Qualità del graft: sottopopolazioni linfocitarie e subsets di cellule staminali.Il ruolo del Plerixafor

Riccardo Saccardi
Careggi University Hospital - Florence, Italy

Plerixafor and quality of the graft

- An accumulating evidence about both efficacy and safety of Plerixafor in rescuing poor mobilizers has been extensively reported
- Beside the increase in circulating/collected CD34+ cells, the use of Plerixafor results in an improvement of the collection condition
- A few data are reported about the impact on cellular subsets of both HSC and cells of the immune system

Plerixafor strategies : Upfront, Preemptive, Salvage

Table 2. Summary of mobilization strategies and outcomes

Author, year	Strategy			>2 × 10^6 CD34+ cells/kg, %	HSCT, %
	Upfront, %	Preemptive, %	Salvage, %		
Arcaini, 2011[25]	0	P+S	P+S	37	17
Attolico, 2012[40]	0	32	68	73	65
Basak, 2011[23]	0	30	66	78	65
Basak, 2011[41]	0	84	16	66	56
Calandra, 2008[31]	0	P+S	P+S	66	76
D'Addio, 2011[26]	0	100	0	100	39
Douglas, 2012[24]	0	19	81	95	71
Duarte, 2011[42]	0	4	96	75	63
Hubel, 2011[28]	0	0	100	75	67
Hubel, 2012[29]	0	0	100	74	NA
Selleslag, 2011[30]	0	0	100	64	59
Shaughnessy, 2013[39]	98	0	0	92	87
Worel, 2011[32]	0	P+S	P+S	63	48

Abbreviations: HSCT – hematopoietic SCT; NA – not available; P+S – preemptive and salvage strategies used although proportions not stated.

- **Plerixafor-based mobilization is effective in perceived poor mobilizers.**
- **The optimal way to incorporate plerixafor into a mobilization strategy, however, remains to be determined.**
- **Centre-specific analysis of resource utilization may help to identify the most cost-effective way to implement various plerixafor-based mobilization strategies.**

Sheppard D et Al. BMT 2014

CD34+ cell subclasses and lymphocyte subsets in blood grafts collected after various mobilization methods in myeloma patients

TRANSFUSION 2013;53:1024-1032.

Ville Varmavuo, Pentti Mäntymaa, Raija Silvennoinen, Tapio Nousiainen, Taru Kuittinen, and Esa Jantunen

- **Cryopreserved grafts collected on the next morning after plerixafor injection in nine MM patients mobilized with G-CSF with (n = 5) or without preceding CY (n = 4).**
- **12 MM patients mobilized with low-dose Cyc + G-CSF were used as controls**

TABLE 2. CD34+ cell counts and CD34+ subclasses in the grafts collected after various mobilization methods in myeloma patients*

Variable	Mobilization with CY 2 g/m² + G-CSF + PLER (Group A), n = 5	Mobilization with G-CSF + PLER (Group B), n = 4	Mobilization with CY 2 g/m² + G-CSF (controls, Group C), n = 12	p value significance		
				Between Group A and the control group	Between Group B and the control group	Between Group A and Group B
Original CD34+ cell content (×10⁶/kg)	2.1 (0.3-2.7)	2.4 (1.2-5.0)	4.0 (1.2-8.4)	0.006	0.212	0.413
CD34+ cell content after cryopreservation without 7-AAD (×10⁶/kg)	1.8 (0.2-2.1)	1.8 (0.9-4.7)	3.5 (1.0-7.4)	0.006	0.170	0.413
CD34+ cell content after cryopreservation with 7-AAD (×10⁶/kg)	1.6 (0.2-2.2)	1.4 (0.5-3.8)	2.9 (0.8-6.1)	0.027	0.212	0.730
Proportion of CD34+ CD133+CD38– cells from CD34+CD133+ cells (%)	3.6 (0.3-7.1)	4.7 (1.7-8.6)	0.8 (0.1-6.2)	0.048	0.020	0.730
Proportion of CD34+ CD133+CD38– cells from all CD34+ cells (%)	2.8 (0.2-6.1)	3.5 (1.4-7.1)	0.6 (0.1-4.9)	0.048	0.020	0.730
The most primitive stem cell (CD34+CD133+CD38–) content of the graft (×10⁶/kg)	0.02 (0.00-0.10)	0.05 (0.01-0.14)	0.02 (0.00-0.084)	0.574	0.212	0.413
Loss of viable CD34+ cells during cryopreservation (%)	24 (10-33)	43 (24-61)	30 (4-65)	0.328	0.379	0.063

CD34+ cell subclasses and lymphocyte subsets in blood grafts collected after various mobilization methods in myeloma patients

TRANSFUSION 2013;53:1024-1032.

Ville Varmavuo, Pentti Mäntymaa, Raija Silvennoinen, Tapio Nousiainen, Taru Kuittinen, and Esa Jantunen

- The proportion of the most primitive stem cells (CD34+CD133+CD38–) from all CD34+ cells in the graft was significantly higher in the PLER group but no significant difference was observed in the graft content of the most primitive stem cells between the groups.
- There was no significant difference in the number of viable CD34+ cells lost during the cryopreservation between the groups either.

CD34+ cell subclasses and lymphocyte subsets in blood grafts collected after various mobilization methods in myeloma patients

TRANSFUSION 2013;53:1024-1032.

Ville Varmavuo, Pentti Mäntymaa, Raija Silvennoinen, Tapio Nousiainen, Taru Kuittinen, and Esa Jantunen

TABLE 3. Lymphocyte subsets and in vitro growth in the grafts collected after various mobilization methods in myeloma patients*

Variable	Mobilization with CY 2 g/m² + G-CSF + PLER (Group A), n = 5	Mobilization with G-CSF + PLER (Group B), n = 4	Mobilization with CY 2 g/m² + G-CSF (controls, group C), n = 12	p value significance: Between Group A and the control group	Between Group B and the control group	Between Group A and Group B
CD3+ cell content (×10⁶/kg)	61.7 (16.9-197.2)	128.8 (11.6-333.9)	40.1 (6.4-90.7)	0.279	0.133	0.730
CD3+CD4+ cell content (×10⁶/kg)	51.9 (10.2-126.5)	56.7 (8.9-206.5)	26.6 (3.6-68.9)	0.160	0.212	1.000
CD3+CD8+ cell content (×10⁶/kg)	10.2 (6.9-71.8)	73.0 (2.7-125.1)	12.6 (2.8-23.10)	0.799	0.170	0.413
CD19+ cell content (×10⁶/kg)	1.8 (0.4-2.7)	15.0 (2.8-56.0)	0.5 (0.1-53.70)	0.160	0.013	0.016
CD4+/CD8+ ratio	2.6 (1.5-5.1)	1.3 (0.6-3.2)	2.0 (0.9-7.5)	0.506	0.262	0.190
NK (CD3–CD16/56+) cell content (×10⁶/kg)	2.5 (1.1-17.6)	23.2 (2.7-27.6)	3.3 (1.0-10.8)	1.000	0.030	0.063
Proportion of nonviable lymphocytes with 7-AAD (%)	19 (7-43)	8 (2-20)	17 (4-28)	0.506	0.262	0.190

