# AUTOLOGOUS GRAFT COMPOSITION: WELCOME TO THE JUNGLE

**Ravenna, 25-Nov-2017**

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Hematology and SCT, Ravenna

# AUTOLOGOUS GRAFT COMPOSITION: BACKGROUND

- Auto-SCT is a potentially curative option for different hematological diseases

- Mobilized PBSC have largely replaced BM as graft source

- Mobilized PBSC has been used for >20 years, however poor knowledge on graft composition

- Impact of different cell subsets on engraftment, immune recovery, anti-tumor activity (??)

- Impact of different mobilizing agents on graft composition (??)

# AUTOLOGOUS GRAFT COMPOSITION - OVERVIEW

- CD34+ stem cell dose
- CD34+ stem cell viability
- CD34+ stem cell functionality
- CD34+, again…no, that's enough! what else besides CD34+?

  → the immune perspective

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# AUTOLOGOUS GRAFT COMPOSITION: CD34+ dose

CD34+ stem cell dose is the most important parameter of graft quality

CD34+ dose → Engraftment potential → Graft “potency” and “efficacy”

# AUTOLOGOUS GRAFT COMPOSITION: CD34+ dose

*Question 7: Which is the target PBPC dose?*

**RECOMMENDATIONS.** *The minimum PBPC dose to be collected and infused to assure a low transplant-related morbidity is $2 \times 10^6$/kg/body weight CD34+ cells per planned transplant.*

*The optimal PBPC dose to be collected and infused to assure a prompt hematopoietic recovery is $5 \times 10^6$/kg/body weight CD34+ cells per planned transplant.*

Pierelli L, Transfusion 2012

# AUTOLOGOUS GRAFT COMPOSITION: CD34+ dose

## Is there an optimal dose of CD34+ cells to be collected for a safe ASCT?

- The **minimal threshold** CD34+ cell dose to be infused is agreed to be ≥ 2-2.5 million CD34 cells/kg for a single ASCT.
- The **optimal dose** for ideal platelet recovery is 4–6 million CD34 cells/kg.
- Reinfusion of high doses of $CD34^+$ cells is associated with:
- long term stable engraftment
- fast platelet and neutrophil engraftment
- reduction in the need for supportive measures, leading to a significant cost sparing
- **reduced toxicity and increased survival rates**

*More is better!*

# Factors That Influence Collection and Engraftment of Autologous Peripheral-Blood Stem Cells

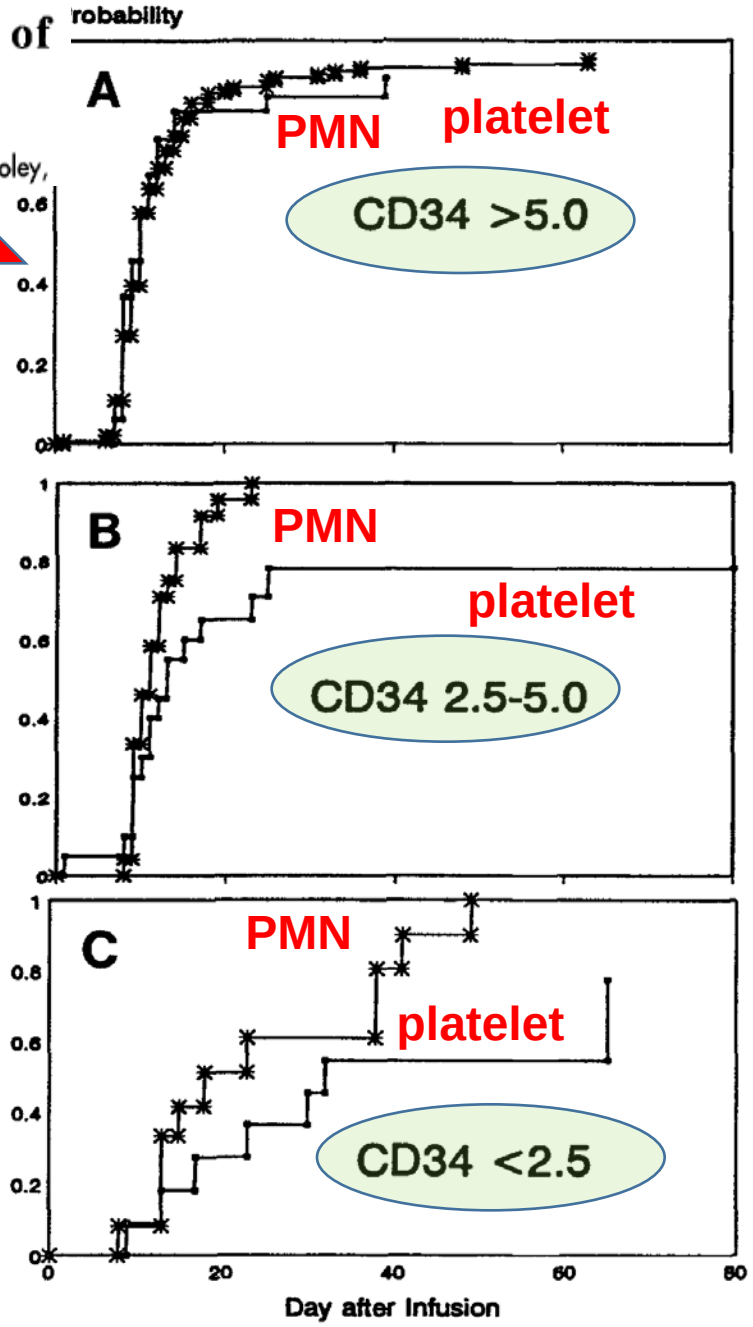
By William Bensinger, Fred Appelbaum, Scott Rowley, Rainer Storb, Jean Sanders, Kathy Lilleby, Ted Gooley,

J Clin Oncol 13:2547-2555. © 1995

tempo of PMN engraftment was indistinguishable between patients who received 2.5 to 5.0 and >5.0 x 10e6 CD34+ cells/kg.

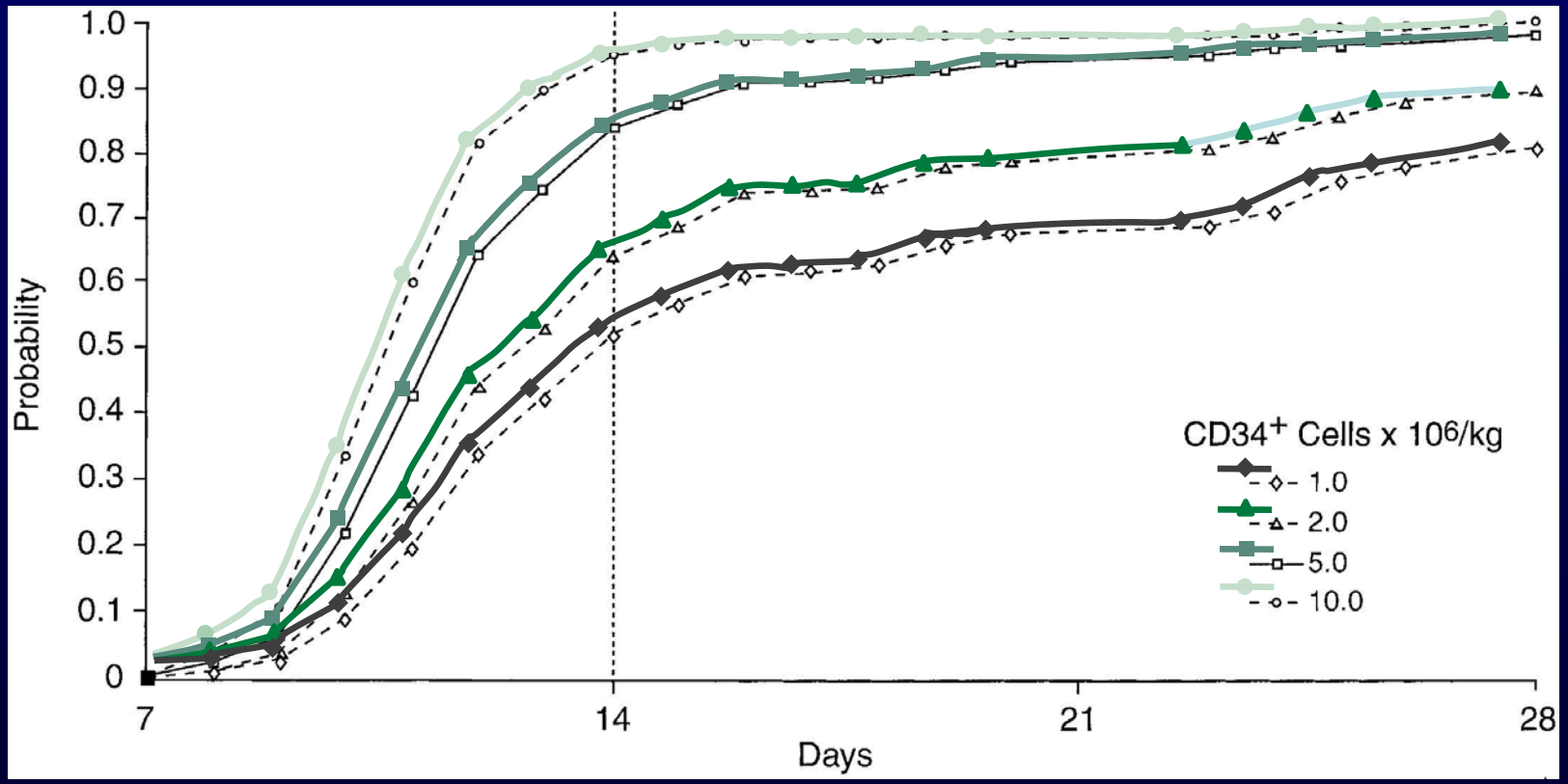
In contrast, the probabilities for achieving platelet independence were distinct for each cell dose level

CD 34+ dose



# Optimal Transplant Cell Dose (CD34+/kg)

- Probability of platelet recovery correlated with the number of CD34+ cells transplanted[1]




- In a retrospective study, lack of full platelet recovery (>150 x $10^9$/L) was associated with lower CD34+ cell doses[2]

Ninan MJ, et al. *Biol Blood Marrow Transplant*. 2007;13:895-904.

# Engraftment and blood cell recovery are not the only clinical end points in autografting

| 8.1. Proposed graded clinical end points in quality assessment | | |
|---|---|---|
| **Objective** | **End point** | **Grading** |
| **Primary: Efficacy** | Days on antibiotics, transfusion of blood components, days in hospital | Favourable: = 7 days on antibiotics and no transfusions |
| | | Intermediate: = 7 days on antibiotics and transfusions OR > 7 days on antibiotics and no transfusions |
| | | Unfavourable: > 7 days on antibiotics and transfusions |
| **Secondary: Toxicity** | Days to ANC >0.5 x 10^6/L and Platelets >20 x 10^6/L | Favourable: ANC and platelets recovery before 14 days |
| | Other organ toxicity if appropriate | Unfavourable: ANC or platelets recovery after 14 days |
| **Tertiary: Safety** | Death or disease recurrence | Favourable: Alive and without disease progression after 12 months |
| | | Unfavourable: Death or disease progression before 12 months |

Lanza et al, bbmt 2013

# Multivariate analysis


1. Pediatric patients resulted to have less toxicity (p=0,0001)
2. 1 or 2 apheresis (p=0,001) predicted good outcome
3. Toxicity increased with higher CD34+volume reinfused (>500ml) (p=0,002)
4. PBSC COLLECTION: CD34+ cells collected > 4 x 10˚ 6/kg in one apheresis (AL excluded)
5. CD34+ cells infused > 5 x 10˚ 6/kg
6. Patients who experienced toxicity had a poor quality transplant (p=0,0001)

**Only a minority of auto-SCT procedures resulted "efficacious"**

Lanza et al, bbmt 2013

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# Evaluating CD34+ cell dose: before or after freezing?

- Currently the amount of harvested CD34+ cells is assessed after completing the aphereses, before cryopreservation

- However, such measurement does not account for the variable loss of viable CD34+ cells which occurs during freezing or thawing processes

We would like to know how many viable CD34+ we are infusing to the patient!

