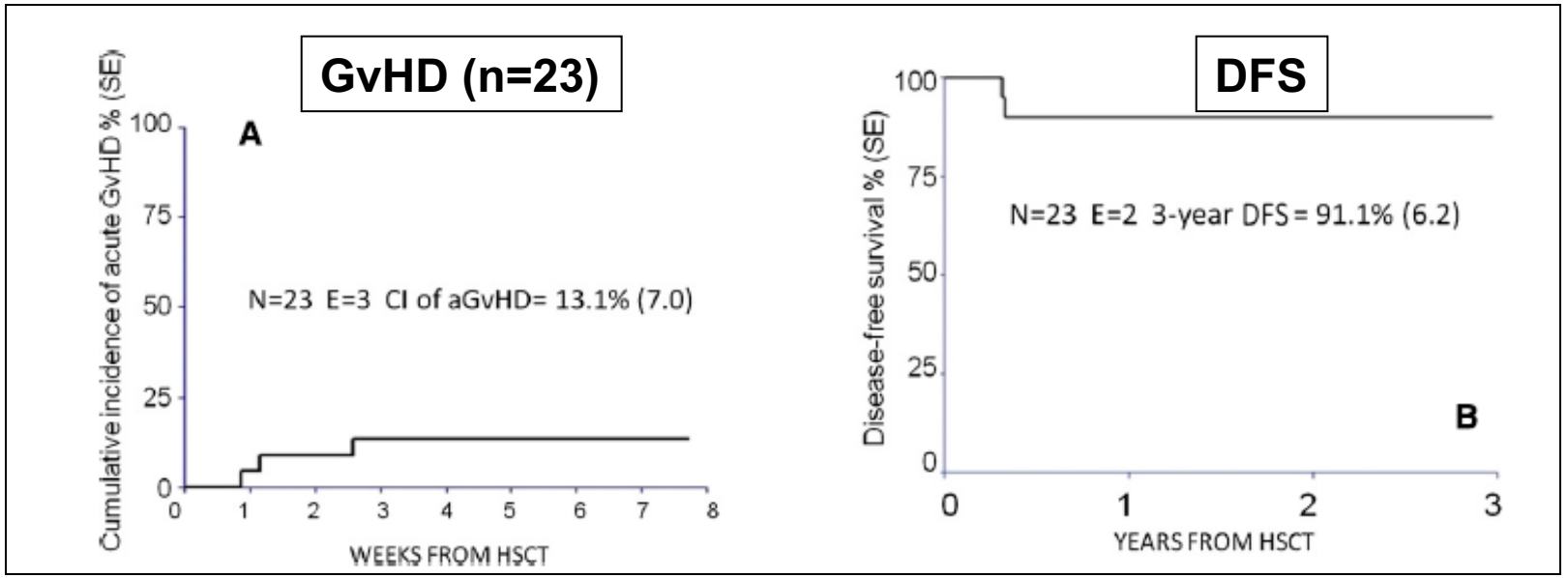
CD34+ Recovery

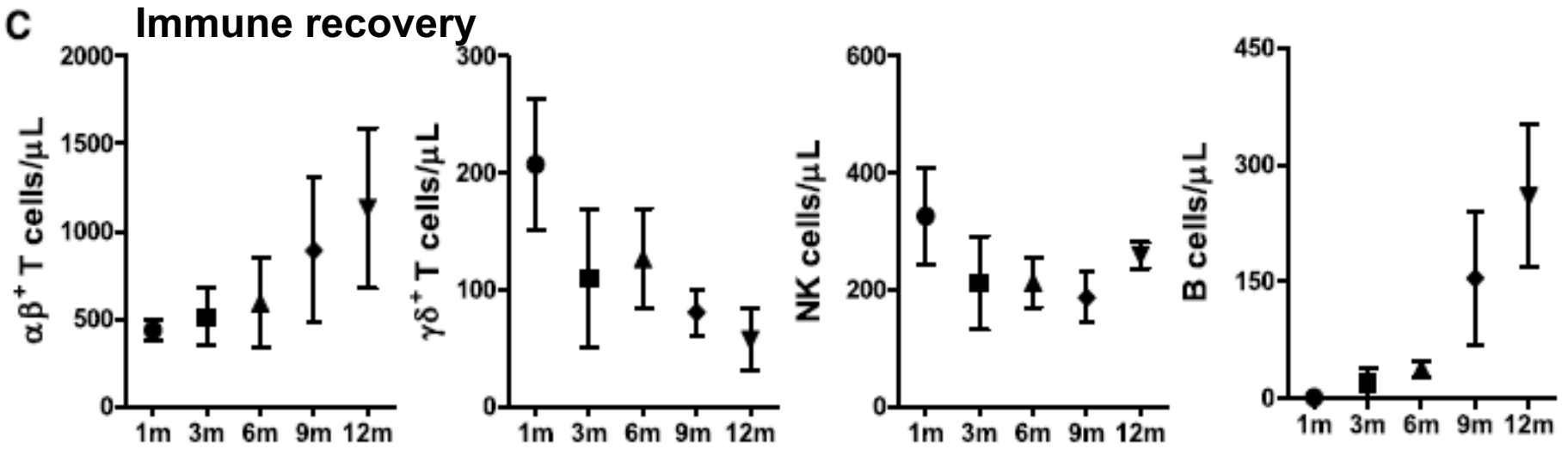


Courtesy of R. Handgretinger

HLA-haploidentical stem cell transplantation after removal of αβ+ T and B cells in children with nonmalignant disorders

Bertaina A et al., Blood 124; 822, 2014.

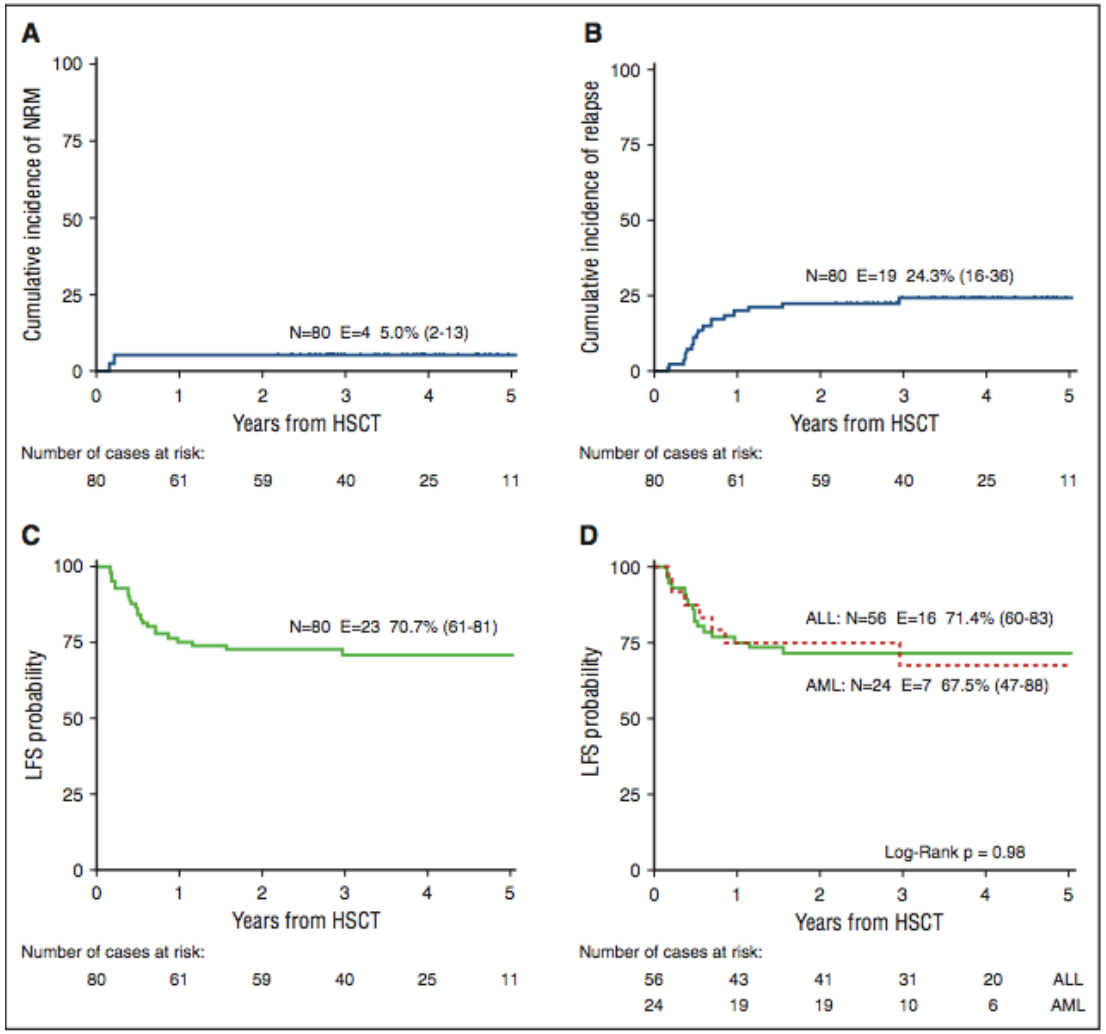




Outcome of children with acute leukemia given HLA-haploidentical HSCT after αβ T-cell and B-cell depletion

PTS = 80
Median age (range) 9.7 (0.9-20.9)
56 ALL - 24 AML

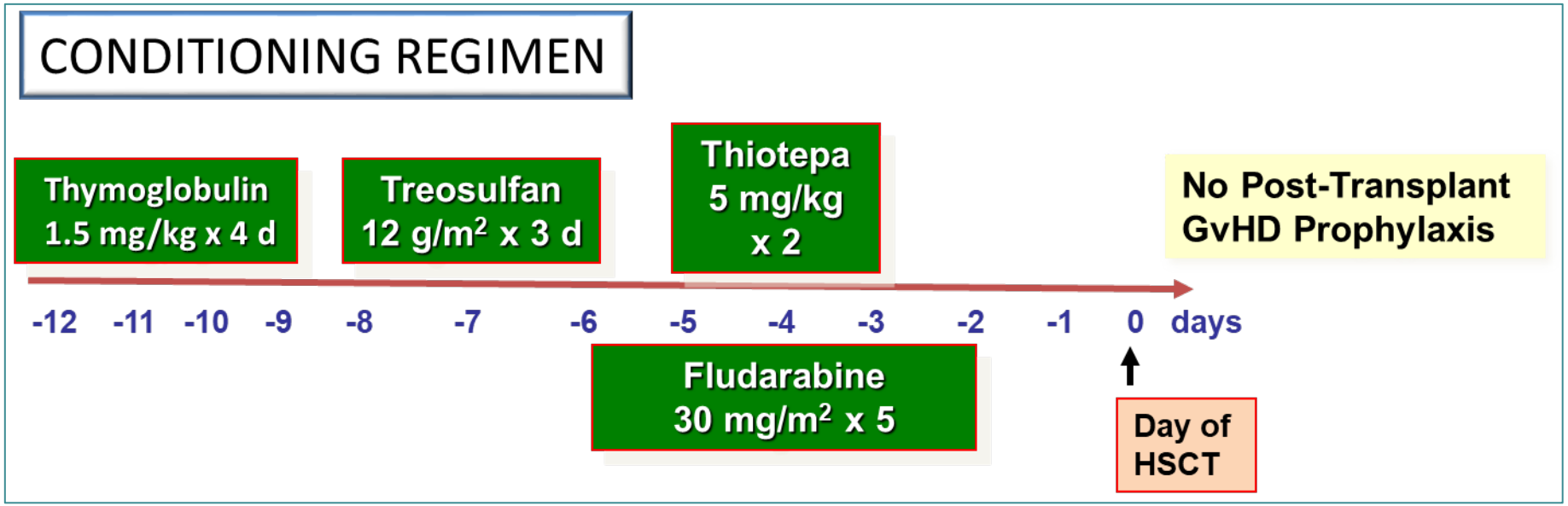
GvHD grade 1-2 : 30% (skin only)



Locatelli et al. Blood. 2017;130(5):677-685)