- The numbers of CD3+, CD4+, and CD8+ lymphocytes were higher in patients mobilized with G-CSF+PLER compared to other groups (not significant).
- The number of CD19+ B-lymphocytes was significantly higher in the patients mobilized with G-CSF and PLER when compared to the control group patients or patients in Group A.
- The number of NK cells was significantly higher in the patients mobilized with G-CSF and PLER when compared to the control group patients.
- No significant differences were reported in the lymphoid subsets between Cy/G+Pler and Cy/G
- No differences in the engraftment and immune recovery were shown across the 3 groups

Blood graft lymphocyte subsets after plerixafor injection in non-Hodgkin's lymphoma patients mobilizing poorly with chemotherapy plus granulocyte–colony-stimulating factor

Ville Varmavuo, Pentti Mäntymaa, Taru Kuittinen, Tapio Nousiainen, and Esa Jantunen

TRANSFUSION 2012;52:1785-1791.

TABLE 2. WBC counts, ALCs, and blood B-CD34+ counts as measured on the morning of the apheresis in plerixafor-treated patients and in the control patients. In two patients both grafts collected before and after plerixafor were included in separate groups

Variable	Stem cell collection with plerixafor* (n = 13)	Stem cell collection without plerixafor* (n = 13)	p value
WBC count ($\times 10^9$/L)	26.4 (9.8-54.9)	5.6 (2.8-35.2)	<0.001
ALC ($\times 10^9$/L)	2.1 (0.6-5.9)	0.9 (0.3-5.3)	0.020
CD34+ cell count ($\times 10^6$/L)	39 (11-81)	25 (11-179)	0.778

* Data are reported as median (range).

ALC= absolute lymphocyte count

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.

- **The median counts of total CD3+ T cells, helper (CD3+CD4+) T subsets, suppressor (CD3+CD8+) T subsets, and NK (CD3-CD16/56+) cells in the graft were significantly higher in the plerixafor group**
- **Graft CD34+ cell count after cryopreservation or CD4+/CD8+ cell ratio did not differ significantly between the groups.**

Characterization of peripheral blood stem cell grafts mobilized by granulocyte colony-stimulating factor and plerixafor compared with granulocyte colony-stimulating factor alone

Cytotherapy, 2013; 15: 861–868

BEATRICE GAUGLER[1,2,3,*], JESSY ARBEZ[1,2,3,*], STEVEN LEGOUILL[4], PIERRE TIBERGHIEN[1,2,3], PHILIPPE MOREAU[4], SOPHIE DERENNE[5], PHILIPPE SAAS[1,2,3] & MOHAMAD MOHTY[4,6,7]

Table I. Study population characteristics.

	G (n = 18)	G + P (n = 18)
Median age (range), years	56 (5–64)	54 (16–64)
Patient gender, male/female	12/6	8/10
Diagnosis, no. (%)		
Multiple myeloma	8 (44.5%)	8 (44.5%)
Non-Hodgkin lymphoma	3 (17%)	6 (33.5%)
Hodgkin lymphoma	2 (11%)	2 (11%)
Solid tumors	2 (11%)	2 (11%)
Chronic lymphocytic leukemia	1 (5.5%)	
Acute myeloid leukemia	1 (5.5%)	
Plasmacytoma	1 (5.5%)	

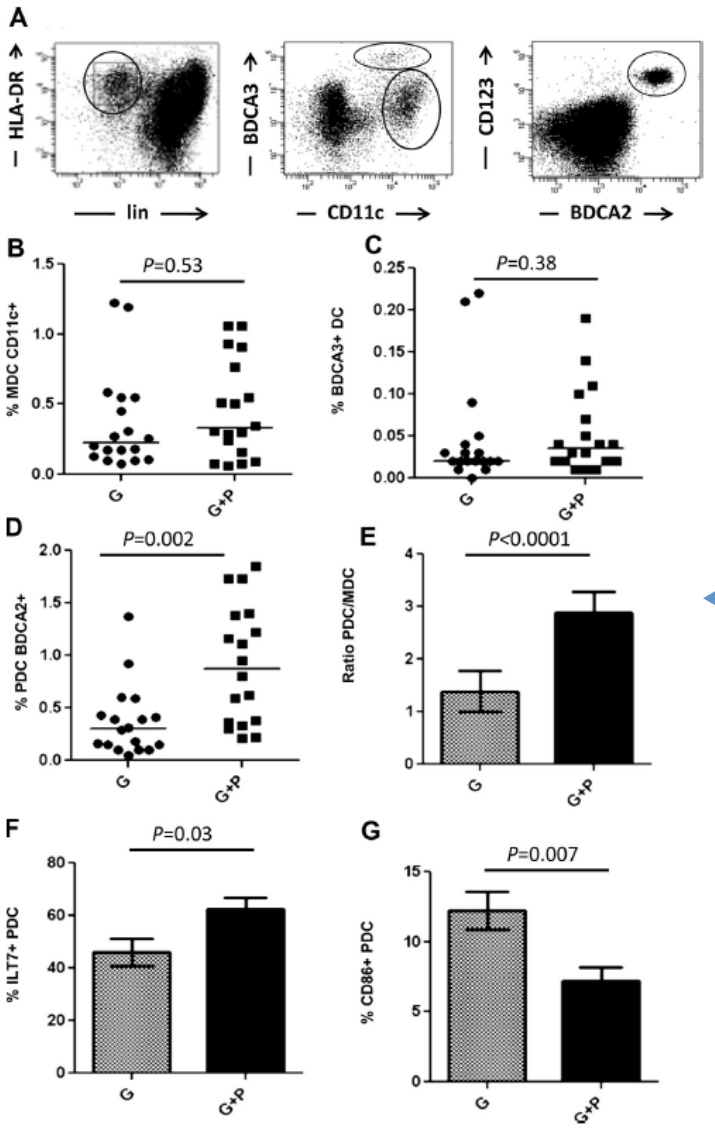
Table II. Lymphocyte graft content.