# EXPECTED VIABLE CD34+ LOSS

**Post-thaw viable CD34$^+$ cell count is a valuable predictor of haematopoietic stem cell engraftment in autologous peripheral blood stem cell transplantation**

S. Lee,[1] *Vox Sanguinis* (2008)

Viable CD34+ (%): 98% (70-100) harvest vs 71% (31-89) post-thaw (27% loss)

Pre infusion, post thaw CD34$^+$ peripheral blood stem cell enumeration as a predictor of haematopoietic engraftment in autologous haematopoietic cell transplantation

*J. D'Rozario et al./Transfusion and Apheresis Science 50 (2014)*

Viable CD34+ (x$10^6$/Kg): 4.9 harvest vs 3.2 post-thaw (33% loss)

Quantifying loss of CD34$^+$ cells collected by apheresis after processing for freezing and post-thaw

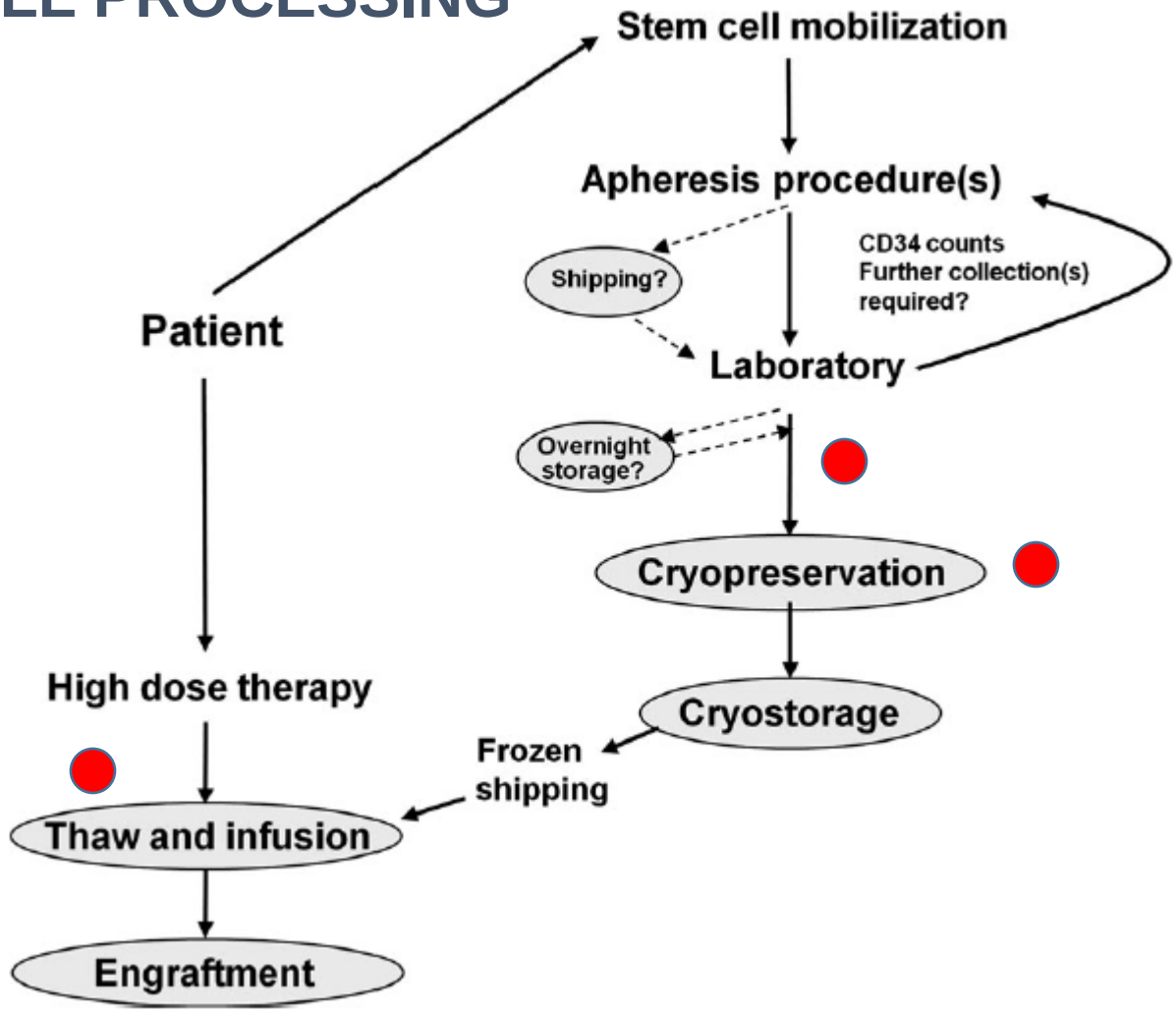
Mariana V. Castelhano Transfusion and Apheresis Science 48 (2013)

Hodgkin vs MM higher CD34+ loss

**Median CD34+ loss: about 30%**

# FACTORS AFFECTING VIABILITY

## STEM CELL PROCESSING




Michael J. Watts[1,2] *British Journal of Haematology*, 2016

# FACTORS AFFECTING VIABILITY

- Which are the main factors affecting frozen HSC viability?

  - Pre-cryopreservation: time-to-freezing, WBC contamination

  - Cryopreservation: controlled vs passive freezing (?), too fast freezing

  - After thawing: delay in reinfusion (acceptable within 2 hours)

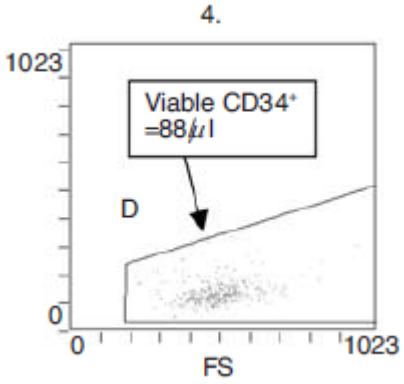
# CD34+ VIABILITY: ENGRAFTMENT

- Is there a correlation between CD34+ viability and engraftment kinetics?

# VIABILITY vs ENGRAFTMENT

## Number of viable CD34+ cells reinfused predicts engraftment in autologous hematopoietic stem cell transplantation

DS Allan[1], Bone Marrow Transplantation (2002)




| | |
|---|---|
| 36 pts | Mixed diseases |
| ISHAGE 7-AAD | |
| CD34+ harvest: 3.6 x$10^6$/Kg | CD34+ post thaw: 2x$10^6$/Kg |

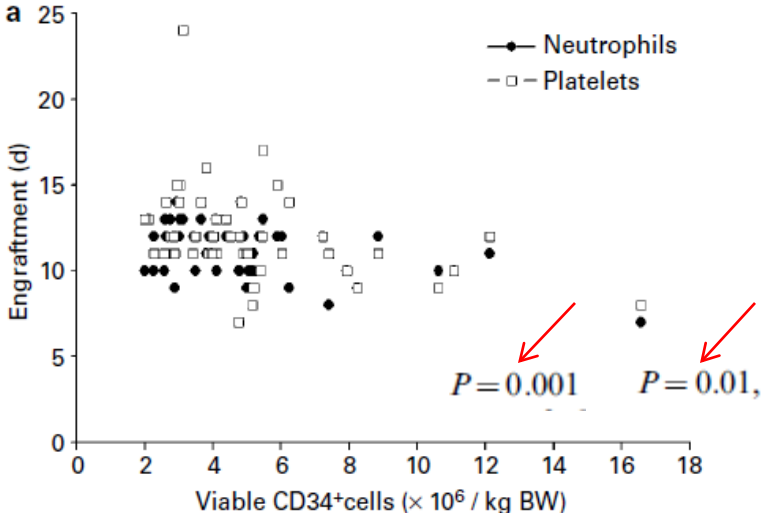
**Table 2** Comparing early and slower platelet engraftment

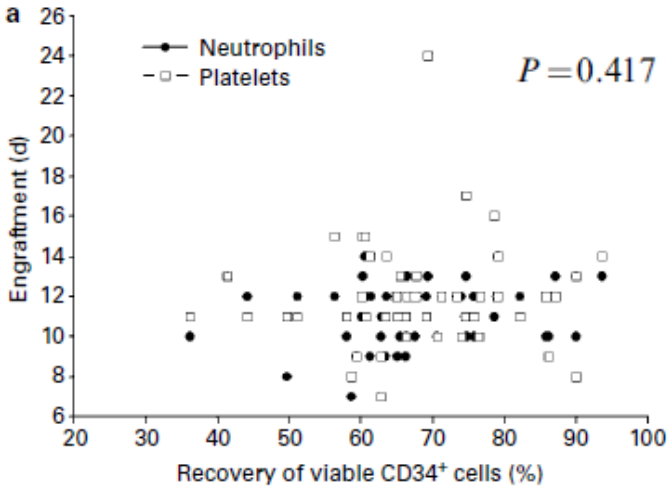
| | *Platelet engraftment* | |
|---|---|---|
| | *≤median (14 d)* | *>median (14 d)* |
| No. patients | 22 | 14 |
| Days to engraftment, median (range) | 12 (9–14) | 18 (15–42) |
| CD34+ cells/kg harvested, median (range) | 5.0 (1.7–182) × $10^6$ | [a]2.9 (1.3–8.5) × $10^6$ |
| CD34+ cells/kg post thaw, median (range) | 3.0 (0.8–110) x$10^6$ | [b]1.7 (0.7–2.7) × $10^6$ |
| No. patients with >2.0 × $10^6$ viable CD34+ cells/kg | | |
| At time of harvest | 20 (91%) | 12 (86%) $P$ = NS |
| Post thaw | 15 (68%) | 2 (14%) $P$ = 0.002 |
| No. of patients with >5.0 × $10^6$ viable CD34+ cells/kg | | |
| At time of harves | 11 (50%) | 2 (14%) $P$ = 0.04 |
| Post thaw | 6 (27%) | 0 ( 0%) $P$ = 0.06 |

# VIABILITY vs ENGRAFTMENT

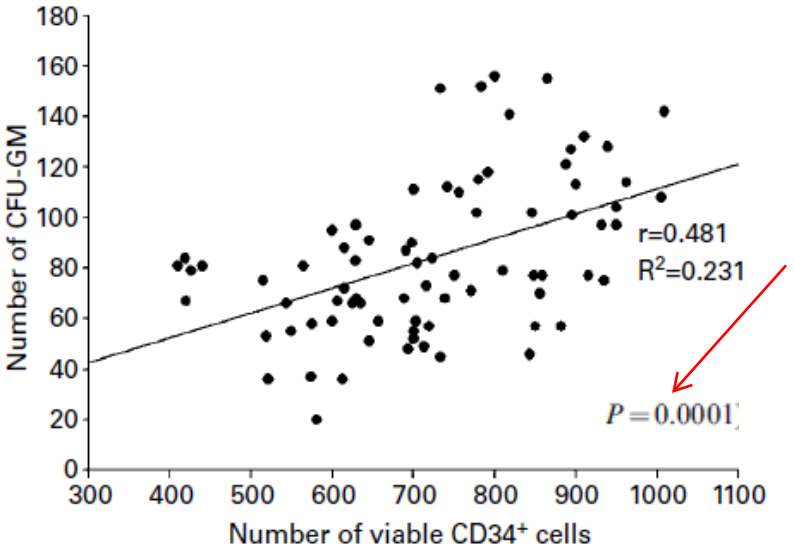
## Association of post-thaw viable CD34$^+$ cells and CFU-GM with time to hematopoietic engraftment

H Yang[1], Bone Marrow Transplantation (2005)




| | |
|---|---|
| 52 pts | Mixed diseases |
| ISHAGE 7-AAD | |
| CD34+ recovery after thawing: 66% | |

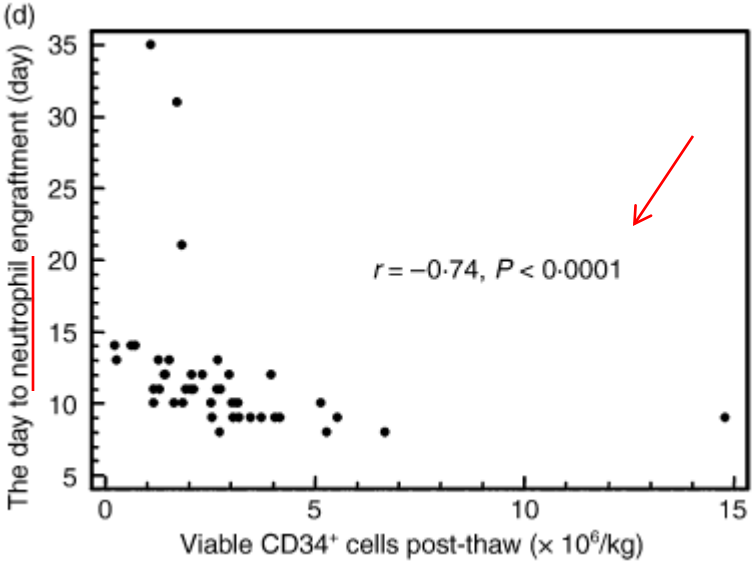


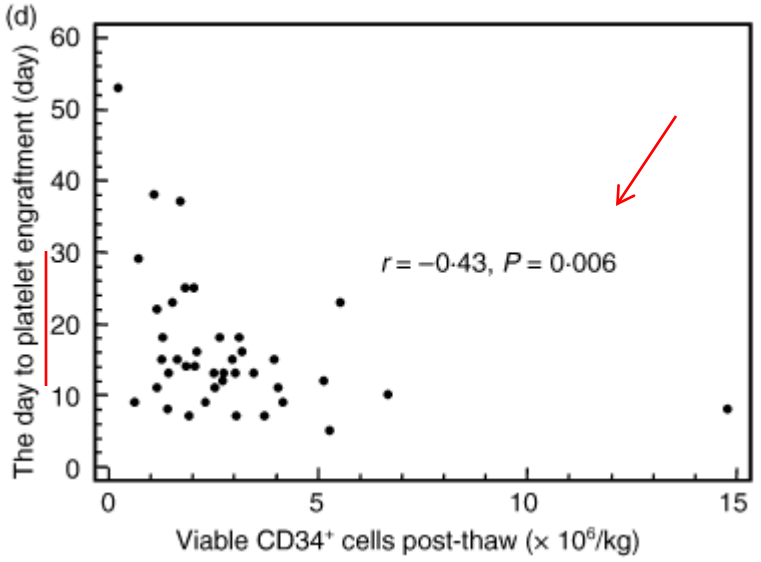
# VIABILITY vs ENGRAFTMENT

**Post-thaw viable CD34$^+$ cell count is a valuable predictor of haematopoietic stem cell engraftment in autologous peripheral blood stem cell transplantation**