TcRαβ / CD19 depleted HaploHSCT in adults

The experience of the BMT Unit
of the University of Parma, Italy



GRAFT COMPOSITION

	CD34	CD3			CD20	NK
		Total CD3	γδ	αβ		
cells/kg Median (Range)	11 x 10^6 (5-19)	4.3 x 10^6 (1-35.7)	4 x 10^6 (1-34)	4,8 x 10^4 (0,4-37)	4.8 x 10^4 (1.8-32)	30 x 10^6 (8-91)

patient and donor characteristics

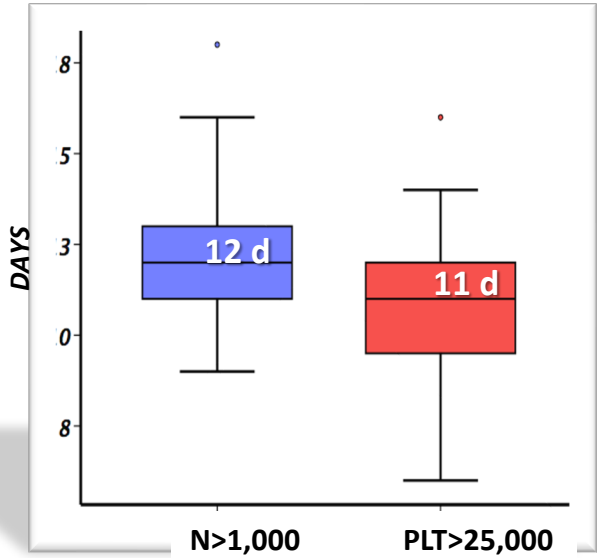
Patients	**44**
Male/Female	**23/21**
Age in years	
Median (range)	**48 (19-73)**
Age, groups	
19 - 50	**23**
51 - 60	**7**
61 - 73	**14**
Disease	
AML	**31**
ALL	**6**
HL	**6**
MM	**1**
Disease Status At Transplant	
CR1	**16**
CR ≥2	**12**
RELAPSE	**16**
CMV status (R/D)	
NEG/NEG	**3**
NEG/POS	**8**
POS/POS	**27**
POS/NEG	**6**

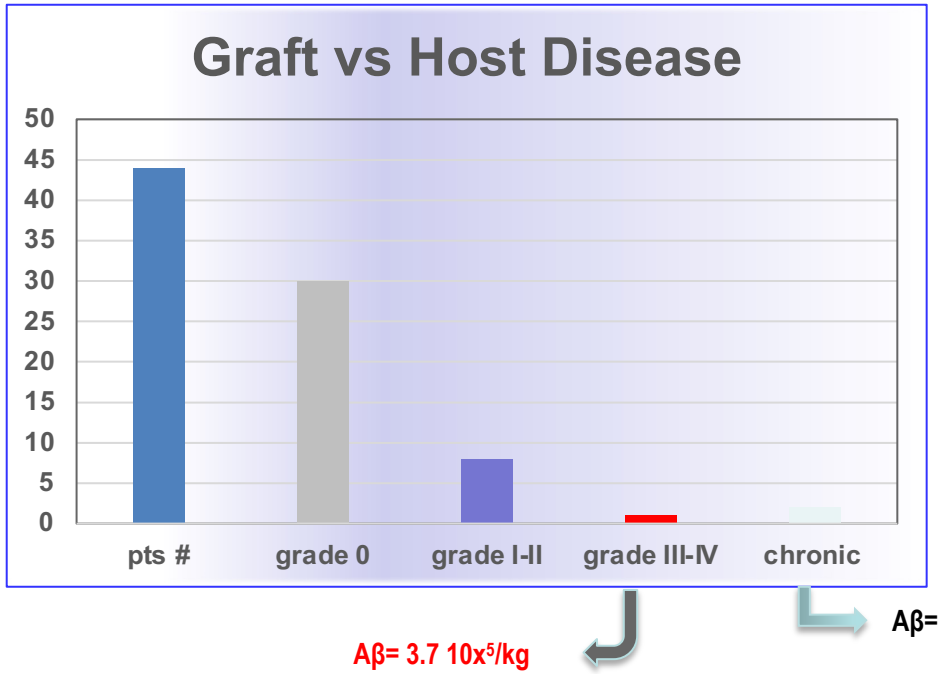
Panel of donors	
Mother	**6**
son	**7**
daughter	**9**
brother or sister	**14**
cousin	**6**
nephew	**1**
uncle	**1**

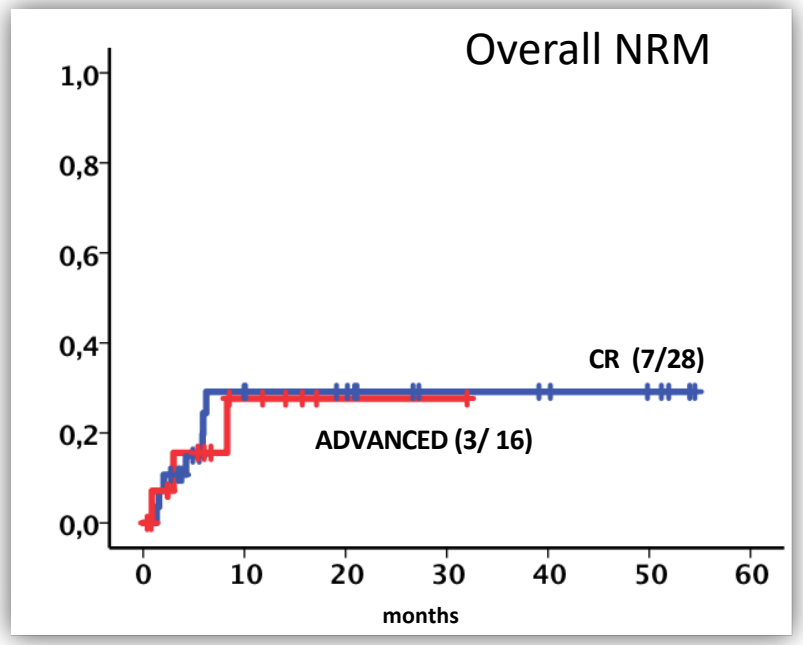
Courtesy of Lucia Prezioso, Parma

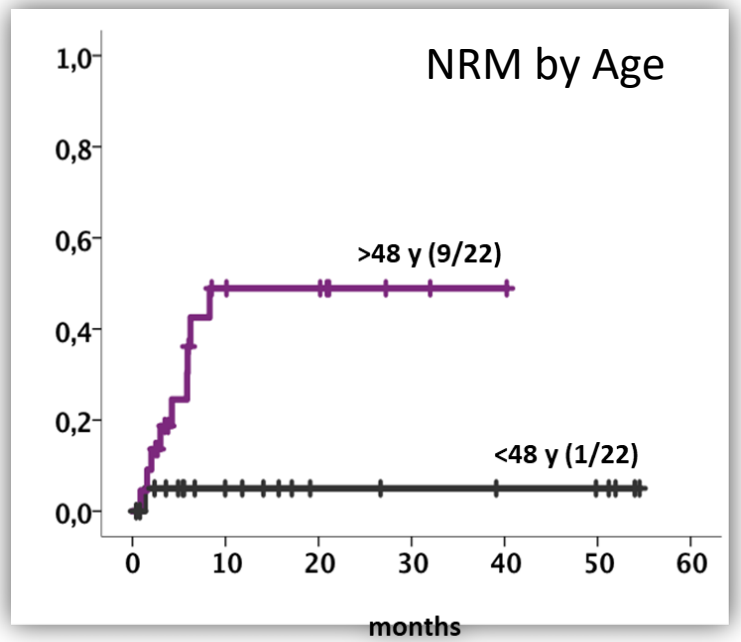
Engraftment	
Primary sustained engraftment	42/44
Overall engraftment	44/44

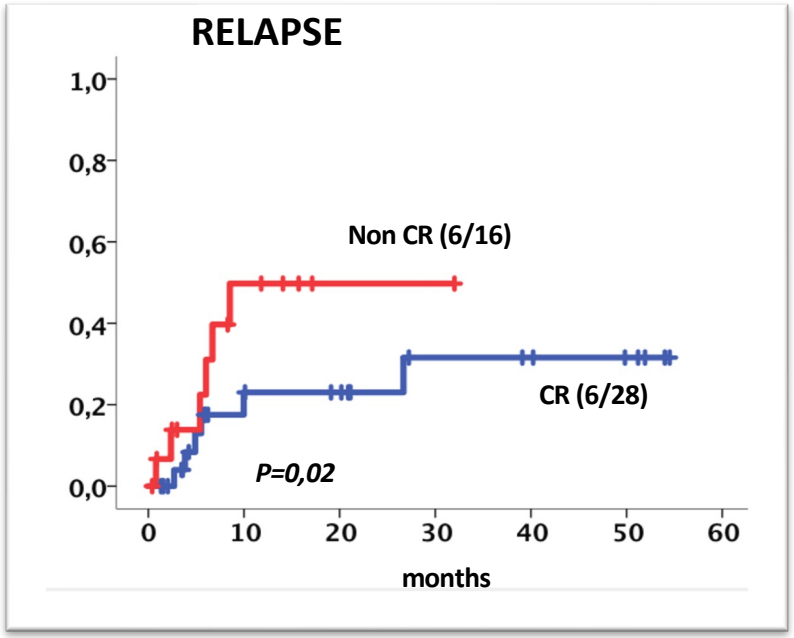
Time to engraftment

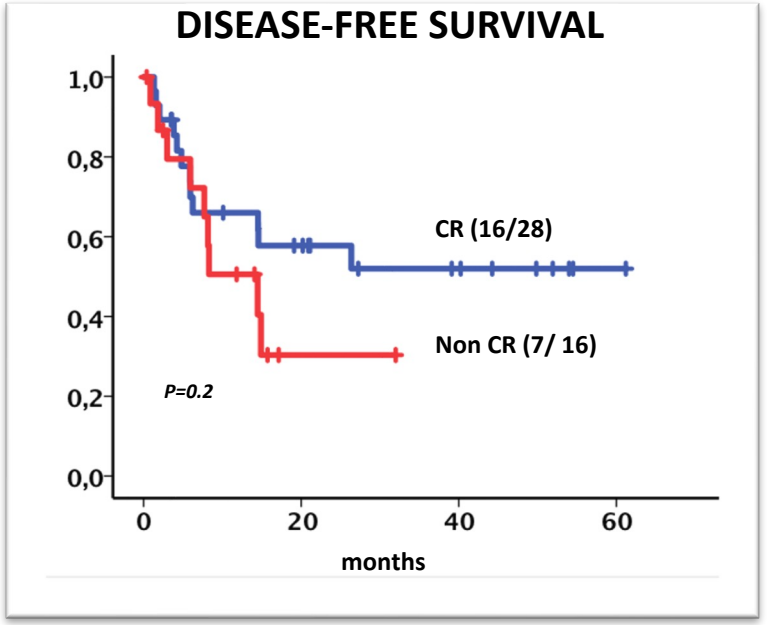





Overall NRM
1,0
0,8
0,6
0,4
0,2
0,0
CR (7/28)
ADVANCED (3/ 16)
0
10
20
30
40
50
60
months


NRM by Age
1,0
0,8
0,6
0,4
0,2
0,0
>48 y (9/22)
<48 y (1/22)
0
10
20
30
40
50
60
months


RELAPSE
1,0
0,8
0,6
0,4
0,2
0,0
Non CR (6/16)
CR (6/28)
P=0,02
0
10
20
30
40
50
60
months


DISEASE-FREE SURVIVAL
1,0
0,8
0,6
0,4
0,2
0,0
CR (16/28)
Non CR (7/ 16)
P=0.2
0
20
40
60
months

Tαβ/CD19-depletion in adults: Comments

- High engraftment rates after a chemotherapy alone based conditioning
- Low incidence and severity of GvHD
  - αβ T cell threshold $<10^5$/kg
  - Skin limited GvHD
- Fast immune reconstitution
- Very low infectious complications
- No benefit in advanced disease status at transplant

Ex vivo T cell depletion

- ***non-selective***
  - all T-cells are removed
    - SBA/ E_N
    - HSC specific monoclonal antibodies in conjunction with magnetic beads: positive selection of CD34+ cells

- ***Selective***
  - **only a given T-cell subset is targeted**
    - Removal of αβ T-cells
    - **Removal of naive T-cells (CD45RA T-cell subset)**
    - Tregs selection