	G	G + P	*P*
Lymphocytes, %[a]			
$CD3^+$	73 (34–93)	81 (53–94)	0.01
$CD4^+$	52 (17–63.5)	48 (17–75)	0.68
$CD8^+$	41 (25–79.5)	43 (18–80)	0.77
Ratio CD4/CD8	1.3 (0.2–2.5)	1 (0.2–4)	0.56
Naïve CD4 ($CD27^+CD45RA^+$)	43 (1.5–80)	36 (2–71)	0.8
Naïve CD8 ($CD27^+CD45RA^+$)	38 (6–90)	43 (5–85)	0.8
$CD19^+$	7 (0–53.5)	1 (0–18)	0.2
NK cells	9 (4–28.5)	9 (1–27)	0.35

[a]Median (range).

- **In grafts collected after P+G, there was a significantly higher percentage of CD3+ T cells compared with samples collected after G. However, the CD4/CD8 ratio was comparable between both groups**
- **When considering the different T-cell subsets, there were no significant differences in the distribution of naïve T cells**

MDC= myeloid DC PDC= Plasmacyotid DC

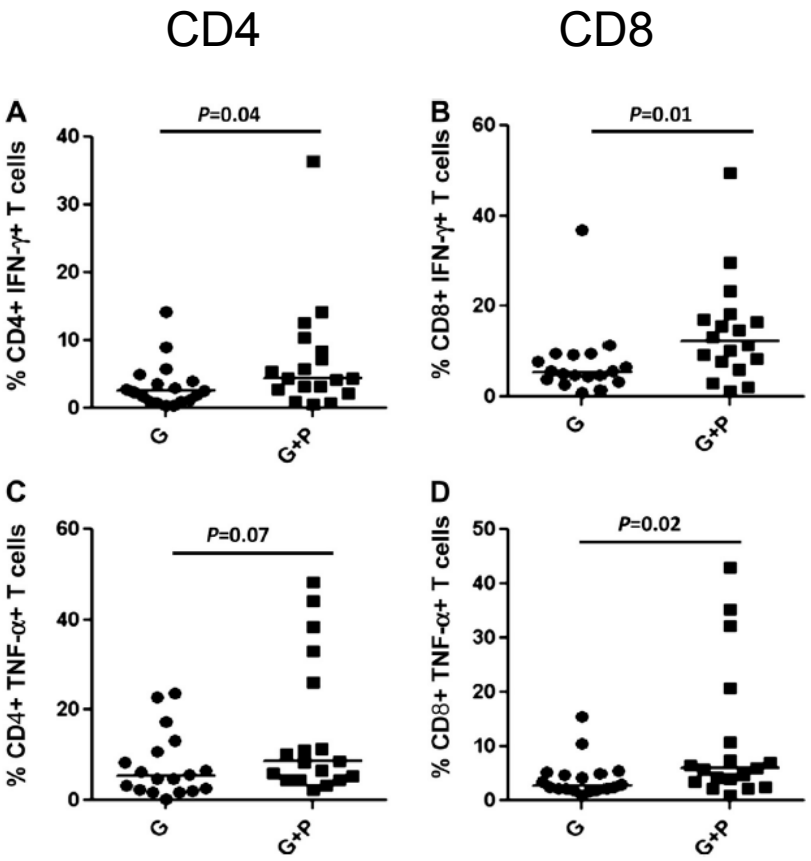


Grafts mobilized with P+G contained similar percentages of MDCs (defined as lin-, HLA-DR+, CD11c+) and blood dendritic cell antigen 3 (BDCA3+) dendritic cells (lin-, HLA-DR+, BDCA3+).

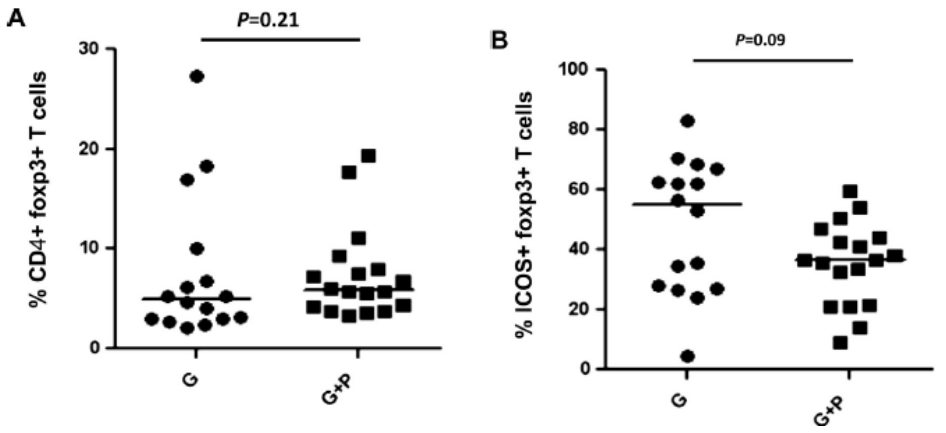
In contrast, the percentage of PDCs (defined as CD123+BDCA2+HLA-DR+) was significantly increased in the P+G grafts

PDCs mobilized by P+G displayed different functional markers compared with PDCs mobilized with G— higher percentage of ILT7+ PDCs and decreased expression of CD86—suggesting a potential regulatory capacity of PDCs mobilized by P+G

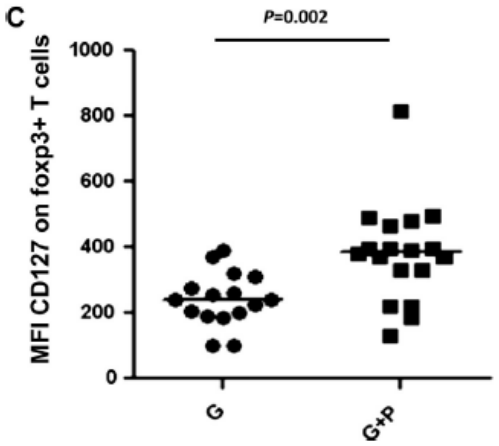
Gaugler et Al, Cytotherapy 2013



Significant increase of IFN-g-secreting and TNF-a-secreting CD8+ T cells in the P+G group compared with the G group



