Mobilizzazione di cellule staminali emopoietiche
«chemo-free» nel Mieloma Multiplo:
è tempo di prime time?

Bologna, 16 marzo 2017

Come il plerixafor modifica la mobilizzazione di cellule staminali e sottopopolazioni linfocitarie nel MM trattato con chemioterapia

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Mobilization of HSC
Key issues

-Understanding bio-molecular mechanisms

To improve mobilization

-Management of poor mobilizers

a)*optimization of a risk-adapted use of Plerixafor*

b)novel mobilizing agents

-Better definition of mobilized PB graft

a)*CD34+ cell subsets*

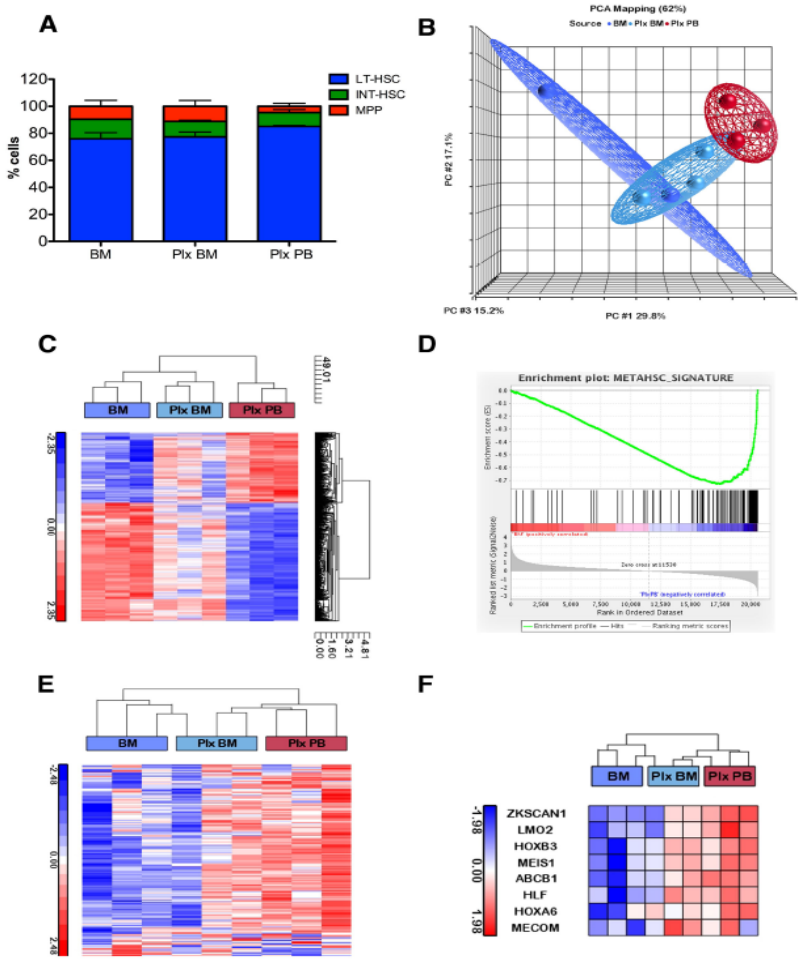
b)the immunological content

Plerixafor: which stem cell?

$CD34^+$ stem cell content in mobilized PB still represents the only and most important parameter of graft quality

Several reports indicate that plerixafor may increase the mobilization of more immature $CD34^+/CD38^-$ stem cell population as compared to G-CSF with or without chemotherapy

Distinct transcriptional profile and function of PLX alone versus PLX plus G-CSF



LT-HSC

Int-HSC

MPPs

Gene expression profiling

Cellular and metabolic related processes

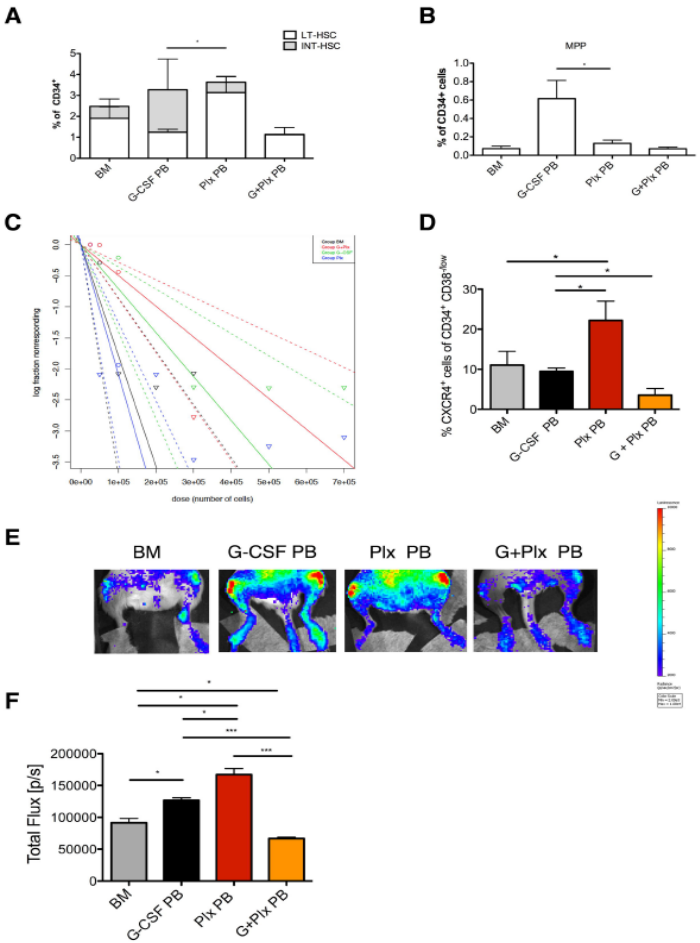
Genes involved in cell-cycle processes

DNA repair genes and Associated to S-phase

PLX PB is enriched in quiescent immature HSCs

Lidonnici et al, Haematologica, 2016

PLX-mobilized HSCs have incresed SCID-repopulating capacity



Lidonnici et al, Haematologica, 2016

Increased SCID-repopulating capacity is due to:

- **Increased migrating cells**
- **Increased LT-repopulating cells**

CXCR4 expression is higher in PLX-mobilized HSCs

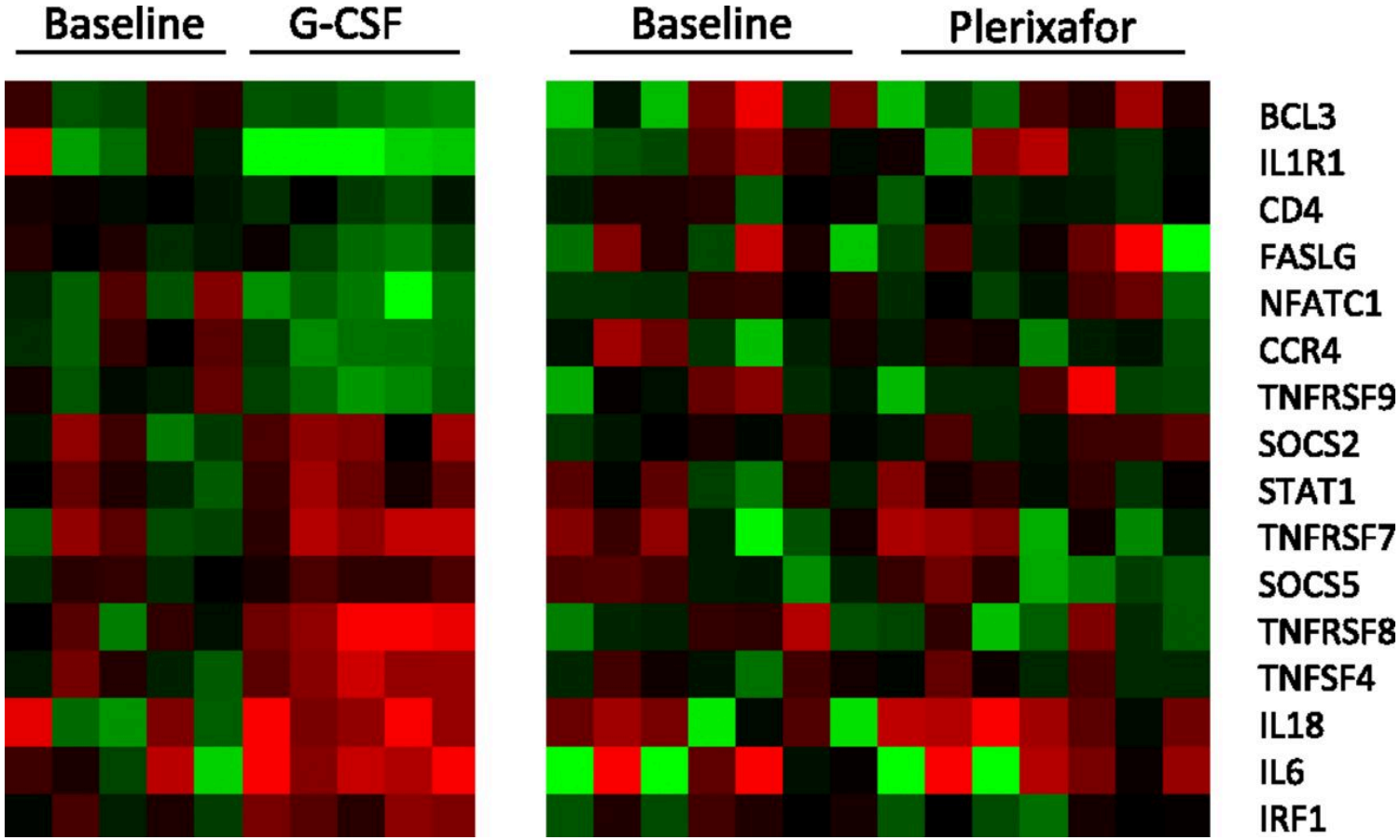
Plerixafor: the question of Immunological graft

Recent studies demonstrate that Plerixafor administration results in increased number of

T and B cells, NK cells in the graft

Biologically, SDF-1/CXCR4 is known to control T and NK cell trafficking from the BM to the PB

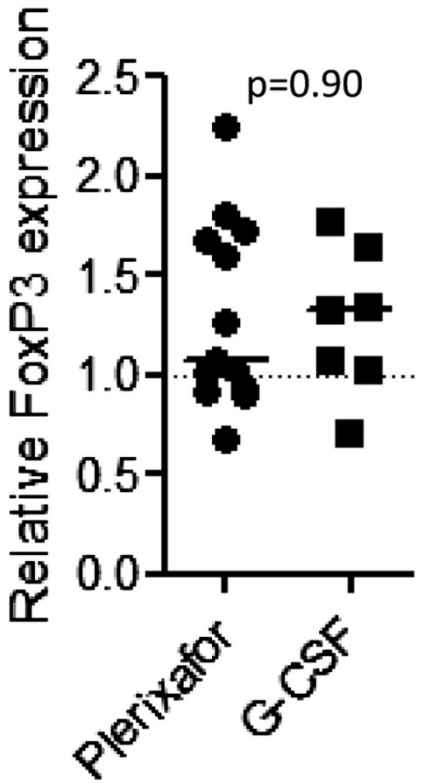
Plerixafor does not alter the phenotype and cytokine polarization of T lymphocytes

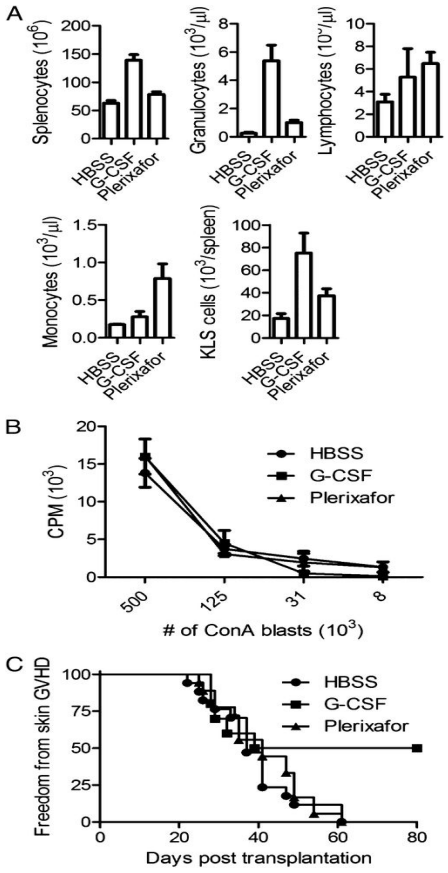


Andreas Lundqvist et al. J Immunol 2013;191:6241-6249

The Journal of Immunology

Reduced alloreactovity of G-mobilized T cells over PLX





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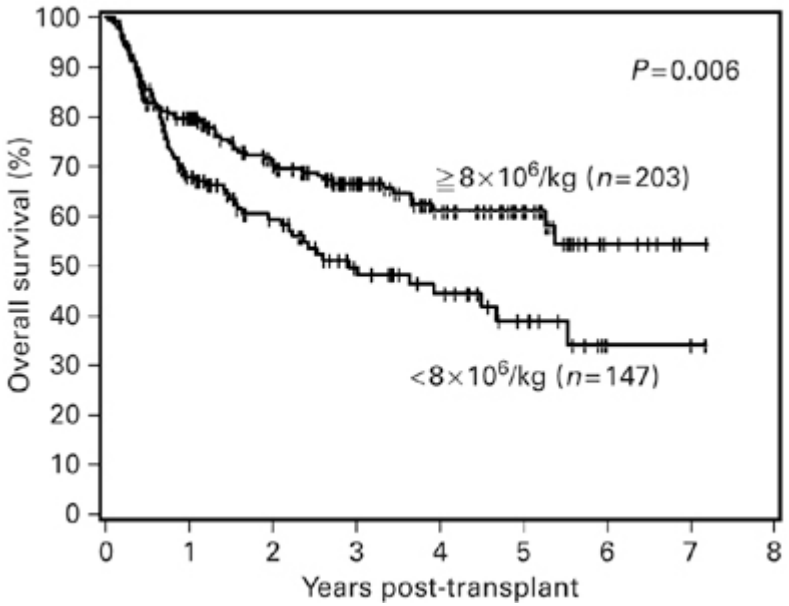
$CD34^+$ cell dose and outcome: clinical studies

Author	Disease	No of patients	Result
Stockerl-Goldestein	NHL	172	No difference
Gordan	NHL, HL	58, 32	Better PFS and OS
Blystad	NHL	43	Better PFS and OS if CD34 > 6.1 x 10e6/kg
Pavone	NHL, HL	205, 57	Better PFS and OS if CD34 > 2 x 10e6/kg
Bolwell	NHL, HL	386, 64	Better PFS and OS if CD34 > 8 x 10e6/kg
Tomblyn	NHL, HL	128, 57	Better PFS and OS if CD34 > 2 x 10e6/kg
Yoon	NHL	97	Better PFS and OS if CD34 > 8.2 x 10e6/kg
Terpos	MM	127	No difference
Wahlin	MM	104	Better OS with high CD34
Toor	MM	104	Better OS with high CD34
O'Shea	MM	211	Better OS with high CD34

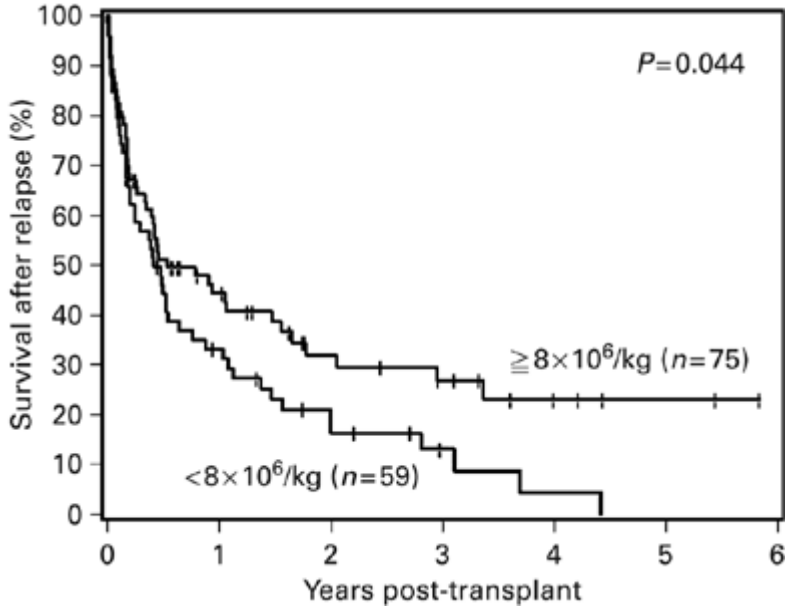
Adapted from Jantunen & Fruehauf, *Bone Marrow Transplantation*, 2011

Patients mobilizing large numbers of CD34+ cells ('super mobilizers') have improved survival in autologous SCT for lymphoid malignancies