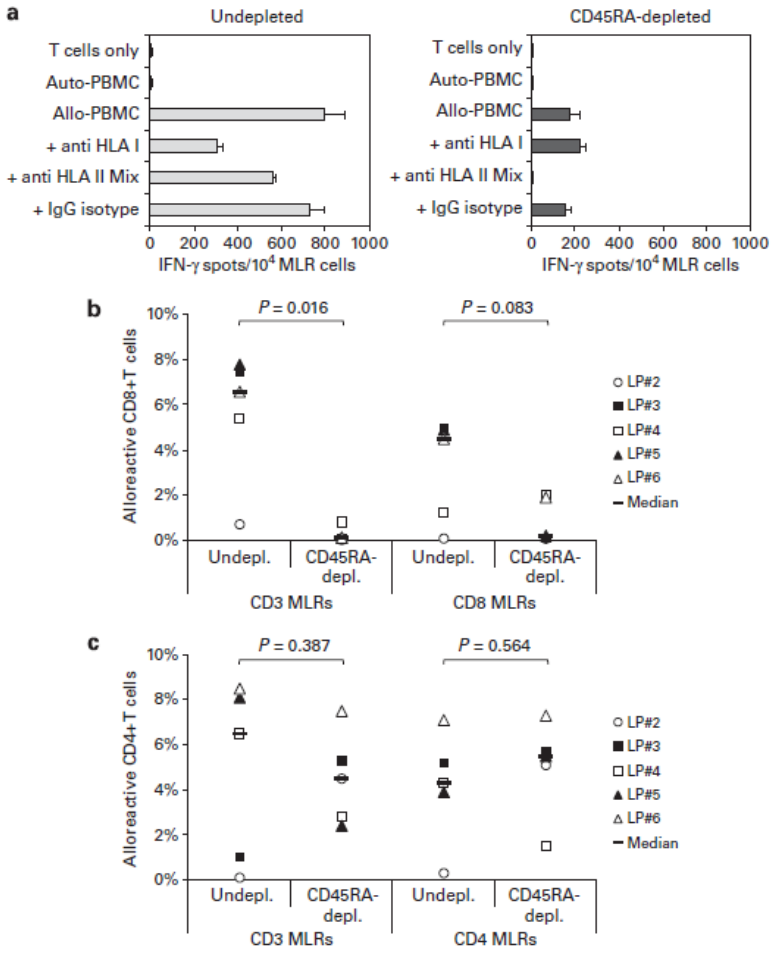
Table 1. Cell numbers (× 10^6) and frequencies (% of WBC) in undepleted versus CD45RA-depleted LPs

Donor	$CD3^{pos}$	$CD3/CD4^{pos}$	$CD3/CD4^{pos}CD45RA^{pos}$	$CD3/CD4^{pos}CD45RA^{neg}$	$CD3/CD8^{pos}$	$CD3/CD8^{pos}CD45RA^{pos}$	$CD3/CD8^{pos}CD45RA^{neg}$	Log depletion	$CD4/CD25/Foxp3^{pos}$	$CD3^{neg}CD16/CD56^{pos}$	$CD19^{pos}$	$CD14^{pos}$	$CD15^{pos}$
Undepleted LPs													
1	6503 (54.9)	4608 (38.9)	1611 (13.6)	2676 (21.8)	1374 (11.6)	537 (4.5)	817 (6.2)	NA	213 (1.8)[a]	NA	NA	NA	NA
2	4461 (40.8)	2941 (26.9)	1268 (11.6)	1585 (14.5)	1400 (12.8)	875 (8.0)	525 (4.8)	NA	197 (1.8)[a]	2810 (25.7)	809 (7.4)	2919 (26.7)	1082 (9.9)
3	6533 (63.9)	3323 (32.5)	1748 (17.1)	1636 (16.0)	2730 (26.7)	1774 (17.4)	951 (9.0)	NA	184 (1.8)[a]	1051 (10.3)	532 (5.2)	1462 (14.3)	90 (0.9)
4	5360 (39.7)	3132 (23.2)	1283 (9.5)	1850 (13.7)	2174 (16.1)	1229 (9.1)	797 (5.9)	NA	230 (1.7)[a]	2923 (21.7)	1715 (12.7)	2619 (19.4)	2336 (17.3)
5	4261 (51.2)	2979 (35.8)	1373 (16.5)	1515 (18.2)	1148 (13.8)	543 (6.5)	531 (6.4)	NA	191 (2.3)[a]	907 (10.9)	599 (7.2)	1831 (22.0)	358 (4.3)
6	5845 (55.2)	3971 (37.5)	1874 (17.7)	2011 (19.0)	1758 (16.6)	1113 (10.5)	566 (5.4)	NA	244 (2.3)[a]	2034 (19.2)	678 (6.4)	1726 (16.3)	286 (2.7)
Median	5603 (53.1)	3228 (34.2)	1492 (15.1)	1743 (17.1)	1579 (15.9)	994 (8.6)	682 (6.0)	NA	205 (1.8)[a]	2034 (19.2)	678 (7.2)	1831 (19.4)	358 (4.3)
CD45RA-depleted LPs													
1	1472 (52.0)	1245 (44.0)	0 (0.0)	1211 (42.8)	175 (6.2)	3 (0.1)	167 (5.9)	4.4	40 (1.4)[a]	NA	NA	NA	NA
2	1198 (28.2)	983 (23.1)	0 (0.0)	954 (22.4)	217 (5.1)	0 (0.0)	204 (4.8)	4.3	60 (1.4)[a]	760 (18.9)	0 (0.0)	1898 (44.6)	891 (20.9)
3	1414 (57.8)	1168 (47.7)	2 (0.1)	1055 (43.1)	233 (9.5)	2 (0.1)	186 (7.6)	4.7	24 (1.0)[a]	56 (2.3)	0 (0.0)	602 (24.6)	93 (3.8)
4	1429 (33.8)	1181 (26.6)	9 (0.2)	1119 (25.2)	244 (5.5)	9 (0.2)	233 (5.5)	3.4	49 (1.1)[a]	1211 (28.6)	0 (0.0)	875 (19.7)	1816 (40.9)
5	1219 (45.6)	1080 (40.4)	3 (0.1)	1015 (38.0)	142 (5.3)	3 (0.1)	120 (4.5)	4.7	59 (2.2)[a]	88 (3.3)	3 (0.1)	871 (32.6)	91 (3.4)
6	972 (50.9)	836 (43.8)	2 (0.1)	815 (42.7)	109 (5.7)	0 (0.0)	84 (4.4)	NA	23 (1.2)[a]	131 (6.9)	2 (0.1)	517 (27.1)	134 (7.0)
Median	1317 (48.3)	1124 (42.1)	2 (0.1)	1035 (40.4)	196 (5.6)	3 (0.1)	177 (4.8)	4.4	45 (1.3)[a]	131 (6.9)	0 (0.0)	871 (27.1)	134 (7.0)

Abbreviations: NA = not applicable; LP = leukapheresis product. [a]Data obtained from thawed cells.

Teschner et al., BMT 2013

Alloreactivity of CD45RA depleted vs undepleted cell fraction



Teschner et al., BMT 2013

CD45RA depleted graft

Graft	
$CD34^+$ cells	
Median (cells/kg × 10^6)	7.4
Range (cells/kg × 10^6)	5.1–19.9
$CD3^+$ cells	
Median (cells/kg × 10^6)	10
Range (cells/kg × 10^6)	1.6–10.0
$CD45RA^+$ $CD45RO^-$ $CD3^+$ cells	
Median (cells/kg × 10^4)	0.36
Range (cells/kg × 10^4)	0.05–7.46
Interquartile range	0.22–0.65

Bleakley et al., JCI 2015

Clinical trials with CD45RA T-cell depletion

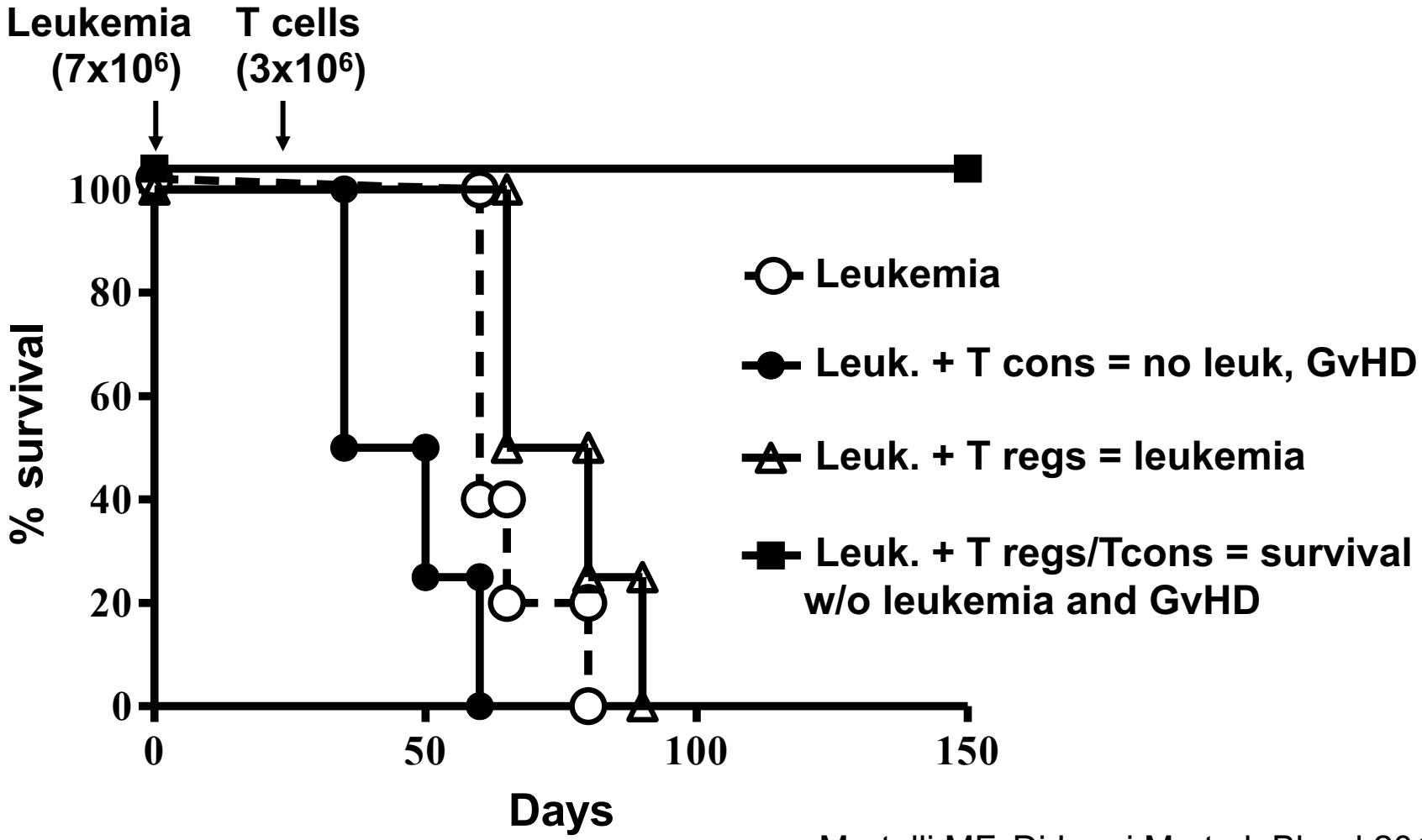
Patients	Disease	GvHD prophylaxis	Acute/Chronic GVHD	TRM	EFS (DFS)/OS	Reference
35	HR-AL	Tacrolimus	66%/9%	9%	70%/78%/2 years	Bleakley et al., JCI 2015
8	Solid Tumors	Sirolimus	0%	1 patient died	NA	Roy et al., Blood 2016
17	Haematological malignancies	Sirolimus and MMF	17,6%/6%	11,7%	76,5% patients alive at 223 days	Triplett et al., BMT 2015

Ex vivo T cell depletion

- ***non-selective***
  - all T-cells are removed
    - SBA/ E_N
    - HSC specific monoclonal antibodies in conjunction with magnetic beads: positive selection of CD34+ cells

- ***Selective***
  - **only a given T-cell subset is targeted**
    - Removal of αβ T-cells
    - Removal of naive T-cells (CD45RA T-cell subset)
    - **Tregs selection**

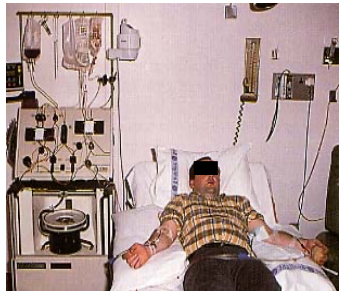
Clearance of human AML by human T regs + T cons in immunodeficient mice



