

Corso di Formazione **GITMO**

Trapianto e terapie cellulari: cellule, donatori e condizionamenti

Ruolo della dose e della composizione del graft nell'outcome del trapianto allogeneico

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Udine, 8-9 Ottobre 2026

Aula Polifunzionale Ospedale di Udine

Disclosures of Name Surname

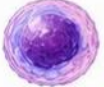
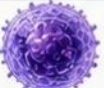
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No disclosures							

Graft composition

BM TNC $1-4 \times 10^8/\text{kg}$

PBSC TNC $6-16 \times 10^8/\text{kg}$

CB TNC $1-2 \times 10^7/\text{kg}$

		Bone Marrow	Peripheral Blood	Cord Blood
	Hematopoietic stem cells	2(0.8-10.4)*- 1-4%	4-6(2-28)*- 0,6-1%	0,1-0,15*- 0,5-1%
	T lymphocytes	23 (14-32)* 5-8%	240(130-409)*-30%	
	Regulatory T cells	10% T-ly 0,3-0,8%	12% T-ly - 1-4%	
	B lymphocytes	8 (5-14)* 2-3 %	48(21-97) 3-6%	
	Gamma-delta T lymphocytes	3 % T-ly 0,1-0,4%	2% T-ly 0,2-0,7%	
	Natural killer cells	1,9(1-3,7)* 0,4-1,4%	18(8-31) 2-4%	
	Dendritic cells	0,7(0,3-2)* 0,2-0,3%	1,2(0,3-3,3) 0,1%	
	Monocytes	 4-10%	 8-10%	
	Myeloid derived suppressor cells	 0,3%	 0,38%	
	Neutrophils	 30%	50%  60%	

*x 10^6 ; % delle TNC

Waller EK et al. JCO 2014; Reshef R Transf and Apher Science 2021; Gu G et al. Cell. Immunol 2018; Borrill R et al Frontiers in pediatrics 2023; AI-created image

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Graft composition: it is not all about CD34+ cell dose

Characteristics	Peripheral Blood	Bone Marrow	Cord Blood
T-ly	PBSC > 1log vs BM (all subset except CD8/69+)		T-ly naive >> EM, CM
R CD4/CD8	3	1,3	CD-4 >> CD8
% T-reg and T- $\gamma\delta$	$\approx$	$\approx$	T-reg enriched
NK	PBSC > 1 log vs BM		T- $\gamma\delta$ << PB
NKT	PBSC vs BM r 6:1		NK enriched
B-ly	PBSC vs BM r 6:1		Expansion, longevity
NK-56bright	2%	0,5%	< alloreactivity
M-DC	n. PBSC $\approx$ BM; % PBSC < BM		0,3-0,4%
P-DC	n. PBSC $\geq$ BM ; % PBSC < BM		P-DC >>> M-DC
MSC	0-1:10 ⁶ TNC	1:10 ⁴ -10 ⁵ TNC	% < BM
MDSC %	$\approx$	$\approx$	>
Telomer lenght	shorter	intermediate	longer
Oct4-stemness marker expression	low	moderate	high

Waller EK et al. JCO 2014; Reshef R Transf and Apher Science 2021; Gu G et al. Cell. Immunol 2018; Borrill R et al Frontiers in pediatrics 2023

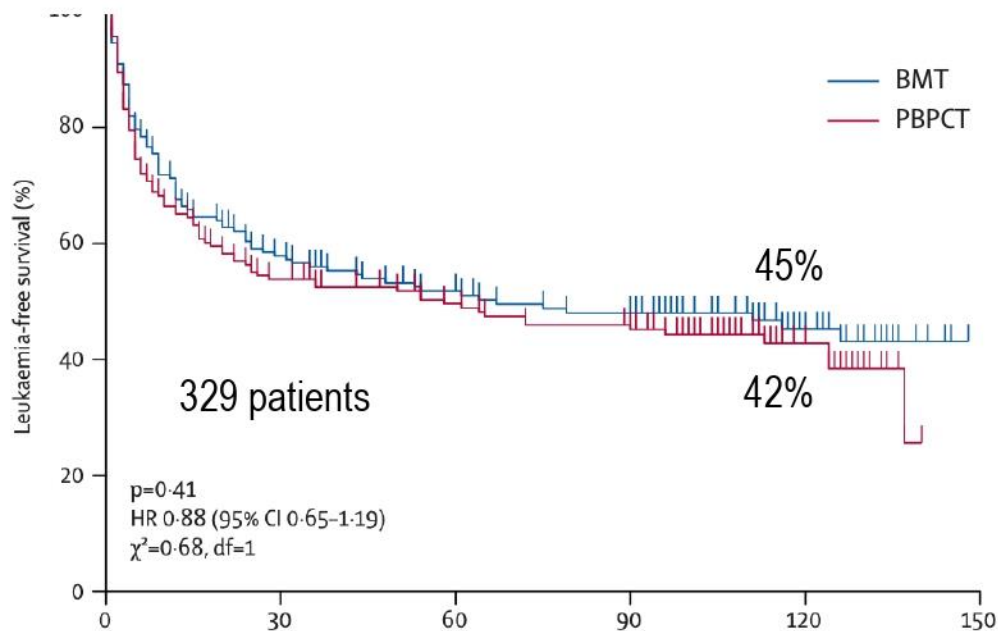
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PBSC vs BM in allo-SCT: matched related donor pre-ATG era

Friedrichs et al. Randomised trial Lancet Oncol 2010



cGVHD 56 vs 64% **p=0.001**

Relapse 24 vs 20% p=NS

Holtick U et al Meta-analysis Cochrane Library 2014

Relapse BM vs PB HR 2,7 **p=0.001**

cGVHD BM vs PB HR 0.72 **p=0.0001**

aGVHD BM vs PB HR 1.03 p=NS

Graft composition and outcome

BM studies (2001-19)	outcome (studies)	PBSC studies (2001-22)	outcome (studies)
CD34 >3.0x10 ⁶ >4.0x10 ⁶	- OS/engraftment (4/8) + aGVHD (1/8)	4-5> CD34 ≤8 x 10 ⁶ >6-8x10 ⁶	+ OS (10/15) - cGVHD (5/15) + aGVHD (3/15; no ATG)
CD3 & subset CD3 >38x10 ⁶ CD4 >10x10 ⁶ CD8 >10x10 ⁶	+ aGVHD (1/6) + aGVHD (1/6) + engraftment (1/6)	CD3 & subset CD8 >133x10 ⁶ CD8 >72x10 ⁶	+aGVHD (1/11) (RIC-ATG) +RFS (1/11) (RIC-no ATG)
CD19 >0.91x10 ⁶ (pro-B)	- aGVHD (1/6)	CD19 >84 x10 ⁶ (mature)	+aGVHD/TRM (2/6)
NK < 0.91x10 ⁶	+ cGVHD	NK < 30x10 ⁶ > 18-32x10 ⁶	+ OS (1/7) + OS/- aGVHD/-Rel (3/7)
DC pDC>0.8x10 ⁶	-EFS/+Rel -cGVHD (1/6)	DC >pDC >mDC	+Rel (2/5) +aGVHD/+TRM - OS (2/5)
>T-reg	+OS/acGVHD (meta-analysis 4/11 BM, 3/4 MRD; 10/11 PBSC 8/10 MRD)		
>T-v/δ	+RFS/-Rel/-virus (meta-analysis 7/11 BM; 7/7 MRD; 8/11 PBSC 4/8 MRD)		