Grafts mobilized with P+G contained a similar percentage of CD4+ Tregs

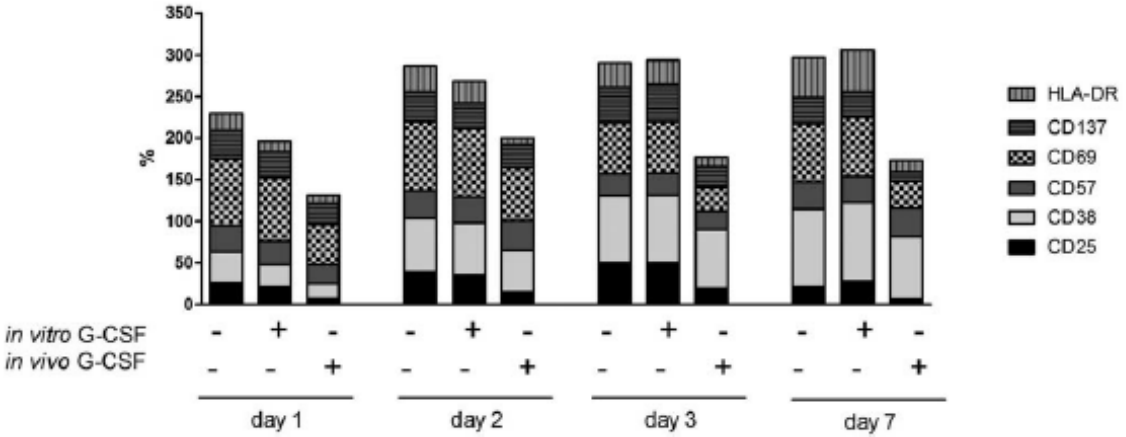


Also, Tregs from the P+G grafts displayed a significantly higher expression of CD127 suggesting that Tregs mobilized with P+G likely exhibit different functional properties.

Gaugler et Al, Cytotherapy 2013

Granulocyte colony-stimulating factor impairs CD8$^+$ T cell functionality by interfering with central activation elements

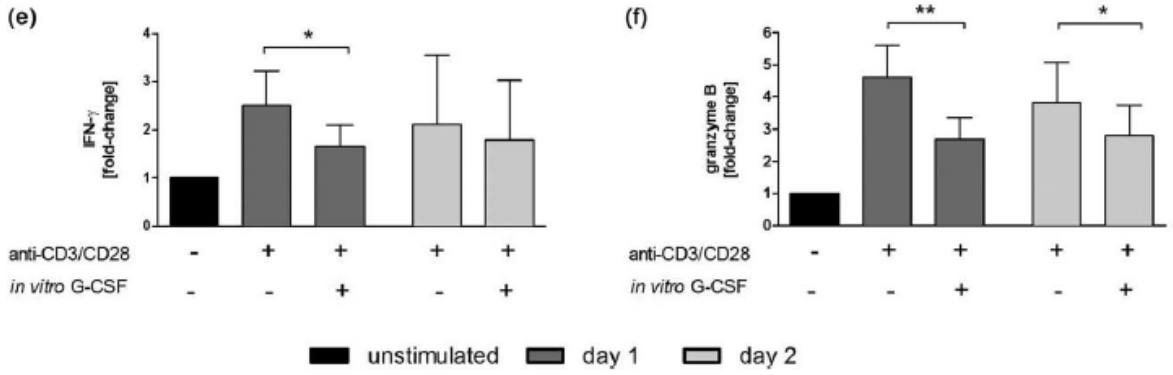
C. E. Bunse,*† S. Tischer,*†
J. Lahrberg,* M. Oelke,‡
C. Figueiredo,* R. Blasczyk*† and
B. Eiz-Vesper*†



CD8+ Cell surface activation markers expression is reduced after *in vitro* and *in vivo* G-CSF treatment

G-CSF treatment of isolated CD8+ T cells alters effector molecule expression of IFN-g (e) and granzyme B (f)

CD8+ T cells treated with G-CSF secrete less IFN-g and release less granzyme B than those expanded in the absence of G-CSF

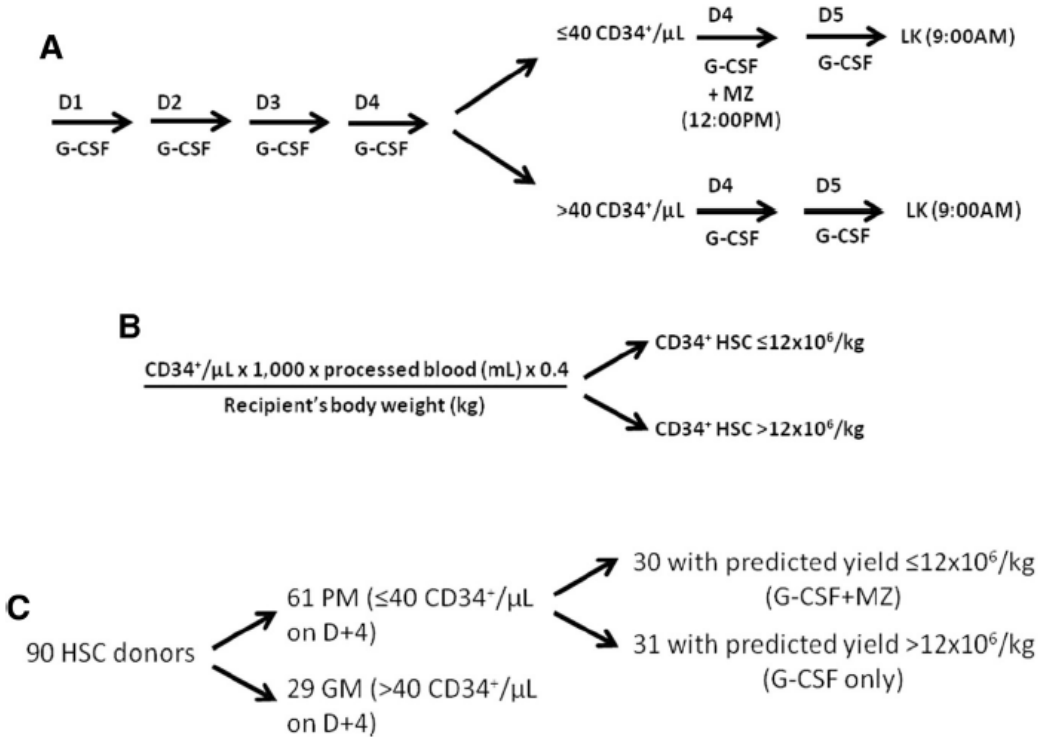


Clinical and Experimental Immunology; 2016, **185**: 107-118

Mobilization of healthy donors with plerixafor affects the cellular composition of T-cell receptor (TCR)-αβ/CD19-depleted haploidentical stem cell grafts

Sergio Rutella[1,5*], Perla Filippini[1], Valentina Bertaina[1], Giuseppina Li Pira[1], Lidia Altomare[1], Stefano Ceccarelli[1], Letizia P Brescia[1], Barbarella Lucarelli[1], Elia Girolami[1], Gianpiero Conflitti[1], Maria Giuseppina Cefalo[1], Alice Bertaina[1], Tiziana Corsetti[2], Lorenzo Moretta[3,4] and Franco Locatelli[1,4]

- 30/90 "poor" mobilizers (CD34/µL<40 @+4 or predicted <12^6 CD34/Kg)
- PLX administered @+4
- MZ significantly increased CD34+ counts in PM.
- Naïve/memory T/B and NK cells, myeloid/ plasmacytoid dendritic cells (DCs), were unchanged compared with baseline.