Martelli MF, Di Ianni M et al. Blood 2014

Selection and Characterization of CD4+CD25+ Regulatory T Cells

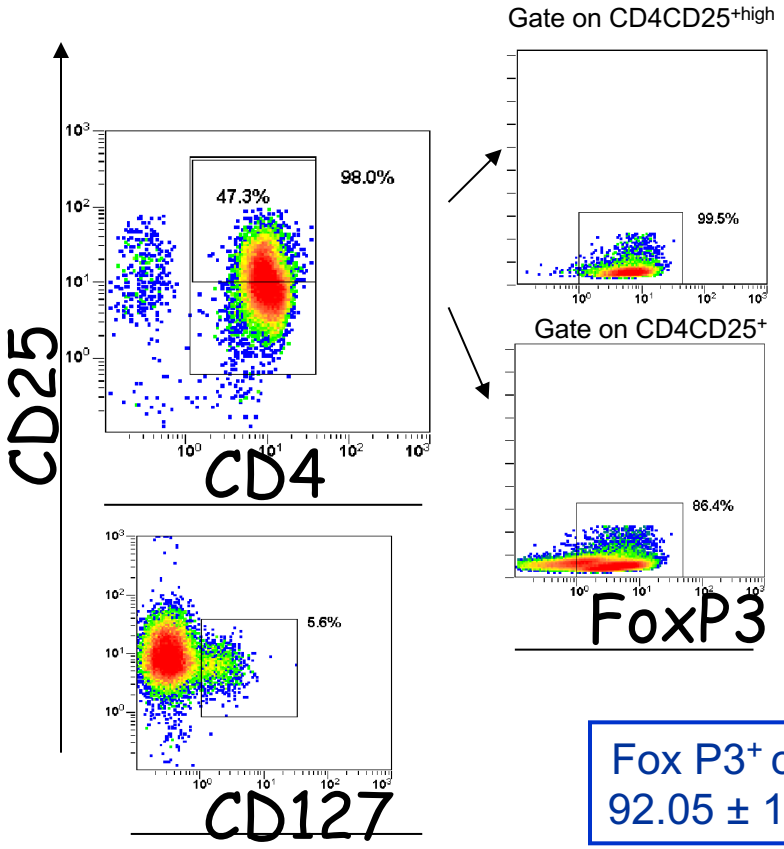
Leukapheresis product



Selection of
$CD4^+CD25^+$Cells

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

2nd step:
Enrichment of $CD25^+$ cells



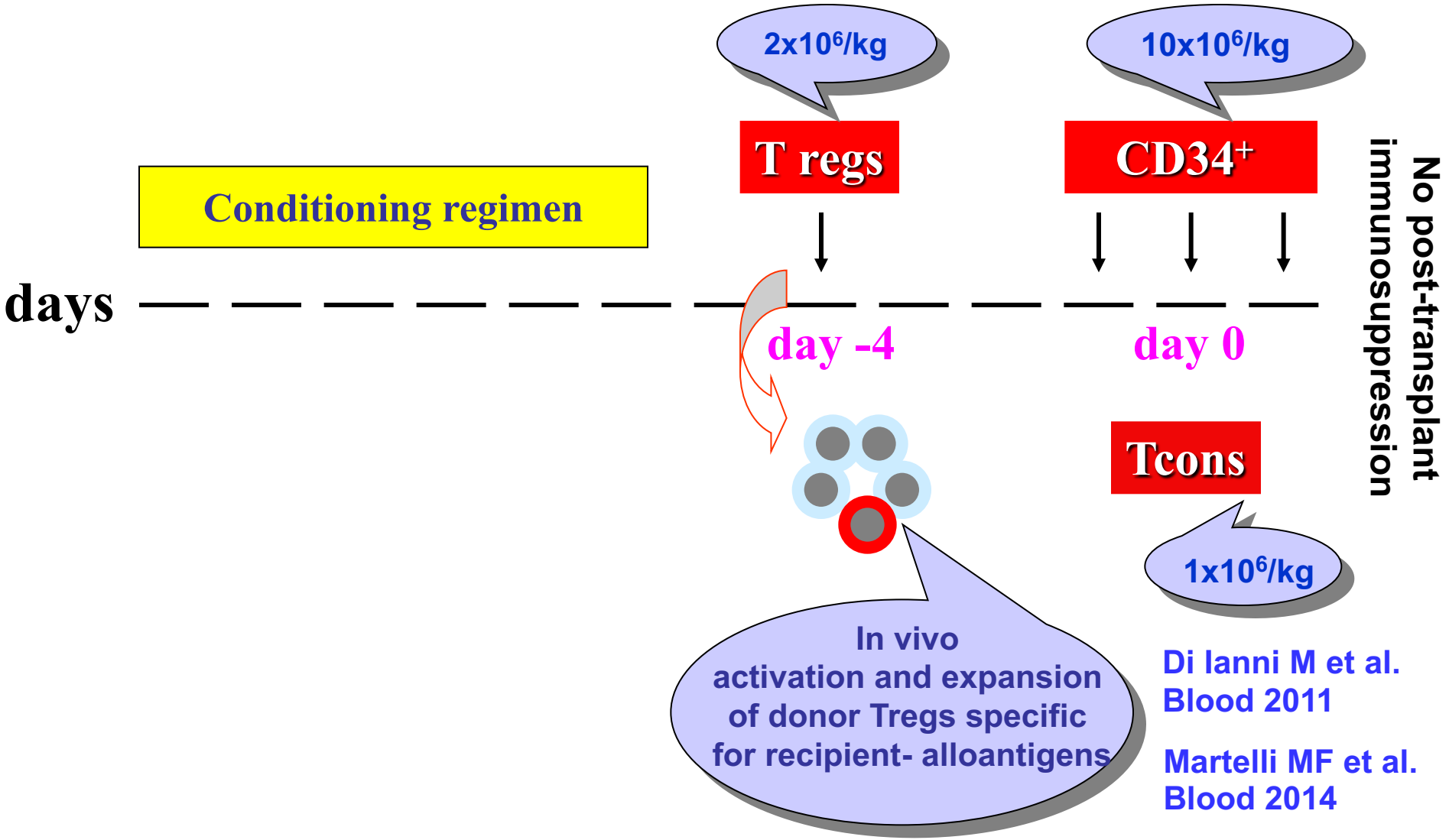
Fox $P3^+$ cells
92.05 ± 15 %

	Starting fraction	Final fraction
Cells ($x10^9$)	1060 (540-1370)	280 (202- 390)
%CD4CD25	3.0 (1.5-7.45)	92.4 (90-97.1)
N° cells ($x\ 10^6$)	330 (221-1020)	256 (185.6-365.4)
$\%CD4CD25^{high}$	0.3 (0.12- 0.89)	33.6 (14.4-39.6)
N° cells ($x\ 10^6$)	36.12 (19.98 - 84)	68.6 (20.9-143)

Di Ianni et al., Exp. Hematology 2008
Di Ianni et al., Clinical and Exp Immunology 2009
Di Ianni et al., Blood 2011
Di Ianni et al., Best Prac Res Clin Haematol 2011
Di Ianni et al., Transfusion Apheresis Science 2012

Treg and Tcon adoptive immunotherapy in haplo HSCT for patients with high risk AL

Conditioning Regimen and Inoculum

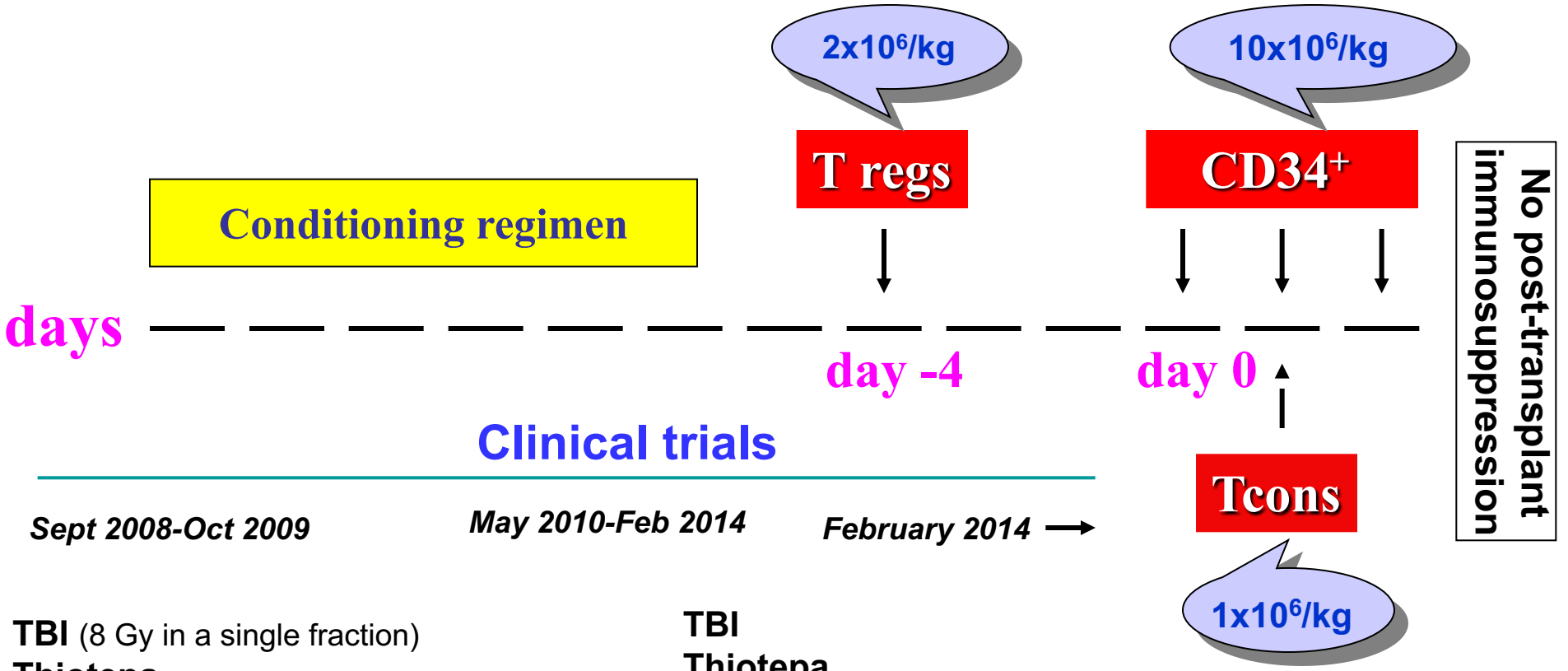


Di Ianni M et al. Blood 2011

Martelli MF et al. Blood 2014

Treg and Tcon adoptive immunotherapy in haplo-HSCT for patients with high risk AL

Conditioning Regimen and Inoculum



Clinical trials

Sept 2008-Oct 2009

TBI (8 Gy in a single fraction)
Thiotepa
(4 mg/kg per day for 2 days)
Fludarabine
(40 mg/m2 per day for 5 days)
Cyclophosphamide
(35 mg/kg per day for 2 days)