Overall Survival

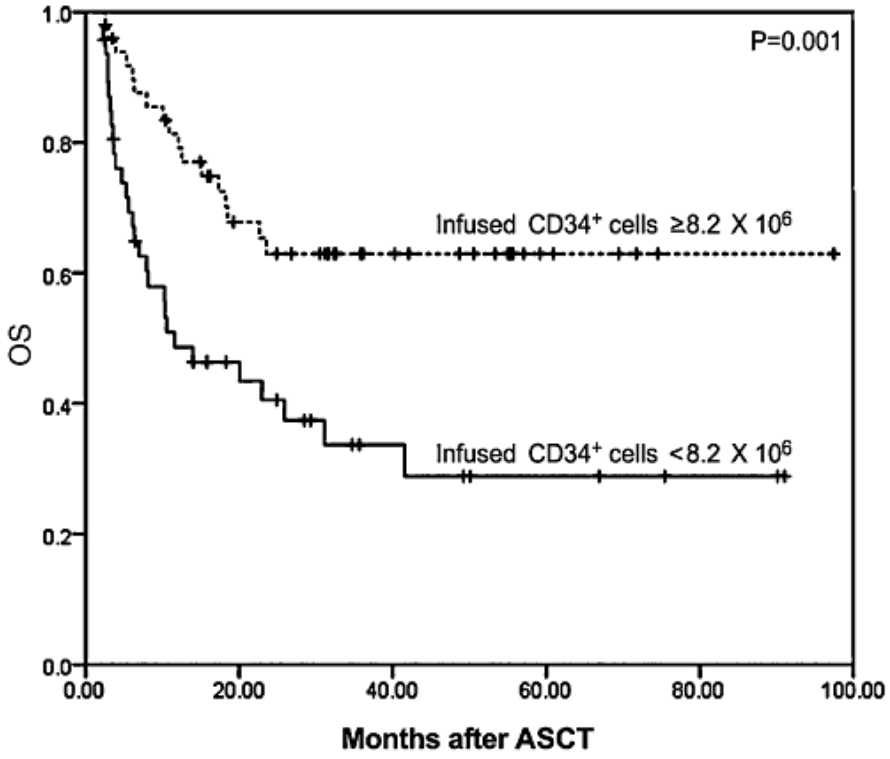


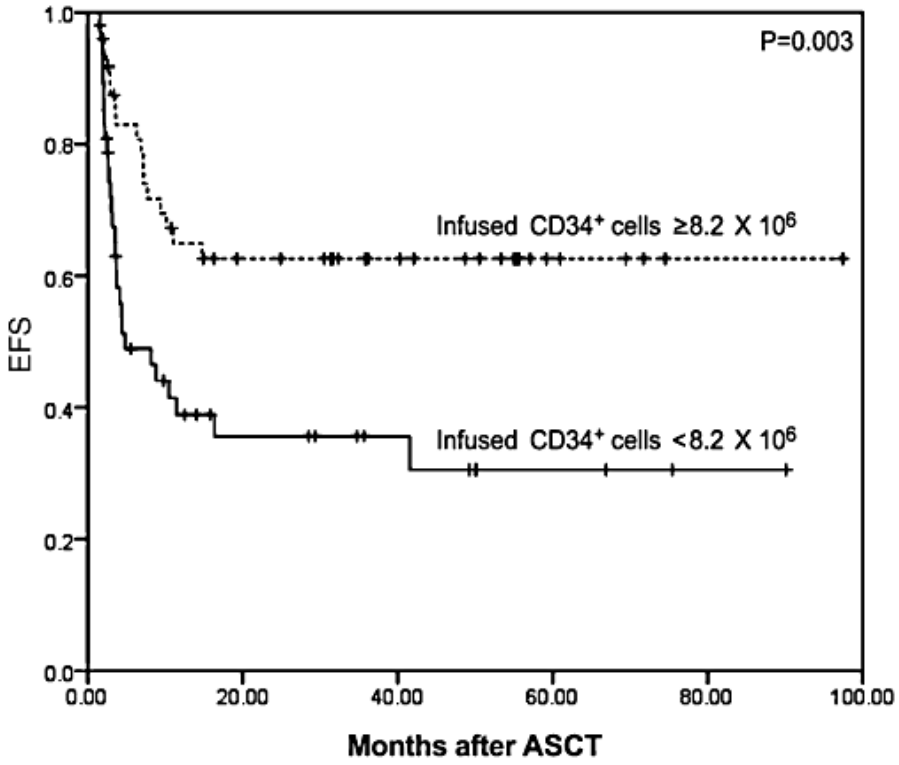
Survival after relapse



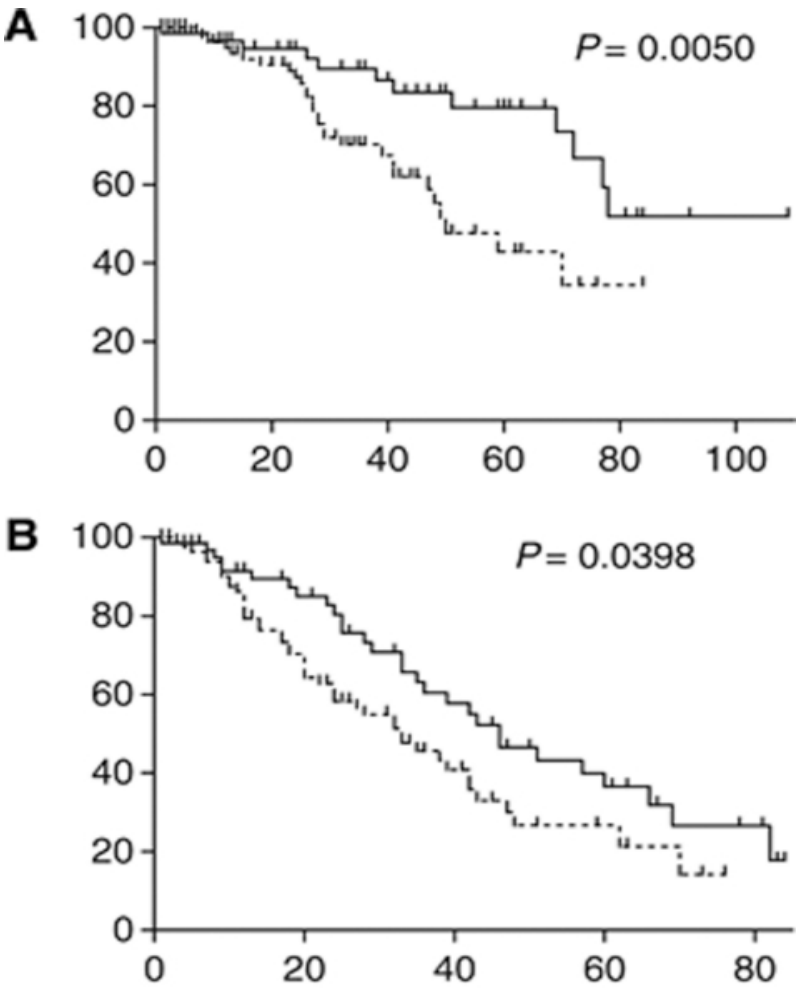
B J Bolwell et al, *Bone Marrow Transplantation* (2007)

Higher infused CD34+ hematopoietic stem cell dose correlates with better clinical outcome after autologous stem cell transplantation in non-Hodgkin's lymphoma





Yoon et al, Transfusion, 2009



High levels of circulating CD34+ cells at autologous stem cell collection are associated with favourable prognosis in multiple myeloma

Better overall survival (A) and longer time to progression (B) in super mobiliser (*n*=69) *vs* normal mobiliser myeloma patients (*n*=89). A value of 100 000 CD34+ cells per ml circulating at the day of stem cell collection was used to stratify between the two groups.

Raschle J et al, Br J Cancer. 105(7): 970–974. 2011

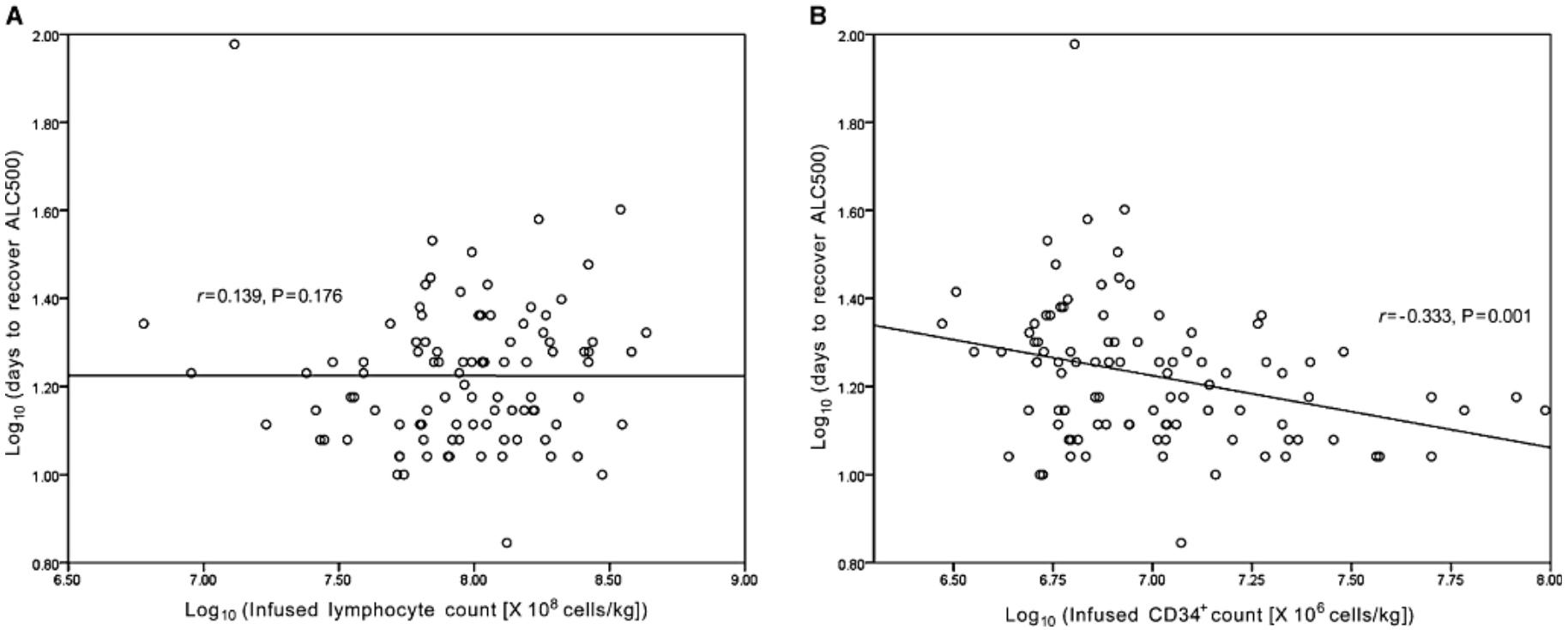
Why does the $CD34^+$ cell dose correlate with clinical outcome?

a) Better and more rapid hemopoietic reconstitution? And then, better non-relapse mortality?

b) Infused $CD34^+$ cells give rise to an increased number of T-cells, which may impact in the immunological recovery post-transplant and in the induction of tumor-specific T-cell repertoire (*immunological skewing*)

c) The higher $CD34^+$ cells, more immune cells.

Higher infused CD34+ hematopoietic stem cell dose correlates with earlier lymphocyte recovery after autologous stem cell transplantation in non-Hodgkin's lymphoma



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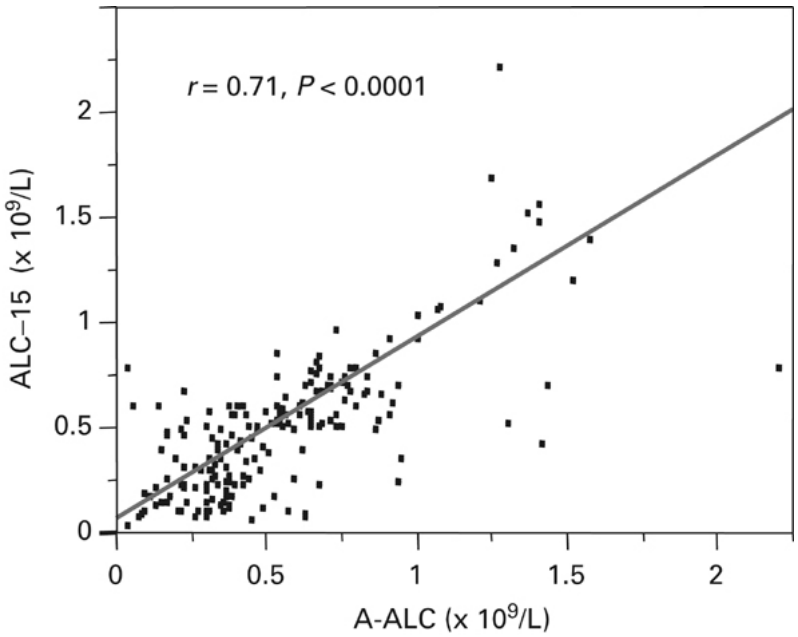
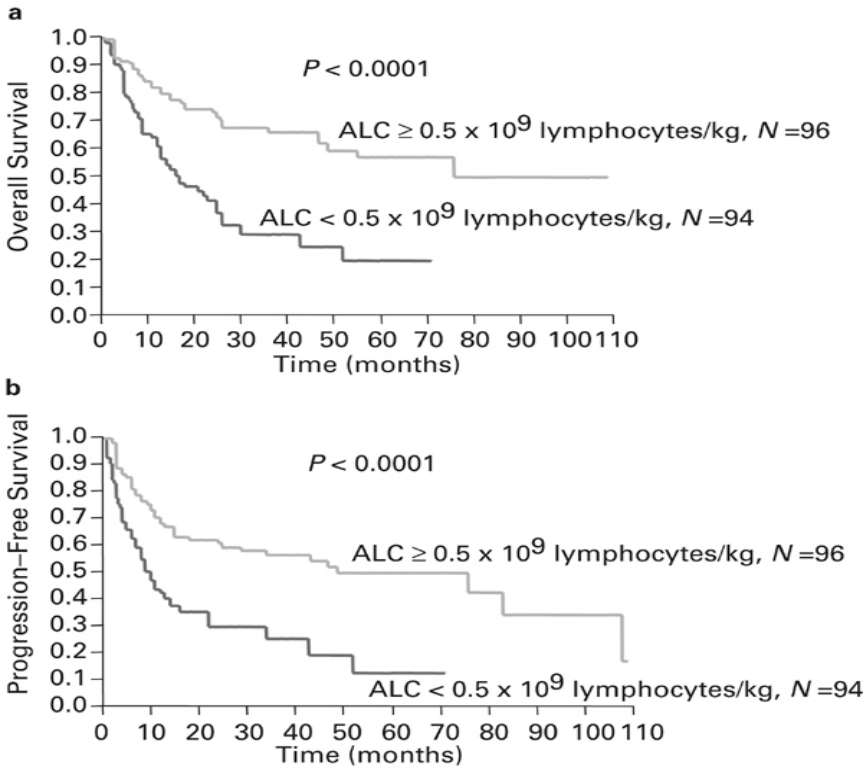
Infused peripheral blood autograft absolute lymphocyte count correlates with day 15 absolute lymphocyte count and clinical outcome after auto-SCT in NHLs





L F Porrata et al, *Bone Marrow Transplantation*, 2004

Plerixafor plus G-CSF after chemotherapy for the mobilization of Peripheral Blood Stem Cells (PBSCs) in Multiple Myeloma (MM) patients undergoing Autologous Stem Cell Transplantation (ASCT).

Phase IV study

Participating centers:

1) Dipartimento di Medicina Specialistica, Diagnostica e Sperimentale, istituto di Ematologia «Seràgnoli», Bologna
2) Clinica Ematologica e Unità di Terapie Cellulari 'Carlo Melzi', Azienda Ospedaliera-Universitaria, Udine
3) Dipartimento di Medicina Interna (Di.M.I), IRCCS San Martino-IST, Cattedra di Ematologia, Genova
4) USC Ematologia A. O. Papa Giovanni XXIII, Bergamo

Objectives

Primary Objectives

- Percentage of patients collecting ≥ 6 x 10^6 $CD34^+$ cells/Kg in three or less apheresis

Secondary Objectives

- Number of apheresis to collect ≥ 6 x 10^6 $CD34^+$ cells/Kg
- Evaluation of engraftment after transplantation of plerixafor-mobilized PB HSCs
- Evaluation of immunological reconstitution after transplantation
- Evaluation of cellular graft content
- Percentage of patients collecting ≥ 4 x 10^6 $CD34^+$ cells/Kg in three or less apheresis following rescue strategy (plerixafor + G-CSF), and number of apheresis required

Patient Population

Eligible patients are those with MM who would benefit from one or tandem autologous HSC transplant and will receive G-CSF plus chemotherapy as mobilizing regimen to collect PBSC.

Mobilization phase

- Chemotherapy regimen: Cyclophosphamide (Cy) at the dose of 4 g/m^2
- G-CSF (Filgrastim or Lenograstim; 10 µg/Kg/day) was administered, starting in the morning of day + 6 after chemotherapy and on each day prior to apheresis, until the completion of PBSC collection, for maximum of 20 days.
- Plerixafor (240 µg/Kg/day) was administered, in the evening (approximately 11 hours prior to initiation of apheresis) and started, during the recovery phase from Cy, when the WBC count was > 1.0 X 10^9/L and the platelet count > 20 X 10^9/L regardless of the concentration of $CD34^+$ cells.

Myeloablative Chemotherapy and Transplantation

- ASCT was performed within 2 months of last apheresis, but not earlier than 4 weeks after Cy

- High dose Melphalan was administered as conditioning regimen as 200 mg/m^2 intravenous dose (creatinine clearance > 30 mL/min) or 140 mg/m^2 (creatinine clearance < 30 ml/min) on day -2, followed by autologous PBSC transplantation on day 0.