Journal of Translational Medicine 2014, 12:240

Plerixafor and graft composition

- Grafts mobilized with Pler+G exhibited some different functional features compared to mobilization with G alone
- However, no significant differences were reported in terms of CD34/Lymphoid cells viability and engraftment
- Lack of detailed data about graft composition collected from prospective, randomized trials may account for this finding

The «ideal collection»

- Large number of $CD34^+$ (possibly for ≥2 ASCT procedures)...
- …in **one** short LK procedure…
- …withouth need of several days of monitoring…
- …withouth reaching exagerate leukocyte count…
- …with low PMN contamination…
- …with low/absent tumor cell contamination…
- …easy to plan (fixed collection day)…
- …no need of toxic mobilizing agents…
- …no SAE during the mobilization/collection time.

Plerixafor and granulocyte colony-stimulating factor for first-line steady-state autologous peripheral blood stem cell mobilization in lymphoma and multiple myeloma: results of the prospective PREDICT trial

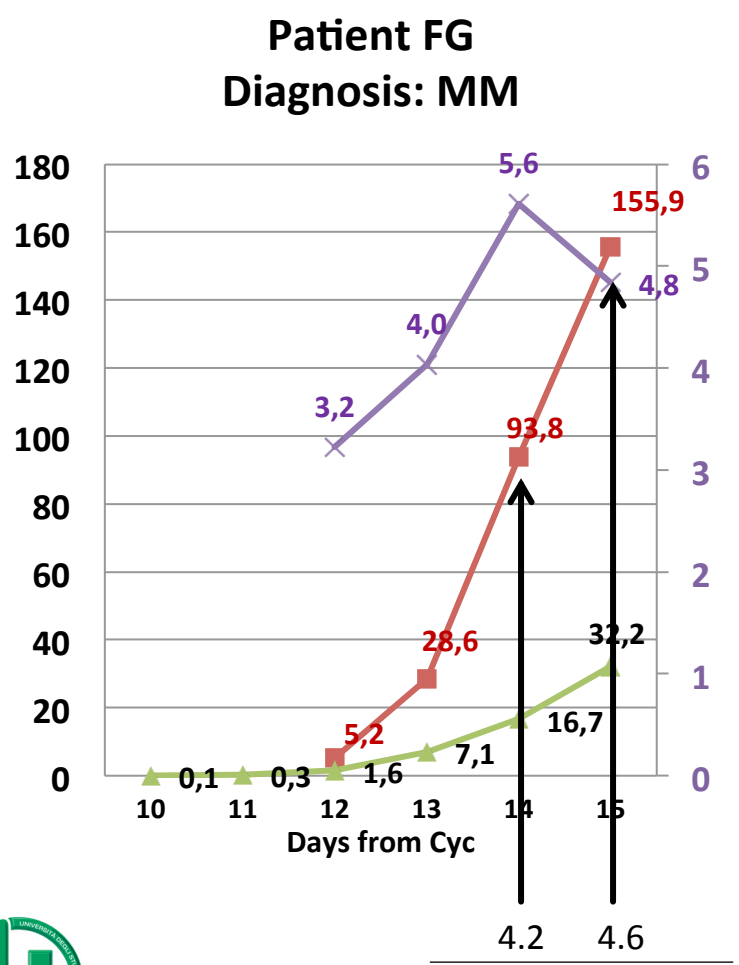
Nigel Russell,[1] Kenny Douglas,[2] Anthony D. Ho,[3] Mohamad Mohty,[4] Kristina Carlson,[5] G.J. Ossenkoppele,[6] Giuseppe Milone,[7] Macarena Ortiz Pareja,[8] Daniel Shaheen,[9] Arnold Willemsen,[10] Nicky Whitaker,[11] and Christian Chabannon[12]

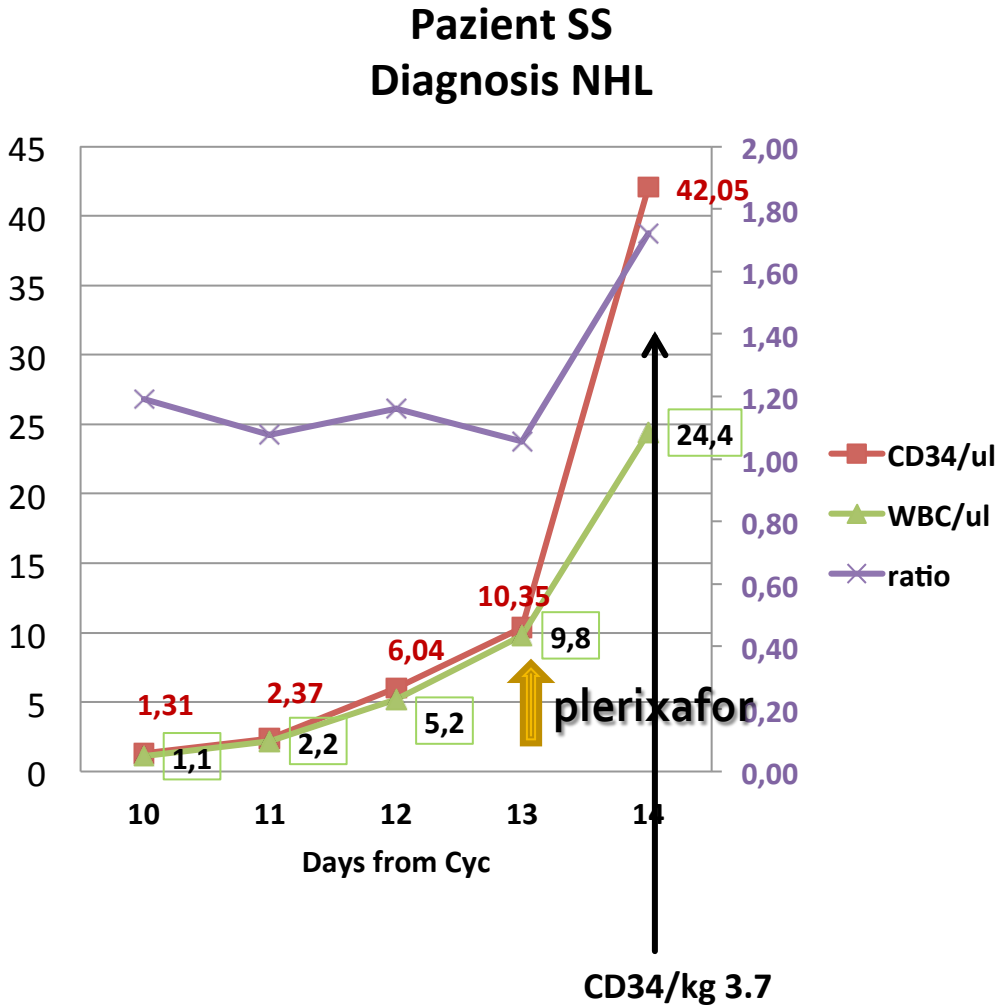
- **Multicenter, open label, single-arm study, Patients received G-CSF (10 mg/kg/day) dd 1-4 + PLX(0.24 mg/kg) on the evening of day 4, Day 5 PBSC apheresis**
- **PLX, G-CSF and apheresis were continued for up to 5 days or until ≥5×10^6 CD34$^+$ cells/kg for lymphoma or ≥6×10^6 CD34$^+$ cells/kg for MM had been collected.**