May 2010-Feb 2014

TBI
Thiotepa
Fludarabine

Anti T antibodies
Alentuzumab/
Thymoglobuline
day-21 before Tregs

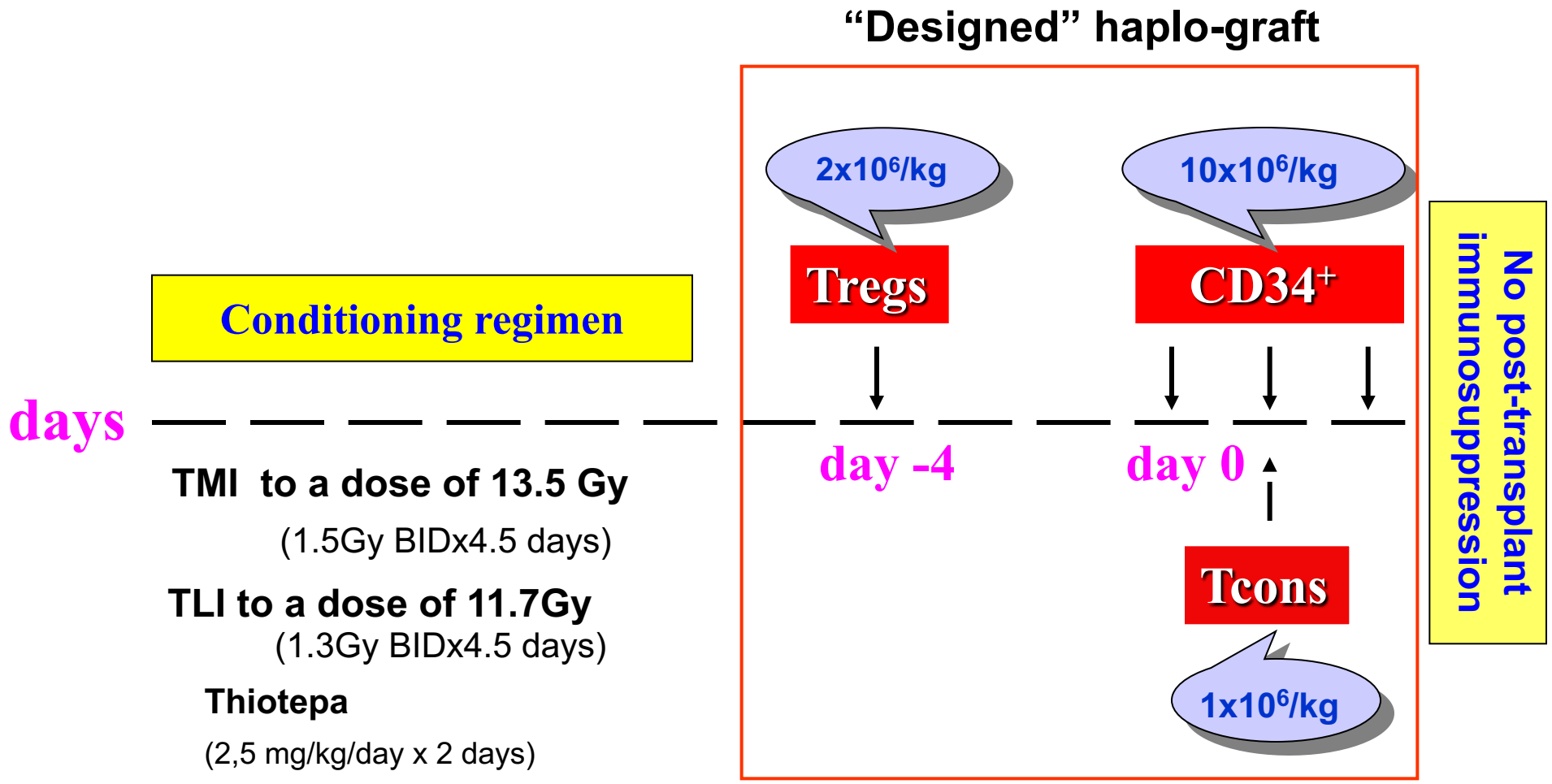
Cyclophosphamide
(15 mg/kg per day
for 2 days)

February 2014 →

Di Ianni et al.
Blood 2011

Martelli, Di Ianni et al.
Blood 2014

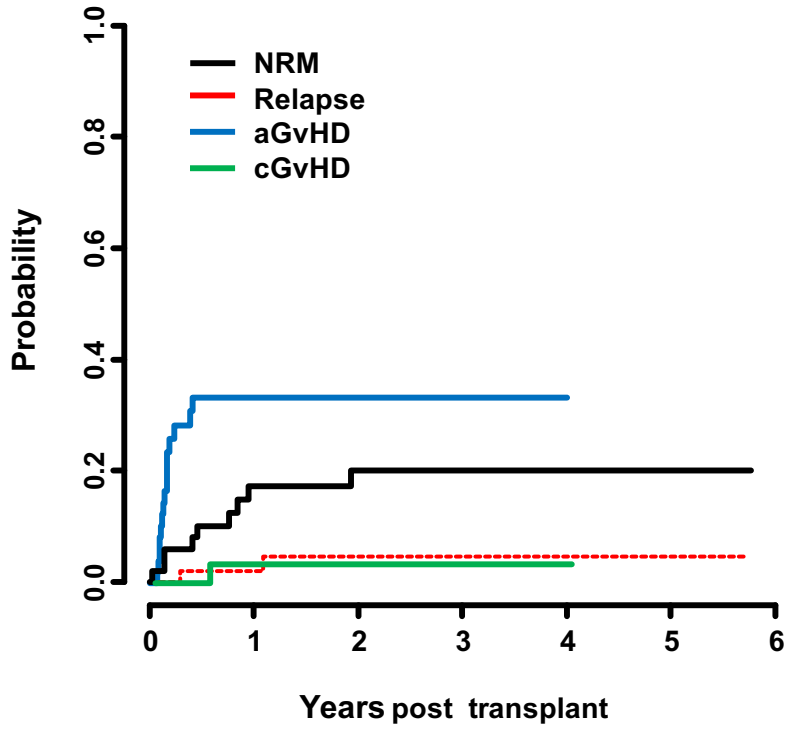
Haplo-HSCT for the elderly with high risk AML



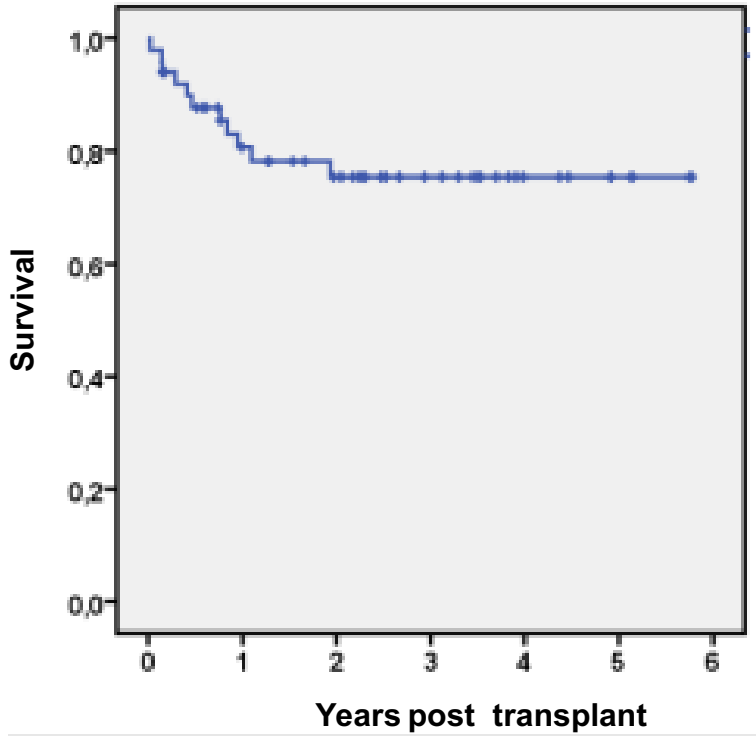
Pierini et al Blood Adv. 2021

Outcome of high-risk AML patients after haploidentical tranplantation with adoptive Tcon and Treg immunotherapy and irradiation-based myeloablative conditioning ragimen

Cumulative incidences



cGvHD/leukemia-free survival

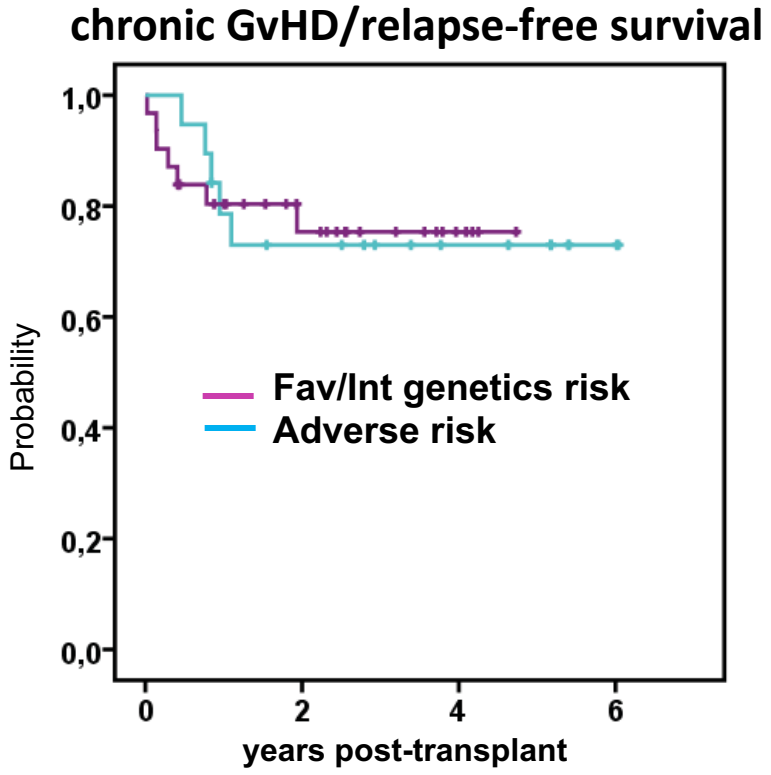


50 high risk AML patients, median age: 53 years,
31 TMLI-based conditioning, 9 sTBI-based conditioning, 10 fTBI-based conditioning

Pierini et al., Blood Adv 2021

Impact of Adverse Genetics

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



Mechanisms underlying Treg suppression of GvHD with no loss of GvL activity

Alloantigen specific Tcon activation (not expansion) triggers GvL activity

Edinger M et al. *Nature Medicine 2003*

In animal models

- Tregs inhibited early expansion of alloreactive donor T cells in lymphoid organs and their capacity to induce GVHD
- Tregs did not inhibit co-transplanted Tcon activation and cytotoxic functions against leukemia and lymphoma cell lines. Thus Tcons conserve their capacity to kill tumor cells.

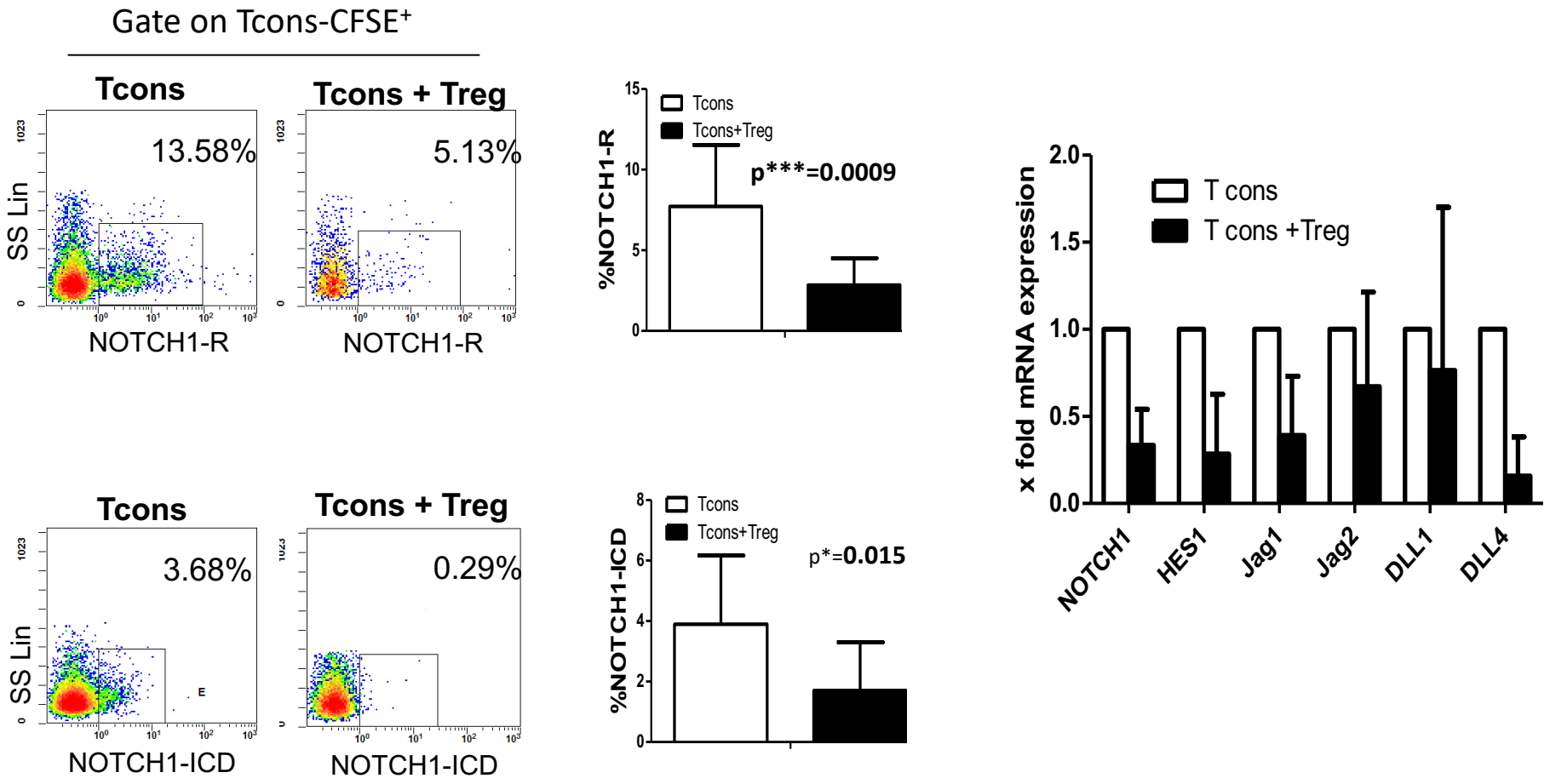
Mechanisms underlying Treg suppression of GvHD with no loss of GvL