Rocha W et al. Exp Hematol 2001; Gu G et al. Cell. Immunol 2018; Gaziev J BMT 2012; Larghero J et al. BJH 2007; Fabrizio V et al Ped Transpl 2018; Vela-Ojeda et al Ann Hematol 2006; Cao TM et al Exp Hematol 2005; Gauntner TD et al Transpl Cell Therapy 2022; Mohty M et al Exp Hematol 2004

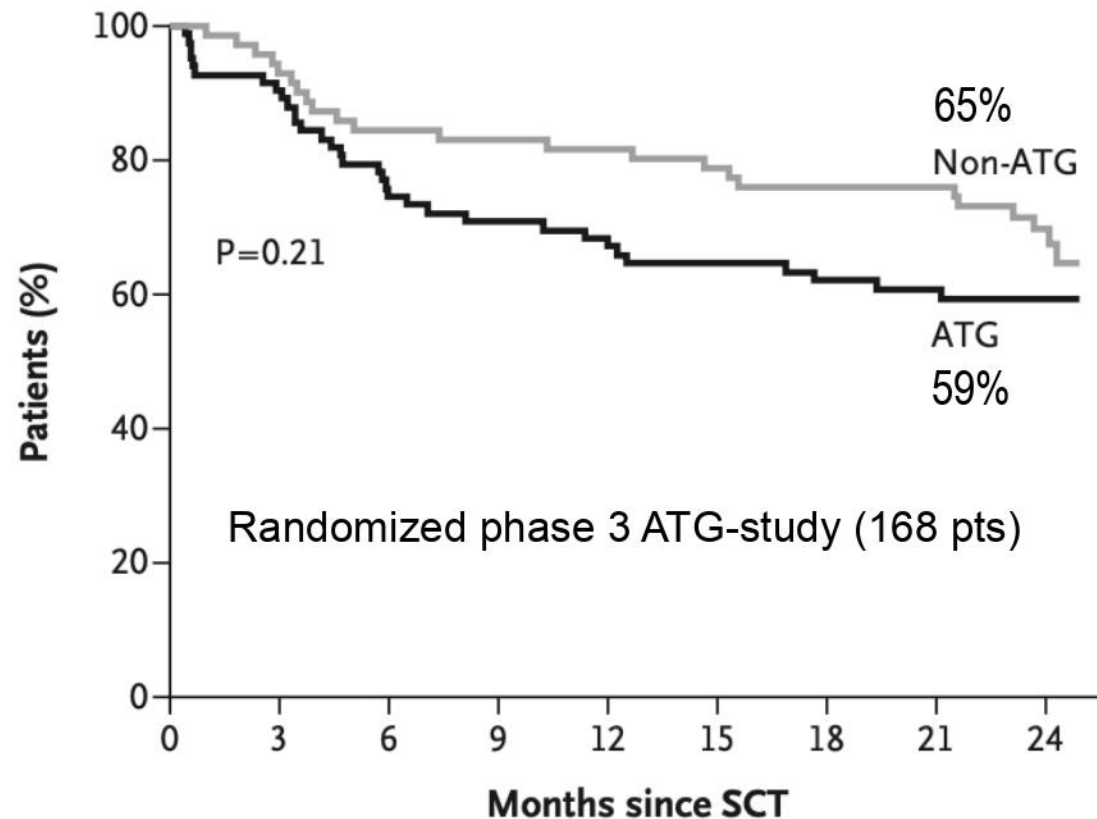
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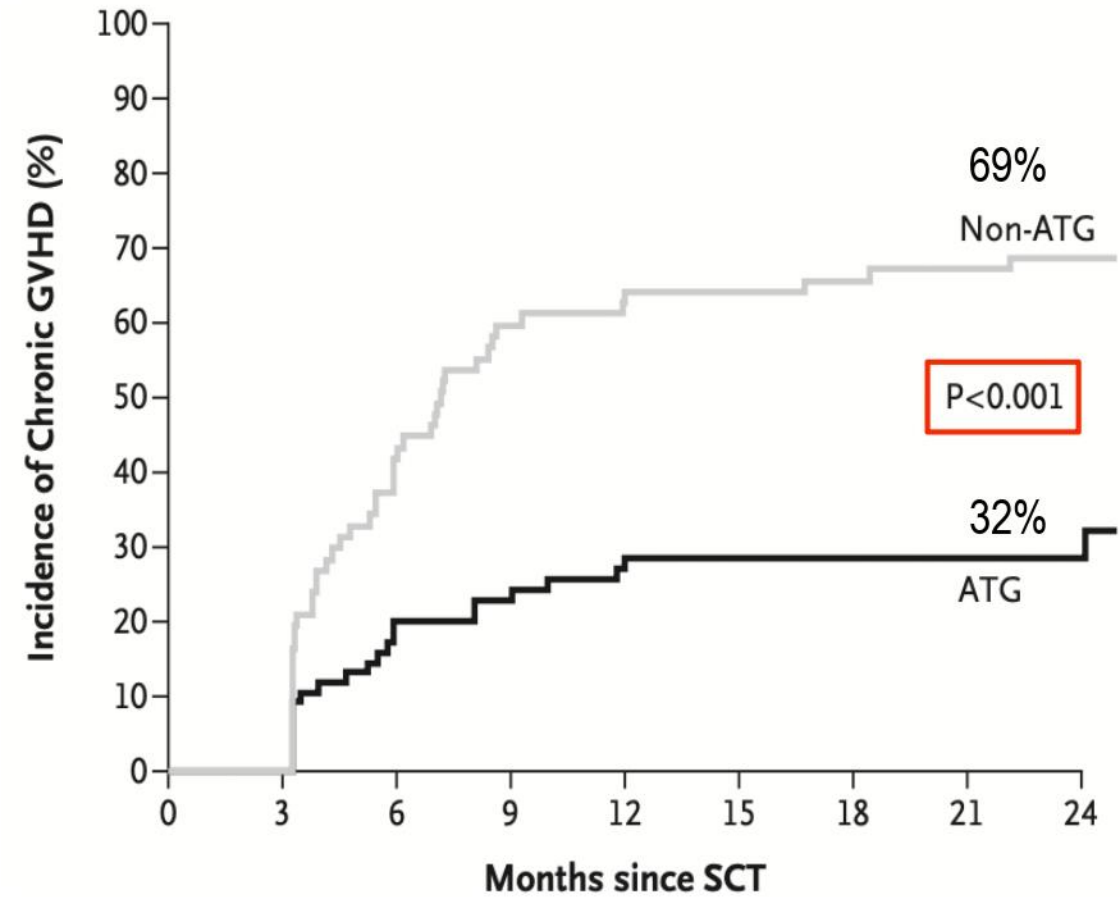
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PBSC allo-SCT matched related donor in ATG era