	MM=90	NHL=25
N. of patients undergoing apheresis (%)	89 (99)	22 (88)
Fold change# in PB CD34+ cells/µL, median (range)	2.6 (0.2-94.0)	2.6 (0.4-5.5)
CD34+ cells/kg x 106 collected, median (range)	7.6 (1.5-24.0)	5.2 (0.2-16.7)
N. of patients yielding **minimal cell dose** (≥2x10^6 /kg)	88 (98%)	20 (80%)
Days to collect minimal cell dose, median (range)	1 (1-3)	1 (1-3)
No. of patients yielding **optimal cell dose** (≥5x106 NHL and ≥6x106 MM CD34+ cells/kg) (%)	80 (89)	12 (48)
Days to collect optimal cell dose, median (range)	1 (1-4)	3 (1-3)

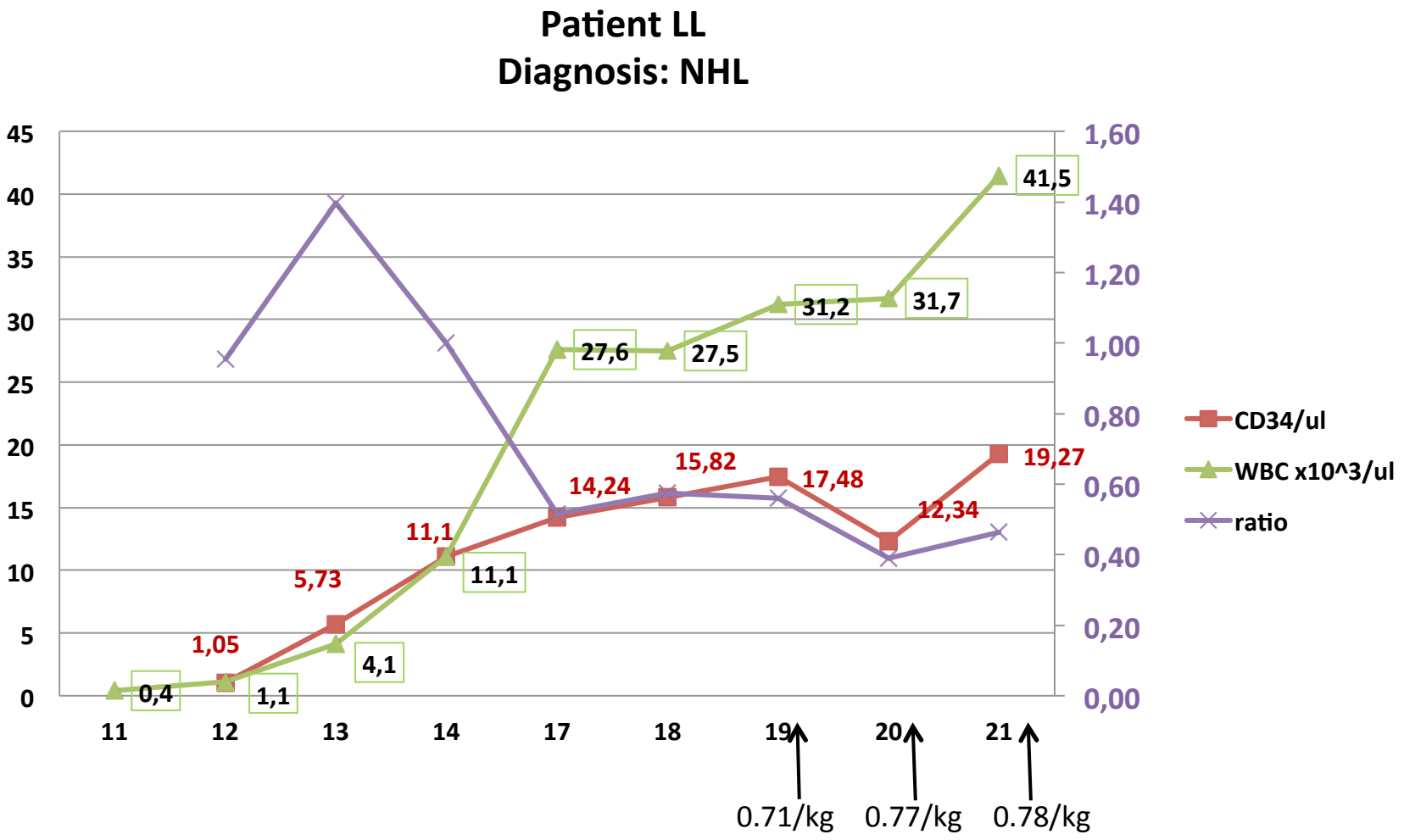
haematologica | 2013; 98(2)