Working hypothesis in clinical haplo-HSCT

- **NOTCH1 inhibition**

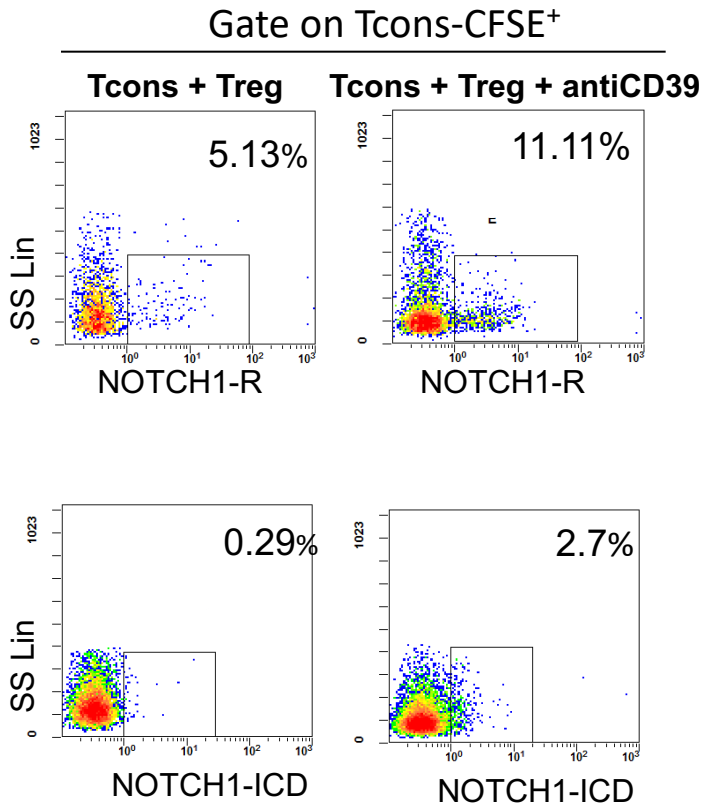
-A pro-inflammatory environment in the BM of Treg transplanted patients

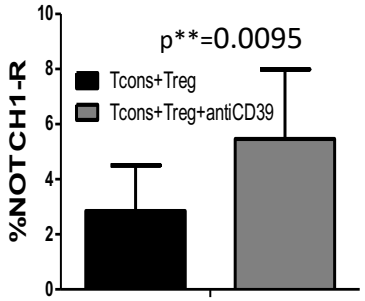
NOTCH1 signalling is down-regulated in Tcons in presence of Tregs: a new major regulator of alloreactivity and tolerance

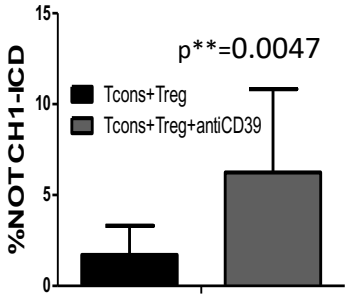


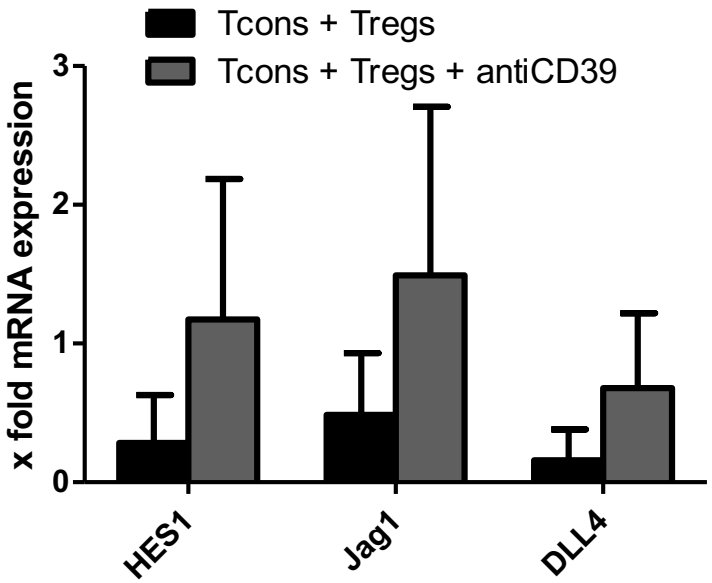
Del Papa et al., Leukemia, 2016

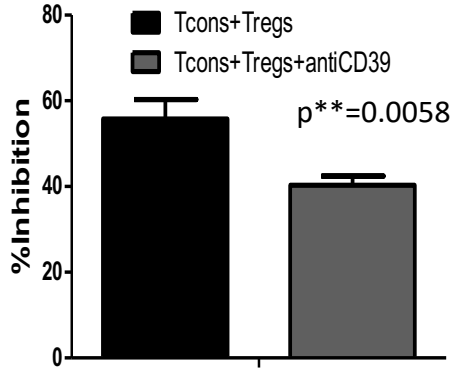
Adding anti-CD39 rescued NOTCH1 signalling





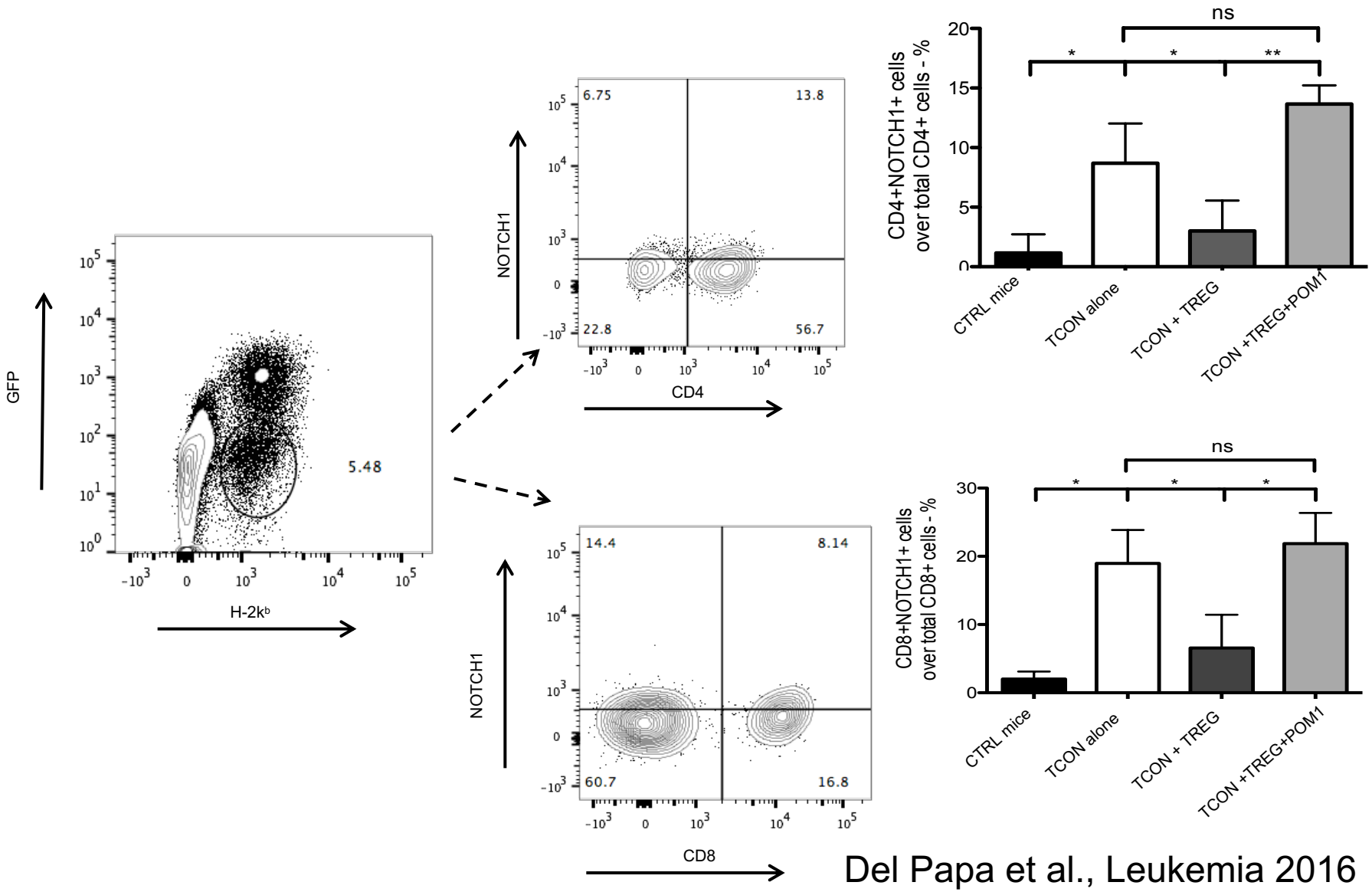






Del Papa et al., Leukemia 2016

Treg prevent NOTCH1 upregulation in TCON after transplantation through a CD39 dependent mechanism



Del Papa et al., Leukemia 2016

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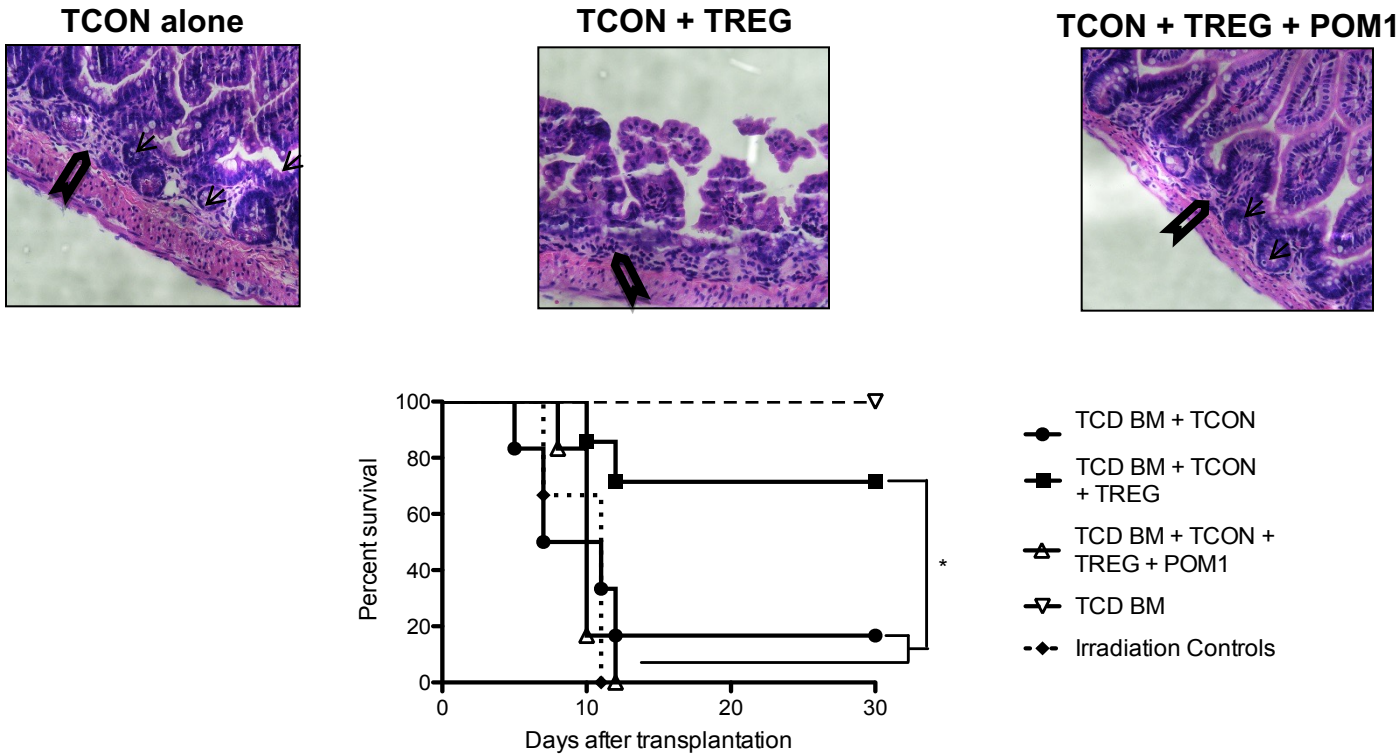