Relapse-free Survival



Relapse 25 vs 32% p=NS

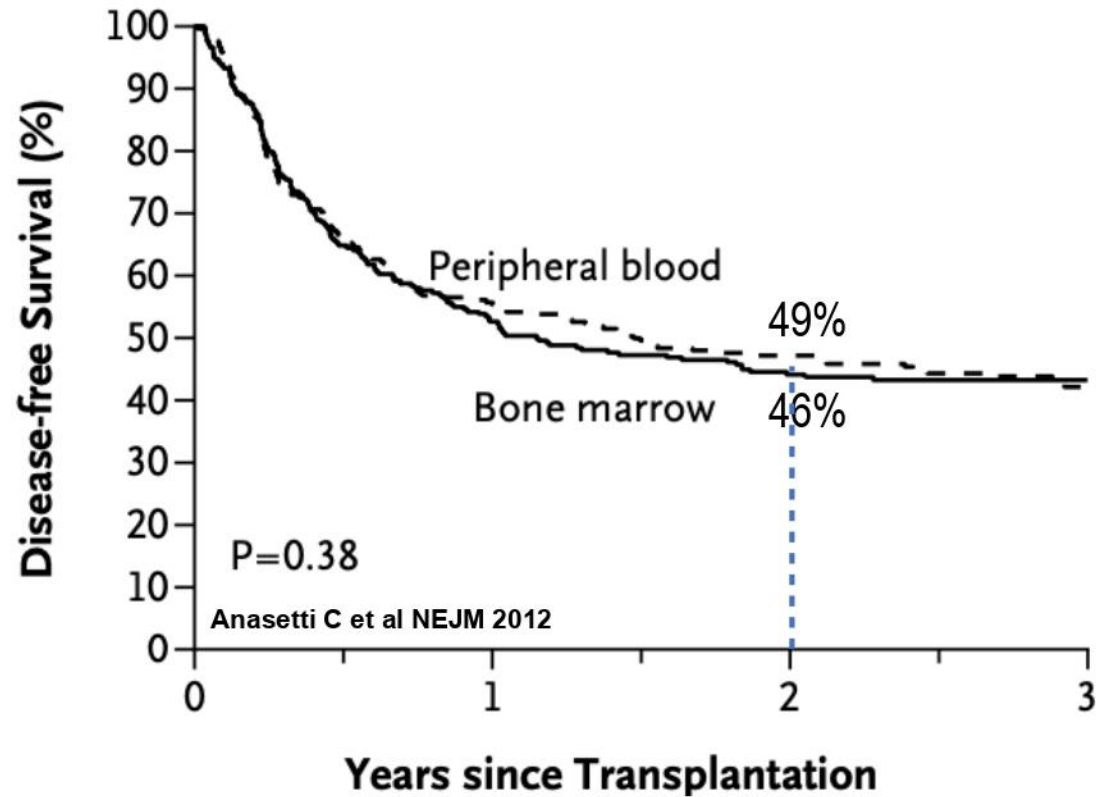


aGVHD 18 vs 11% p=NS

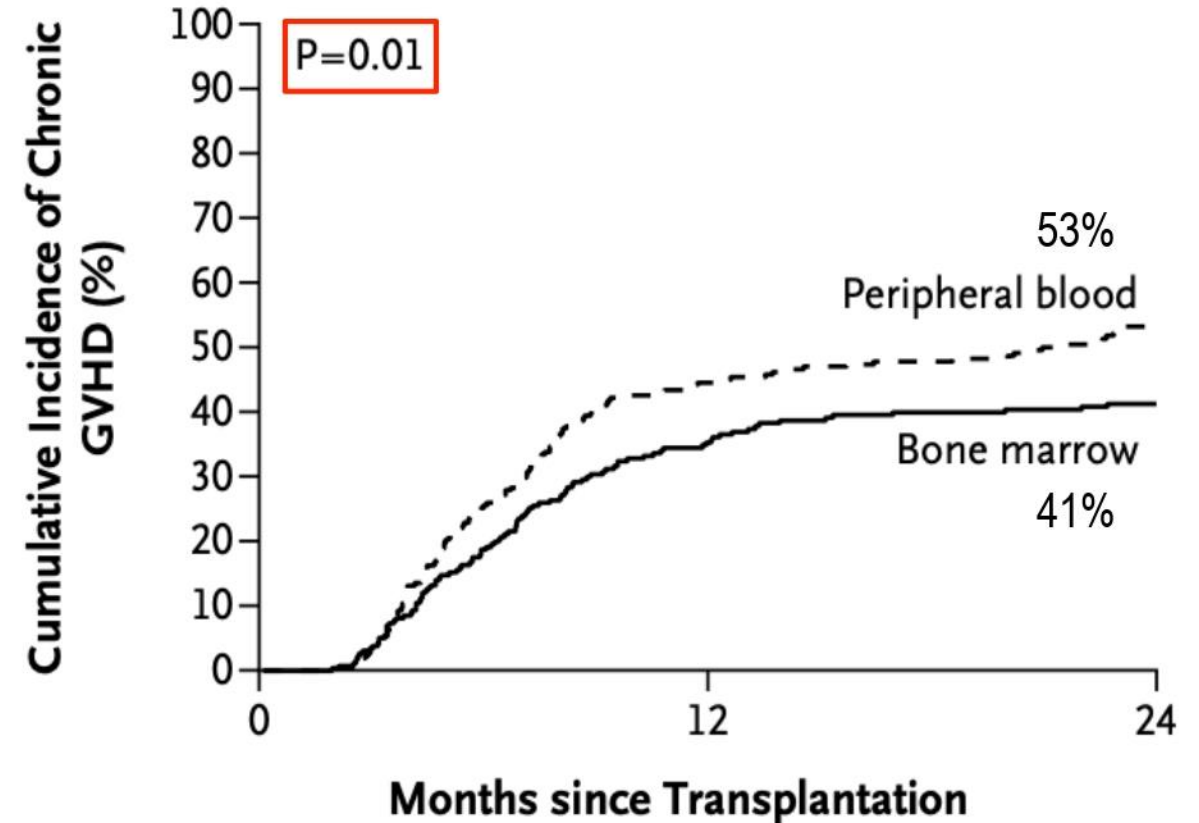
Kroger N et al NEJM 2016

PBSC vs BM in allo-SCT: matched unrelated donor pre-ATG era

Randomized phase 3 BM vs PBSC (551 pts)



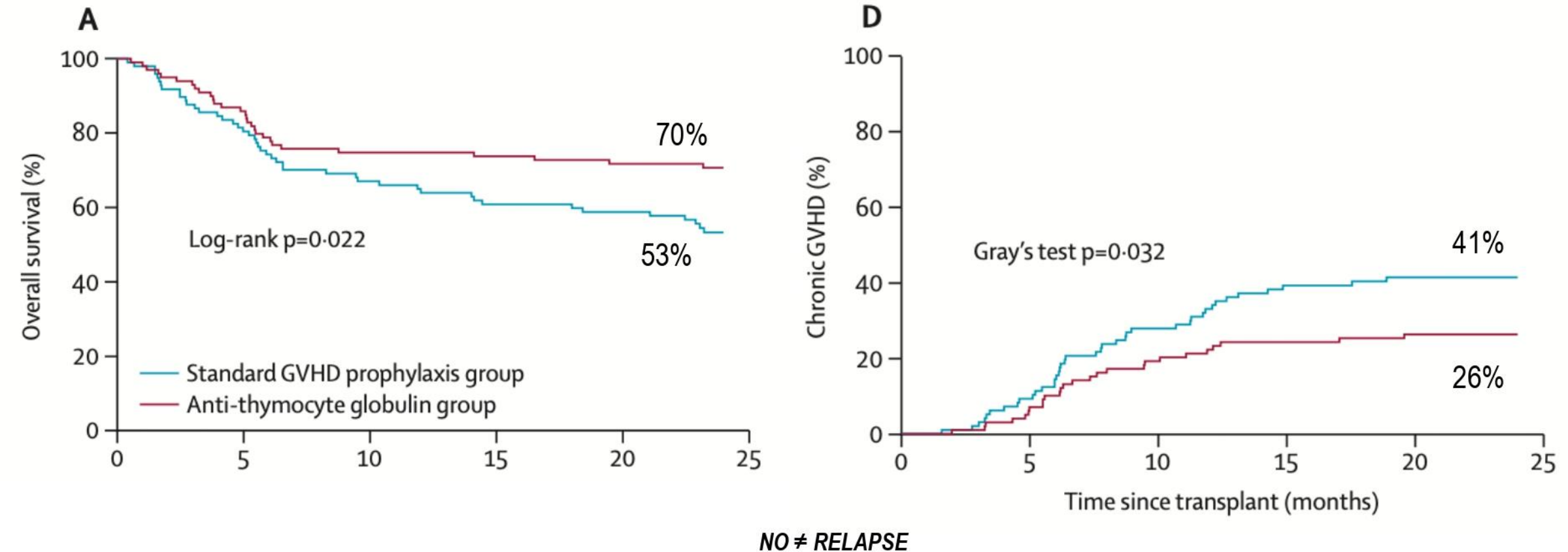
Jo T 2022 Japan Registry DFS 3y 52 vs 48% p=NS (ATG 11%)
Ramahi JS 2023 USA DFS 1y 63 vs 62% p=NS (no ATG)



aGVHD 31 vs 37% p=NS (III-IV<11%); cGVHD 40 vs 31% **p<0.025**
aGVHD 52 vs 50% p=NS (III-IV≤12%); cGVHD 55 vs 39% **p<0.014**