Mobilization monitoring: WBC, $CD34^+$, ratio and collection





Mobilization monitoring: "slow" mobilizers



3 collections; TOT CD34⁺/kg 2,26

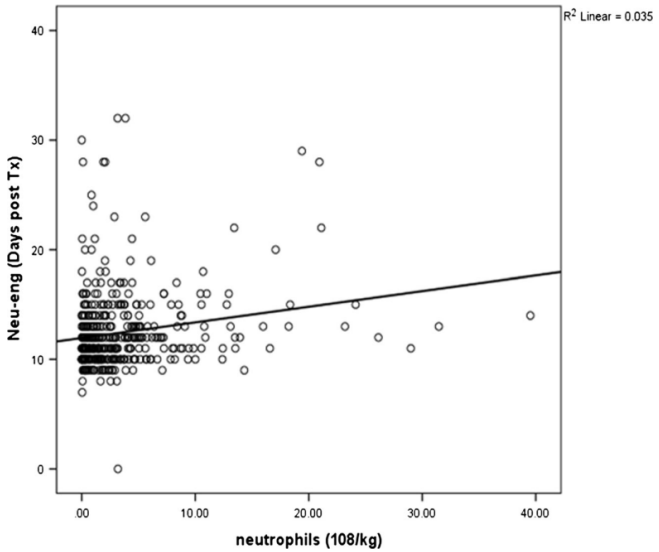
Aiming to the ideal collection I

- The algorithms targeted to the optimal use of Plexirafor are mostly aimed to the early identification of poor mobilizers
- Both circulating and collected CD34+ cells minimum thresholds are utilized to a decision making
- Strategies to achieve a condition of very good mobilizers might be considered

Aiming to the ideal collection II

- The count of circulating $CD34^+$ after 3 days of G-CSF administration in SS is a good predictor of mobilization at peak day (+5)
- In chemo+GF schedules, the CD34+ count and WBC/CD34 ratio at WBC recovery predict the mobilization kynetics
- The early use of Plerixafor in a setting of poor/slow mobilizers can be targeted to further optimizing the PBSC collection

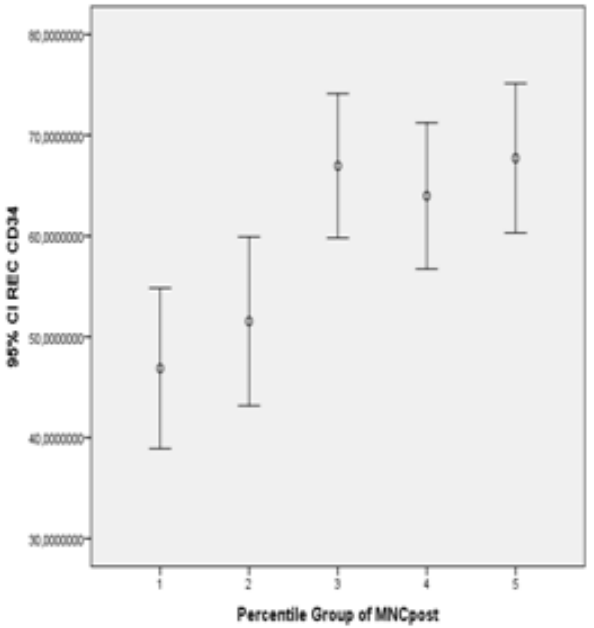
Impact of the graft quality on the clinical outcome: CD34^{+} content and PMN contamination



•Time to platelet engraftment was significantly delayed in those receiving low versus medium or high CD34+ doses.

•Increasing neutrophil contamination of HPC-A was strongly associated with slower neutrophil recovery

Lijun Bai et Al, Ann Hematol (2014) 93:1655–1664



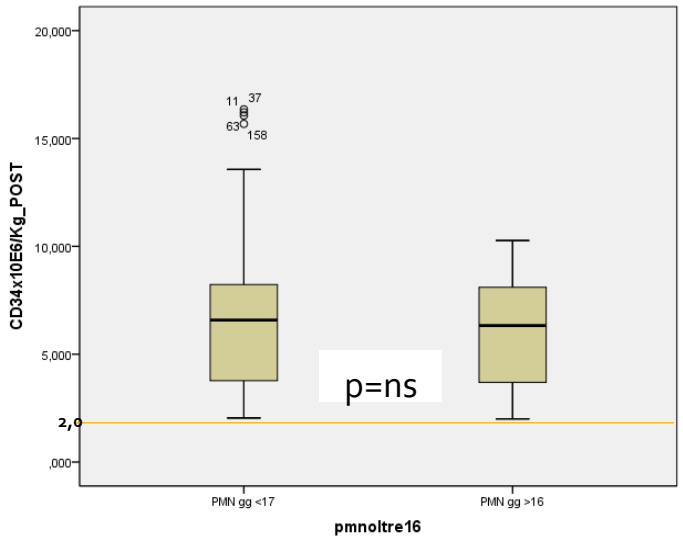
Recovery of viable CD34+ cells is proportional both to TNC and MNC content of the frozen product

Urbani et Al, EBMT2016

Impact of the graft quality on the clinical outcome: need for a safe CD34⁺ cells dose

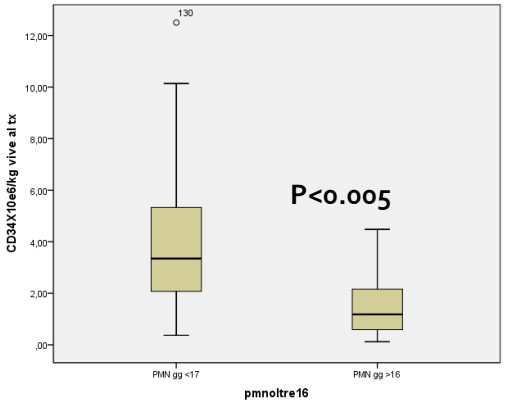
Loss of viable CD34+ cells in the freezing/thawing process may have a detrimental effect on the engraftment