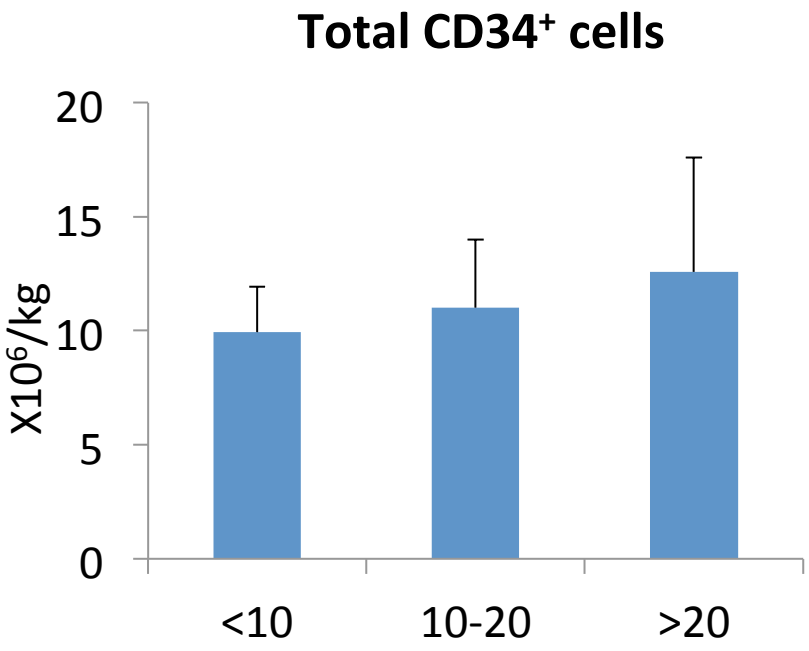
Patients characteristics

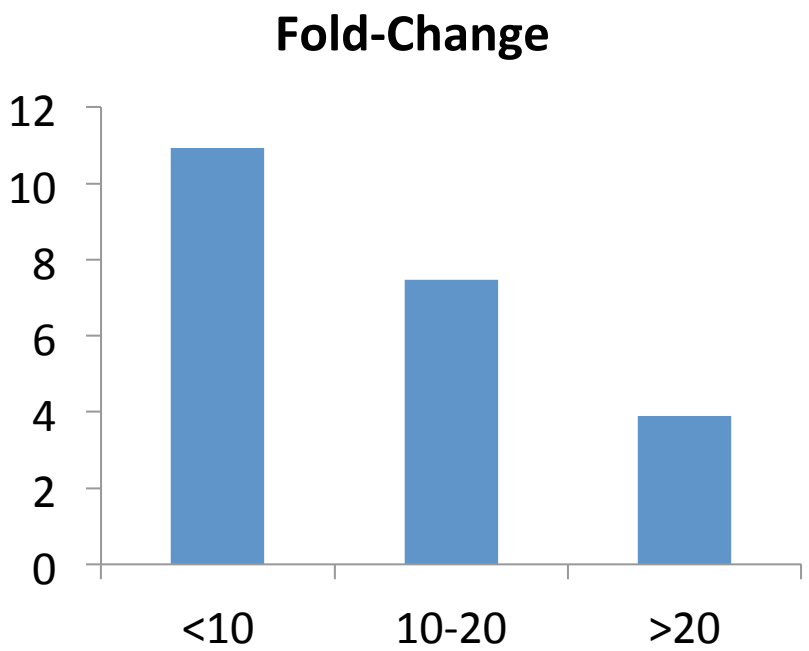
37 patients	Median
Age (yrs)	58 (41-72)
Sex (M/F)	19/18
ISS at diagnosis	
1	16 (43%)
2	13 (35%)
3	8 (22%)
Disease status at enrollment	
PR	7 (18%)
VGPR	17 (45%)
nCR	4 (11%)
sCR	4 (11%)
CR	5 (15%)
Prior chemotherapy or radiotherapy	
VTD	24 (66%)
TD+EDX+ASCT+VTD+IFN+DEX	3 (8%)
VCD+EDX+ASCT+Lena+VTD	1 (3%)
VTD+EDX+TD	3 (8%)
VTD+EDX	5 (15%)
Radiotherapy	7 (18%)
ASCT	4(11%)

Mobilization and Collection Results

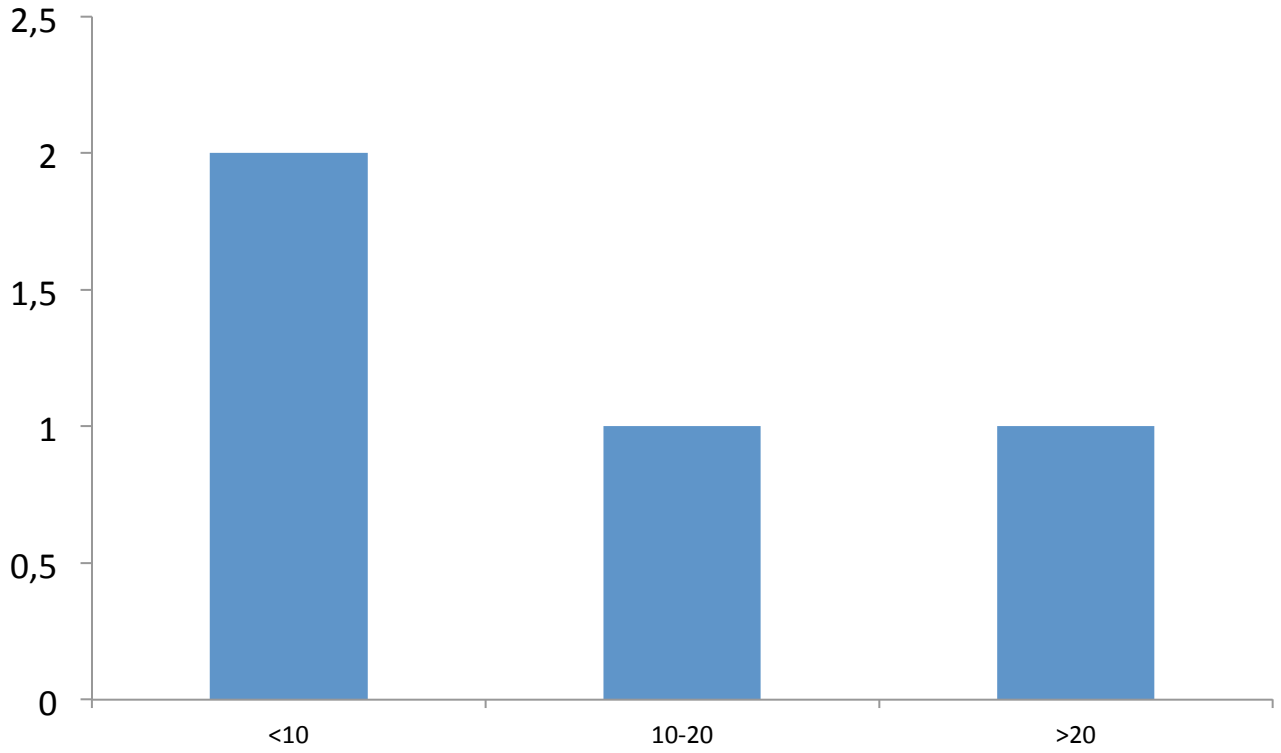
34 patients	Median
CD34+ number before PLX	27 (3.1-224)
WBC before PLX	3.500 (1.300-19.400)
PLT before PLX	58.000 (21.000-282.000)
CD34+ number before first apheresis	125.87 (16.4-519.8)
Total collected MNC (x 10^8/kg)	4.12 (1.6-15.75)
Total collected CD34+ cells (x 10^6/kg)	10.4 (2.9-32.65)
Number of aphereses	2 (1-3)

$CD34^+$ cell collection accordingly to CD34 count before PLX

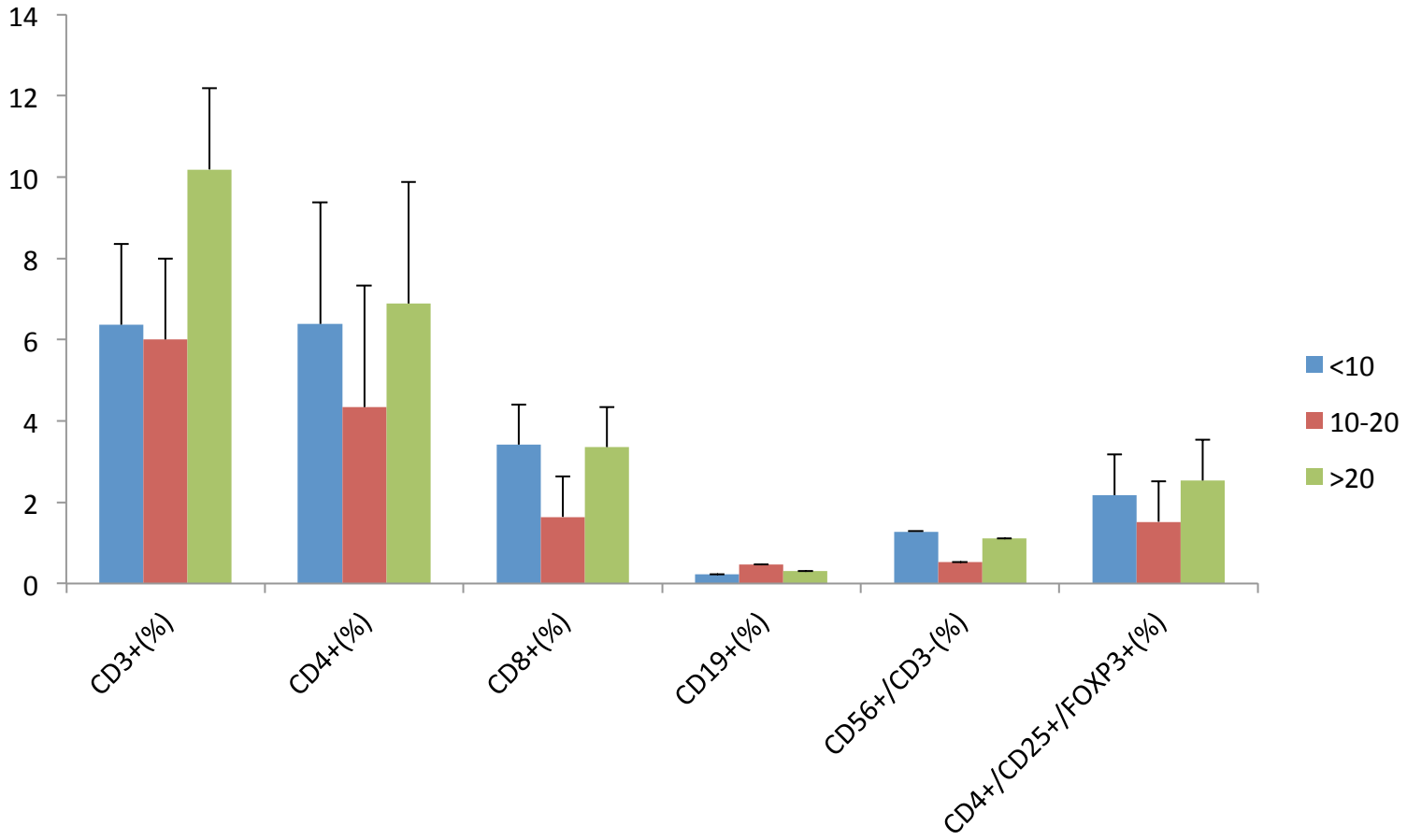




Number of aphereses accordingly to CD34 count before PLX

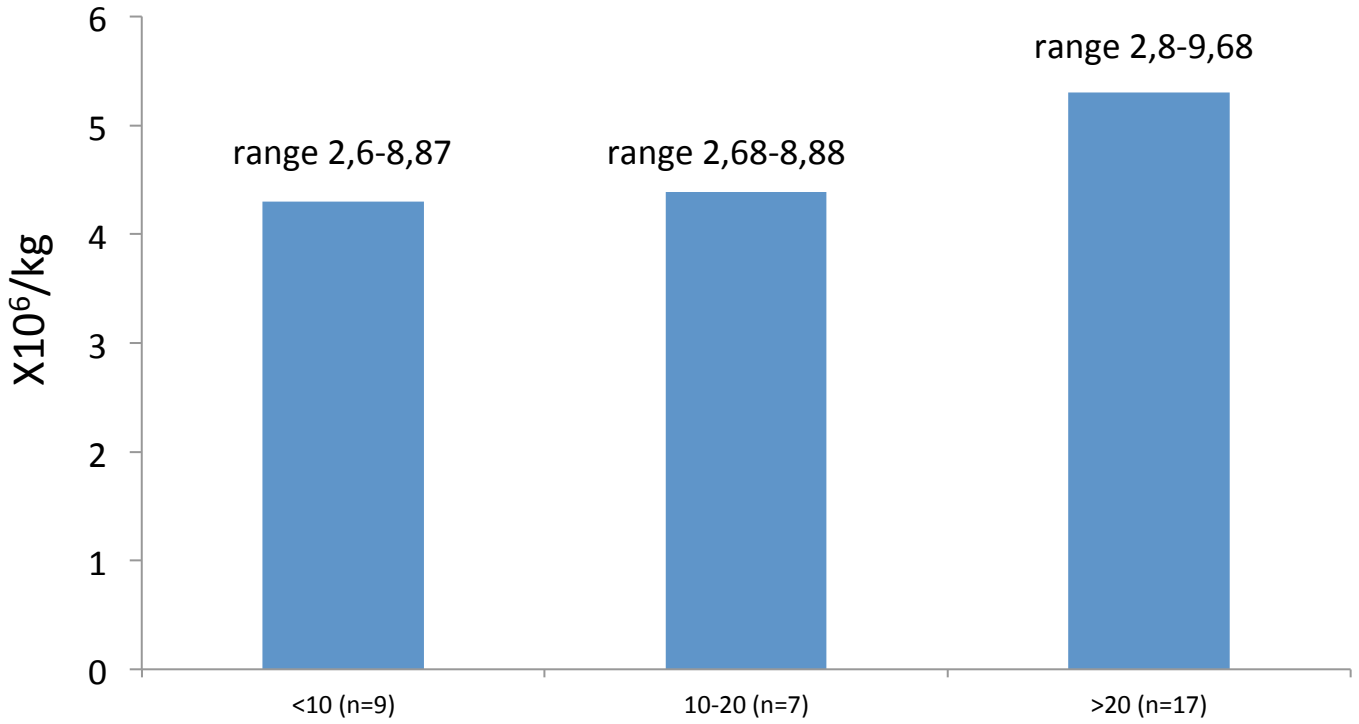


Immune graft composition accordingly to CD34 count before PLX



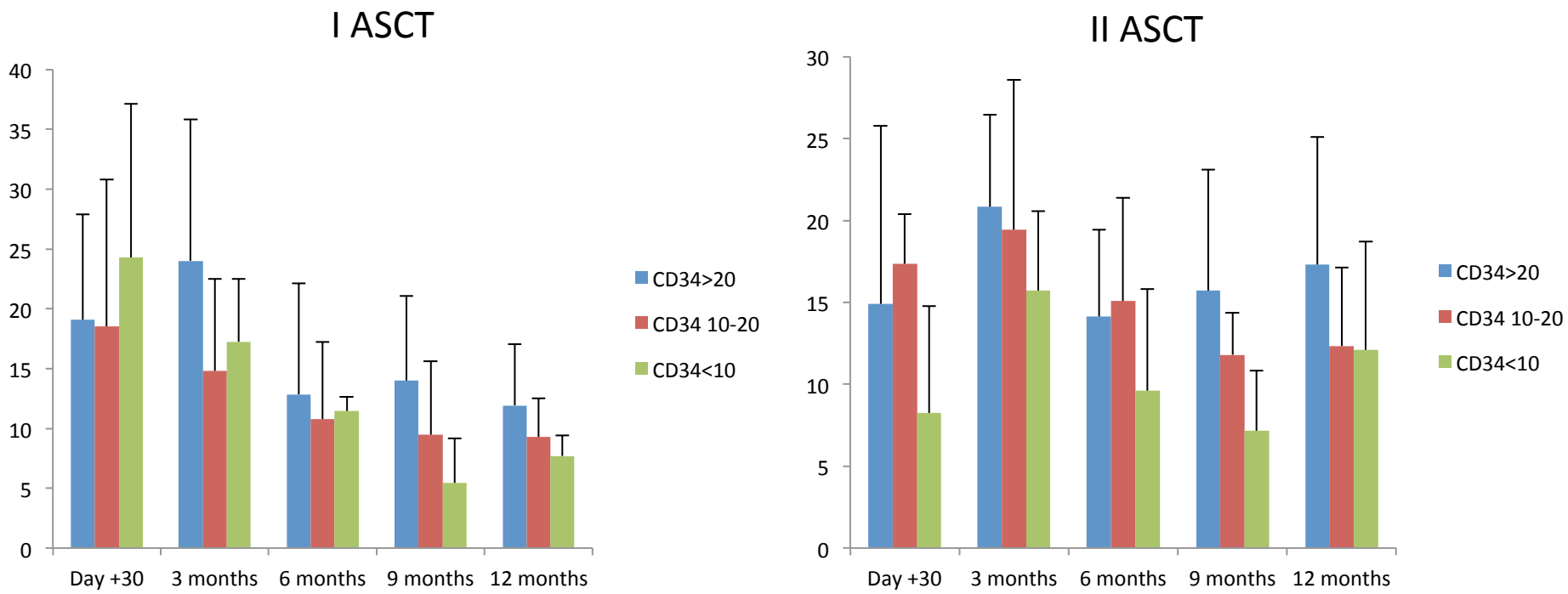
Infused CD34^{+} cells accordingly to CD34 count before PLX

1 ASCT n=19
2 ASCT n=15



Immunological recovery accordingly to CD34 count before PLX (1)

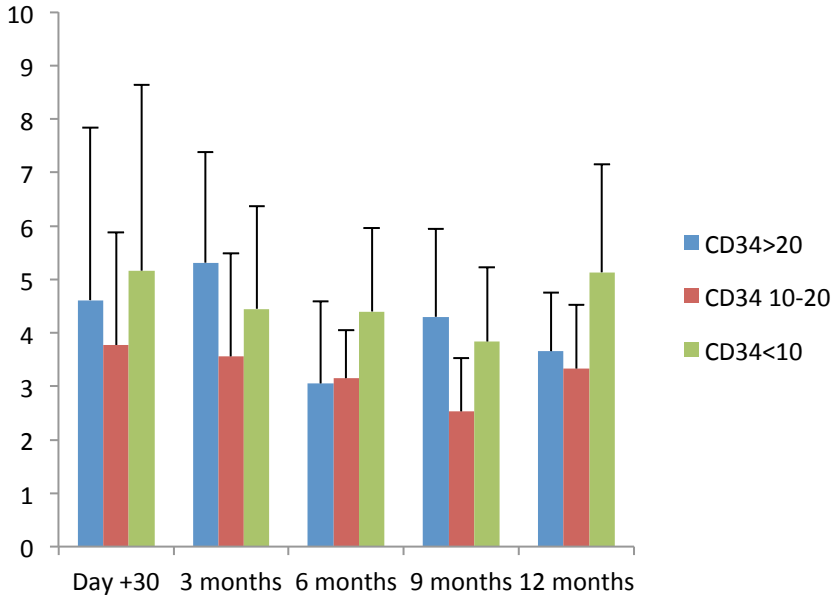
% CD3+ T cells in PB



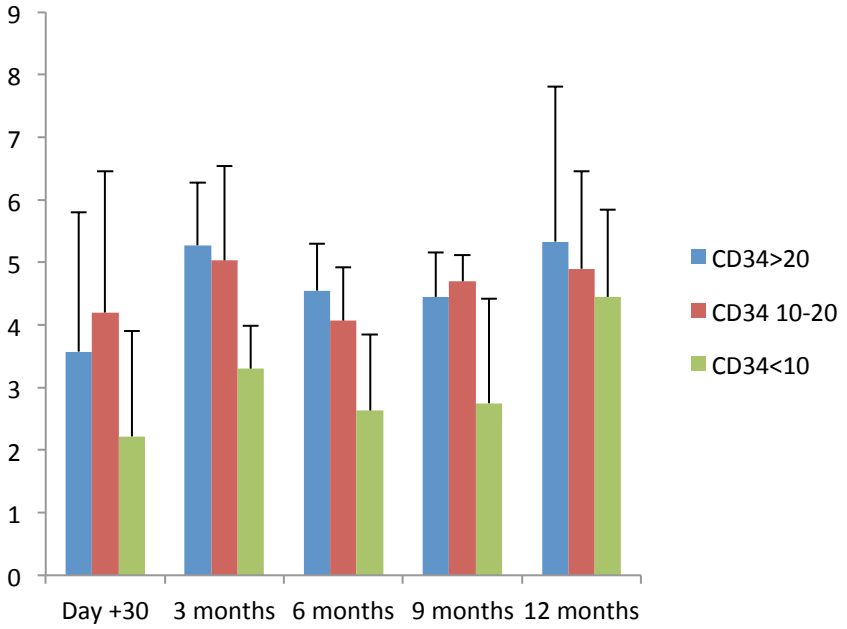
Immunological recovery accordingly to CD34 count before PLX (2)