PBSC vs BM in allo-SCT: matched unrelated donor in ATG era

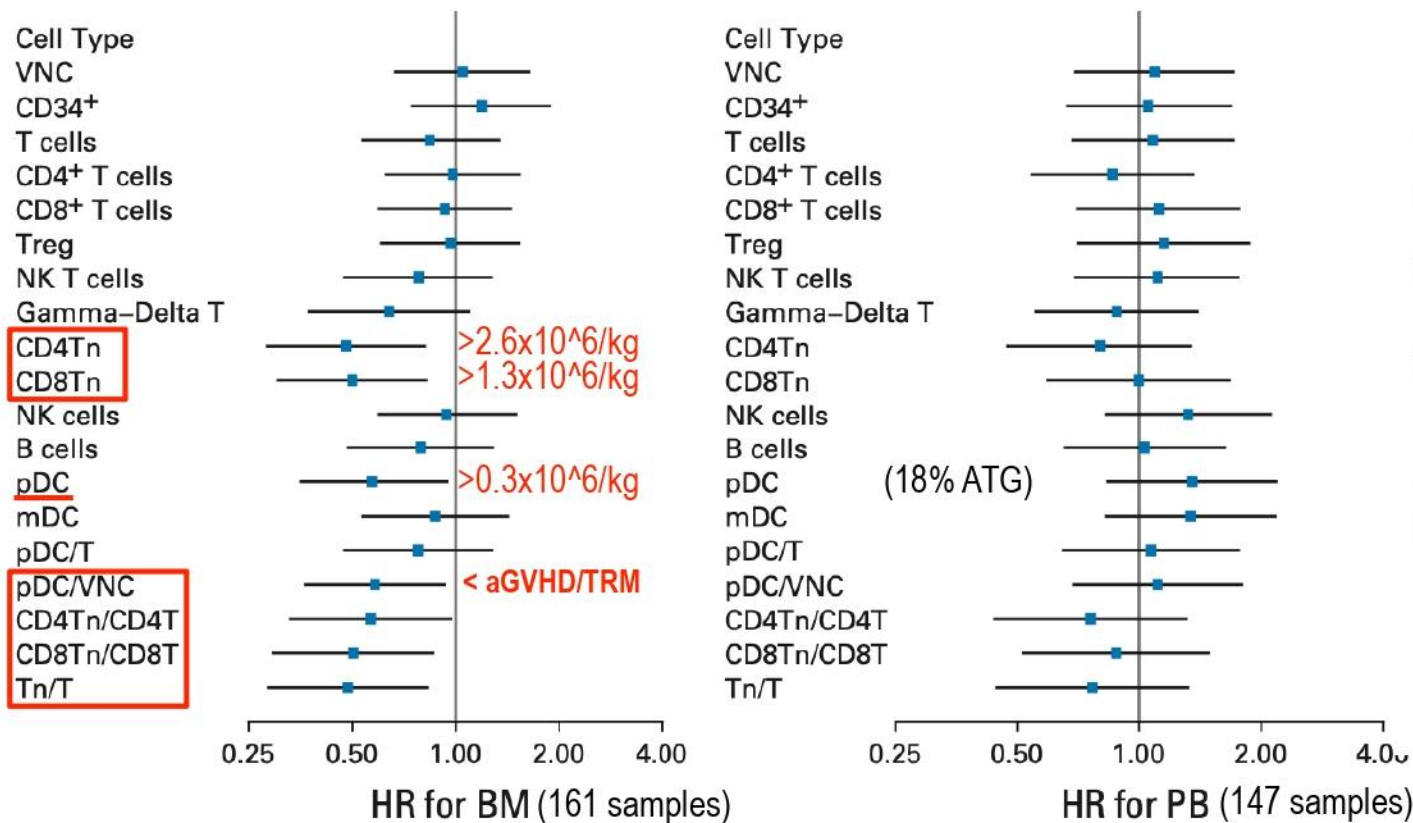
Randomized phase 3 ATG-study (203 pts MMUD <20%; 67-68% MAC)