S. Lee,[1] *Vox Sanguinis* (2008)

| | |
|---|---|
| 36 pts, mixed diseases | ISHAGE 7-AAD |
| Viable CD34+ (%): 98% (70-100) harvest vs 71% (31-89) post-thaw | |
| Viable CD34+ (x$10^6$/Kg): 3.6 vs 2.2 | |

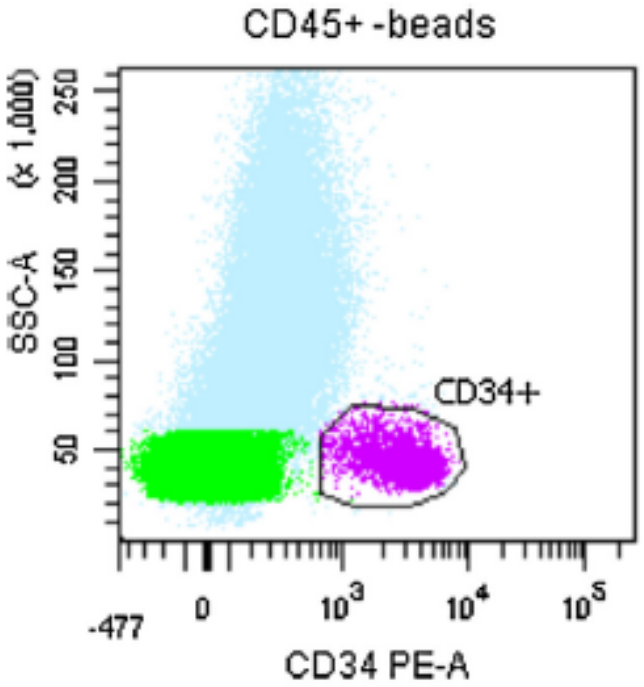


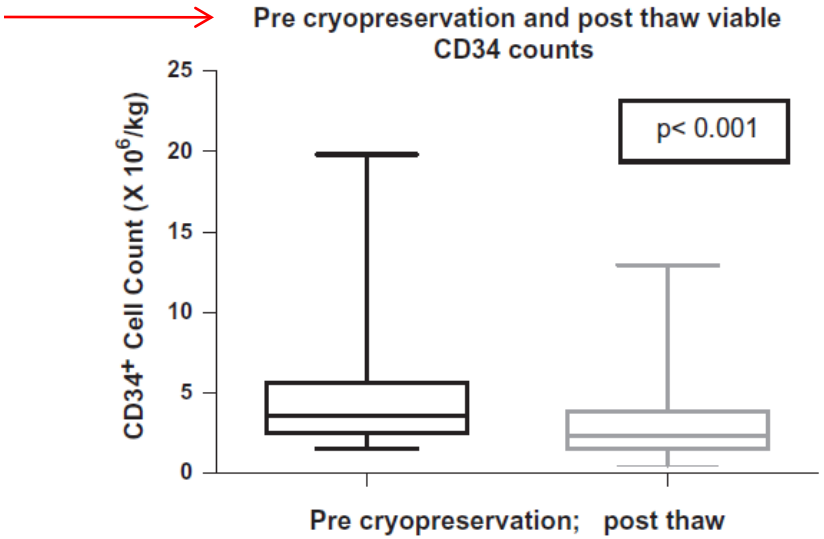
# VIABILITY vs ENGRAFTMENT

Pre infusion, post thaw CD34$^+$ peripheral blood stem cell enumeration as a predictor of haematopoietic engraftment in autologous haematopoietic cell transplantation

*J. D'Rozario et al./Transfusion and Apheresis Science 50 (2014)*




| | |
|---|---|
| 106 SCT procedures | mixed diseases |
| ISHAGE 7-AAD | |
| Viable CD34+ (x$10^6$/Kg): 4.9 harvest vs 3.2 post-thaw (33% loss) | |
| CD34+ post thaw vs PLT: correlation (no N) | |



# CD34+ VIABILITY AFTER THAWING

- Is there a correlation between CD34+ viability and engraftment kinetics?

  -Yes, mostly with PLT engraftment

# WHAT CLINICAL ENDPOINT FOR GRAFT QUALITY?

## Pre infusion, post thaw CD34$^+$ peripheral blood stem cell enumeration as a predictor of haematopoietic engraftment in autologous haematopoietic cell transplantation

*J. D'Rozario et al./Transfusion and Apheresis Science 50 (2014)*

| Post thaw CD34$^+$ count | Mean LOS (d) | |
|---|---|---|
| >3.9 × 10$^6$/kg | 14.2 ± 0.7 | |
| 2.3 to ⩽3.9 × 10$^6$/kg | 15.1 ± 1.0 | $P < 0.001$ |
| 1.6 to ⩽2.3 × 10$^6$/kg | 16.5 ± 0.9 | |
| ⩽1.6 × 10$^6$/kg | 17.8 ± 0.9 | |
| | Mean red cell units/patient | |
| >3.9 × 10$^6$/kg | 2.0 ± 0.1 | |
| 2.3 to ⩽3.9 × 10$^6$/kg | 2.7 ± 0.4 | $P < 0.001$ |
| 1.6 to ⩽2.3 × 10$^6$/kg | 3.6 ± 0.5 | |
| ⩽1.6 × 10$^6$/kg | 3.7 ± 0.4 | |
| | Mean platelet concentrate units/patient | |
| >3.9 × 10$^6$/kg | 1.6 ± 0.2 | |
| 2.3 to ⩽3.9 × 10$^6$/kg | 1.9 ± 0.3 | $P < 0.001$ |
| 1.6 to ⩽2.3 × 10$^6$/kg | 2.4 ± 0.3 | |
| ⩽1.6 × 10$^6$/kg | 2.7 ± 0.2 | |
| | Mean days of G-CSF administration | |
| >3.9 × t10$^6$/kg | 9.6 ± 0.4 | |
| 2.3 to ⩽3.9 × 10$^6$/kg | 10 ± 0.4 | $P < 0.001$ |
| 1.6 to ⩽2.3 × 10$^6$/kg | 10.5 ± 0.5 | |
| ⩽1.6 × 10$^6$/kg | 11.2 ± 0.5 | |
| | Mean days of IV antibiotic administration | |
| >3.9 × 10$^6$/kg | 3.8 ± 0.5 | |
| 2.3 to ⩽3.9 × 10$^6$/kg | 4.9 ± 0.6 | $P < 0.001$ |
| 1.6 to ⩽2.3 × 10$^6$/kg | 6.6 ± 0.8 | |
| ⩽1.6 × 10$^6$/kg | 6.7 ± 0.9 | |

# AUTOLOGOUS GRAFT COMPOSITION - OVERVIEW

- CD34+ stem cell dose
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  → the immune perspective

# CD34+ VIABILITY vs FUNCTIONALITY

Does CD34+ viability test represent a valid surrogate of graft functionality?

# CD34+ VIABILITY vs FUNCTIONALITY


VIABLE

VIABLE

FUNCTIONAL?

# CD34+ VIABILITY vs FUNCTIONALITY

## Post-thaw viability of cryopreserved peripheral blood stem cells (PBSC) does not guarantee functional activity: important implications for quality assurance of stem cell transplant programmes

Daniel A. Morgenstern,[1] *British Journal of Haematology*, 2016,

**Table III.** Results of four split-harvest cryopreservation procedures comparing different methods

| PBSC sample | Cryopreservation method | WBC ($\times 10^9$/l) | Viability | CFU-GM/well | CFU-GM $\times 10^6$/ml of product (thawed yield) |
|---|---|---|---|---|---|
| Sample 1 | Fresh | 145 | 100% | 32·8 | 0·38 (100%) |
| | UCLH −80°C freezer | 166 | 78% | 17·0 | 0·23 (59%) |
| | GOSH −80°C freezer | 175 | 79% | 17·5 | 0·25 (64%) |
| | GOSH CRF | 176 | 77% | 0·0 | 0·00 (0%) |
| Sample 2* | Fresh | 204 | 99% | 5·5 | 0·09 (100%) |
| | UCLH −80°C freezer | 239 | 90% | 5·0 | 0·10 (106%) |
| | GOSH −80°C freezer | 239 | 78% | 5·25 | 0·10 (112%) |
| | GOSH CRF | 242 | 79% | 0·0 | 0·00 (0%) |
| Sample 3* | Fresh | 210 | 98% | 3·25 | 0·06 (100%) |
| | UCLH −80°C freezer | 237 | 82% | 3·5 | 0·07 (118%) |
| | GOSH −80°C freezer | 234 | 64% | 2·25 | 0·04 (47%) |
| | GOSH CRF | 251 | 80% | 0·0 | 0·00 (0%) |
| Sample 4 | Fresh | 87 | 99% | 47·0 | 0·33 (100%) |
| | GOSH −80°C freezer | 100 | 76% | 28·5 | 0·23 (70%) |
| | GOSH CRF | 97 | 89% | 0·0 | 0·00 (0%) |

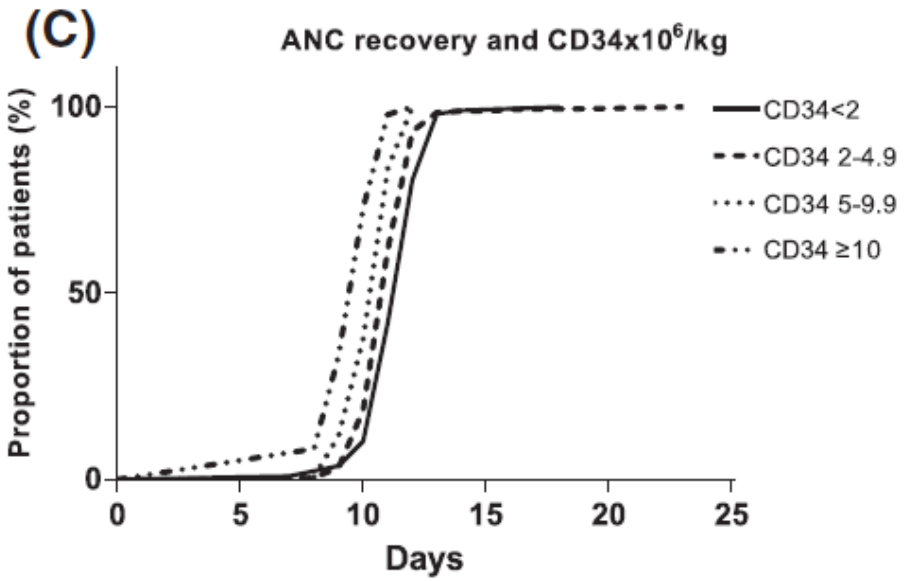
# CD34+ VIABILITY vs FUNCTIONALITY

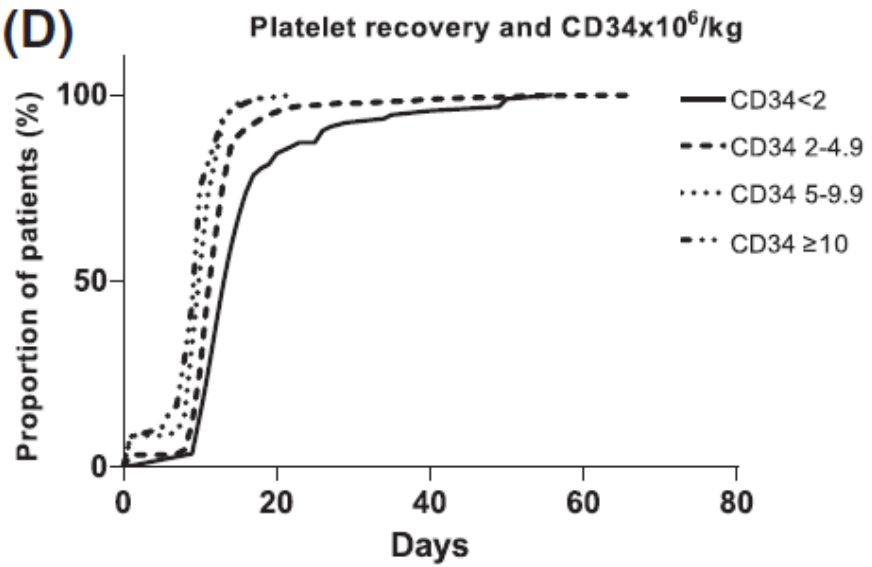
**Re-evaluation of progenitor thresholds and expectations for haematopoietic recovery based on an analysis of 810 autologous transplants: Implications for quality assurance**