% CD4+ T cells in PB

I ASCT



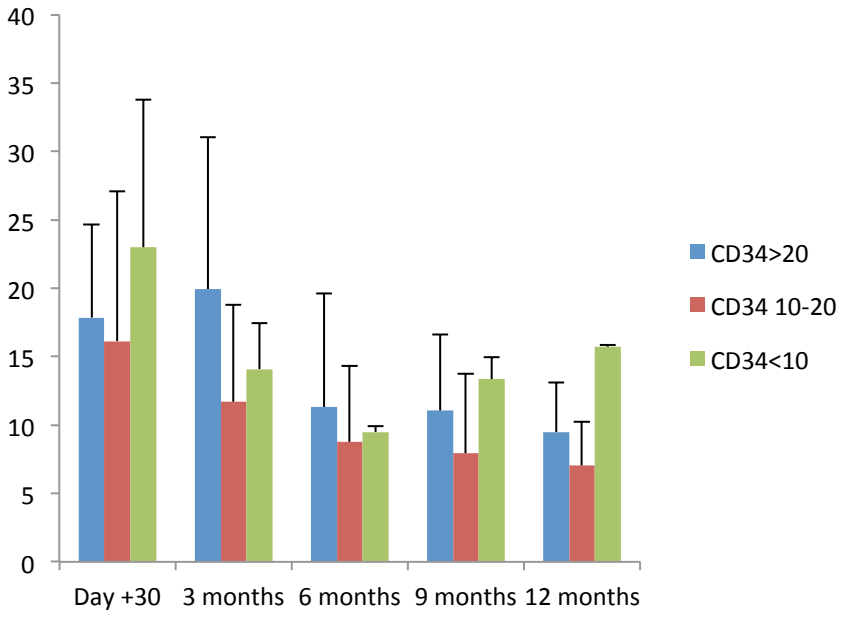
II ASCT



Immunological recovery accordingly to CD34 count before PLX (3)

% CD8+ T cells in PB

I ASCT



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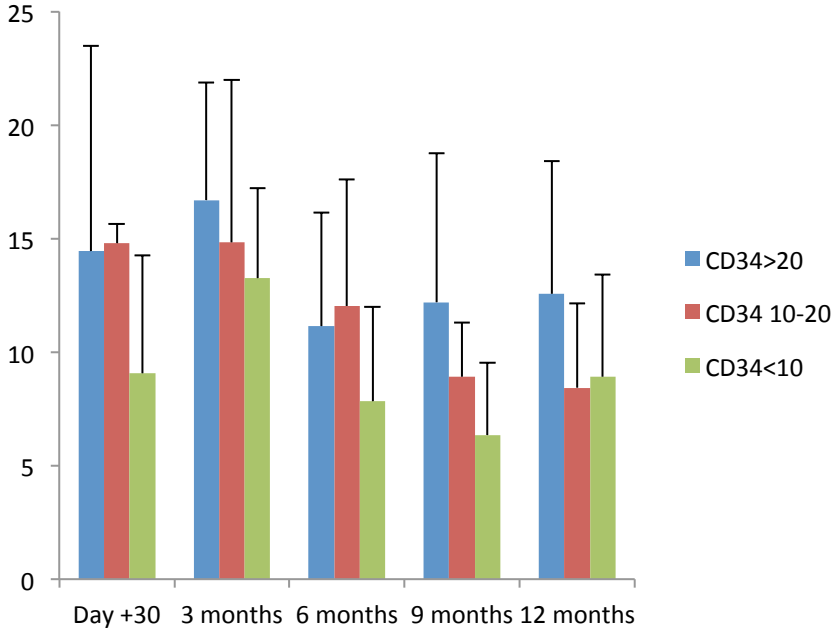
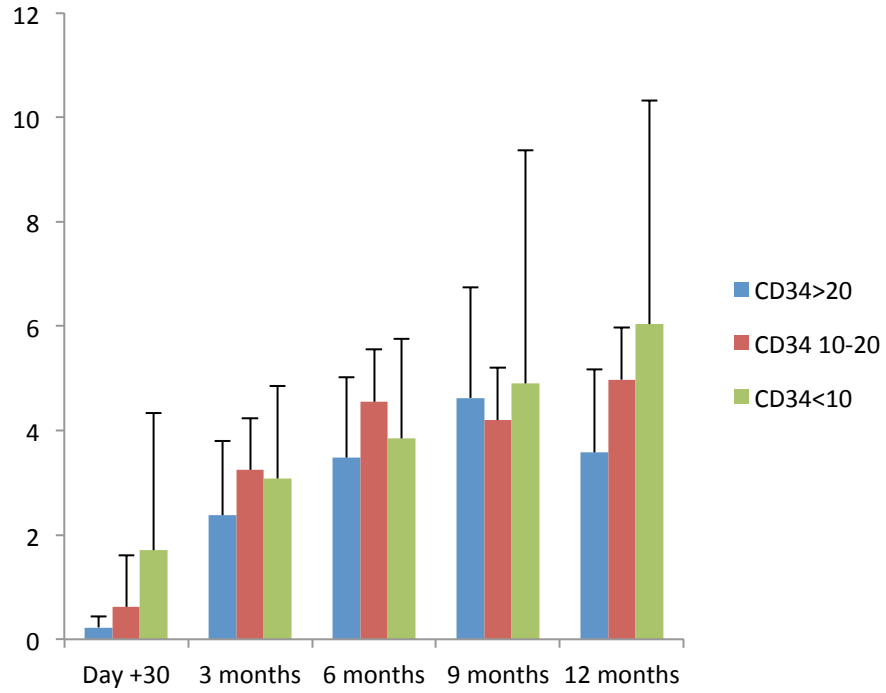


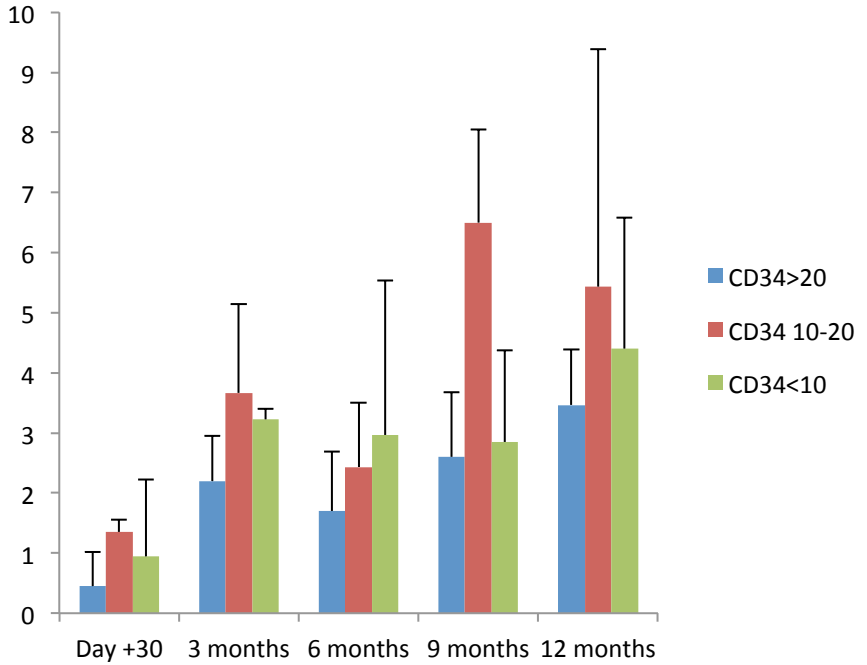
Immunological recovery accordingly to CD34 count before PLX (4)

% CD19+ cells in PB

I ASCT



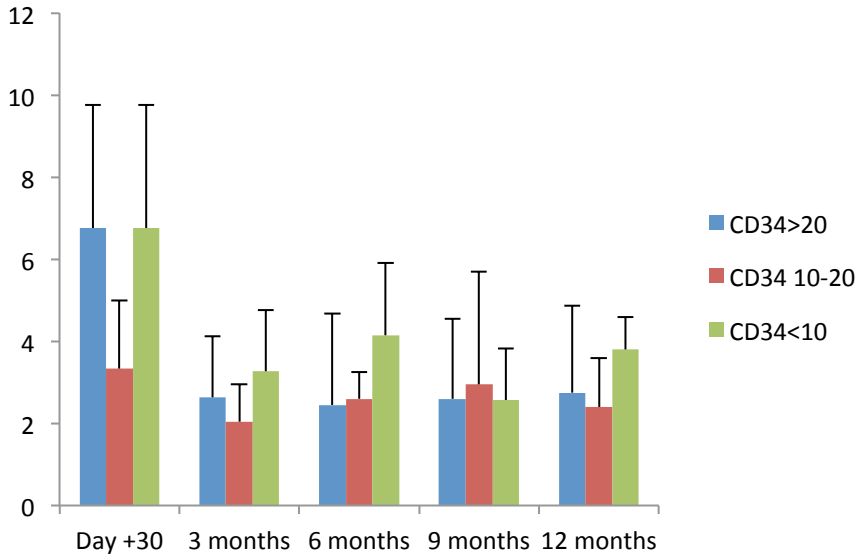
II ASCT



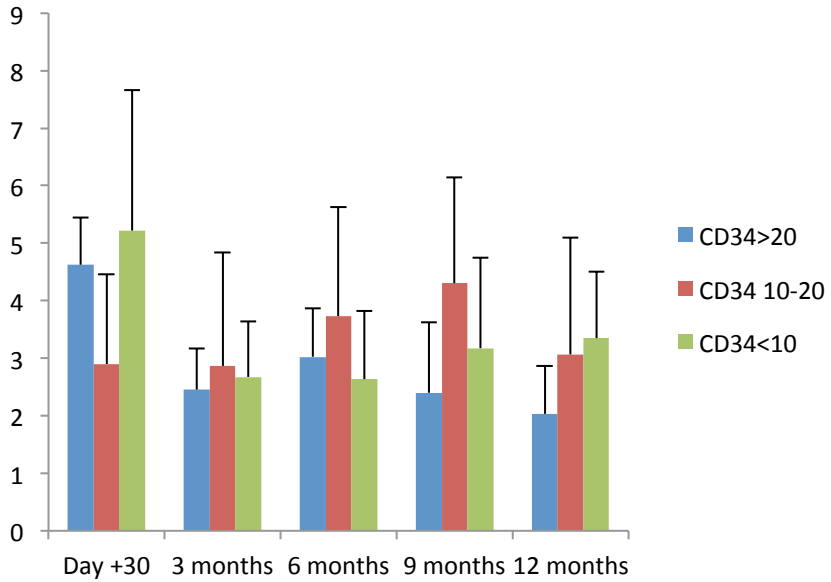
Immunological recovery accordingly to CD34 count before PLX (5)

% $CD56^{+}CD3^{-}$ NK cells in PB

I ASCT



II ASCT



Discussion and future prespectives

Recent studies demonstrate that Plerixafor (and novel agents) is capable of increasing the number of T and B cells, NK cells in the graft, thus providing the rationale for considering mobilization as a whole and comprehensive strategy to collect cells, and not only stem cells.

If the $CD34^{+}$ cell count really has a role in clinical outcome, whatever the mechanisms are, increasing stem-cell mobilization should be an important issue for the future, beside their use in poor-mobilizers.

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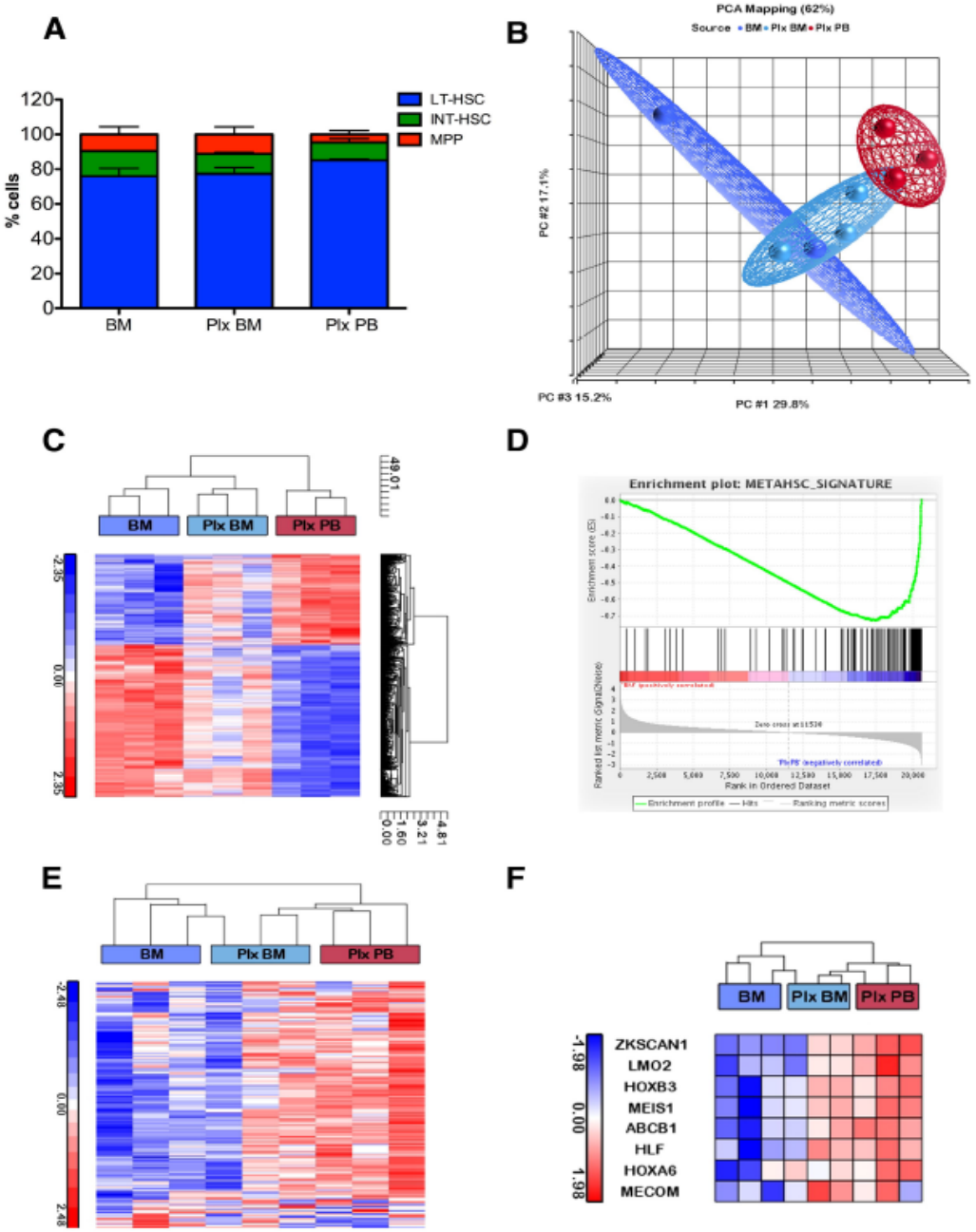
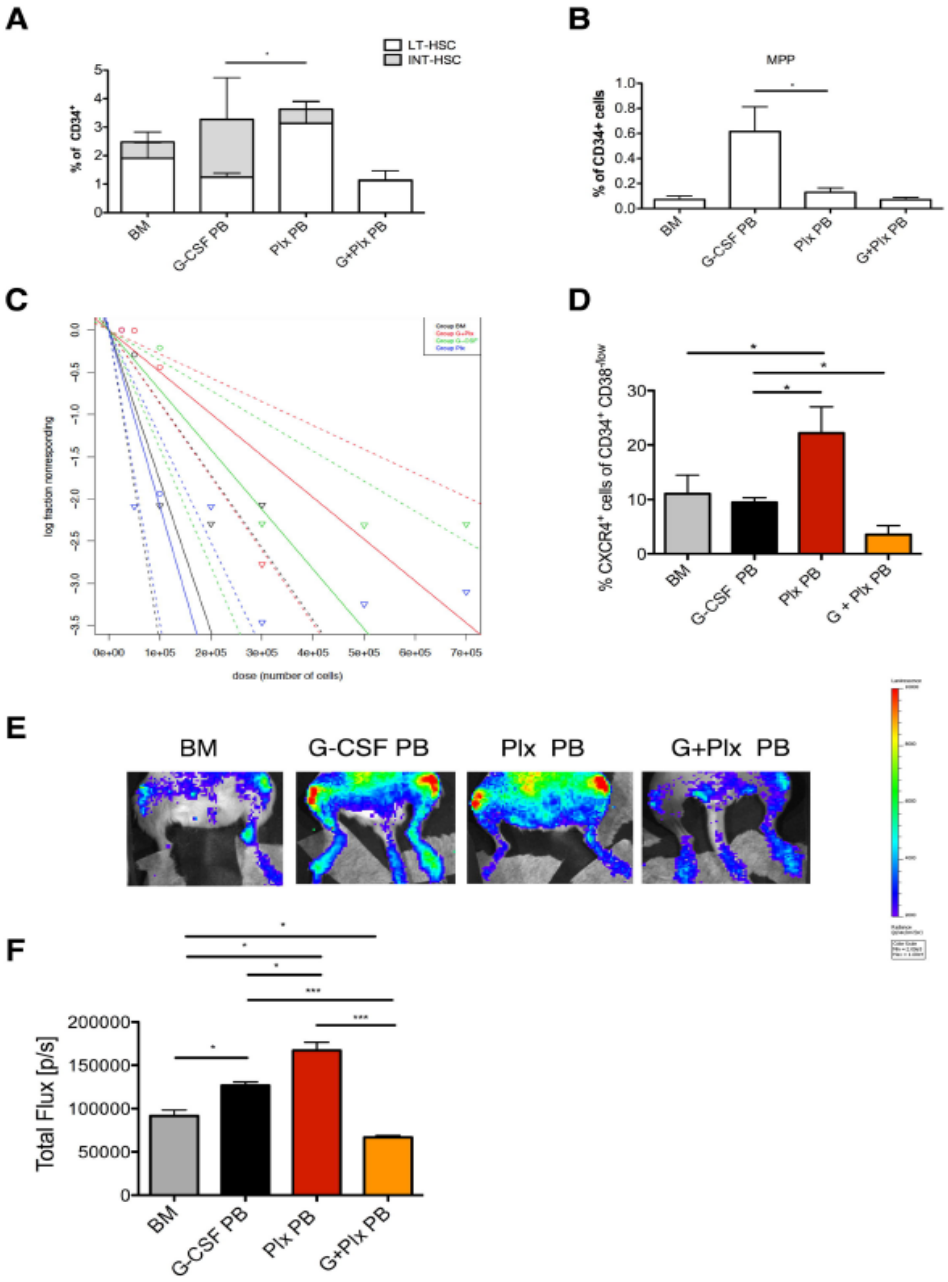