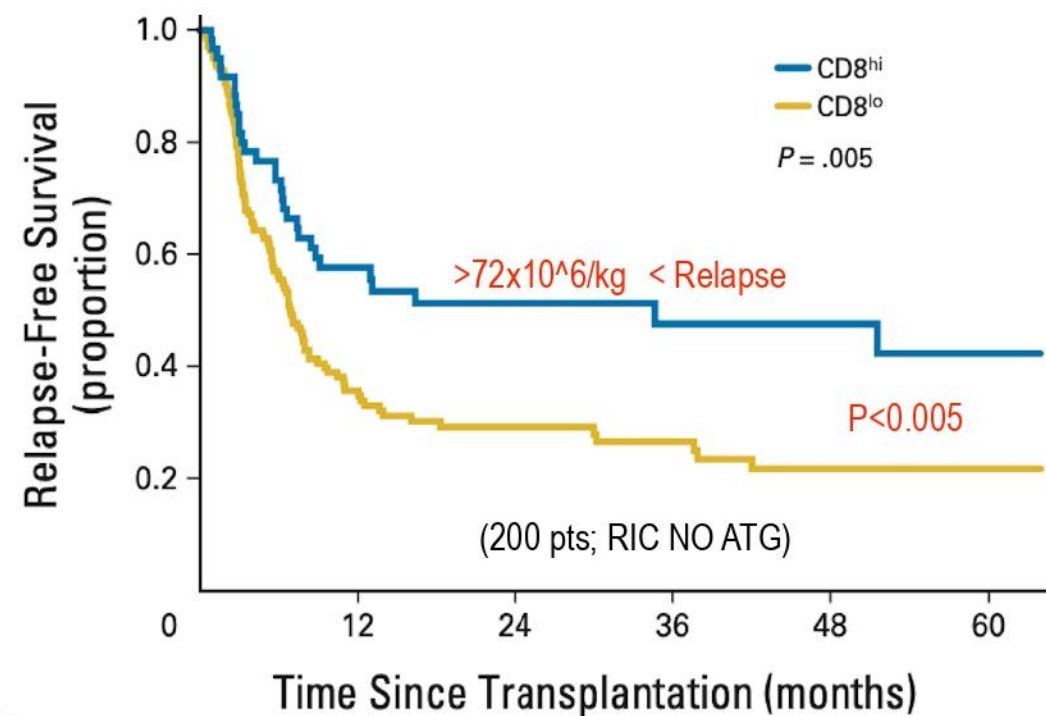
Walker I et al Lancet Hematol 2020

Graft composition and outcome: BM vs PBSC in unrelated donor

OS Multivariate analysis of BM and PBSC graft constituents Waller JCO 2014



DFS Multivariate analysis of PBSC graft constituents Reshef R JCO 2015



Czerw et al 2016 (RIC; 82% ATG) CD3 > 347x10⁶/kg > aGVHD

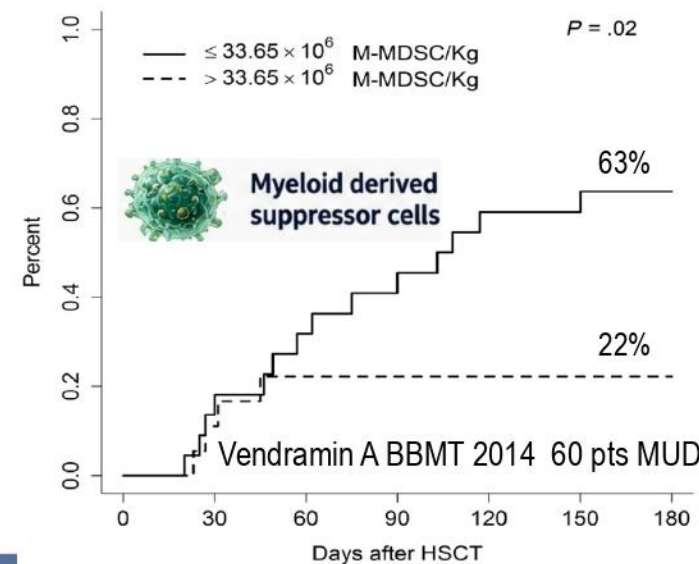
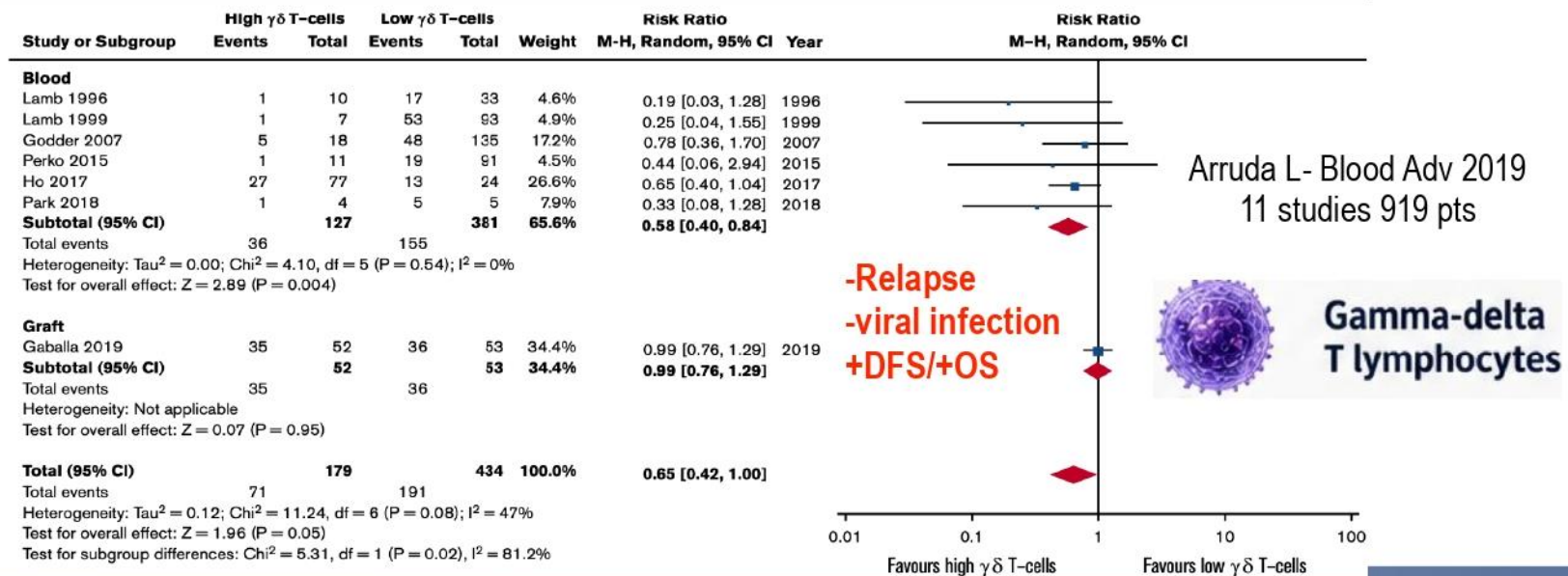
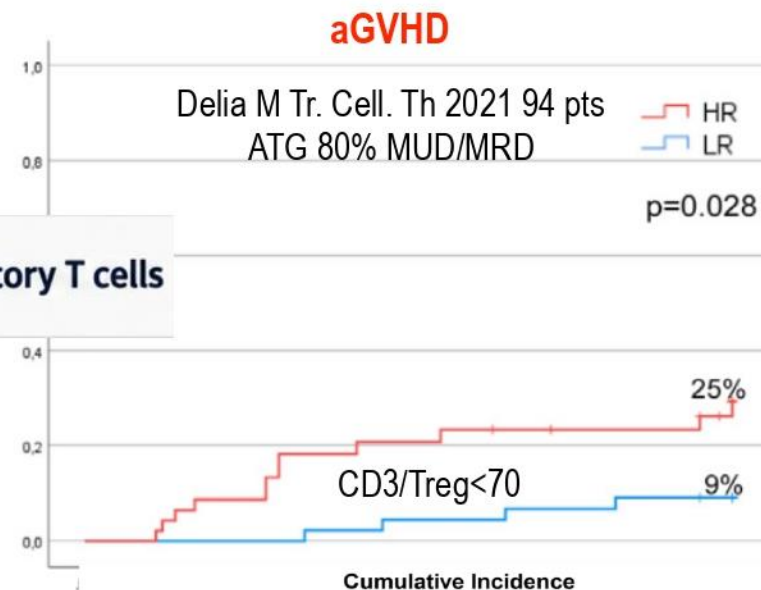
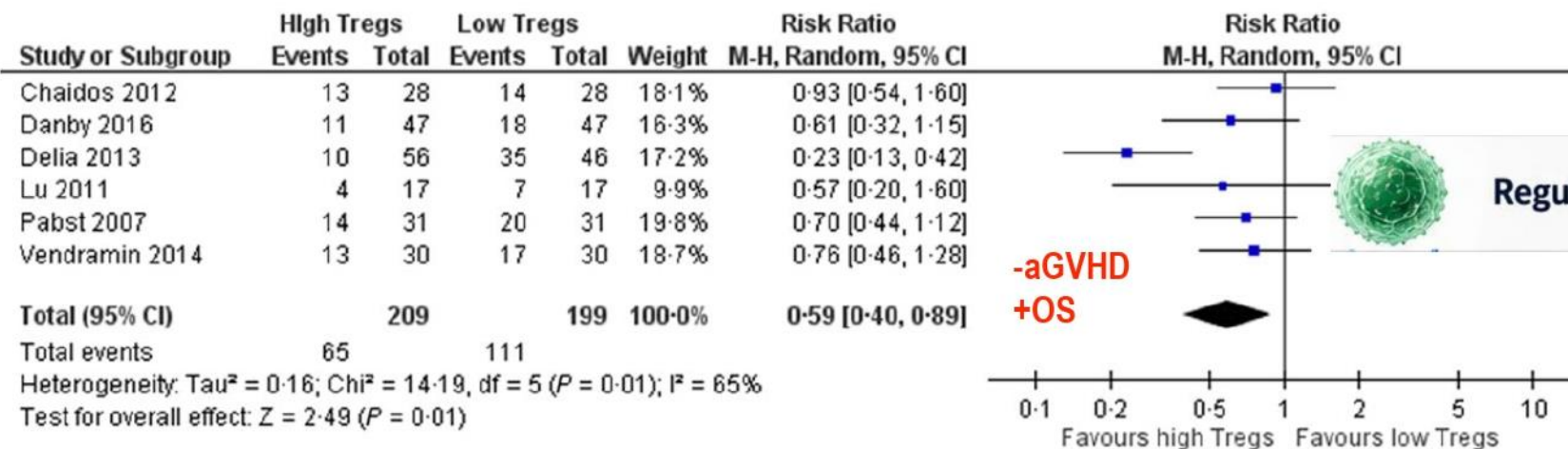
Svenberg et al 2019 (RIC; ATG) CD8 Relapse HR 0.94