Michael J. Watts,[1] *British Journal of Haematology*, 2016,

$2x10^{6}$/Kg OK

<$2x10^{6}$: check with colony forming units: CFU>$2x10^{5}$/Kg OK



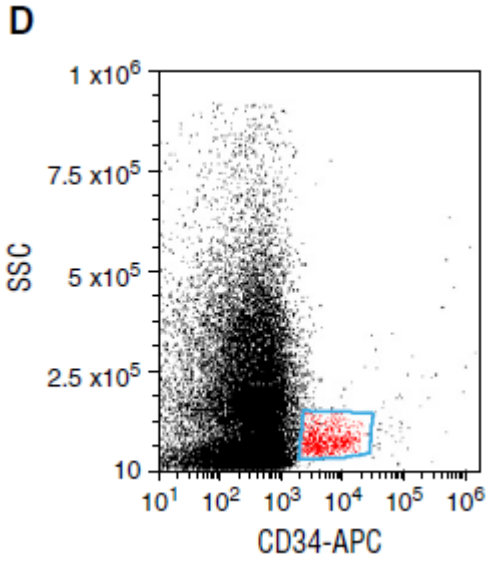



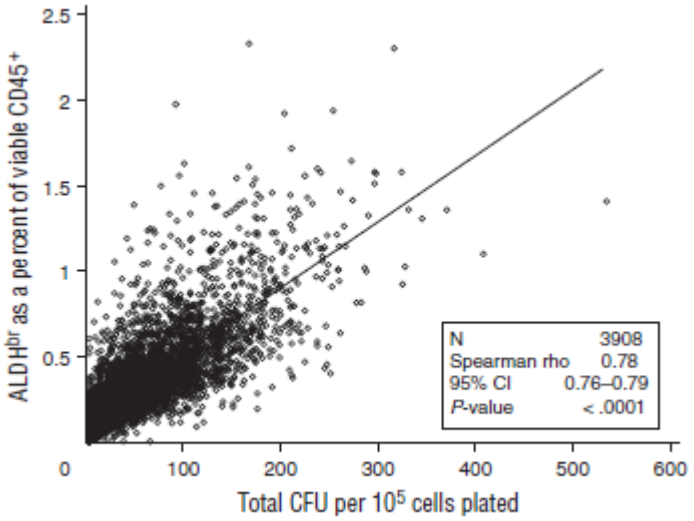

1 $x10^{6}$CD34+/Kg is enough if part of a greater collection

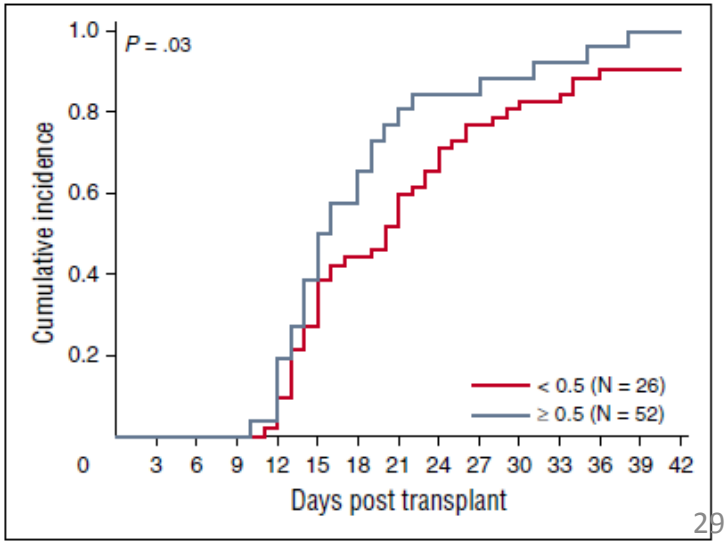
# CD34+ VIABILITY vs FUNCTIONALITY

## Development and validation of a rapid, aldehyde dehydrogenase bright–based cord blood potency assay

Kevin Shoulars,[1] BLOOD, 12 MAY 2016



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  → the immune perspective

# CD34+ / 38- stem cells

- Among the CD34+ stem cells, the CD34+/CD133+/CD38− most primitive subsets are known to have high self-renewal and repopulation capacity
- CD34+ / 38- and are thought to be responsible of rapid engraftment after auto-SCT.
- Unclear impact on long-term stable engraftment

# AUTOLOGOUS GRAFT COMPOSITION - OVERVIEW

- CD34+ stem cell dose
- CD34+ stem cell viability and outcome
- CD34+ stem cell subsets
- CD34+ stem cell functionality

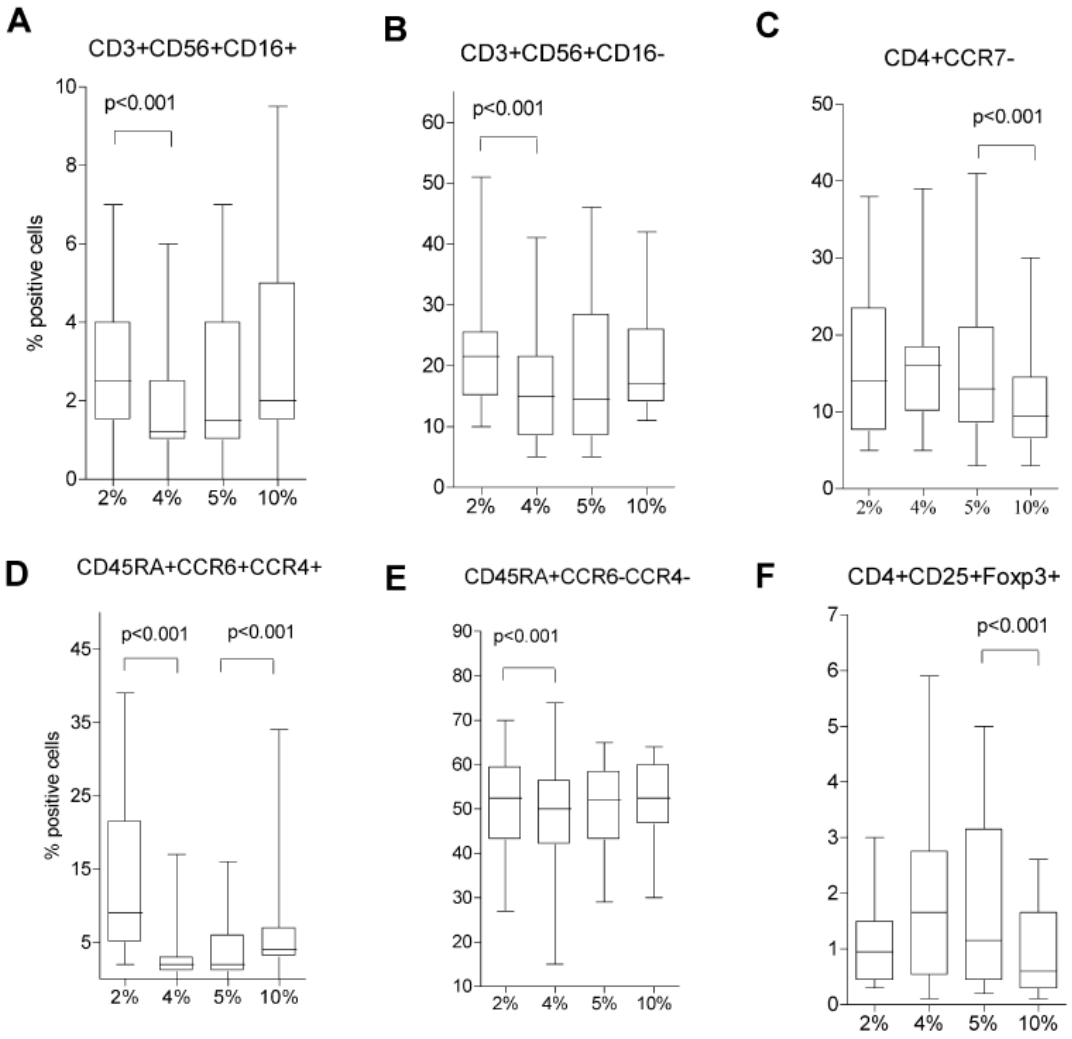
- CD34+, again…no, that's enough! what else besides CD34+?

→ the immune perspective

# What else besides CD34+? the immune perspective

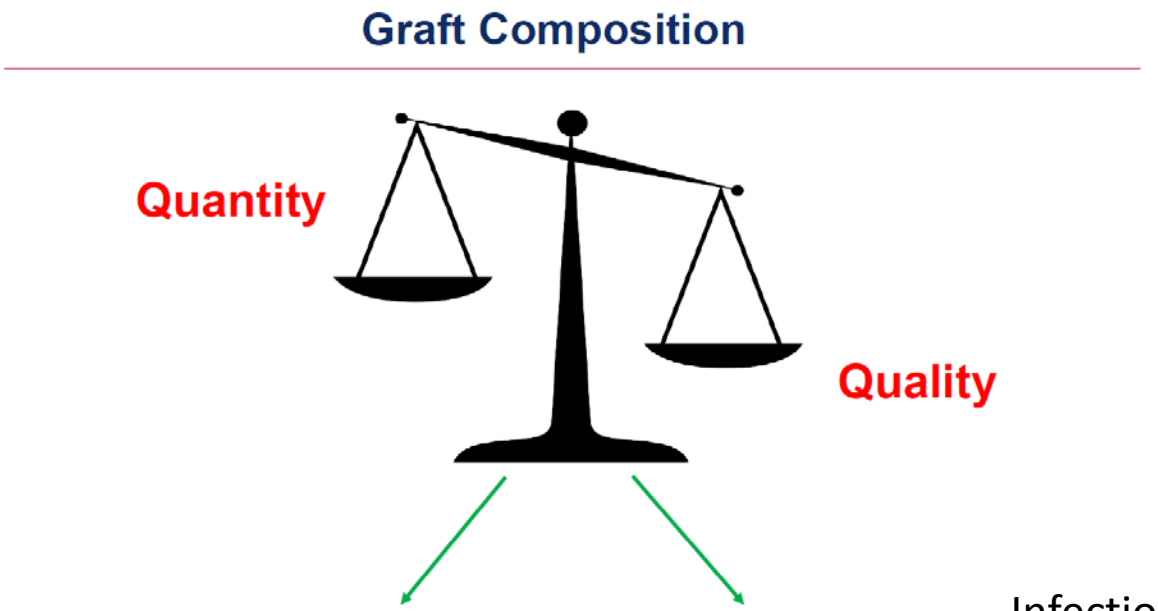
We might be interested in these, as well:

-CD3+/4+
-CD3+/8+
-CD19+
-CD3+/56+
-pDC
[…]




*Knut Liseth,*
TRANSFUSION 2009;

# What else besides CD34+? the immune perspective




- Infection
- Disease

Achieving a sufficient CD34+ stem cell dose is certainly an essential goal, but…
…graft quality in terms of immune cell subsets and immune recovery is acquiring increasing importance

# What else besides CD34+? the immune perspective

- The number of lymphocytes infused within the graft (A-ALC), has been shown to be strictly related to Absolute lymphocyte count on day 15 (ALC-15)