Giuliana Ferrari, Haematologica, 2016



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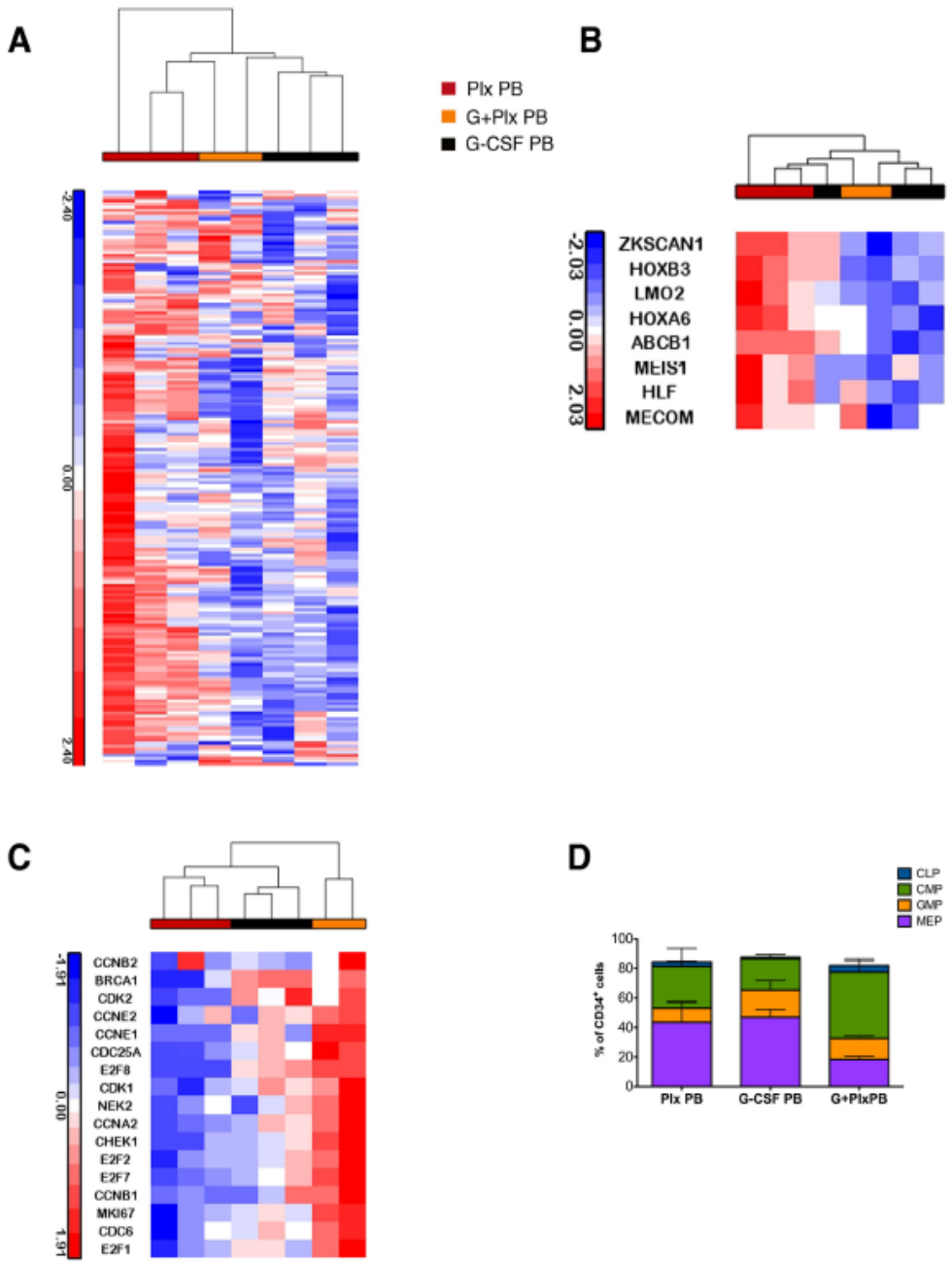

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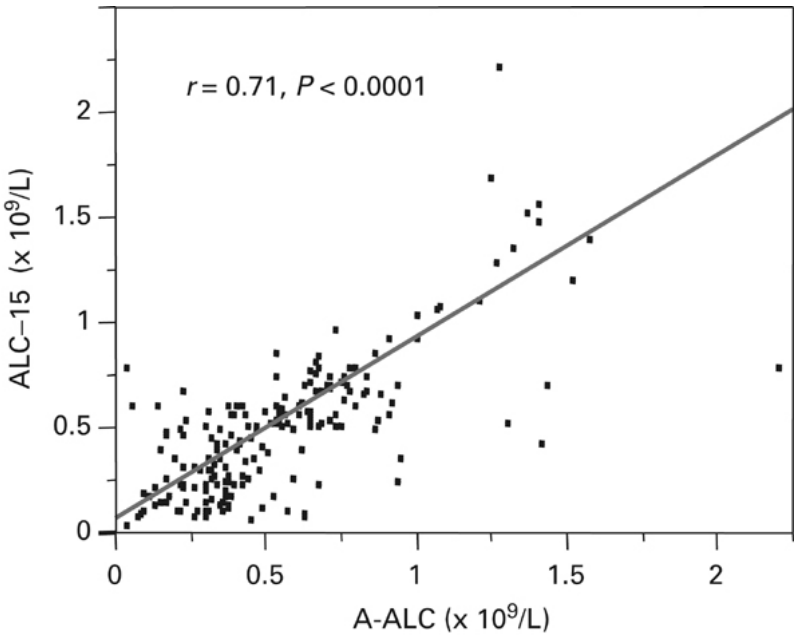
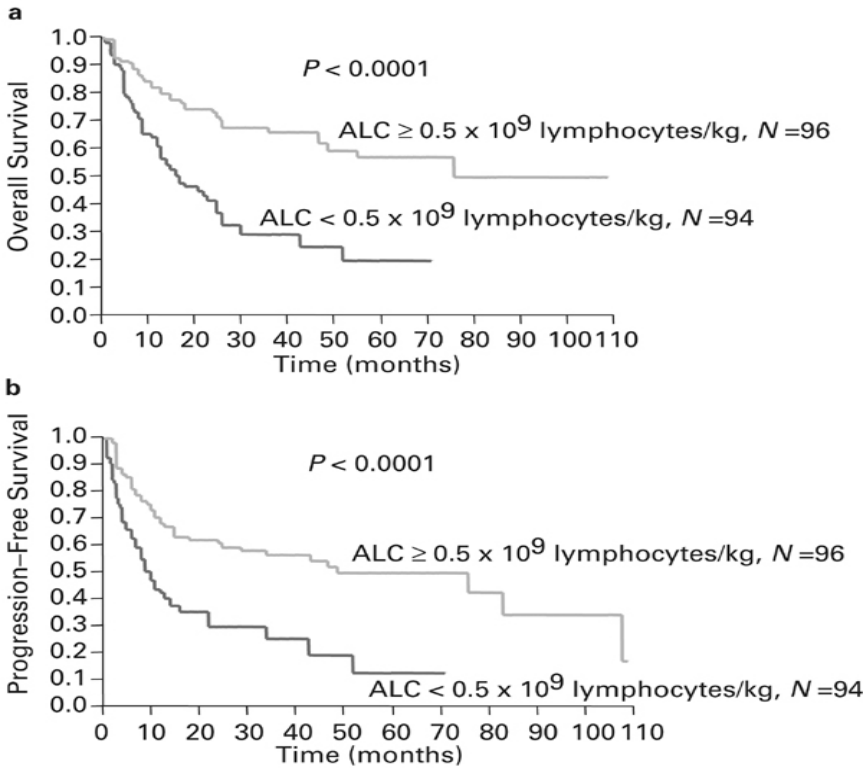
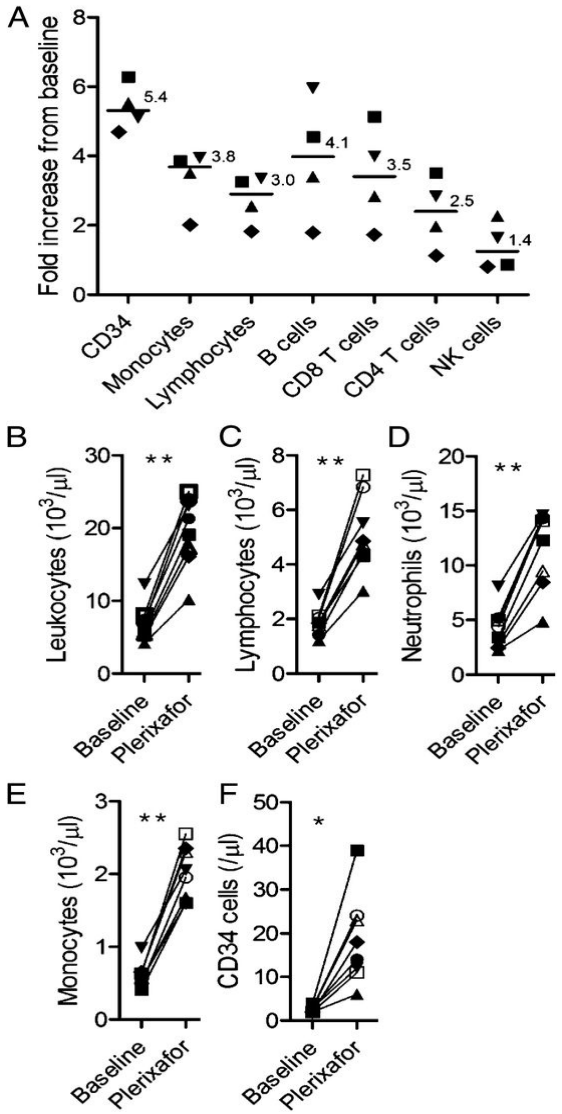




L F Porrata et al, *Bone Marrow Transplantation*, 2004

Mobilization of blood mononuclear cells after a single dose of plerixafor in healthy subjects.



Apheresis products were collected from eight healthy subjects mobilized with a single 240 μg/kg injection of plerixafor. Relative to the weight of the subjects mobilized, apheresis collections after plerixafor mobilization (median 19.6 l apheresed; range 15–22 l) contained a median 81 × 10^6 $CD19^+$ B cells/kg, a median 274 × 10^6 $CD3^+$ T cells/kg, and a median 1.6 × 10^6 $CD34^+$ cells/kg (Table I). Plerixafor preferentially mobilized $CD34^+$ cells followed by monocytes and lymphocytes (Fig. 1A). Within the lymphocyte compartment, B cells were preferentially mobilized followed by T cells and NK cells. Among $CD19^+$ B cells, CD20, κ, and λ expression did not change from baseline, although the percentage of B cells expressing CD27 declined significantly in seven of eight donors, consistent with plerixafor preferentially mobilizing naive type B cells; the median percentage of $CD27^+CD19^+$ B cells was 35.1% at baseline and 19% after plerixafor mobilization (p = 0.011). The total WBC count and the absolute numbers of blood neutrophils, monocytes, lymphocytes, and $CD34^+$ cells increased significantly from baseline after plerixafor administration

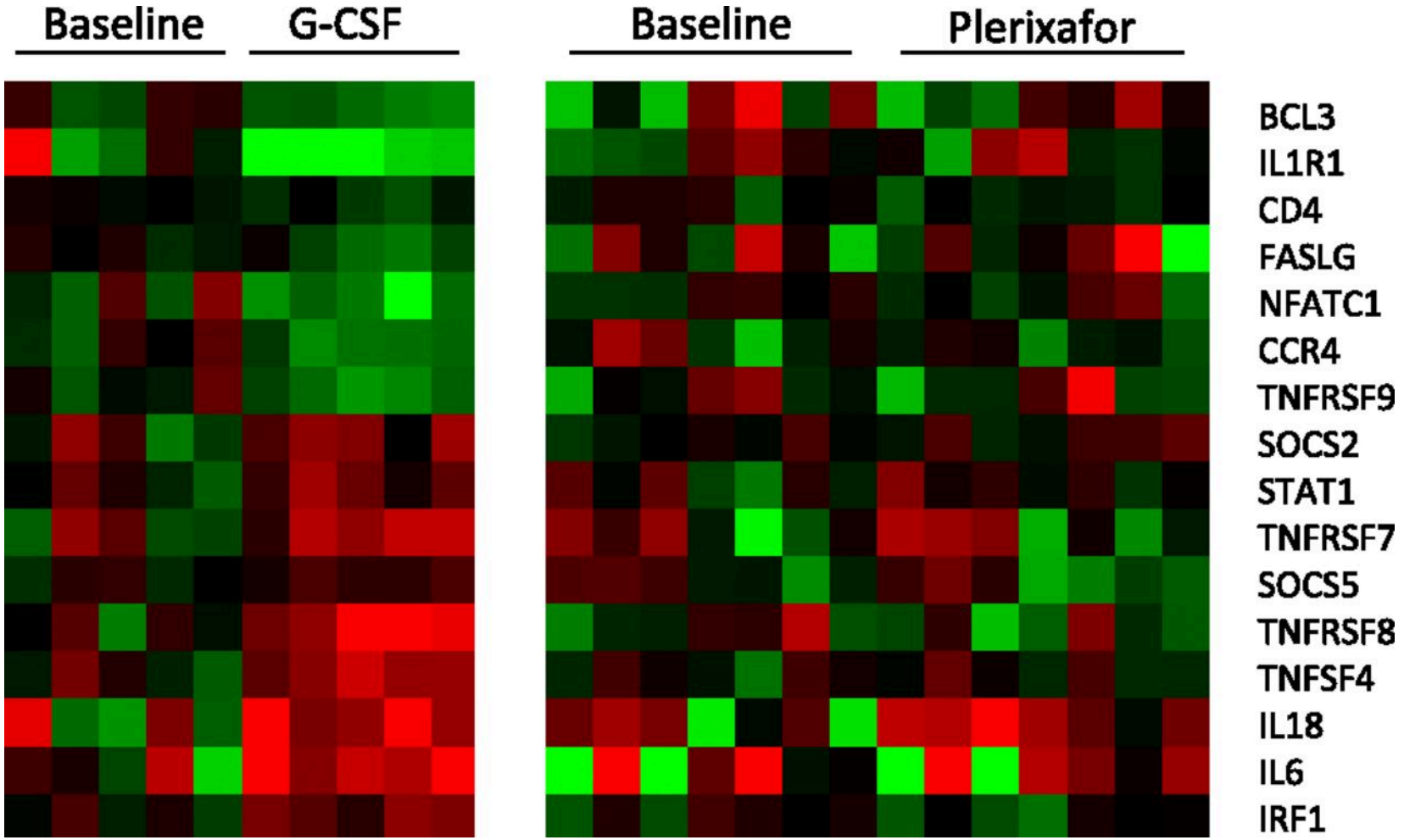
A detailed phenotypic analysis using six-color flow cytometry of $CD4^+$ and $CD8^+$ lymphocyte subsets at baseline and 6 h after a single injection of plerixafor or 2 h after the fifth dose of G-CSF is shown in Table II. No significant change from baseline was observed after mobilization with plerixafor in the percentage of $CD4^+$ and $CD8^+$ T cells expressing the majority of surface markers analyzed including CD45RA, CD45RO, CD34, CD56, CD57, CD27, CD71, and CD62L. Although the phenotype also did not change after G-CSF mobilization in most $CD4^+$ and $CD8^+$ T cell populations, there was a significant decline in the percentage of CD4 and CD8 T cells that expressed CD62L and in CD8 T cells that expressed CD27 (Table II).

Andreas Lundqvist et al. J Immunol 2013;191:6241-6249

The Journal of Immunology

Commento dalla Discussione del paper alla figura della slide precedente
In particolare, importante il punto sul CD62L e sul CD27. Correlazione clinico-biologica

G-CSF and plerixafor mobilized a similar number of T cells into circulation. As reported by others, we observed the expression of CD62L on T cells to decline after G-CSF mobilization (30). In contrast, we observed no change in T cell phenotypic markers including CD62L expression after plerixafor mobilization. Normally, $CD62L^-$ T cells are effector memory cells; in contrast with $CD62L^+$ T cells, transplantation of $CD62L^-$ T cells does not appear to cause GVHD in murine models (31). The impact of transplanting higher numbers of $CD62L^+$ T cells using allogeneic grafts is unknown. Although animal models might suggest the incidence of GVHD could increase, it is important to consider that CD62L expression on $CD3^+$ T cells alone may not reflect the true subtype of T cells. Studies have also shown that serum-soluble l-selectin levels are increased after G-CSF mobilization (32) and that some CD62L loss can be reversed with in vitro incubation (30). Based on this observation, it is possible that the lower levels of CD62L found on G-CSF mobilized T cells occur as a consequence of shedding, rather than reflecting preferential mobilization of an effector type T cell. Although no kinetic studies on CD62L status were performed in our study, CD62L shedding after G-CSF treatment has previously been established to be a temporary phenomenon. Importantly, after mobilization with either plerixafor versus G-CSF, no differences in the percentage of T cells expressing CD45RA or CCR7, markers of naive (both) and central memory (CCR7) cells, were observed. Although the expression of CD27 on plerixafor mobilized T cells did not change, a significant decrease in CD27 expression was observed in G-CSF mobilized $CD8^+$, but not $CD4^+$, T cells. The functional consequence of higher numbers of $CD27^-$ CD8 T cells using allogeneic grafts is unknown. CD27 binds CD70 and is required for generation and long-term maintenance of T cell immunity, and was recently shown to augment $CD8^+$ T cell activation (33, 34). One might therefore speculate that allografting of G-CSF mobilized $CD8^+$ T cells results in reduced GVHD.