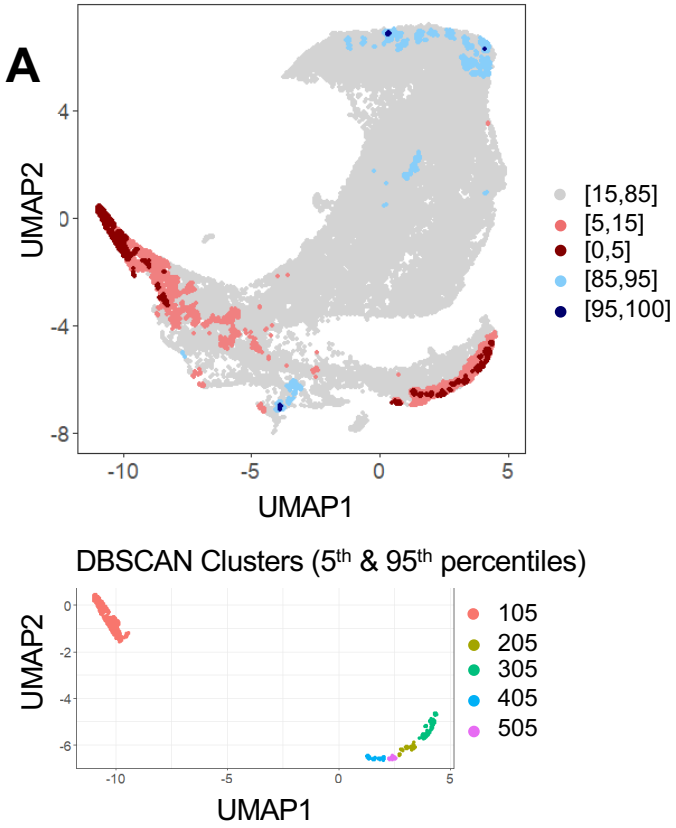
Mechanisms underlying Treg suppression of GvHD with no loss of GvL

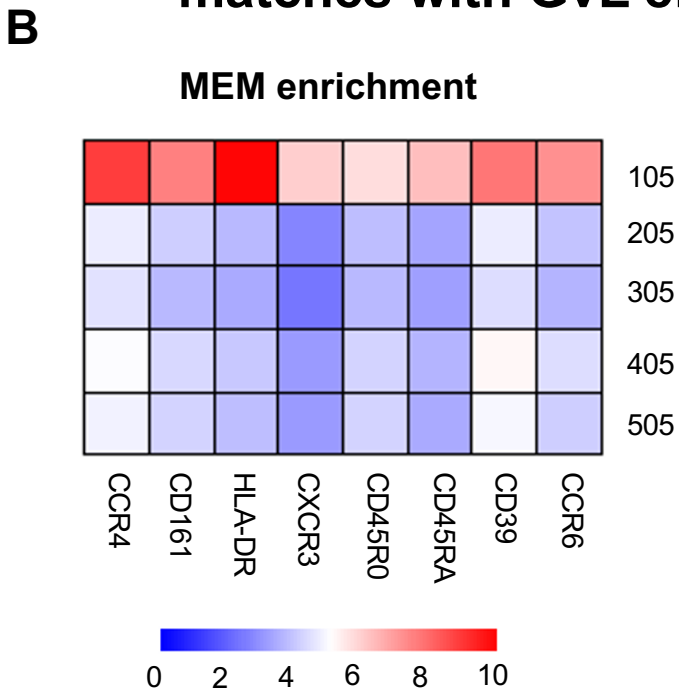
Working hypothesis in clinical haplo-HSCT

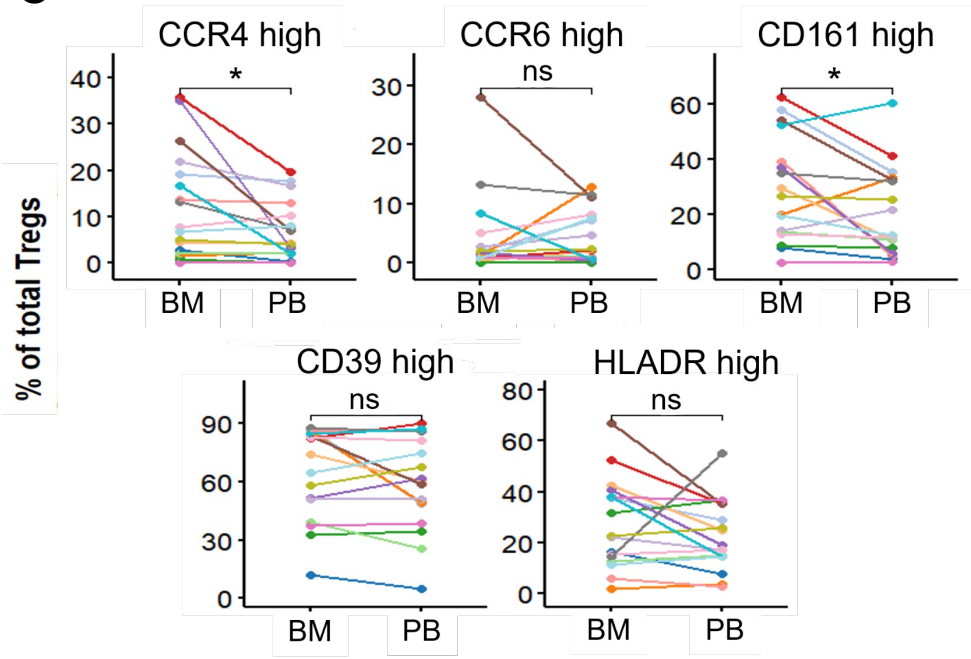
- NOTCH1 inhibition

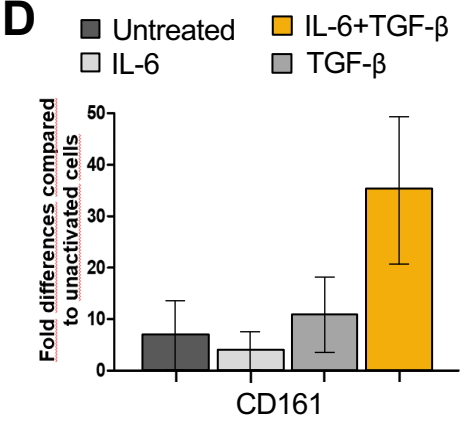
-A pro-inflammatory environment in the BM of Treg transplanted patients

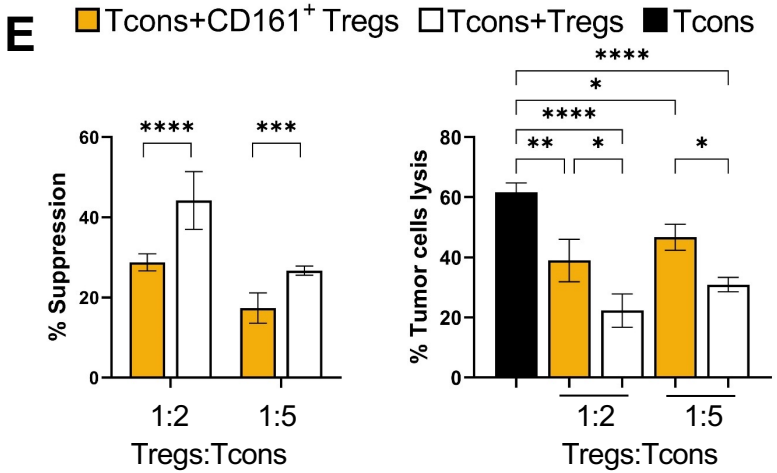
Treg CD161+ is as new marrow pro-inflammatory population that matches with GvL effect

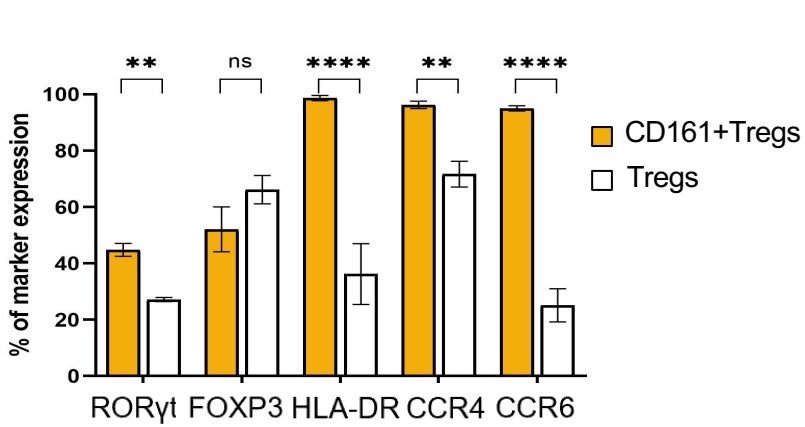












Guardalupi et al., Leukemia 2023

Tregs in haploidentical BMT: how to proceed to standardize?

Naturally occurring Tregs

Pros: Easy purification from a leukapheretic product using a fully automated immunomagnetic procedure

Cons: Relatively low number of Tregs (2-3 x10^6/Kg) can be collected from a donor

Treg in vitro activation

Pros: IL-2/TNF-α priming enhances Treg suppressive function and improves Treg gut homing for better GvHD prevention.

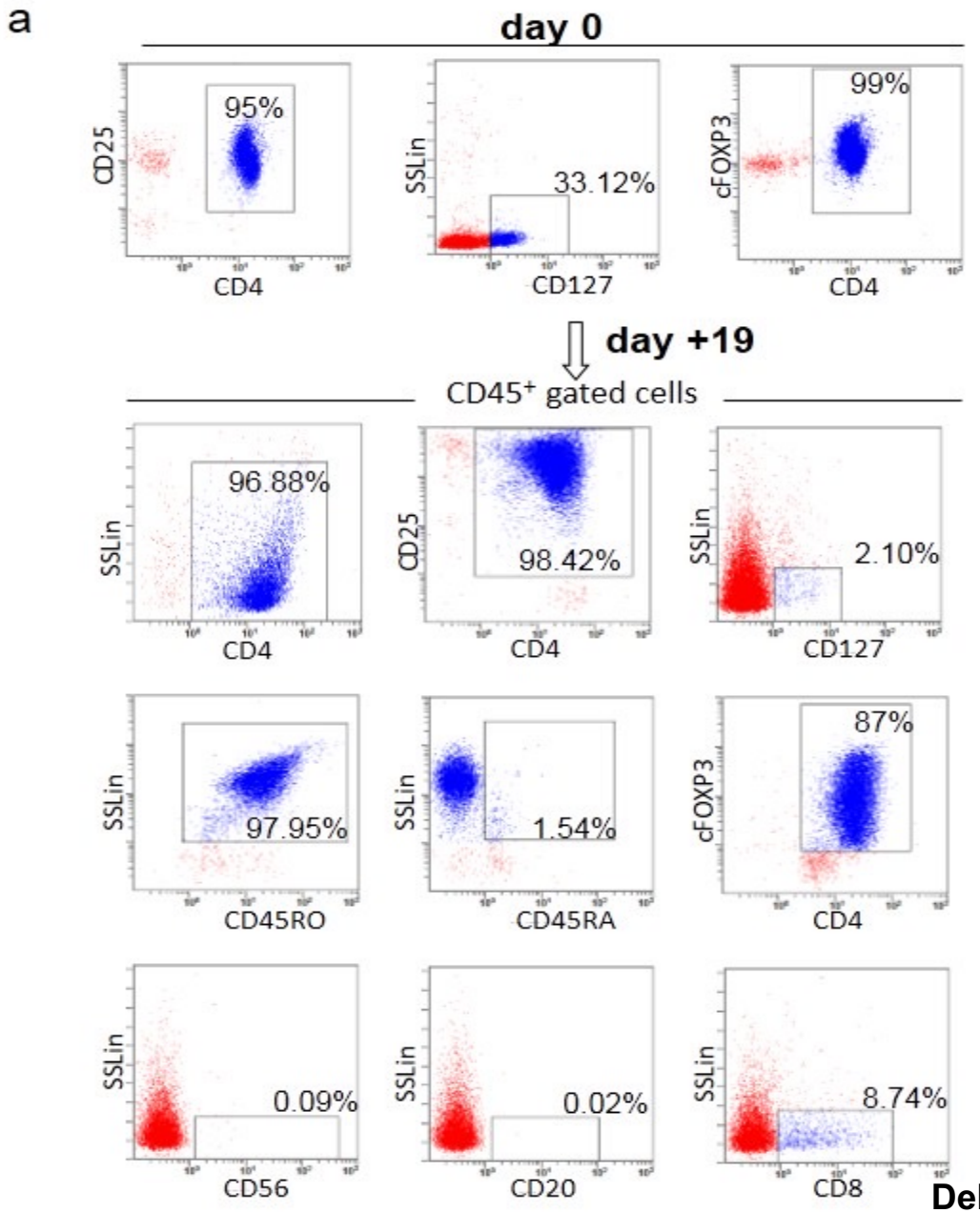
Cons: Studies are needed to assess whether IL-2/TNF-α priming can be used to safely infuse higher number of Tcons