Maggs et al 2017 (MAC/RIC; all TCD ATG 26%) NK-CD56^{dim} > 6.3x10⁶/kg < Relapse

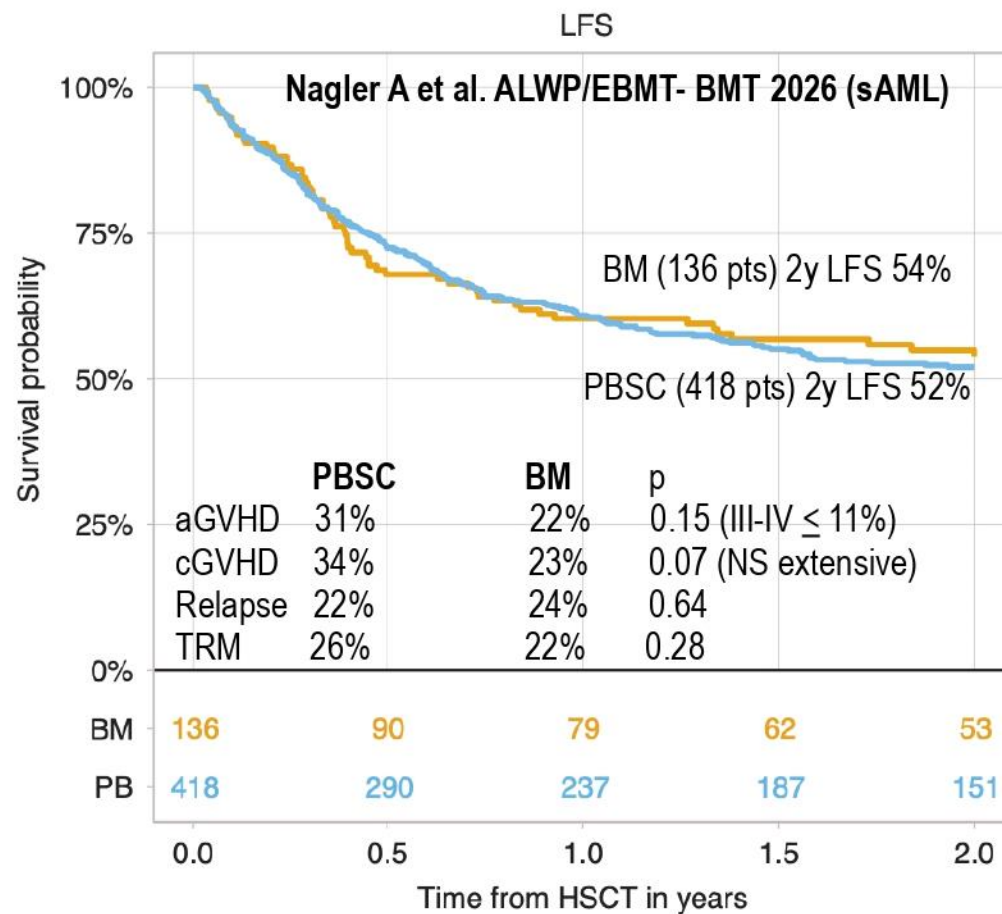
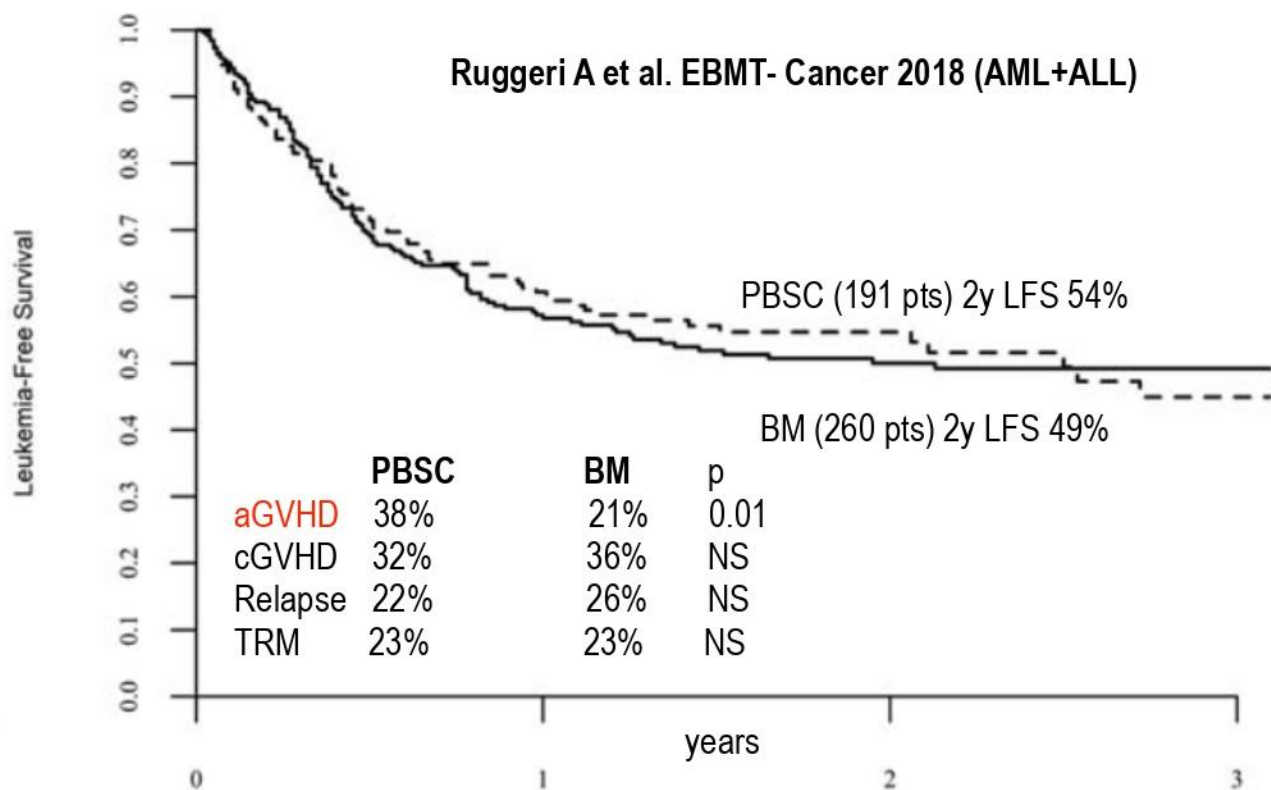
Waller EK et al. JCO 2014; Reshef R et al JCO 2015; Urbini et al Exp Hematol 2003