Andreas Lundqvist et al. J Immunol 2013;191:6241-6249

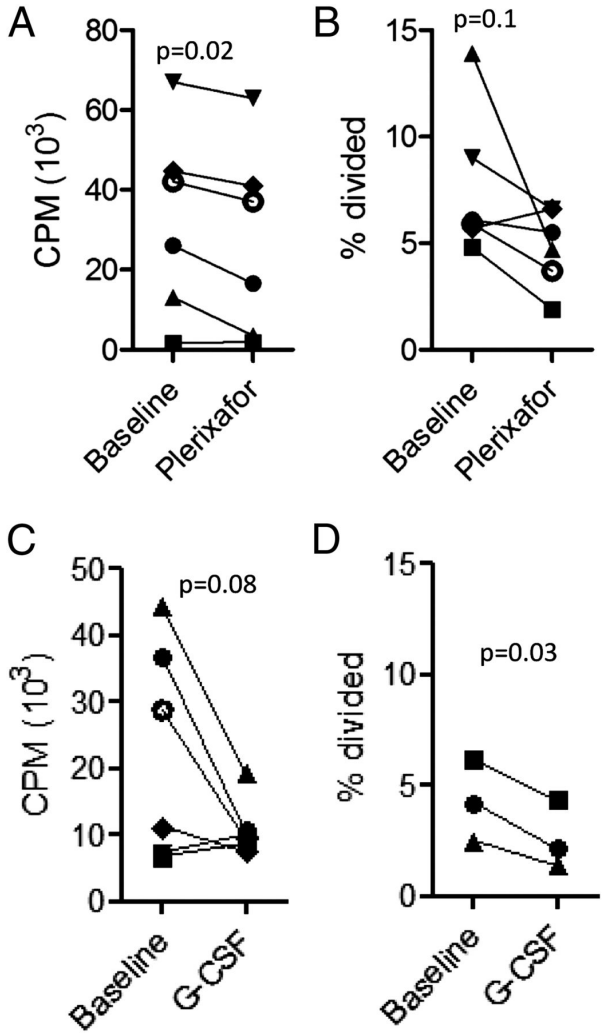
The Journal of Immunology

Commento dalla Discussione del paper alla figura della slide precedente sul cyokine pattern

G-CSF mobilization has been shown to skew T cells toward a Th2-type phenotype characterized by increased expression of IL-4 and IL-10, and decreased IFN-γ production. Th2-type T cells are thought to be associated with a lower risk for causing acute GVHD but may play a part in contributing to the increased incidence of chronic GVHD, which occurs with the use of G-CSF mobilized allografts compared with bone marrow transplants. We found that T cells mobilized with plerixafor did not display any changes in Th1- and Th2-type cytokines after nonspecific mitogen stimulation (data not shown). However, similar to G-CSF mobilized T cells, we did observe a small reduction in responsiveness to allogeneic stimuli in vitro by [^{3}H]thymidine uptake MLR in plerixafor mobilized T cells. Only minor changes in serum levels of IL-4, IL-10, and IFN-γ were found in mice receiving G-CSF compared with HBSS-treated controls. However, we did observe significant decrease in serum levels of IL-8 in donors mobilized with G-CSF (data not shown). Similar to this observation, investigators have previously reported that IL-8 levels decline in patients with esophageal cancer after treatment with G-CSF (35). rIL-8 is known to directly suppress the spontaneous production of IL-4 by CD4$^+$ T cells (36). Taken together, these data suggest that G-CSF–mediated reductions in serum levels of IL-8 may lead to a shift toward a Th2 phenotype in CD4$^+$ cells potentially accounting for the reduced incidence of GVHD observed in this cohort.

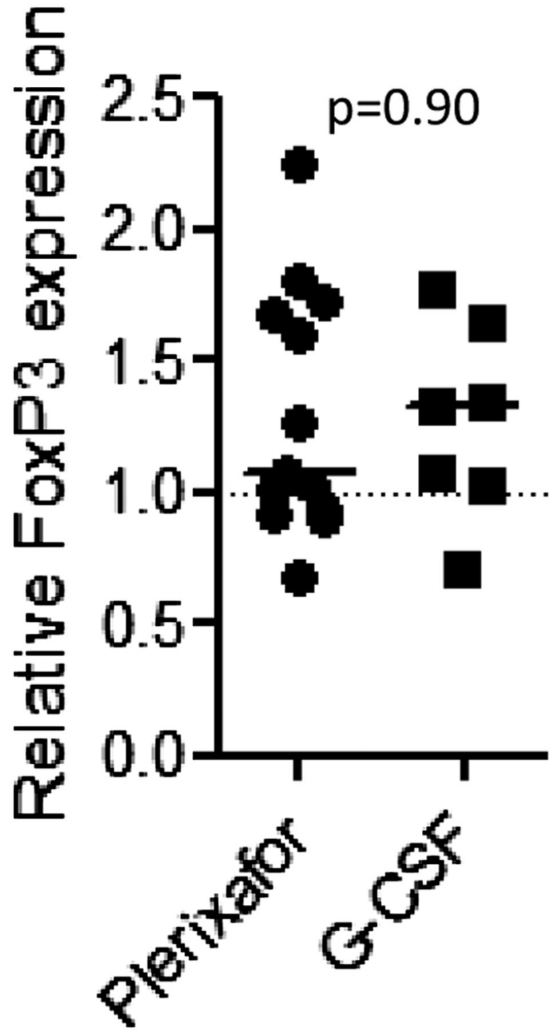
Remarkably, the cytokine gene expression profiles related to Th1/Th2/Th3 pathways differed significantly in T cells mobilized with these two different agents. Real-time PCR assays showed T cells mobilized with plerixafor had cytokine gene expression patterns that were similar to nonmobilized T cells. In contrast, G-CSF altered a number of cytokine-related genes in mobilized CD3$^+$ T cells including both TH1 and TH2 cytokine-related genes, transcription factors, and genes regulating T cell activation. Numerous prior studies have also reported that G-CSF induces alterations in cytokine-related genes. G-CSF mobilized allografts contain more than a log higher T cell dose than bone marrow allografts but are not associated with an increased incidence of acute GVHD. This has led investigators to speculate that G-CSF–induced alterations in cytokines inhibit T cells from causing acute GVHD. Franzke et al. (37) showed G-CSF mobilization increased gene expression of the Th2-related gene GATA3 in CD4$^+$ T cells, with this effect being less apparent in nonsorted CD3$^+$ cell fractions. In our analysis, GATA3 levels in T cells did not change significantly in CD3$^+$ T cells after G-CSF mobilization, although our genotype study did not include an analysis that was specific for the CD4$^+$ T cell subset. Recent data suggest reductions in Th17 cells may be partially responsible for the avoidance of acute GVHD in G-CSF mobilized allografts (38). However, using a Luminex array analysis, we observed no changes in serum IL-17 levels after G-CSF mobilization. Instead, serum levels of G-CSF, IL-1Rα, IL-4, and MIP-1β increased significantly from baseline after G-CSF mobilization. In contrast with these findings, we observed no significant changes in any of these or other serum cytokines or chemokines measured after plerixafor mobilization (data not shown).

The impact of mobilization on alloreactivity.



Andreas Lundqvist et al. J Immunol 2013;191:6241-6249

The impact of mobilization on CD4+ Foxp3 expression.



Increasing data suggest Tregs play an important role in regulating GVHD after allogeneic hematopoietic cell transplantation (a-HCT). Transplanting PBSC allografts that contain higher doses of Tregs has been associated with a reduced incidence of GVHD (39–41). In preclinical murine models of a-HCT, injection of $CD4^+CD25^+$ Tregs was shown to suppress the early expansion of alloreactive donor T cells and their capacity to induce GVHD without abrogating graft-versus-tumor effector function (42).Furthermore, data in humans have shown that supplementation of allografts with donor Tregs reduces the risk for acute GVHD after haploidentical a-HCT (43). Consistent with these observations, a number of studies have recently reported that Treg numbers are decreased in patients who develop chronic GVHD (29, 44). Therefore, we measured the percentage of Tregs mobilized in mice and also measured Foxp3 gene expression in human $CD4^+$ T cells after plerixafor versus G-CSF administration. In mice, there was no significant difference in the percentage of regulatory $CD4^+/Foxp3^+$ T cells mobilized with either G-CSF or plerixafor. Furthermore, HBSS, G-CSF, or plerixafor mobilized murine Tregs did not differ in their suppressive activity of anti-CD3–stimulated T cells in $[^3H]$thymidine proliferation assays (data not shown). Furthermore, in humans, Foxp3 gene expression levels did not change significantly from baseline and were similar in G-CSF and plerixafor mobilized $CD4^+$ T cells. Based on these data, one would expect any impact that Tregs will have on GVHD would not differ significantly between allografts mobilized with plerixafor versus G-CSF.

Andreas Lundqvist et al. J Immunol 2013;191:6241-6249

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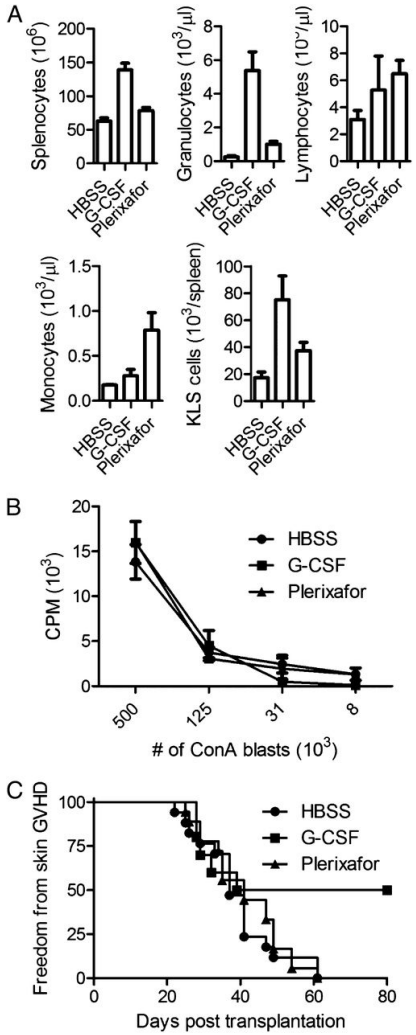
Animal transplant model.



Mobilized PBSCs were collected from spleens of mice harvested 6 h after the last injection of G-CSF versus plerixafor. Compared with HBSS (saline) controls, there was a trend toward an increase in the absolute number of monocytes ($p = 0.09$) and lymphocytes ($p = 0.09$) in the blood after plerixafor mobilization (Fig. 5A). The absolute number of circulating lymphocytes in the blood after G-CSF mobilization was similar to plerixafor mobilization ($p = 0.68$), although there was a trend toward a higher number of monocytes being mobilized with plerixafor compared with G-CSF ($p = 0.07$). Both mobilizing agents increased absolute circulating granulocyte numbers, but granulocyte numbers in the blood after G-CSF mobilization were significantly higher compared with saline controls ($p = 0.02$) and plerixafor mobilized mice ($p = 0.007$). Similarly, absolute numbers of c-Kit$^+$/Lin-/sca-1$^+$ (KLS) cells were highest in spleens of G-CSF–treated mice. Spleens of G-CSF–treated mice contained a 3.6-fold and a 1.7-fold higher number of KLS cells compared with HBSS controls ($p = 0.002$) and plerixafor-treated mice ($p = 0.07$), respectively. Spleens from plerixafor-treated mice contained 2.2-fold higher numbers of KLS cells compared with HBSS controls ($p = 0.02$).

BALB/c recipient mice were conditioned with total body irradiation and then were transplanted i.v. with 15×10^6 splenocytes after mobilization with G-CSF versus plerixafor. To compensate for the higher CD4$^+$ T cell numbers in the spleens of plerixafor mobilized mice, we also conducted experiments in which transplant recipients had the lymphocyte dose adjusted to 2×10^6 CD4$^+$ T cells combined with 13×10^6 CD4$^-$ splenocytes mobilized with either G-CSF or plerixafor. Lymphoid and myeloid chimerism assessed 30 d after transplantation by flow cytometry was >90% donor in origin in BALB/c recipients of G-CSF ($n = 6$) and plerixafor mobilized splenocytes ($n = 6$). The incidence of skin GVHD in recipients of plerixafor and saline mobilized splenocytes was comparable. In contrast, recipients of G-CSF mobilized splenocytes had a reduced incidence of skin GVHD compared with recipients of either plerixafor or saline mobilized splenocytes ($p = 0.02$, Fisher test; $p < 0.05$, log-rank test; Fig. 5C). Furthermore, recipients of G-CSF mobilized splenocytes had significantly less weight loss on day 15 posttransplantation compared with mice transplanted with HBSS mobilized splenocytes ($p = 0.002$) and mice transplanted with plerixafor mobilized splenocytes ($p = 0.04$). When the number of transplanted CD4$^+$ T cells was normalized to 2×10^6 cells across all 3 groups, recipients of G-CSF mobilized splenocytes still had a significantly lower incidence (7/15 recipients developed GVHD within day +60; $p < 0.05$) of skin GVHD compared with plerixafor (16/17 recipients developed GVHD within day +60) or saline mobilized mice (16/17 recipients developed GVHD within day +60). Furthermore, total clinical GVHD scores were significantly lower ($p < 0.05$) in recipients of G-CSF mobilized splenocytes (1.6 ± 0.3) compared with recipients of plerixafor (2.9 ± 0.5) or saline mobilized splenocytes (2.7 ± 0.4).

Andreas Lundqvist et al. J Immunol 2013;191:6241-6249

The Journal of Immunology

Commento dalla Discussione del paper alla figura della slide precedente sul modello animale

Because plerixafor does not alter the phenotype and cytokine polarization of T lymphocytes, one might postulate the T cell–mediated immune sequelae of allogeneic transplantation may differ when allografts are mobilized with plerixafor compared with G-CSF. To better interpret the clinical relevance of transplanting T cells with these differences, we explored a murine model of PBSC transplantation in which transplant recipients received a T cell replete plerixafor versus G-CSF mobilized allograft from MHC-matched, minor histocompatibility–mismatched donors. A single injection of plerixafor resulted in the rapid mobilization of KLS hematopoietic stem cells into the peripheral blood of B10.d2 donor mice. The phenotype and MLR reactivity to alloantigens of T cells mobilized into the spleen with plerixafor was similar to T cells mobilized with G-CSF and nonmobilized T cell splenocytes. However, the $CD4^+$ T cell content was lower in G-CSF mobilized splenocytes, and recipients of G-CSF mobilized cells displayed a lower incidence of GVHD compared with recipients transplanted with plerixafor mobilized cells. Importantly, the cumulative incidence of GVHD was still lower in recipients of G-CSF mobilized grafts even when the transplanted number of $CD4^+$ T cells was matched between cohorts. Of note, the incidence of GVHD did not differ between recipients of plerixafor mobilized splenocytes compared with recipients of nonmobilized splenocytes. Transplanting large numbers of T cells that do not undergo changes in their cytokine gene expression profiles in contrast with transplanting T cells that undergo cytokine changes after G-CSF mobilization could potentially account for the higher incidence of acute GVHD observed in this analysis with plerixafor mobilized allografts. Because CXCR4 is expressed on many different hematopoietic cells, changes in the homing capacity of leukocytes mobilized with plerixafor or G-CSF might also have an impact on GVHD induction. To the best of our knowledge, no study has investigated whether CXCR4 antagonists impact the risk for GVHD by altering leukocyte migratory patterns. However, the magnitude and clinical significance of this effect with plerixafor mobilized lymphocytes would likely be low given the rapidly reversible nature of CXCR4 inhibition with this agent.

In conclusion, our results show important differences in the cellular composition of products mobilized with plerixafor compared with G-CSF. Although in vitro alloreactivity by MLR was similar, phenotypic, genotypic, and functional differences characterize T cells mobilized with plerixafor compared with G-CSF. Based on these data, one could hypothesize that immune reconstitution, graft-versus-tumor effects, and acute and chronic GVHD might differ in recipients receiving PBSC transplants mobilized with plerixafor compared with G-CSF. A pilot trial evaluating plerixafor for the mobilization and transplantation of HLA-matched sibling donor hematopoietic stem cells was recently reported. Although preliminary results from this trial have reported a similar incidence of acute and chronic GVHD and relapse rates as observed with historical controls receiving allografts mobilized with G-CSF (45), larger patient numbers will be needed to evaluate the true impact of plerixafor mobilization on engraftment, immune reconstitution, and other immune-mediated transplant events. Finally, because it is possible that plerixafor administered concurrently with G-CSF may be used for allogeneic stem cell donors who fail to mobilize sufficient $CD34^+$ cells with G-CSF alone, the effect of combining both mobilizing agents on T cell function will also need to be analyzed.

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

Beatrice Gaugler, Jessy Arbez, Steven Legouill, Pierre Tiberghien, Philippe Moreau, Sophie Derenne, Philippe Saas, Mohamad Mohty

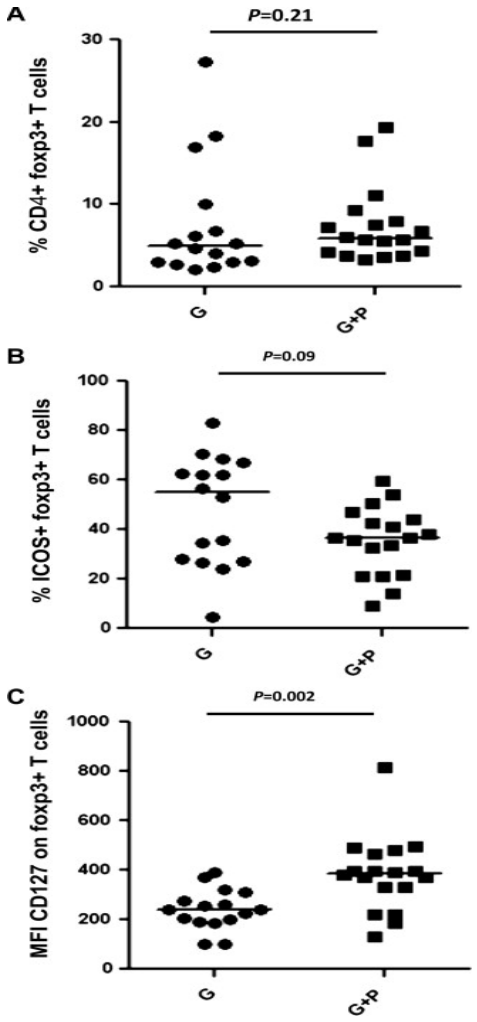


Figure 2. Phenotypic analysis of Tregs from grafts. Tregs were quantified in the G and P+G grafts by flow cytometry by the expression of CD4 and CD25 surface markers and intracellular analysis of Foxp3 (A). ICOS (B) and CD127 (C) were analyzed among CD4+Foxp3+...