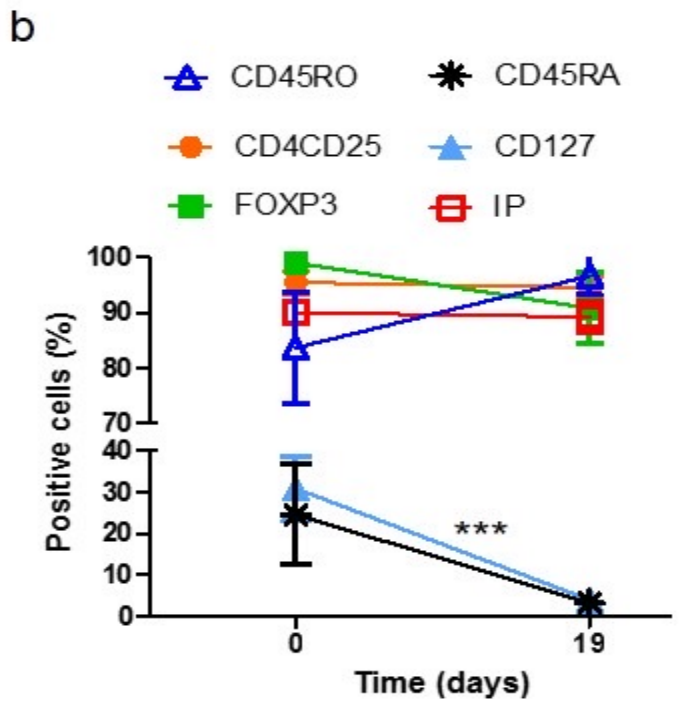
Ex vivo expanded Tregs

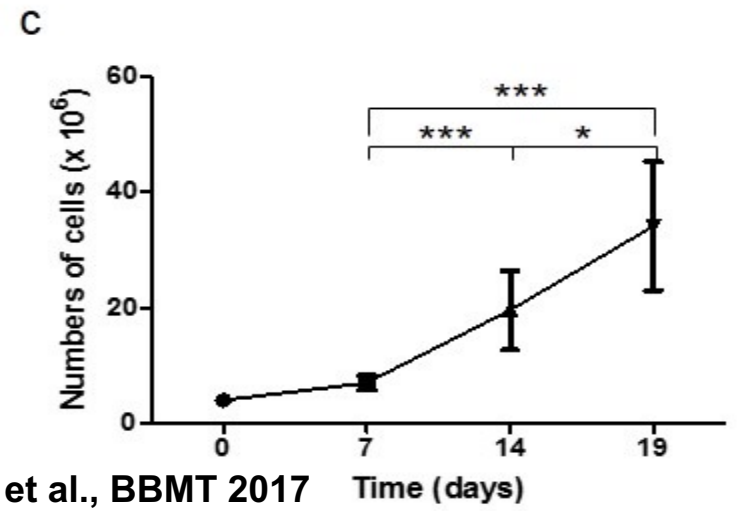
Pros: Feasibility of present technology to produce large numbers of cGMPgrade Tregs. For instance, a “designed” graft could easily include 1x10^7/Kg Tregs and 0.5x10^7/Kg Tcons

Cons: Requirement of GMP manufacture which is expensive, not always available and requires expert, dedicated laboratory staff

Expanded non automated Tregs are FoxP3^{+} and CD127^{-}

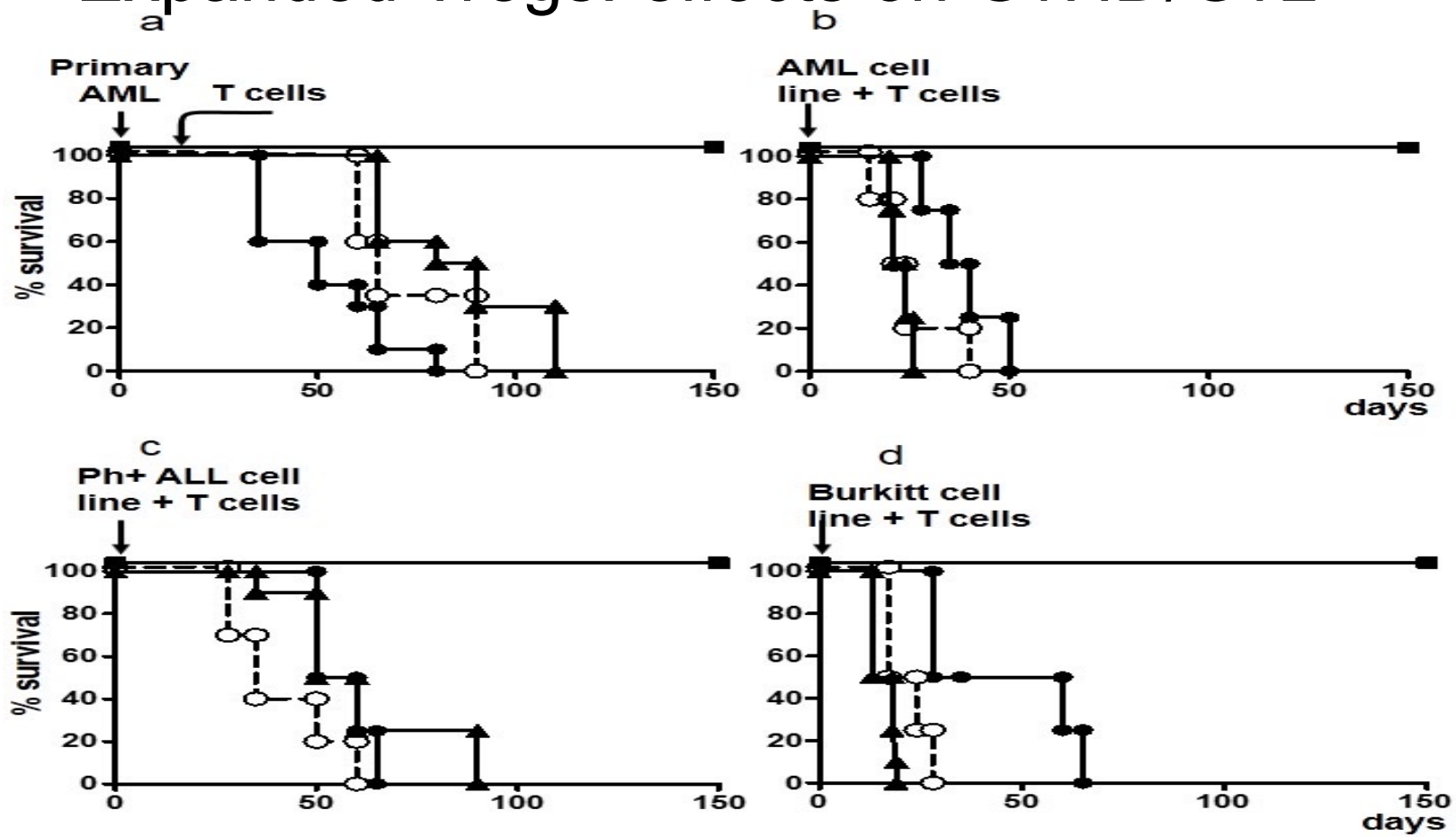






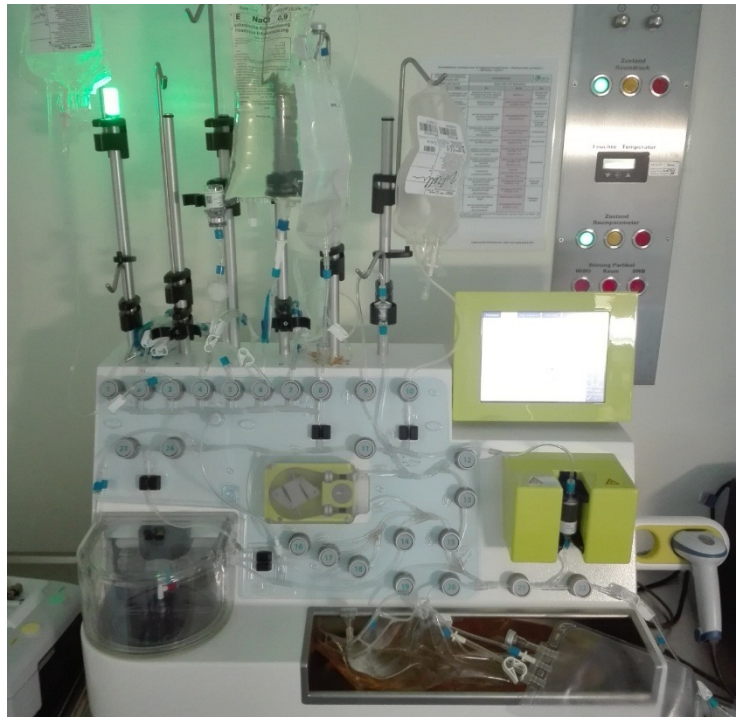
Del Papa et al., BBMT 2017

Expanded Tregs: effects on GvHD/GvL



Del Papa et al., BBMT 2017

Tregs expansion through CliniMacs Prodigy Technology

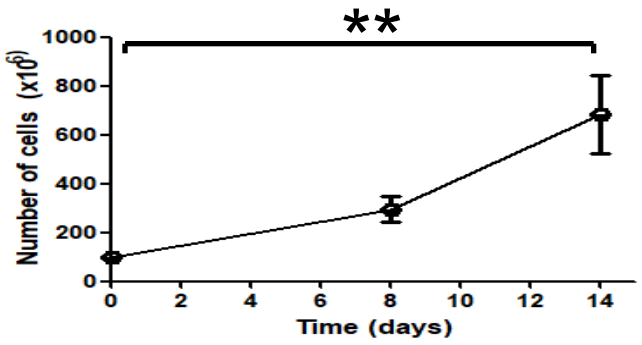


-Leukapheresis product

-CD8-CD19neg/CD25pos selection by CliniMacs

-$100x10^6$ immunoselected Tregs as starting fraction

Tregs are polyclonally activated with colloidal polymeric nanomatrix covalently attached to humanized recombinant agonists against human CD3 and CD28 and cultured for up to 14 days in presence of Rapamycine (100 nm/ml) and IL-2 (1000 U/ml)



Ulbar et al., BBMT 2020

Cellular therapies: next steps

- Haplo-Treg for AML; gamma-delta option
- For ALL?
- **Treg/Tcon HSCT could serve as a platform to allow for further post-transplant immunotherapy such as donor CAR-NK cells to cure refractory lymphoid malignancies**
- immunotherapies that engage natural killer (NK) cells are particularly intriguing as NK cells are expected not to cause GvHD (Ruggeri et al., Science 2002)
- **we hypothesize donor NK cells that have been engineered to express a CAR directed against CD19 combined with Treg/Tcon HSCT may represent a powerful tool against relapsed/refractory lymphoid malignancies such as ALL without causing GvHD**
- As already showed (Liu et al., NEJM 2020) CAR-NK may found a place in any malignant cancer CD19 positive outside of the transplantation procedure