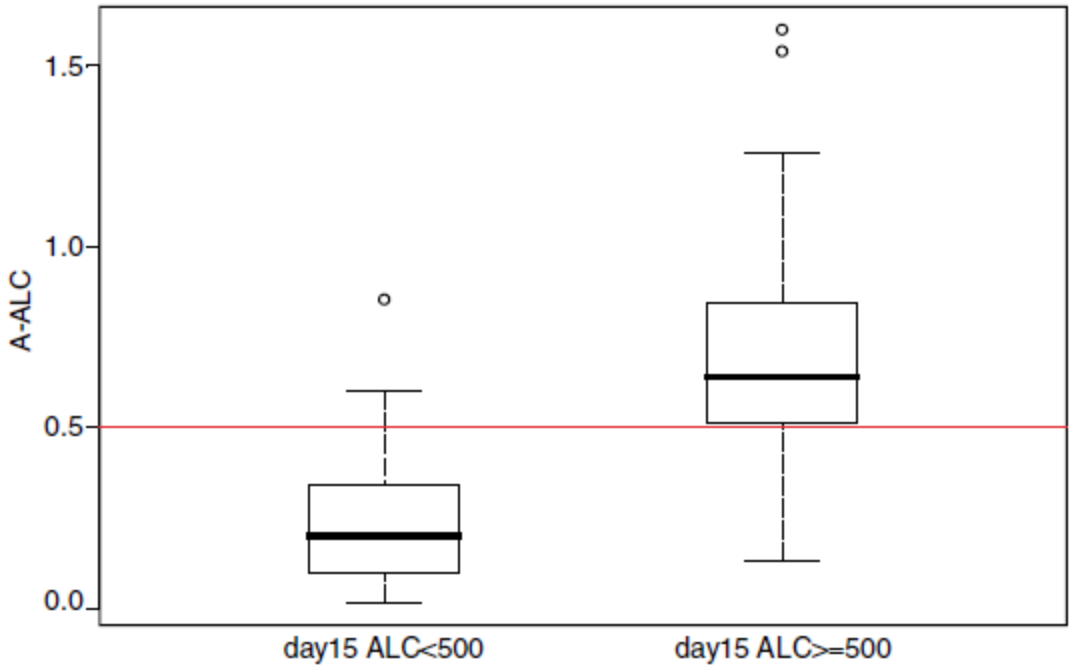
- Absolute lymphocyte count on day 15 has been reported to be an independent prognostic factor for OS in patients undergoing auto-SCT, both in multiple myeloma and NHL patients.

<u>CD34+ stem cell dose does not affect immune recovery!</u>


Porrata LF, Leuk Lymphoma 2003
Porrata LF, Leukemia 2004
Atta EH, Am Journ Hematol, 2009
Porrata LF, Biol Blood Marrow Transplant 2008
Porrata LF, Biol Blood Marrow Transplant 2014
Porrata LF, J Hematol Oncol 2015

# What else besides CD34+? the immune perspective

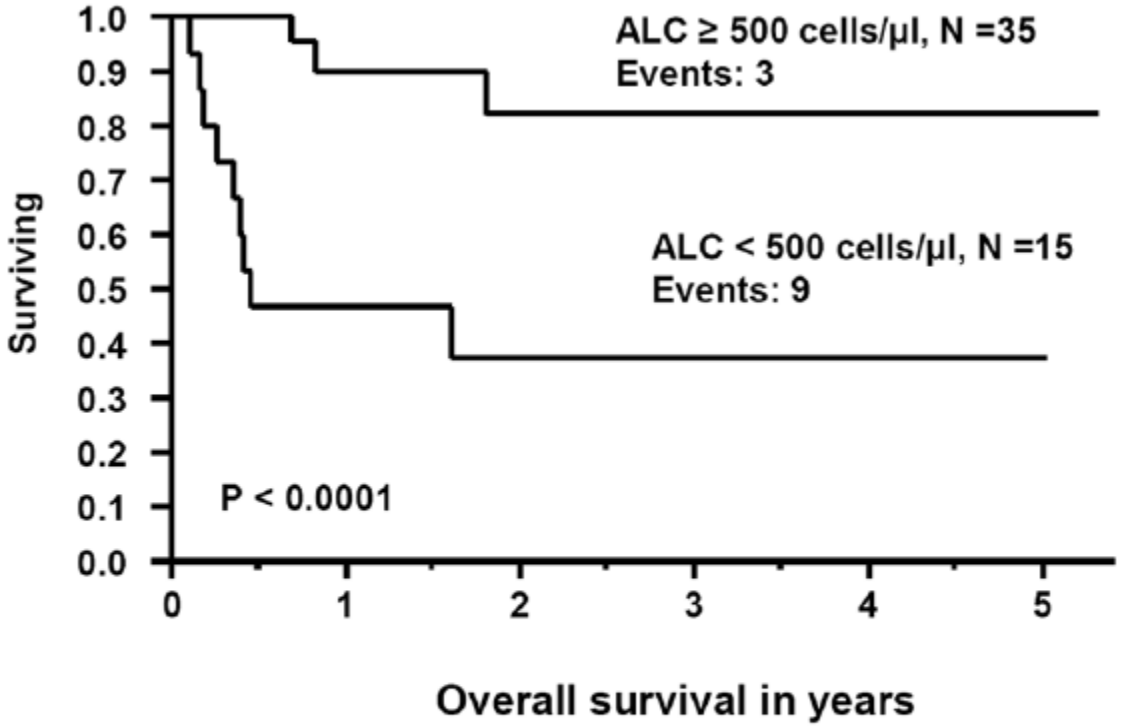
A-ALC vs day15 ALC




Porrata LF, Leukemia 2004

# What else besides CD34+? the immune perspective

NHL patients



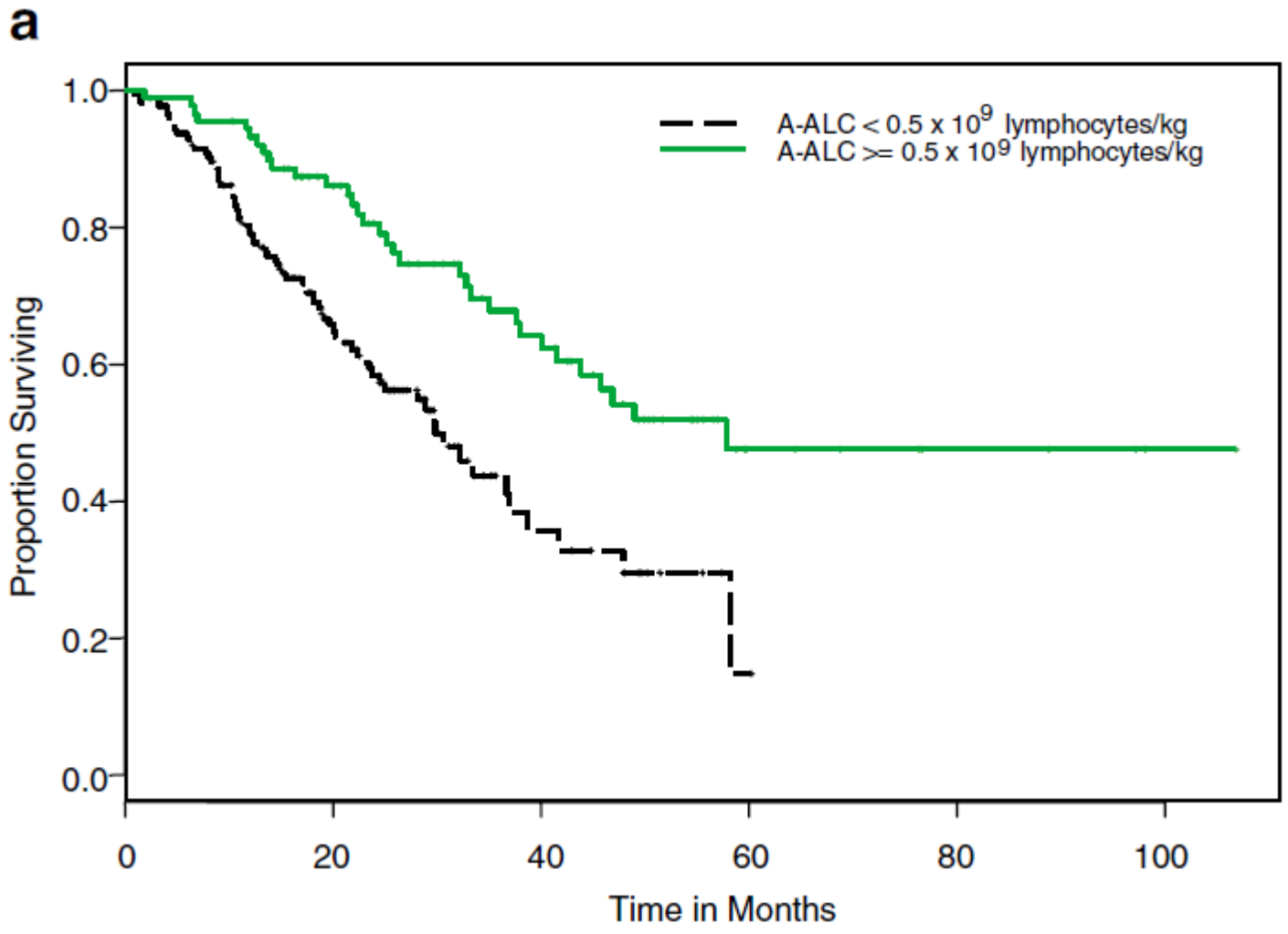

| Number at risk | | | | | | |
|---|---|---|---|---|---|---|
| ALC ≥ 500 cells/µl | 35 | 16 | 11 | 6 | 5 | 3 |
| ALC < 500 cells/µl | 15 | 6 | 4 | 3 | 2 | 1 |

NK cell count at day 15 post auto-SCT predicts better OS

Porrata LF, Biol Blood Marrow Transplant 2008

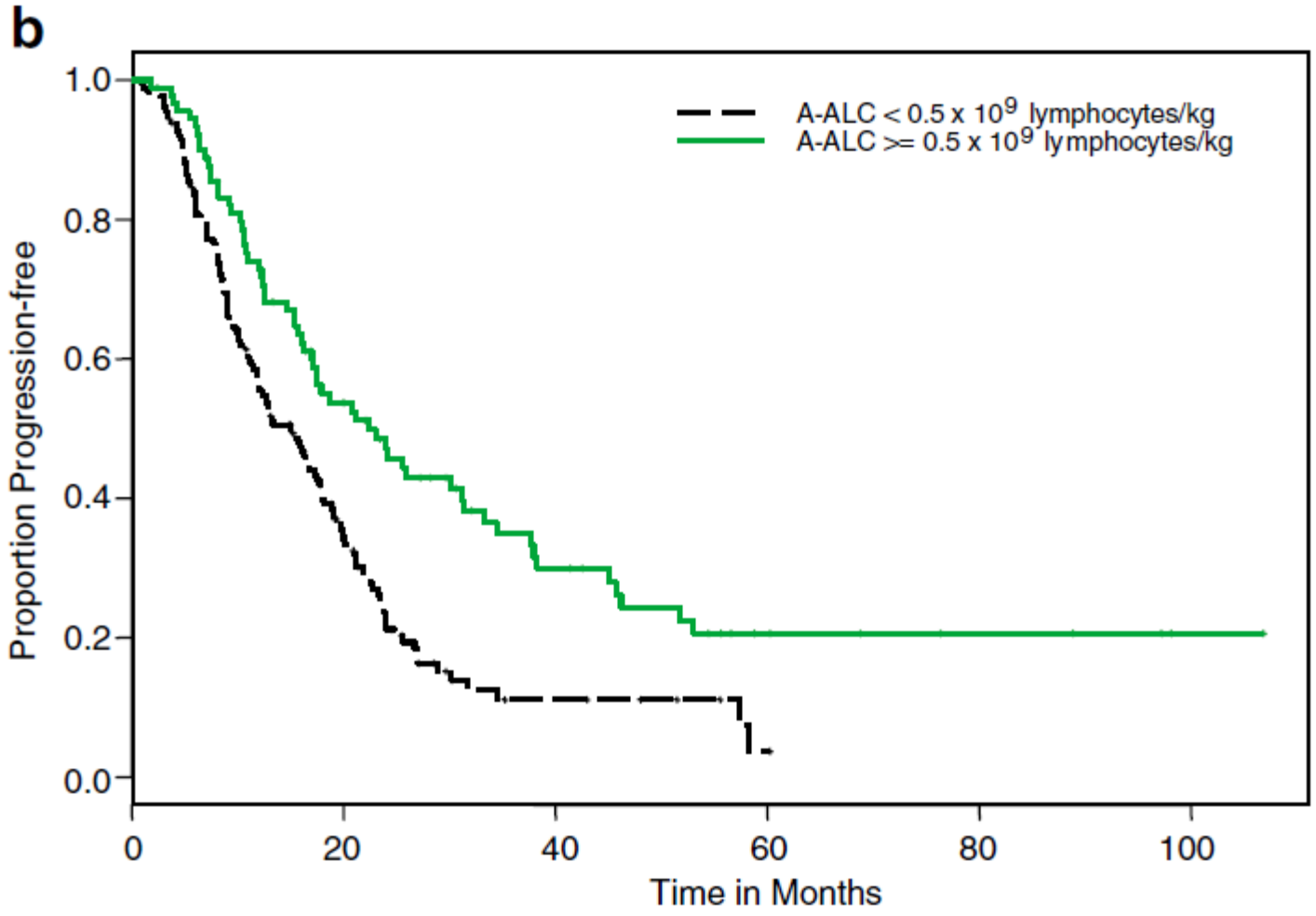
# What else besides CD34+? the immune perspective