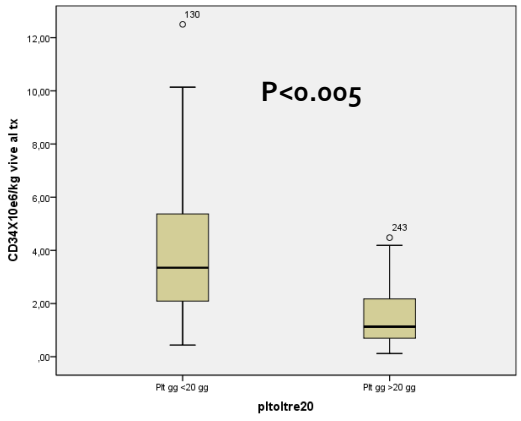
Viable CD34⁺ at freezing



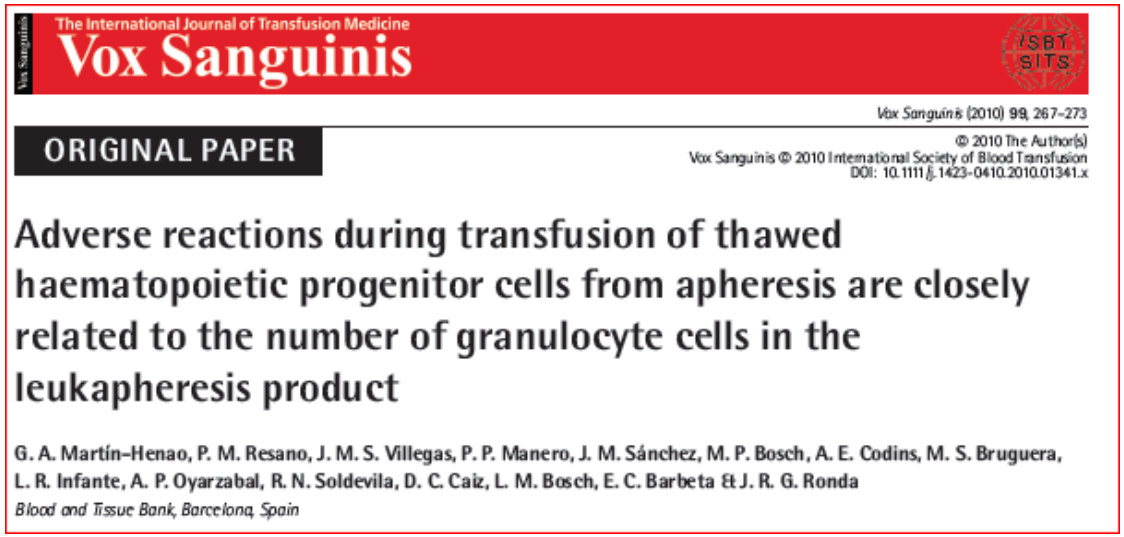
PMN ≤ 16 days PMN > 16 days

Viable CD34⁺ after thawing





N	Diag	PMN eng	Plt eng
241	MM, NHL, HL, MS	12 (8-31)	13 (8-43)



The International Journal of Transfusion Medicine
Vox Sanguinis

Vox Sanguinis (2010) 99, 267–273

ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2010.01341.x

Adverse reactions during transfusion of thawed haematopoietic progenitor cells from apheresis are closely related to the number of granulocyte cells in the leukapheresis product

G. A. Martín-Henao, P. M. Resano, J. M. S. Villegas, P. P. Manero, J. M. Sánchez, M. P. Bosch, A. E. Codins, M. S. Bruguera, L. R. Infante, A. P. Oyarzabal, R. N. Soldevila, D. C. Caiz, L. M. Bosch, E. C. Barbeta & J. R. G. Ronda
Blood and Tissue Bank, Barcelona, Spain

- 423 **unmanipulated** infusions of 398 patients
- **24.8%** of adverse events, mostly moderate to severe

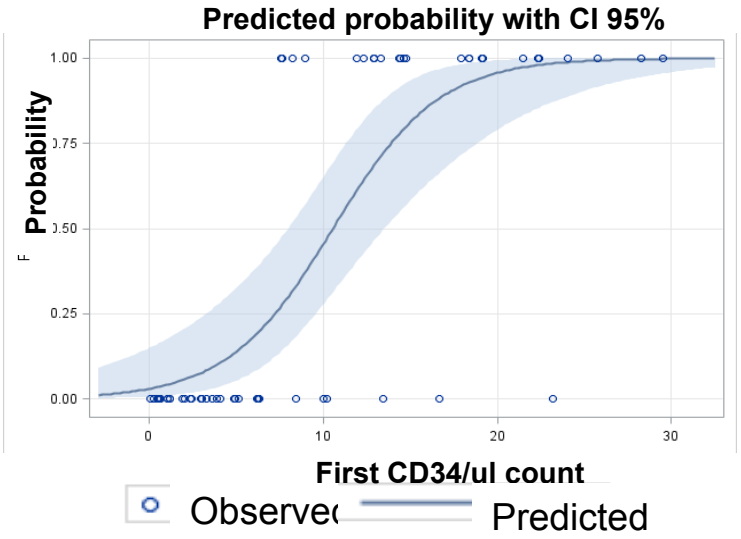
- ✓ The volume of DMSO/kg ($P < 0.001$),
- ✓ volume of red-blood-cells/kg ($P = 0.02$)
- ✓ number of nuclear cells (NCs)/kg ($P < 0.001$)
- ✓ number of granulocytes/kg ($P < 0.001$)

  in the infused graft were significant in the **univariate** analysis for the occurrence of ARs.

- The amount of granulocytes/kg remained significant in the **multivariate** analysis

Prediction of very good vs poor mobilizers in a Chemo+G-CSF model

- 102 patients (59 MM, 21 NHL, 9 HD, 12SM)
- Mobilization Cyc 4 g/sqm, G-CSF 10 µG/Kg from +5
- First CD34 count at WBC recovery (≥1x10^9/L)



First_CD34_count	False Positive	False Negative	Sensitivity	Specificity
29,53	0	25	3,84	100
28,26	0	24	7,69	100
25,75	0	23	11,53	100
24,06	**0**	**22**	**15,38**	**100**
23,22	1	22	15,38	96,875
22,42	1	21	19,23	96,875
.....	....	....	....	...
8,97	5	3	88,46	84,37
8,45	6	3	88,46	81,25
8,28	6	2	92,3	82,25
7,68	6	1	96,15	81,25
7,56	**6**	**0**	**100**	**81,25**
6,35	7	0	100	78
6,28	8	0	100	12
6,19	9	0	100	75
5,16	10	0	100	71,875
.....	....	....	....	...

First CD34/ul count ≥ 24.06 :
100% true "very good mobilizer"

First CD34/ul count ≤ 8 :
100% true NOT good mobilizer

Both CD34 count and WBC/CD34 ratio at WBC recovery can predict the probability of a CD34 count either <20 or >40/µL the following day

PERSONALIZATION OF MOBILIZATION: CONCLUSIONS

- Many decision-making algorithms aimed to target the PBSC mobilization were published in the last five years
- The optimization of the mobilization kynetics can improve both the clinical outcome and the collection/processing logistics
- An appropriate use of Plerixafor will help in achieving the ideal collection in a high percentage of patients, also resulting cost-effective

CELLULAR THERAPY AND TRANSFUSION MEDICINE UNIT

Blood and Marrow Transplant Section

Director: Riccardo Saccardi

- Clinical Unit:
  - Ilaria Cutini
  - Irene Donnini
  - Antonella Gozzini
  - Stefano Guidi
  - Chiara Nozzoli
- Collection Unit:
  - Francesca Pagliai
- Immunogenetic Unit:
  - Gianni Rombolà

- Processing lab:
- Director:Serena Urbani
- Personale operativo:
  - Lara Ballerini
  - Lucia Bianchi
  - Paola Bufano
  - Alessia Gelli
  - Francesca Materozzi
  - Michela Santosuosso
  - Valentina Sbolci
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