Cytotherapy, Volume 15, Issue 7, 2013, 861–868

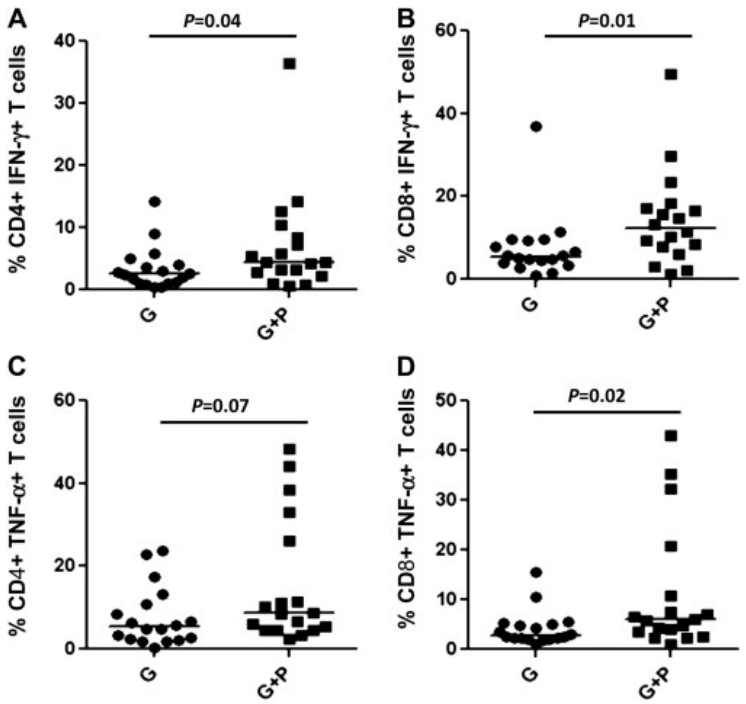


Figure 1. Functional analysis of T cells from grafts. PBMCs from G and P+G grafts were stimulated with PMA and ionomycin for 4.5 h, and gated CD3+CD4+ T cells or CD3+CD8+ T cells were analyzed for their frequency of IFN-γ (A, B) and TNF-α production (C, D) by ...

Beatrice Gaugler, Jessy Arbez, Steven Legouill, Pierre Tiberghien, Philippe Moreau, Sophie Derenne, Philippe Saas, Mohamad Mohty

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

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http://dx.doi.org/10.1016/j.jcyt.2013.03.013

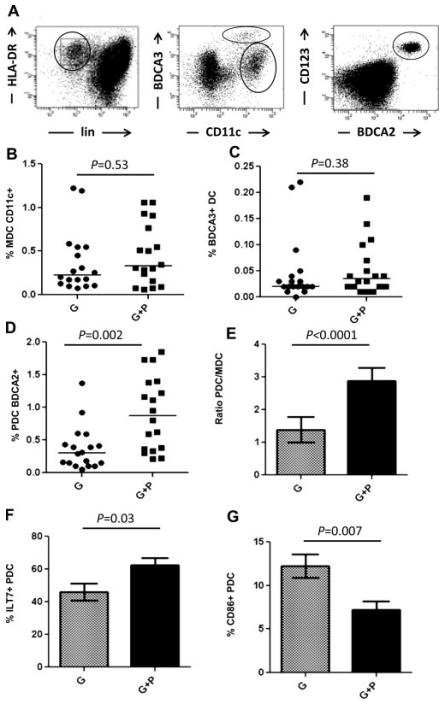

Figure 3. Quantitative and phenotypic differences in dendritic cell subsets from G and P+G grafts. Dendritic cell subsets were identified by flow cytometry according to specific markers. PBMCs were gated on lin− and HLA-DR+ cells. PDCs were characterized by th...

Beatrice Gaugler, Jessy Arbez, Steven Legouill, Pierre Tiberghien, Philippe Moreau, Sophie Derenne, Philippe Saas, Mohamad Mohty

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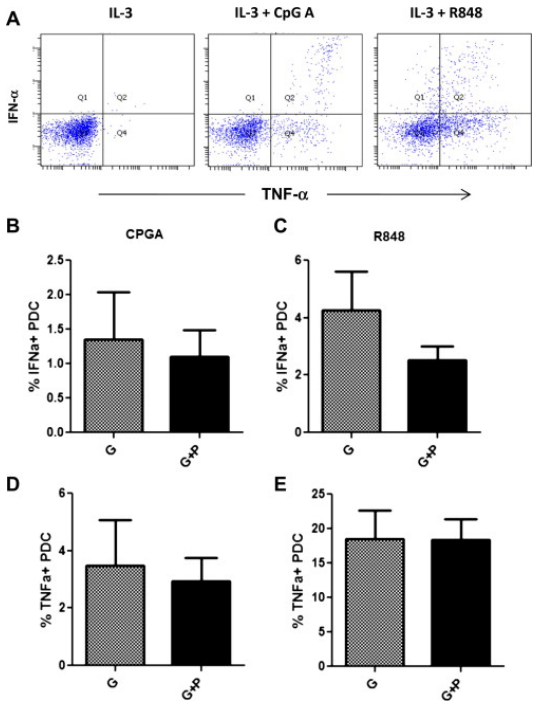

Figure 4. Functional analysis of PDCs from G and P+G. PBMCs from G and G+P grafts were stimulated with CpGA or R848 or with IL-3 only for 6 h, and gated BDCA2+CD123+ PDCs were analyzed for their frequency of IFN-α and TNF-α production by flow cytometry. (A) A ...

Beatrice Gaugler, Jessy Arbez, Steven Legouill, Pierre Tiberghien, Philippe Moreau, Sophie Derenne, Philippe Saas, Mohamad Mohty

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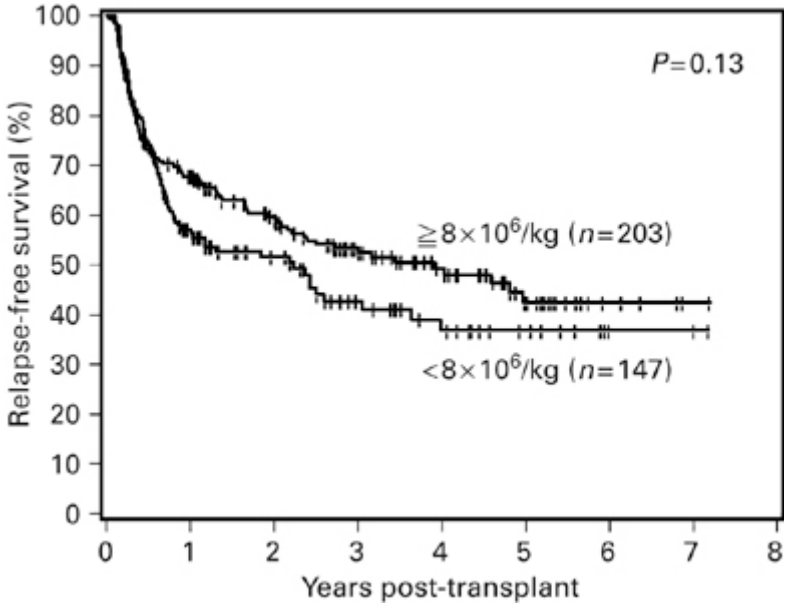
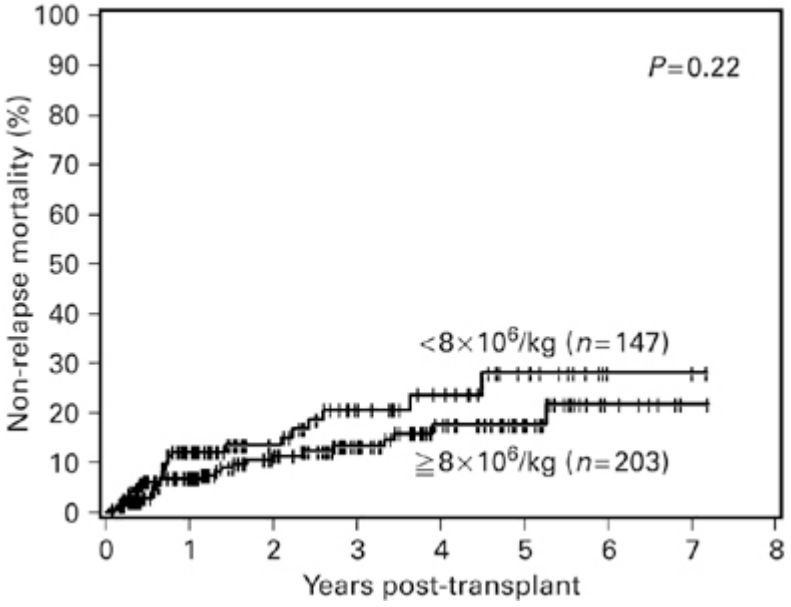
Patients mobilizing large numbers of CD34+ cells ('super mobilizers') have improved survival in autologous stem cell transplantation for lymphoid malignancies

B J Bolwell et al, *Bone Marrow Transplantation* (2007) **40,** 437–441

Relapse-free Survival



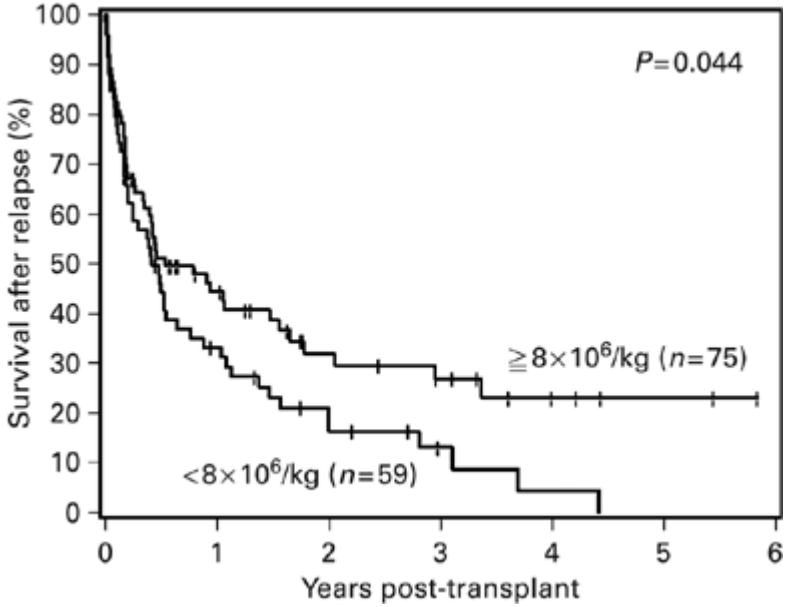
Risk of non-relapse mortality.



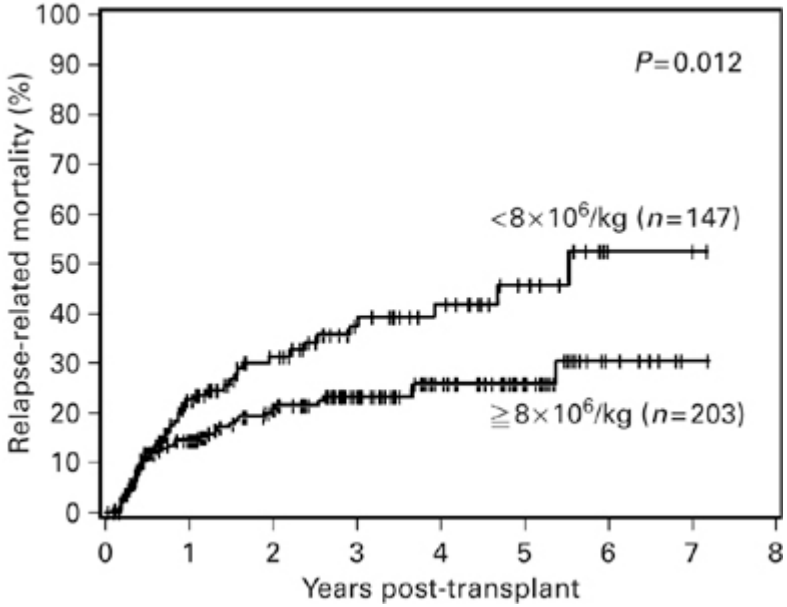
Patients mobilizing large numbers of CD34+ cells ('super mobilizers') have improved survival in autologous stem cell transplantation for lymphoid malignancies

B J Bolwell et al, *Bone Marrow Transplantation* (2007) **40,** 437–441

Survival after relapse



Relapse-related mortality



Abstract

Send to:

J Clin Oncol. 2000 Mar;18(6):1360-77.

Therapeutic relevance of CD34 cell dose in blood cell transplantation for cancer therapy.

Siena S[1], Schiavo R, Pedrazzoli P, Carlo-Stella C.

Author information

Abstract

PURPOSE:

To review recent advances in peripheral-blood progenitor-cell (PBPC) transplantation in order to define the optimal cell dose required for autologous and allogeneic transplantation.

MATERIALS AND METHODS:

A search of MEDLINE was conducted to identify relevant publications. Their bibliographies were also used to identify further articles and abstracts for critical review.

RESULTS:

The CD34(+) cell content of a graft is regarded as an accurate predictor of engraftment success. Postchemotherapy autologous PBPC transplantation with >/= 5 x 10(6) CD34(+) cells/kg body weight leads to more rapid engraftment than does transplantation of lower cell doses. Further increases in transplant cell dose further accelerate platelet but not neutrophil engraftment. Evidence that long-term hematopoietic recovery may be more accurately predicted by the subpopulation of primitive progenitors transplanted suggests that the content of CD34(+)CD33(-) and long-term culture-initiating cells in cell collection samples may be important for predicting successful engraftment, particularly in patients with poor mobilization. Allogeneic transplantation has been limited by concerns regarding graft-versus-host disease and the use of hematopoietic growth factors in donors. The risk of graft rejection and engraftment failure after HLA-mismatched allogeneic transplantation may be overcome by intensive chemoradiotherapy and the infusion of large numbers of T cell-depleted hematopoietic stem cells.

CONCLUSION:

An optimal cell dose of >/= 8 x 10(6) CD34(+) cells/kg seems to be recommended for autologous PBPC transplantation. This dose facilitates the administration of scheduled chemotherapy on time and reduces the demand for other supportive therapies. A combination of growth factors may enable patients with poor mobilization to achieve a collection sufficient to allow transplantation. The optimum PBPC dose for allogeneic transplantation remains to be defined.

Comment in

Optimal CD34(+) cell dose in autologous peripheral-blood stem-cell transplantation. [J Clin Oncol. 2000]

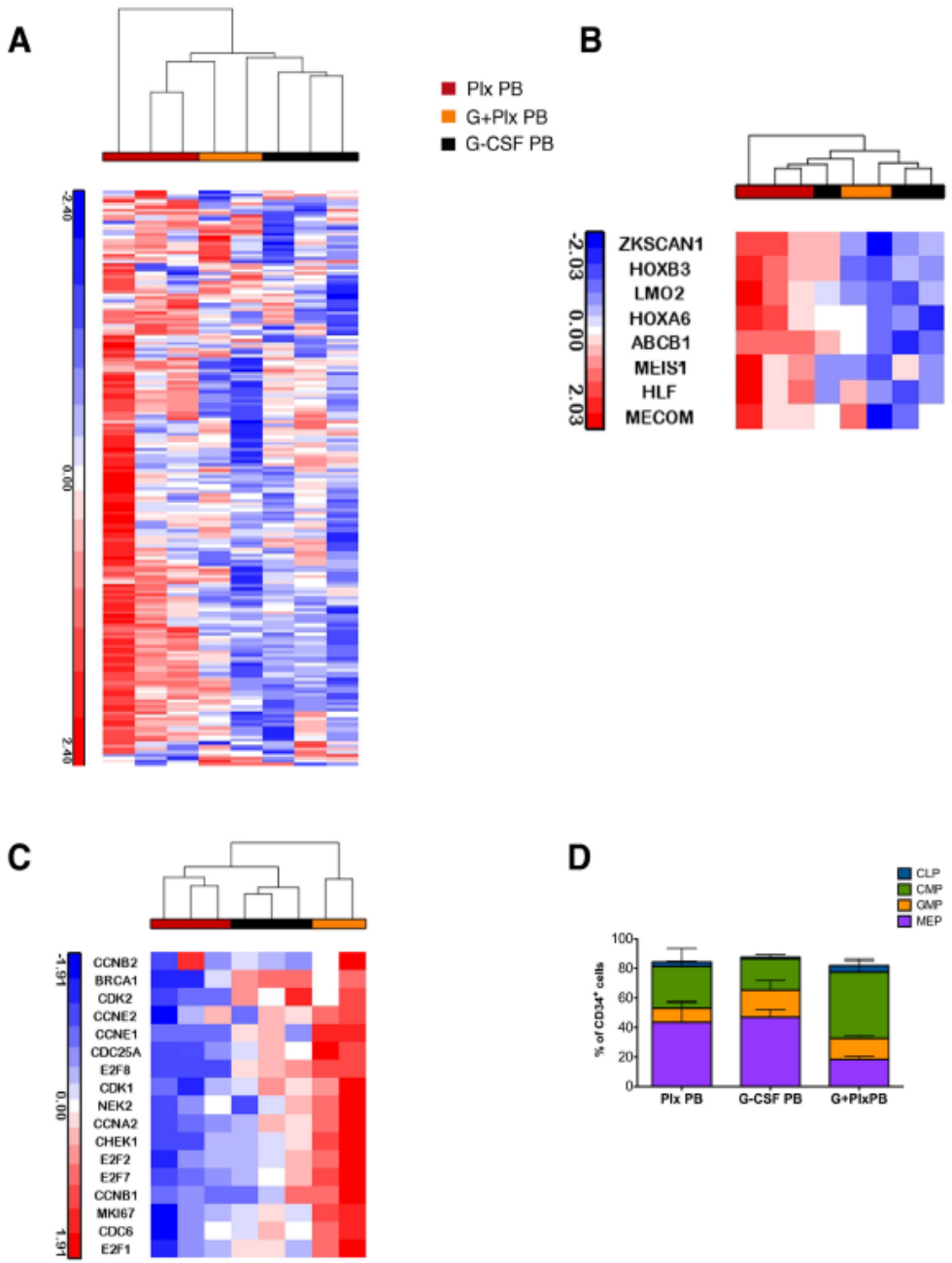
Appunti per la presentazione e schema di massima:

1) Il Plerixafor mobilizza cellule staminali e cellule immunitarie diverse da quelle che mobilizza il G-CSF. A supporto di ciò, presentare i dati del paper della Ferrari sulle HSCs e di Lundqvist sulle cellule immunitarie

2) e' possibile trovare una connessione tra compoente staminale e immuniyaria. Ovvero, come si può legare il fenomeno della mobilizzzaione delle cellule staminali Con quello delle cellule immunitarie? Anche e perché il graft è un tutt'uno e quindi è Importante provare a capire la dinamica di mobilizzazione di entrambi i compartimenti In corso di PLX

3) Dati di correlazione tra numero di CD34 e numenro dei linfociti (vedi presentazione COSTEM)