MM patients




Porrata LF, Leukemia 2004

# What else besides CD34+? the immune perspective




Porrata LF, Leukemia 2004

# What else besides CD34+? the immune perspective

## Graft Composition

- Main focus of the clinician is the CD34+ stem cell dose → the only generally accepted indicator of graft quality[1]
- Increasing knowledge in graft composition [1,2,3,4,5,6]:
  - Role of CD34+ subpopulations
  - Immune cell subsets
  - Influence on engraftment
  - Immune recovery
  - Anti-tumor activity[5]
  - Impact on patient's outcome
- Effect of different mobilization agents on graft composition[6]


1. Mohty M *et al.* Bone Marrow Transplant. 2014;49(7):865-72
2. Saraceni F *et al.* Bone Marrow Transplant. 2015;50(7):886-91.
3. Porrata LF *et al.* Biol Blood Marrow Transplant. 2014;20(11):1804-12
4. Jantunen *et al.* Expert Rev Hematol. 2016;9(8):723-32
5. Kohrt *et al.* Eur. J. Immunol. 2010. 40: 1862–1869
6. Fruehauf *et al.* Biol Blood Marrow Transplant 2010;16:1629-1648

# What else besides CD34+? the immune perspective

**Table 1.** Studies comparing G-CSF and plerixafor mobilization of the most primitive $CD34^+/38^-$ stem cell subset

| *Author* | *Study* | *Disease(s)* | *No. of patients* | *Proportion of $CD34^+/38^-$ mobilized (% of all $CD34^+$ stem cells)* | | P *value* |
|---|---|---|---|---|---|---|
| | | | | *G-CSF* | *G-CSF+plerixafor* | |
| Fruehauf *et al.*[10] | Prospective | MM, NHL | 15 | 0.5 | 4 | 0.004 |
| Varmavuo *et al.*[11] | Prospective | NHL | 34 | 1.6[a] | 2.9[a] | 0.09 |
| Varmavuo *et al.*[12] | Retrospective | MM | 21 | 0.6[a] | 3.5 | 0.02 |
| Roug *et al.*[13] | Prospective | MM, NHL, HD | 22 | | Higher mobilization[b] | 0.03 |
| Taubert *et al.*[9] | Prospective | MM | 8 | | 2,8-Fold increase compared with G-CSF only | n.a. |

**Plerixafor may increase mobilization of CD34+/CD38– stem cell subpopulation when compared with G-CSF alone or G-CSF combined with chemotherapy**

Saraceni F et al. *Bone Marrow Transplantation* 2015

# What else besides CD34+? the immune perspective

**Table 2.** Impact of mobilizing regimens on autologous graft immune effector cells and patients' outcome

| *Graft cell subset* | *Authors* | *Mobilizing regimen used and relative efficacy (if compared)* | | | | *Outcome implications (if evaluated)* |
|---|---|---|---|---|---|---|
| | | *CT+ G-CSF* | *G-CSF* | *G-CSF +MZ* | *CT+GCSF +MZ* | |
| T CD3$^+$ | Porrata *et al.*[22] | | ● | | | A-ALC $>0.5\times10^9$/kg predicts better OS and PFS<br>A-ALC independent prognostic factor for OS and DFS[22] |
| | Porrata *et al.*[21] | ●[a] | | | | A-ALC $>0.5\times10^9$/kg predicts better OS and PFS<br>A-ALC independent prognostic factor for OS and PFS[21] |
| | Holtan *et al.*,[32] Varmavuo *et al.*[33] and Gaugler *et al.*[27] | | ● | ●● | | No relapses in G-CSF+MZ group, 15/19 in the G-CSF group[32]<br>Not significative[33] |
| | Varmavuo *et al.*[11,29] | ● | | | ●● | |
| B | Varmavuo *et al.*[12] | ● | | | ●●[b] | |
| NK | Porrata *et al.*[20] | | ● | | | NK-15 $>80/\mu$L predicts better OS and DFS<br>NK-15 independent prognostic factor for OS[20] |
| | Varmavuo *et al.*[11,12,29] | ● | | | ●●[b] | |
| Treg | Gaugler *et al.*[27] | | ● | ● | | |
| DC | Gaugler *et al.*[27] and Gazitt *et al.*[28] | | ● | ●● | | |

Abbreviations: A-ALC = absolute lymphocyte count in the graft; ALC-15 = absolute lymphocyte count at day 15 post autologous transplant; B = B lymphocytes; CT = chemotherapy; DFS = disease free survival; MZ = plerixafor; NK = natural killer cells; NK-15 = NK cell count at day 15 post autologous transplant; T CD3$^+$ = T lymphocytes; Treg = regulatory T cells; ●● = higher mobilization compared with ●. [a]Patients received either G-CSF or GM-CSF, 8/267 received only GM-CSF. [b]Four of nine patients did not receive chemotherapy but only G-CSF+plerixafor.

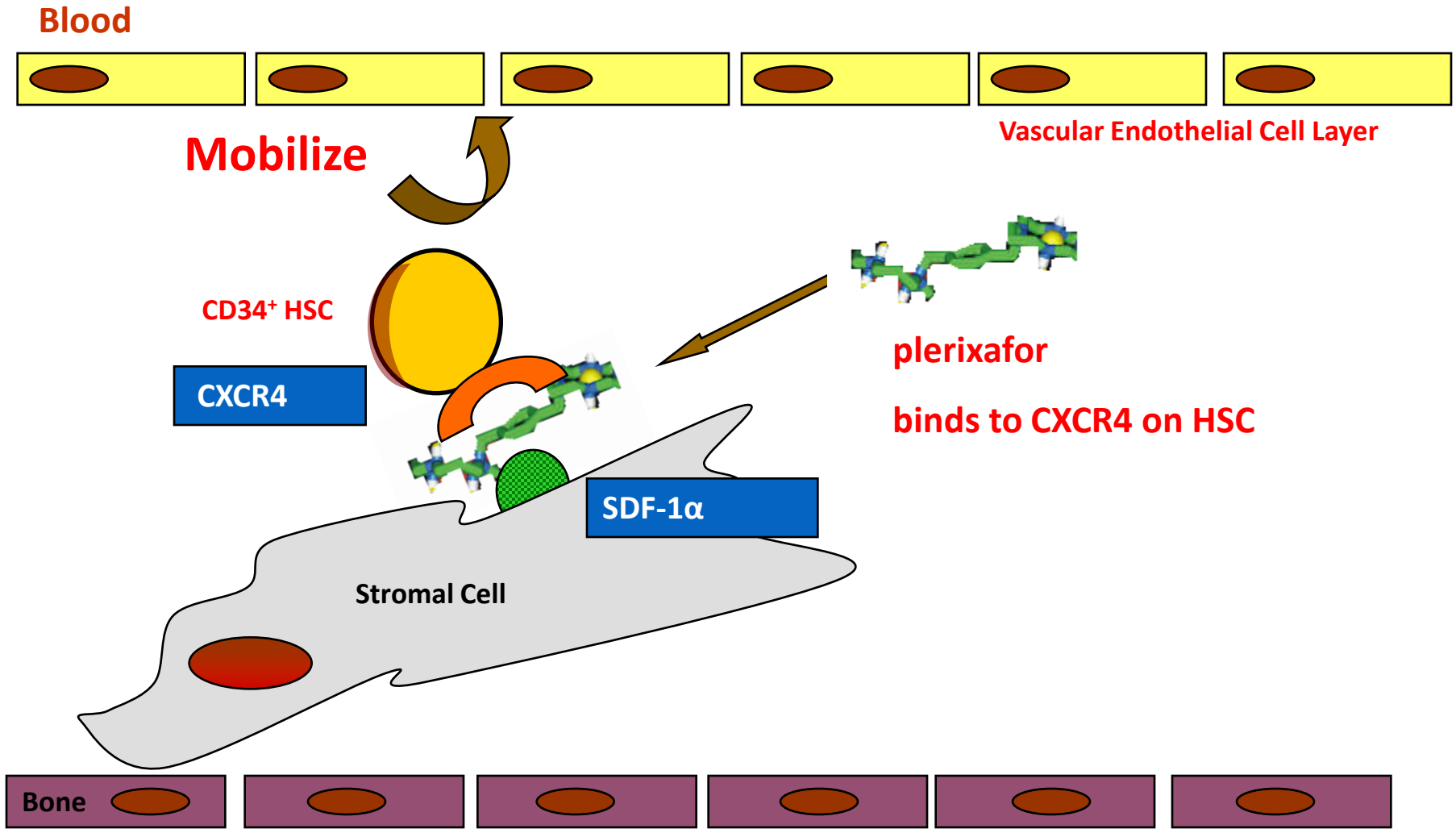
**plerixafor may increase mobilization of immune effectors when compared with G-CSF alone or G-CSF combined with chemotherapy**

CHT is heavily toxic to lymphocytes

Saraceni F et al. *Bone Marrow Transplantation* 2015

# What else besides CD34+? the immune perspective

## PLERIXAFOR: Mechanism of HPC Mobilization



# What else besides CD34+? the immune perspective

Mobilization strategy can influence graft cellular composition, therefore *maybe* graft-vs-disease activity and anti-infectious potential and *maybe* patient outcome?

This would be fantastic!!

Higher median A-ALCs were observed in the AMD3100 group compared with the control group (4.16 x 10(9) lymphocytes/kg vs. 0.288 x 10(9) lymphocytes/kg; P < 0.0001). With a median follow-up of 20 months (range, 4-24 months), no relapses were reported in the AMD3100 group compared with 15 of 29 in the control group (P < 0.02).

***Holtan SG, Clin Lymphoma Myeloma 2007***

# What else besides CD34+? the immune perspective

Initial flow cytometry characterization of graft cell subsets

**TABLE 3. Graft volume, sample preservation time, lymphocyte subsets, and CD34+ content of the grafts. In two patients both grafts collected prior and after plerixafor were included**

| Variable | Stem cell collection with plerixafor* (n = 13) | Stem cell collection without plerixafor* (n = 13) | p value |
|---|---|---|---|
| Graft volume (mL) | 100 (43-190) | 80 (45-140) | 0.280 |
| Graft sample preservation time (days) | 299 (31-450) | 291 (103-397) | 0.898 |
| CD34+ cell content ($\times 10^6$/kg) after 7-AAD | 1.45 (0.40-4.40) | 1.80 (0.31-4.74) | 0.858 |
| CD3+ cell content ($\times 10^6$/kg) | 75.3 (14.6-327.3) | 21.3 (9.1-159.4) | 0.004 |
| CD3+CD4+ cell content ($\times 10^6$/kg) | 32.7 (10.6-132.8) | 12.4 (6.9-51.5) | 0.002 |
| CD3+CD8+ cell content ($\times 10^6$/kg) | 33.4 (4.2-200.5) | 8.8 (2.2-125.0) | 0.006 |
| CD19+ cell content ($\times 10^6$/kg) | 0 | 0 | NA |
| NK (CD3–CD16/56+) cell content ($\times 10^6$/kg) | 5.1 (0.2-30.40) | 1.5 (0.3-8.0) | 0.045 |
| CD4+/CD8+ cell ratio | 0.98 (0.34-3.04) | 1.41 (0.28-5.06) | 0.228 |

* Data are reported as median (range).
7-AAD = 7-aminoactimycin D.