Graft composition and outcome: role of T-reg and “unconventional” cells

Acute Graft versus Host Disease Fisher SA - BJH 2017 11 studies 899 pts



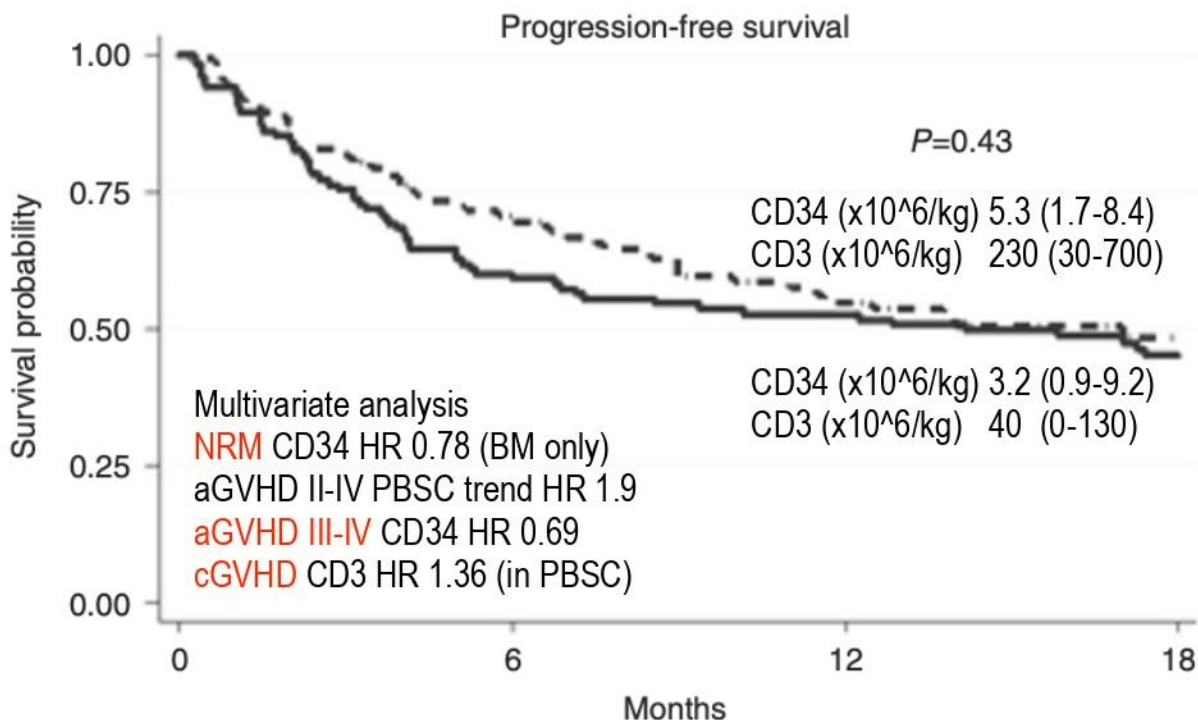
PBSC vs BM in haploidentical transplant using post-transplantation Cyclophosphamide (ptCY)



Castagna L et al. BBMT 2014 (NMA mixed diagnosis) No $\neq$ in outcome
Shahzad M et al. Blood 2024 meta-analysis (12 studies, 11604 pts, mixed diagnosis):
DFS PBSC=BM; Relapse PBSC<BM (2y; = 3y); aGVHD PBSC>BM (III-IV p=NS)

Graft composition and outcome: BM vs PBSC in haploidentical transplant with pTCy

Mussetti A et al. - BMT 2018 (234 pts)



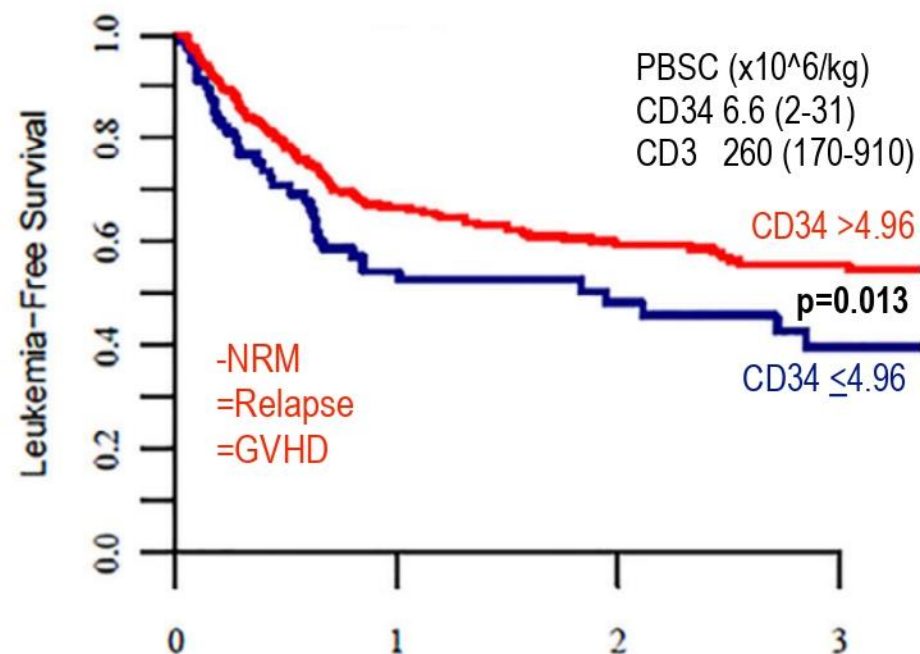
Saliba RM et al Haematologica 2021 (BM 147 pts)

+PFS: balanced CD4/CD8 (0.85-1.5), $\text{NK} \geq 2.5 \times 10^6/\text{kg}$

-NRM early: CD4/CD8 < 0.85, CD19 < $2.5 \times 10^6/\text{kg}$; NRM late CD4/CD8 > 1.5