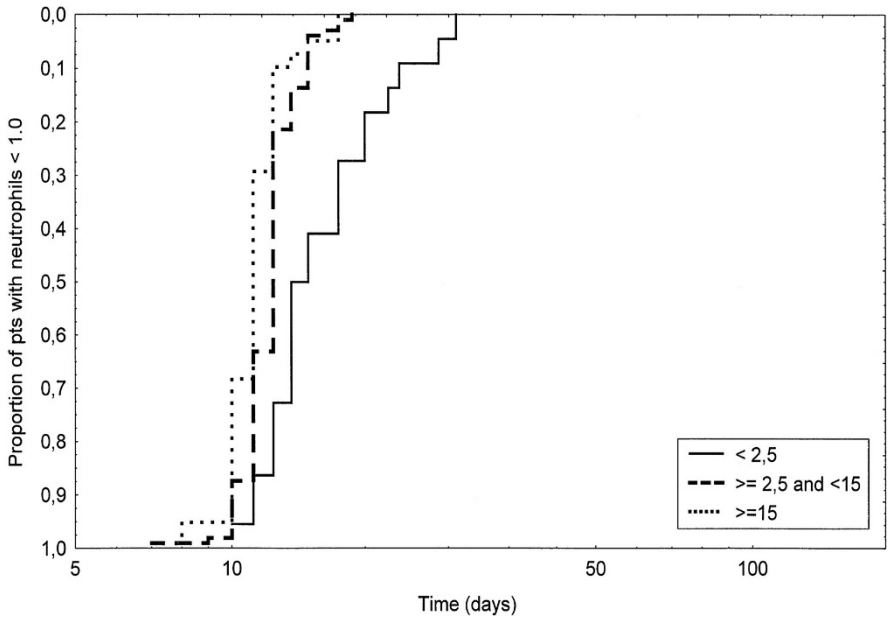
4) Su questo background, il nostro studio…



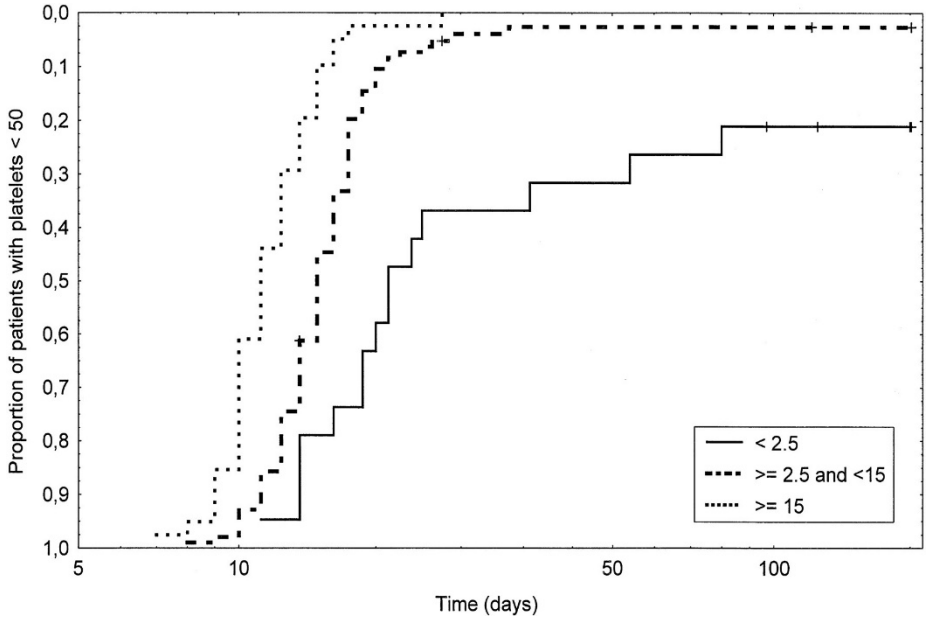
Giuliana Ferrari, Haematologica, 2016

CD34+ cell dose and engraftment: clinical studies

Time to recovery to an ANC greater than 1.0 × 109/L



Time to recovery to a PLT count greater than 50 × 109/L



Nicolas Ketterer et al. Blood 1998;91:3148-3155

High-dose CD34+ cells are not clinically relevant in reducing blood component consumption following autologous SCT as compared with standard dose

TABLE 6. Blood transfusions required following PBPC transplantation*

	Group 1	Group 2
Median day for the last RBC transfusion (range)	10 (5–30)	9.5 (0–72)
Median number of days with packed RBC transfusion (range)	2 (0–19)	2 (0–6)
Median number of RBCs transfused (range)	4 (0–39)	4 (0–12)
Number of patients without RBC transfusion	5	5
Median day for the last APC transfusion (range)	10.5 (6–300)	9 (3–32)
Median number of days with APC transfusion (range)	3 (0–20)	3 (0–11)
Median number of platelet units transfused (range)	22.5 (0–158)	20 (0–104)
Number of patients without platelet transfusion	2	2

* p values not significant for any measure.

Lefrere F, Transfusion 2002 pages 443-450

Results correlating CD34 dose with engraftment are contrasting, although a confirmation of the relation between CD34 dose and enfraftment, especially PLT recovery, has been established. However, the optimum dose has not been properly evaluated in prosepctive studies (most of them are registry-based and retropsective) and, more importantly, these studies did not take into considerations other clinically relevant parameters, such as durable enfraftment, cost of supportive care, costs of mobilization, collection and logistics in the stem cell lab

Beyond $CD34^+$ cell count as the only parameter of stem cell graft quality

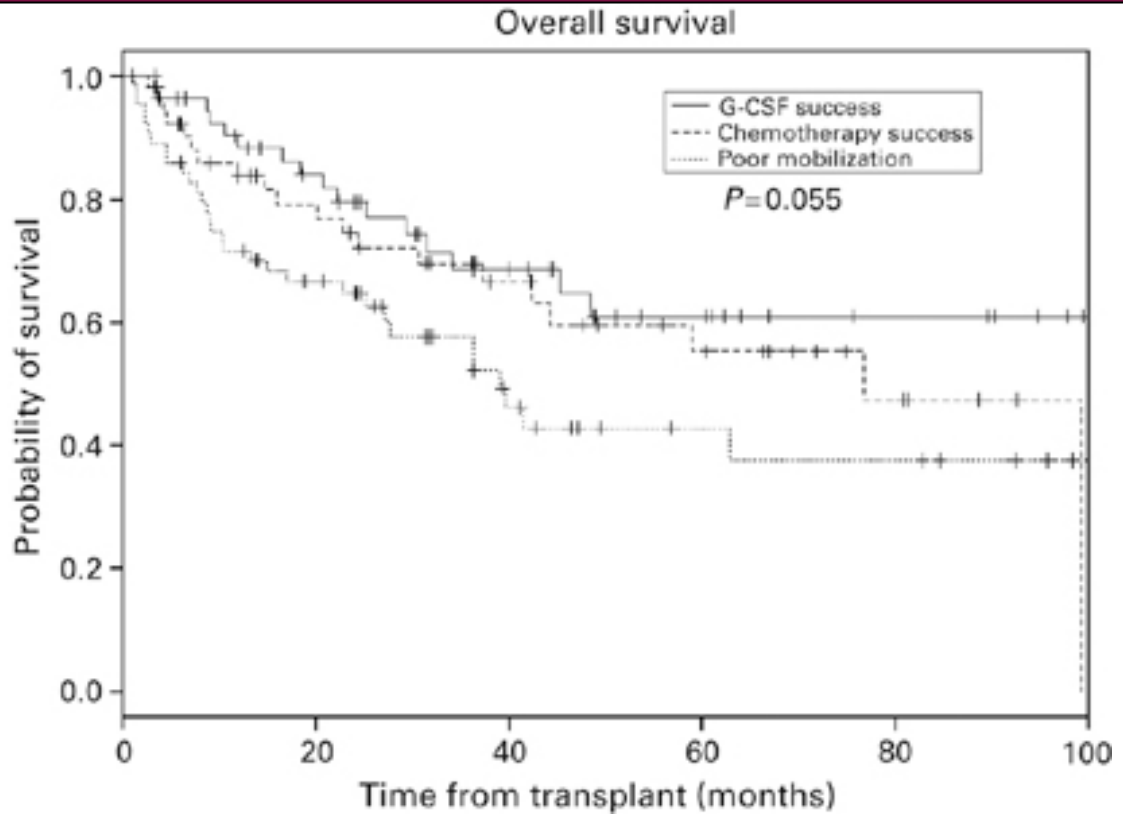
Subsets of $CD34^+$ cells are likely to be very important for engraftment after autologous SCT

The number of $CD34^+CD38^-$HLA-DR^- are correlated with faster early engraftment *(Zubair et al, Cytotherapy, 2006)*

The number of $CD34^+CD110^+$ cells is associated with more rapid long-term platelet engraftment *(Wellington-Beddoe, BBMT, 2009)*

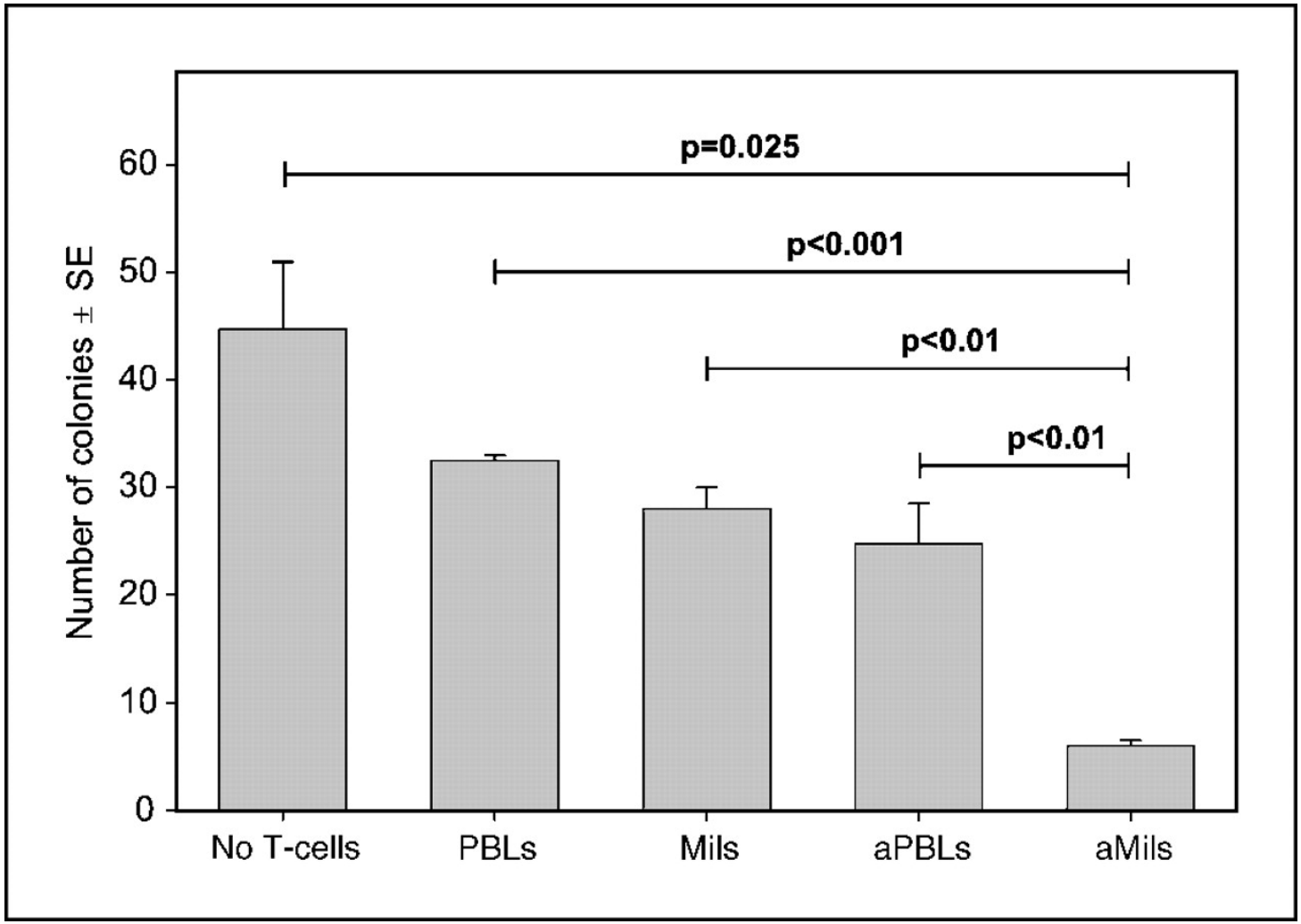
Several reports indicate that plerixafor may increase the mobilization of $CD34^+CD38^-$ stem cell population as compared to G-CSF with or without chemo *(Fruehauf, Cytotherapy, 2009)*

Difficult stem cell mobilization despite adequate CD34^{+} cell dose predicts shortened progression free and overall survival after autologous HSCT for lymphoma



Tomblyn et al, Bone Marrow Transplantation, 2007

Activated MILs significantly impair tumor outgrowth in a myeloma cell clonogenic assay.

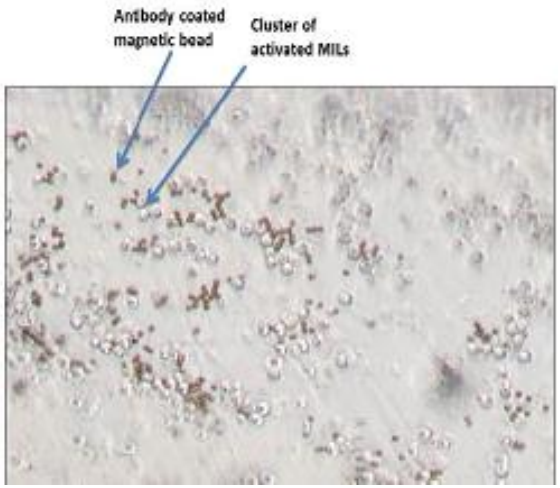


Kimberly Noonan et al. Cancer Res 2005

AACR American Association for Cancer Research
Cancer Research

Adoptive transfer of activated marrow-infiltrating lymphocytes (MILs) induces measurable antitumor immunity in the bone marrow in multiple myeloma

Kimberly A. Noonan & Ivan Borrello, *Science Translational Medicine, May 2015*



Newly diagnosed or relapsed MM 25 patients received infusion of autologous MILs after myeloablative therapy.

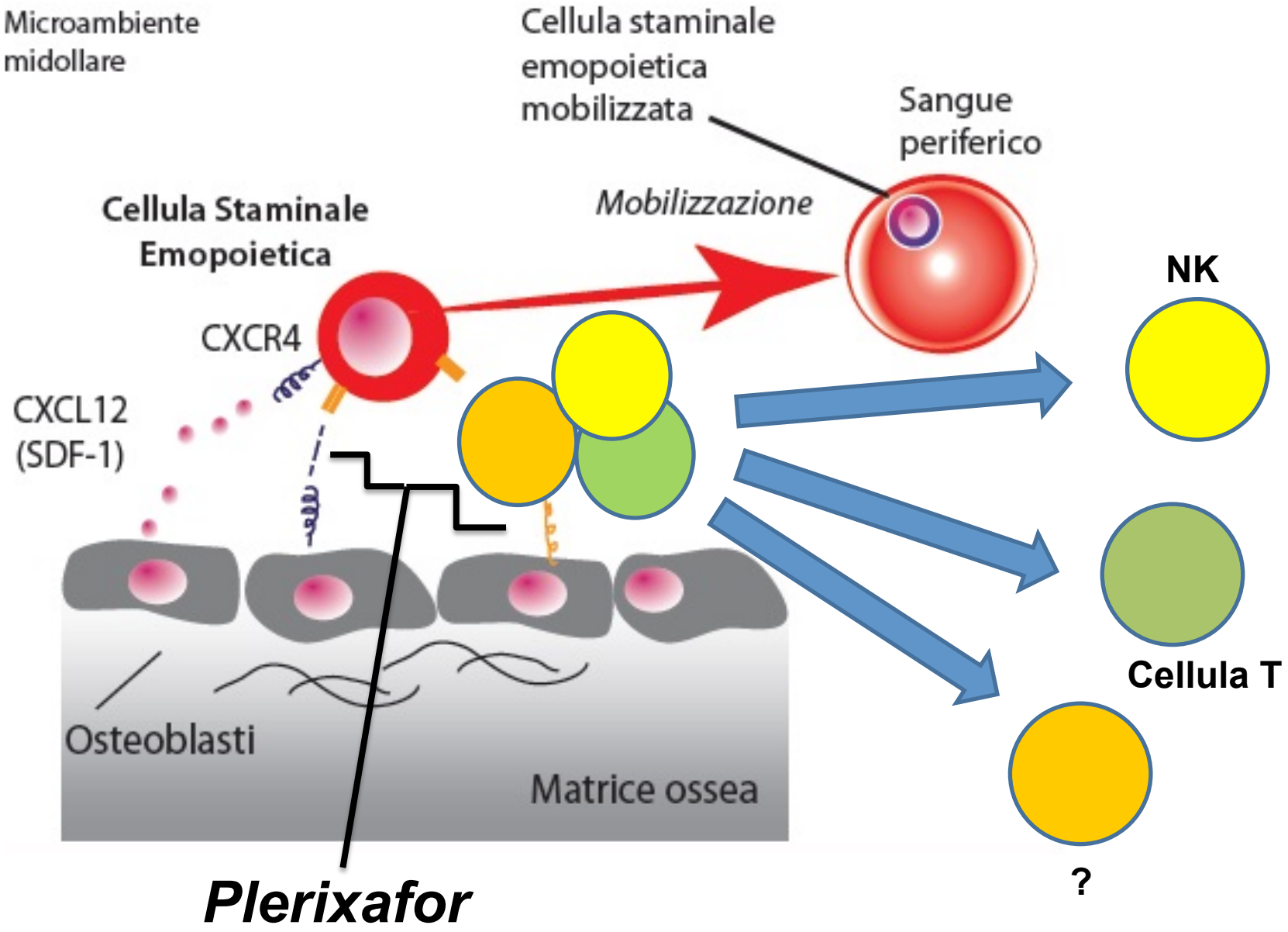
Response: 13/27 >> 50% reduction
7/27>> 90% reduction (DFS 25.1 months).
DFS of all patients 11.8 months.

Achieving at least a 90% reduction in disease burden significantly increased the progression-free survival (25.1 months versus 11.8 months; $P = 0.01$).

Better response in case of:

- measurable myeloma-specific activity of the ex vivo expanded product,
- low endogenous bone marrow T cell interferon-γ production at baseline
- a $CD8^+$ central memory phenotype at baseline,
- the generation and persistence of myeloma-specific immunity in the bone marrow at 1 year after ACT.

This study demonstrates the feasibility and efficacy of MILs as a form of ACT with applicability across many hematologic malignancies and possibly solid tumors infiltrating the bone marrow


Microambiente midollare
Cellula staminale emopoietica mobilizzata
Sangue periferico
Mobilizzazione
Cellula Staminale Emopoietica
CXCR4
CXCL12 (SDF-1)
NK
Cellula T
?
Osteoblasti
Matrice ossea
Plerixafor