Finnish group, early plerixafor era
Chemomobilization w ot w/o plerixafor. NHL.
Initial observation of higher CD3+/NK mobilization with plerixafor

Varmavuo, Transfusion 2012

# What else besides CD34+? the immune perspective



**TABLE 2. Analysis of cryopreserved grafts***

| Blood graft content ($\times 10^6$ cells/kg) | Mobilization with plerixafor (n = 15) | Mobilization w/a plerixafor (n = 26) | p value† |
|---|---|---|---|
| CD34+ without 7-AAD | 2.2 (0.8-6.7) | 3.5 (1.9-14.9) | 0.001 |
| CD34+ with 7-AAD | 2.0 (0.6-5.7) | 3.1 (1.5-14.3) | 0.006 |
| CD34+CD133+CD38– | 0.07 (0.01-0.17) | 0.05 (0.11-0.35) | NS |
| Proportion of CD34+CD133+CD38– cells from all CD34+ cells (%) | 3.5 (0.8-10.8) | 1.7 (0.44-5.3) | <0.001 |
| CD3+ | 160.9 (49.2-454.4) | 58.6 (10.9-415.4) | <0.001 |
| CD3+CD4+ | 81.1 (29.1-267.1) | 35.2 (7.7-114.3) | <0.001 |
| CD3+CD8+ | 75.3 (16.5-279.1) | 21.0 (3.1-301.8) | 0.001 |
| CD19+ | 0.0 (0.0-0.01) | 0.0 (0.0-3.2) | NS |
| NK | 20.4 (0.4-39.5) | 4.8 (0.6-20.7) | <0.001 |

* Data are reported as median (range).
† p values 0.05 or more are designated as NS.

46 NHL pts
Chemomobilization w ot w/o plerixafor
higher CD34+/38-, CD3+/NK mobilization with plerixafor

Valtola, Transfusion 2015

# What else besides CD34+? the immune perspective

**TABLE 4. Immunologic recovery after auto-SCT***

| Variable | Mobilization with plerixafor (n = 14) | Mobilization without plerixafor (n = 17) | p value† |
|---|---|---|---|
| Blood flow cytometry 1 month after auto-SCT ($\times 10^9$/L) | | | |
| CD3+ | 1.2 (0.5-3.1); 14 | 1.1 (0.2-3.6); 17 | NS |
| CD3+CD4+ | 0.3 (0.1-0.6); 14 | 0.2 (0.1-1.5); 17 | NS |
| CD3+CD8+ | 0.9 (0.3-2.5); 14 | 0.9 (0.1-2.8); 17 | NS |
| NK | 0.4 (0.1-0.6); 14 | 0.2 (0.02-0.7); 17 | 0.001 |
| CD19+ | 0.0 (0.0-0.0); 14 | 0.0 (0.0-0.0); 17 | NS |
| CD4/CD8– ratio | 0.3 (0.2-0.8); 14 | 0.3 (0.2-1.0); 17 | NS |
| Blood flow cytometry 3 months after auto-SCT ($\times 10^9$/L) | | | |
| CD3+ | 1.4 (0.4-3.3); 13 | 1.7 (0.3-4.8); 16 | NS |
| CD3+CD4+ | 0.4 (0.2-0.7); 13 | 0.3 (0.1-0.9); 16 | NS |
| CD3+CD8+ | 1.1 (0.2-2.8); 13 | 1.2 (0.1-4.2); 16 | NS |
| NK | 0.2 (0.1-0.9); 13 | 0.2 (0.1-0.3); 16 | NS |
| CD19+ | 0.0 (0.0-0.2); 13 | 0.0 (0.0-0.3); 16 | NS |
| CD4/CD8– ratio | 0.3 (0.1-0.9); 13 | 0.3 (0.1-1.1); 16 | NS |
| Blood flow cytometry 6 months after auto-SCT ($\times 10^9$/L) | | | |
| CD3+ | 1.3 (0.5-4.0); 12 | 1.2 (0.3-2.4); 14 | NS |
| CD3+CD4+ | 0.3 (0.2-0.6); 12 | 0.3 (0.1-0.6); 14 | NS |
| CD3+CD8+ | 0.8 (0.3-3.4); 12 | 1.0 (0.2-2.1); 14 | NS |
| NK | 0.2 (0.1-1.1); 12 | 0.1 (0.03-0.3); 14 | NS |
| CD19+ | 0.0 (0.0-0.3); 12 | 0.0 (0.0-0.3); 14 | NS |
| CD4/CD8– ratio | 0.4 (0.2-1.1); 12 | 0.4 (0.1-1.3); 14 | NS |
| IgG (g/L) at 6 months | 5.1 (1.4-5.7); 6 | 6.2 (1.3-13.0); 12 | NS |

* Data are reported as median (range); number of patients. Reference values of blood flow cytometry reported by the Laboratory of Eastern Finland: B-T-CD3, 0.7 $\times$ $10^9$ to 2.1 $\times$ $10^9$/L; B-CD19, 0.1 $\times$ $10^9$ to 0.5 $\times$ $10^9$/L; B-T-CD4, 0.3 $\times$ $10^9$ to 1.4 $\times$ $10^9$/L; B-T-CD8, 0.2 $\times$ $10^9$ to 0.90 $\times$ $10^9$/L; B-NK, 0.09 $\times$ $10^9$ to 0.6 $\times$ $10^9$/L; plasma IgG, 7 to 15 g/L.

† p values 0.05 or more are designated as NS.

Valtola, Transfusion 2015

# What else besides CD34+? the immune perspective

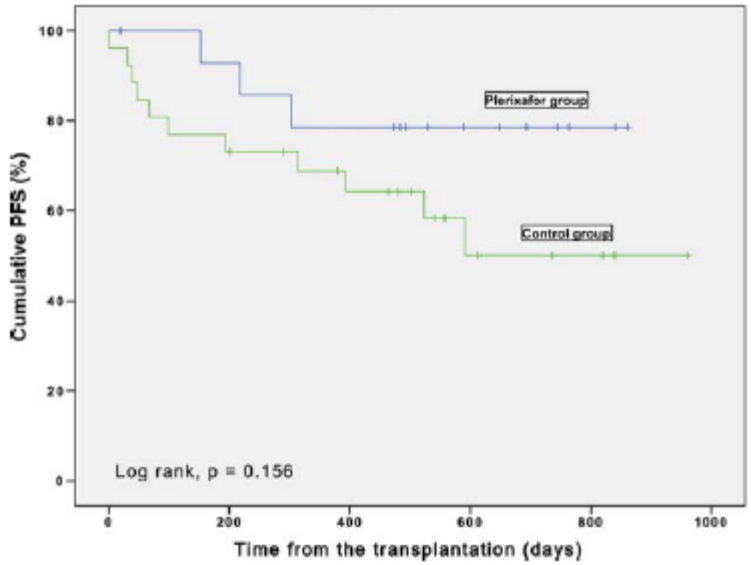



Fig. 1. Cumulative PFS of the patients.

**plerixafor**

**No plerixafor**

Valtola, Transfusion 2015

# What else besides CD34+? the immune perspective

**TABLE 2. Flow cytometry analysis of the lymphocyte subsets of the cryopreserved grafts***

| Blood graft content ($\times 10^6$ cells/kg) | Arm A (CY plus G-CSF), n = 17 | Arm B (G-CSF), n = 19 | p value† |
|---|---|---|---|
| CD3+ | 65.1 (28.3-283.0) | 215.2 (50.4-683.6) | <0.001 |
| CD3+CD4+ | 45.2 (12.6-156.4) | 116.3 (29.4-502.5) | 0.001 |
| CD3+CD8+ | 23.1 (5.3-133.9) | 90.5 (20.8-197.3) | 0.001 |
| CD19+ | 2.03 (0.46-11.6) | 8.7 (0.3-76.7) | <0.001 |
| NK | 6.8 (0.9-36.1) | 31.7 (15.5-144.7) | <0.001 |

* Data are reported as median (range).
† p values of 0.05 or more are designated as NS.

38 MM pts
CY vs G mobilization, randomized
Higher lympho mobilization in no-CHT arm

Valtola, Transfusion 2016

# What else besides CD34+? the immune perspective

**TABLE 4. Immunologic recovery after auto-SCT***

| Variable | Arm A (CY plus G-CSF), n = 17 | Arm B (G-CSF), n = 19 | p value† |
|---|---|---|---|
| Blood flow cytometry 1 month after auto-SCT ($\times 10^9$/L) | | | |
| CD3+ | 1.44 (0.7-5.9); 9 | 1.09 (0.3-2.5); 14 | 0.124 |
| CD3+CD4+ | 0.31 (0.2-0.5); 9 | 0.33 (0.1-0.6); 14 | 0.829 |
| CD3+CD8+ | 1.21 (0.5-5.4); 9 | 0.77 (0.2-2.2); 14 | 0.123 |
| NK | 0.27 (0.1-0.8); 9 | 0.46 (0.2-0.9); 14 | 0.083 |
| CD19+ | 0.001 (0.0-0.06); 9 | 0.01 (0.0-0.03); 14 | 0.877 |
| CD4/CD8– ratio | 0.21 (0.0-0.56); 9 | 0.4 (0.0-1.2); 14 | 0.109 |
| Blood flow cytometry 3 months after auto-SCT ($\times 10^9$/L) | | | |
| CD3+ | 1.37 (0.29-2.61); 14 | 1.06 (0.37-2.95); 19 | 0.038 |
| CD3+CD4+ | 0.35 (0.11-0.55); 14 | 0.32 (0.16-0.75); 19 | 0.358 |
| CD3+CD8+ | 1.1 (0.17-2.1); 14 | 0.69 (0.22-2.42); 19 | 0.035 |
| NK | 0.17 (0.07-0.36); 14 | 0.25 (0.14-0.51); 19 | 0.005 |
| CD19+ | 0.15 (0.0-0.38); 14 | 0.09 (0.05-0.29); 19 | 0.760 |
| CD4/CD8– ratio | 0.26 (0.0-0.64); 14 | 0.4 (0.0-0.86); 19 | 0.142 |
| Blood flow cytometry 6 months after auto-SCT ($\times 10^9$/L) | | | |
| CD3+ | 1.04 (0.48-1.8); 14 | 0.97 (0.35-1.61); 14 | 0.427 |
| CD3+CD4+ | 0.34 (0.2-0.62); 14 | 0.37 (0.18-0.71); 14 | 0.874 |
| CD3+CD8+ | 0.67 (0.24-1.44); 14 | 0.62 (0.18-1.27); 14 | 0.511 |
| NK | 0.15 (0.07-0.44); 14 | 0.26 (0.1-0.75); 14 | 0.014 |
| CD19+ | 0.13 (0.0-0.52); 14 | 0.07 (0.01-0.24); 14 | 0.352 |
| CD4/CD8– ratio | 0.4 (0.0-1.31); 14 | 0.6 (0.0-1.1); 14 | 0.164 |

* Data are reported as median (range); number of patients. Reference values of blood flow cytometry reported by the Laboratory Center of Eastern Finland: B-T-CD3 0.85 $\times$ $10^9$–2.28 $\times$ $10^9$/L; B-CD19 0.12 $\times$ $10^9$–0.43 $\times$ $10^9$/L; B-T-CD4 0.458 $\times$ $10^9$–1.406 $\times$ $10^9$/L; B-T-CD8 0.24 $\times$ $10^9$–0.98 $\times$ $10^9$/L; B-NK 0.08 $\times$ $10^9$–0.57 $\times$ $10^9$/L.

† p values of 0.05 or more are designated as NS.