-aGVHD: CD56 < 6%

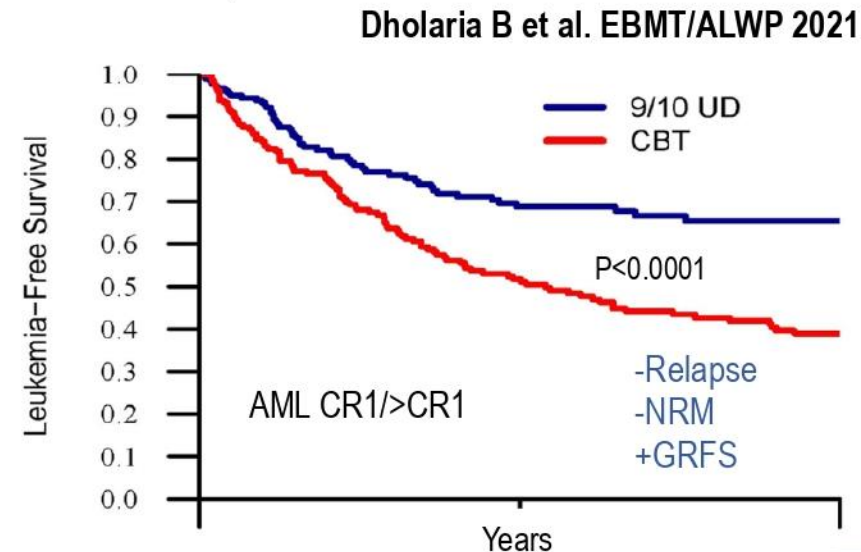
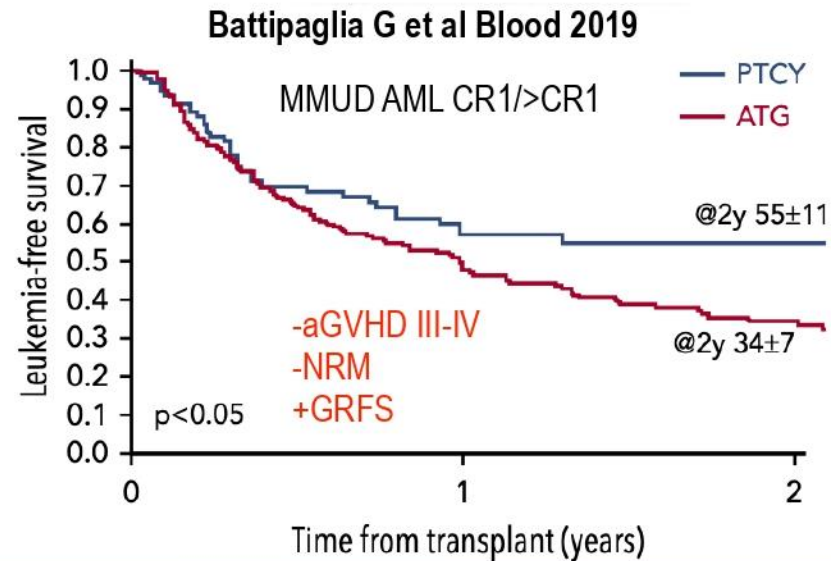
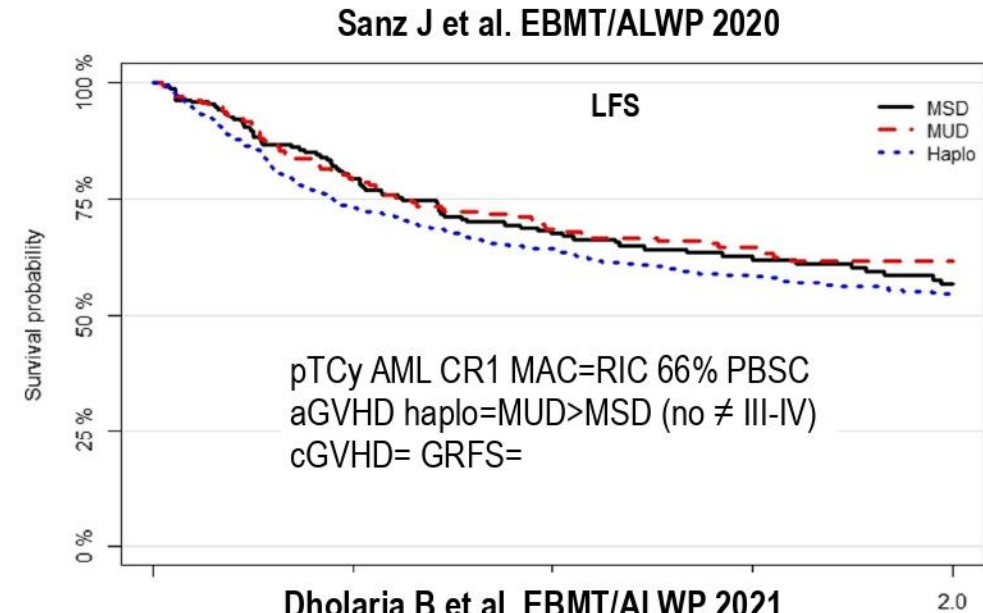
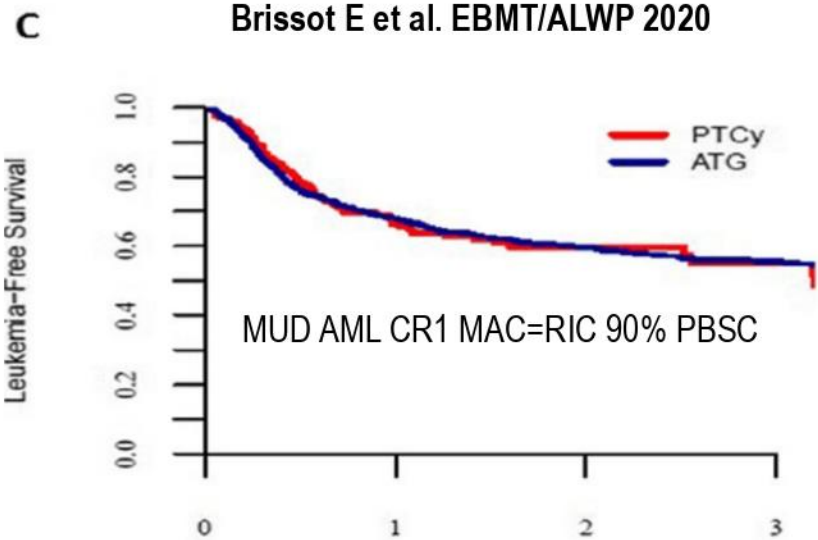
Maffini E et al. - AJM 2020 (414 pts AML; 30% ATG)



Garnier A et al. Ann. Hem 2019 (77 pts; 73% haplo RIC): no effect of CD3 or CD34

Pedraza A et al. Transp. Cell. Th. 2023 (221 pts; 20% haplo): no effect CD3 but CD34 capped at 8 and CD 3 < 400

ATG and pTCy effect: is the gap between MSD, MUD and haplo-SCT closing?



Devine S et al
EBMT abstr. 2025
CIBMTR 13.336 pts

	2y-OS
MSD	70%
MUD	66%
MMUD pTCy	66%
Haplo pTCy	62%
CB	57%
GRFS MMUD>haplo>CB	

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Overview

- Optimal CD34 dose for PBSC: range $4-8 \times 10^6/\text{kg}$ (5-8 in haplo-SCT?)
- No agreement on optimal CD3 cell dose for BM or PBSC (? MUD-PBSC $>350 \times 10^6/\text{kg}$ +aGVHD; risk for cGVHD in haplo-PBSC with pTCy)
- Correlation between lymphocyte subsets and other “unconventional cells” with outcome:
- **T-reg** < aGVHD and **T- γ/δ** < Relapse and viral infections (meta-analysis) (< haplo-SCT)
- **?CD8** < Relapse in PBSC MUD > MRD (RIC) and balanced CD4/CD8 ratio in haplo-BM (few studies)
- **?>NK** < aGVHD, relapse, >OS in PBSC MUD, MRD and haplo-BM (? >NK CD56dim); **<NK** < aGVHD, relapse, >OS in MRD and haplo-BM (?NK CD56bright) —> few studies, limited research on subsets
- **?>pDC** -aGVHD+/-relapse in MRD and MUD BM **?>mDC** + aGVHD in MRD and MUD PBSC —> few studies
- **? MDSC** in PBSC and *mature B-ly* reduce risk of aGVHD (few studies).

Conclusions

- Agreement for optimal range of CD34 dose in PBSC, little or no evidence for CD3 role and dose
- Limited evidences/agreement on role of T subsets, except maybe for T-reg and T- γ/δ , NK cell, B cell and other “unconventional cells”.
- Limits of the studies: they are heterogeneous, overlooking Cell-to-Cell crosstalk & Synergy, and are blind on interactions with the “modified” BM-environment of the patient.
- ATG and pTCy are pleiotropic agents with slight differences on immunomodulatory effects:
ATG—>T-reg expansion, depletion of other T-cell, B-cell, NK cell and DC (sparing T- γ/δ ?)
pTCy—> T-reg sparing, > PD1 memory cell (<**GVHD**); apoptosis CD4>CD8 (>**GVT**); reduced NK (prevailing NKCD56 bright) and naive T-cell (<**GVT**).
- ATG and pTCy are closing the gap between donors and BM vs PBSC (highly competitive over CB).
- However, the precise role and the optimal dose of conventional lymphocytes and unconventional cells represent an ongoing challenge in order to further improve the outcome.