Faster NK cell recovery in no-CHT arm

Valtola, Transfusion 2016

# What else besides CD34+? the immune perspective

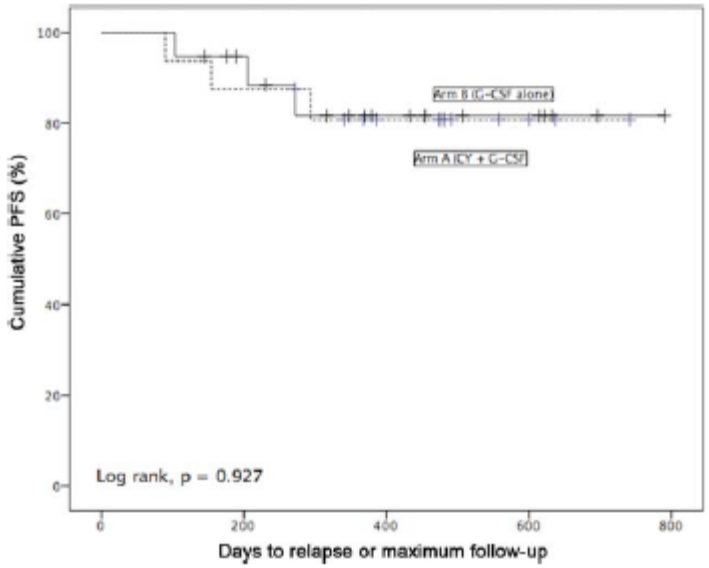



**Fig. 1. PFS of the patients after auto-SCT according to the method of mobilization.**

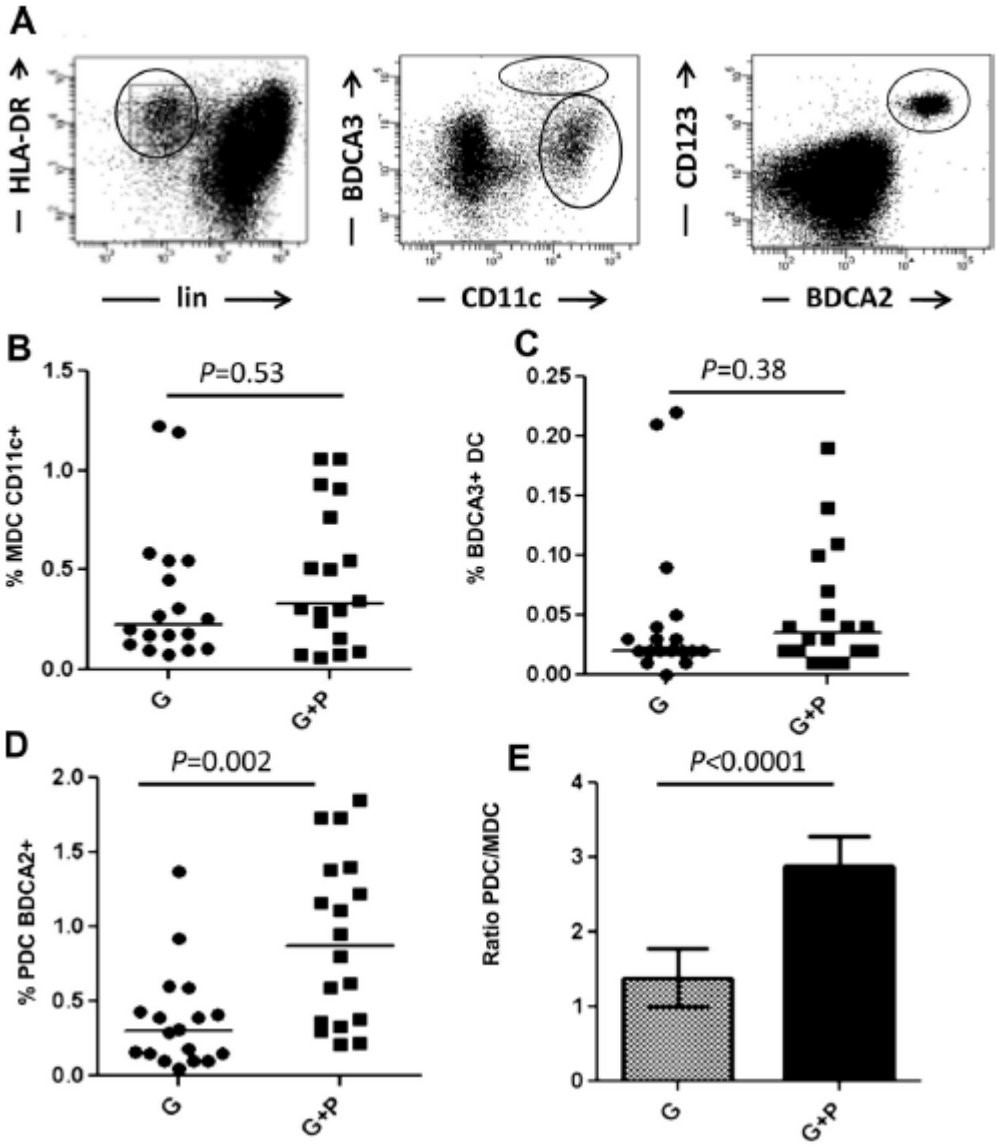
Similar outcome

Valtola, Transfusion 2016

# What else besides CD34+? the immune perspective

**Plasmacytoid dendritic cells** (PDCs: CD123+BDCA2+HLA-DR+)

- Significant mobilization with PLX
- Unknown implications in anti-disease activity and immune regulation




Gaugler, cytotherapy 2013

# What else besides CD34+? the immune perspective

**P033**

**Effects of plerixafor in the lymphocyte count in aphaeresis product**

*T Arias Fernandez[1], LR Morais Bras[2], J Zanabili Al-Sibbai[2], JM Garcia Gala[2], E Martinez Revuelta[2], LF Avila Idrovo[2], E Colado Varela[2] and F Garcia Menendez-Tevar[2]*

*[1]Hematology, Hospital Universitario Central de Asturias, Spain and [2]Hematology, Hospital Universitario Central de Asturias, Spain*

38 MM and NHL pts
G vs G+PLX
Higher CD34+ in NHL
No difference in MM

**Table 1. Results of CD3+ cell in product.**

| Diagnosis | Mobilization | n | CD3+ x $10^6$/kg (mean ± DS) | P |
|---|---|---|---|---|
| NHL | G-CSF | 8 | 171.18 (± 145.47) | 0.017 |
| | Plerixafor+ G-CSF | 10 | 205.89 (± 145.472) | |
| MM | G-CSF | 4 | 440.16 (± 197.52) | 0.063 |
| | Plerixafor+ G-CSF | 16 | 278.89 (± 94.45) | |

# What else besides CD34+?

**P039**

**Impact of lenalidomide induction in the mobilization of CD34+ cells, blood graft cellular composition and post-transplant recovery in myeloma patients: a prospective multicenter study**

*A Partanen[1], J Valtola[1], R Silvennoinen[2], A Ropponen[3], T Siitonen[4], M Putkonen[5], M Sankelo[6], J Pelkonen[3,7], P Mäntymaa[7], V Varmavuo[8] and E Jantunen[1]*

60 MM pts

Prior lena yes vs no

Similar mobilization of immune subsets

**Table1. Mobilization and harvesting results in myeloma patients according to the previous lenalidomide use**

| Variable | LEN(+) n=26 | LEN(-) n=34 | p-value |
|---|---|---|---|
| Peak B-CD34$^+$ cell count x10$^6$/L, mean(range) | 85(12-291) | 122(17-415) | 0.477 |
| Peak CD34$^+$ count >100x10$^6$/L, N (%) | 5(19) | 15(44) | 0.333 |
| Peak CD34$^+$count <20x10$^6$/kg, N (%) | 1(3) | 3(8) | 0.261 |
| B-CD34$^+$ cells x10$^6$/L at the time of first apheresis, mean (range) | 73(13-291) | 104(13.4-415) | 0.391 |
| CD34$^+$ cell yield x10$^6$/kg with first apheresis, mean (range) | 4.2(0.9-14.7) | 7.0(0.8-17.8) | 0.362 |
| Total yield CD34$^+$ cells x10$^6$/kg harvested, mean (range) | 7.1(2-14.7) | 8.5(2-17.8) | 0.854 |
| CD34$^+$ cells yield > 4x10$^6$/kg, N (%) | 21(80) | 28(82) | 0.821 |
| CD34$^+$ cells yield > 6x10$^6$/kg, N (%) | 12(46) | 21(61) | 0.663 |
| The number of apheresis, mean (range) | 2.0(1-4) | 1.5(1-3) | 0.039 |

# What else besides CD34+? the immune perspective

**P635**
**Blood graft composition and post-transplant recovery in myeloma patients mobilized with plerixafor**

*J Valtola[1], R Silvennoinen[2,3], A Ropponen[4], T Siitonen[5], M Säily[6] M Sankelo[7], M Putkonen[8], A Partanen[3], M Pyörälä[1] E-R Savolainen[9], P Mäntymaa[10], J Pelkonen[11,10], E Jantunen and V Varmavuo[12]*

-87 MM pts
-Chemomob +/- PLX
-no difference CD34+ dose
-Higher primitive CD34+ 38- higher in PLX
-Higher CD3+ , NK in PLX
**-Faster CD3+CD4+ T cell recovery!**

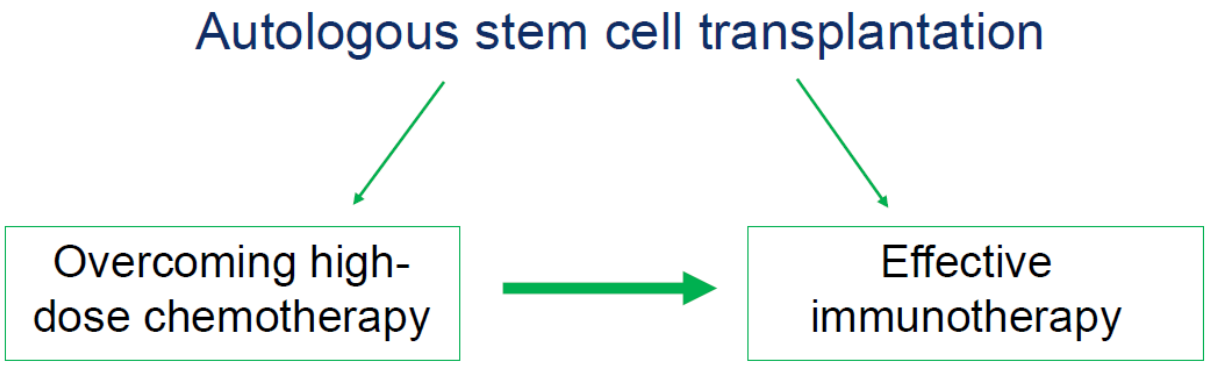
Table 1. Graft cellular composition according to the use of plerixafor.

| Variable | Collected grafts | | | Infused grafts | | |
|---|---|---|---|---|---|---|
| | Patients mobilized w plerixafor (n = 10) | Patients mobilized w/a plerixafor (n = 77) | p value | Patients mobilized w plerixafor (n = 10) | Patients mobilized w/a plerixafor (n = 77) | p value |
| CD34⁺ w/a 7-AAD (x10⁶/kg) | 4.9 (1.9-13.1) | 5.0 (1.7-17.4) | 0.545 | 3.2 (1.9-7.6) | 3.2 (1.0-10.3) | 0.680 |
| CD34⁺w 7-AAD (x10⁶/kg) | 3.2 (1.2-10.0) | 3.6 (0.2-14.3) | 0.284 | 1.8 (1.2-4.7) | 2.4 (0.2-7.2) | 0.581 |
| CD34⁺CD133⁺ CD38⁻ (x10⁶/kg) | 0.1 (0.04-0.75) | 0.09 (0.005-106.0) | 0.754 | 0.08 (0.04-0.35) | 0.07 (0.005-103.0) | 0.269 |
| Proportion of CD34⁺CD133⁺ CD38⁻ from all CD34⁺ cells (%) | 4.3 (2.6-7.5) | 3.0 (0.3-22.1) | 0.001 | 3.1 (1.9-5.6) | 1.9 (0.2-11.1) | <0.001 |
| CD3⁺ (x10⁶/kg) | 292.7 (58.3-683.6) | 89.4 (5.5-496.5) | <0.001 | 210.6 (29.2-388.3) | 54.8 (2.75-345.1) | <0.001 |
| CD3⁺CD4⁺ (x10⁶/kg) | 206.2 (37.6-502.5) | 54.8 (3.4-249.9) | <0.001 | 128.2 (18.8-290.3) | 31.6 (2.1-156.4) | <0.001 |
| CD3⁺CD8⁺ (x10⁶/kg) | 86.6 (21.2-194.1) | 25.6 (1.5-242.9) | 0.004 | 74.1 (10.6-1941) | 18.1 (0.75-195.0) | 0.001 |
| CD19⁺ (x10⁶/kg) | 17.6 (1.7-76.7) | 2.2 (0.01-61.59) | 0.001 | 14.1 (0.87-66.61) | 1.2 (0.005-61.590) | <0.001 |
| NK cells (x10⁶/kg) | 28.3 (2.3-65.3) | 9.2 (0.5-144.7) | 0.015 | 27.1 (1.15-59.86) | 6.9 (0.24-144.7) | 0.008 |

w/a = without; w = with, 7-AAD = 7-aminoactinomycin

# What else besides CD34+? the immune perspective

## Shift of paradigm




Graft design? Not quite there yet

# What else besides CD34+? the immune perspective

- Is there something more besides CD34+?
  - YES, autologous graft is a crowded and variegate family of cell subsets

**Autologous graft:**

-Not only a bag full of CD34+!

-Powerful cell therapy

-High immunological properties

-Theoretic possibility to engineer immunocompetent graft

-Platform for post-SCT immunotherapy

# CONCLUSIONS

• **CD34+ stem cell dose**: at least $2x10^6$, target $5x10^6$ for rapid PLT recovery and higher efficacy of auto-SCT procedure

• **CD34+ stem cell viability:** of great importance for quality check, not enough to predict graft functionality

•**CD34+ stem cell functionality:** the best parameter to be assessed, but how to do that?

• **CD34+, again…no, that's enough! what else besides CD34+?** Much more than just CD34+:

- •CD34+/38- dose predicts rapid engraftment
- • immune cell subsets are associated with patient outcome
- • mobilization strategy may influence graft composition and potential

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**THANK YOU!**