	N Units	N (%) with < 75% CD34+ Cell Viability	Univariate p value*	Multivariate p value**
FACT accreditation at time of unit collection				
Yes	259	8 (3%)	Ref.	< 0.001
No	143	25 (18%)	< 0.001	
Year of cryopreservation				
1997 - 2002	61	11 (18%)	0.009	NS
2003 - 2012	341	22 (7%)	Ref.	
Cryopreserved volume (ml)				
< 24.5	14	5 (36%)	< 0.001	0.028
24.5 - 25.5	274	8 (3%)	Ref.	
25.6 - 30	59	5 (9%)	0.06	
> 30	55	15 (27%)	< 0.001	
Method of processing				
Manual	178	22 (12%)	0.003	NS
Semi-automated	30	2 (7%)	0.62	
Automated	183	7 (4%)	Ref.	
Not RBC depleted	6	1 (17%)	0.23	
Unknown	5	1 (20%)	0.20	

* Fisher's exact test **Linear Regression

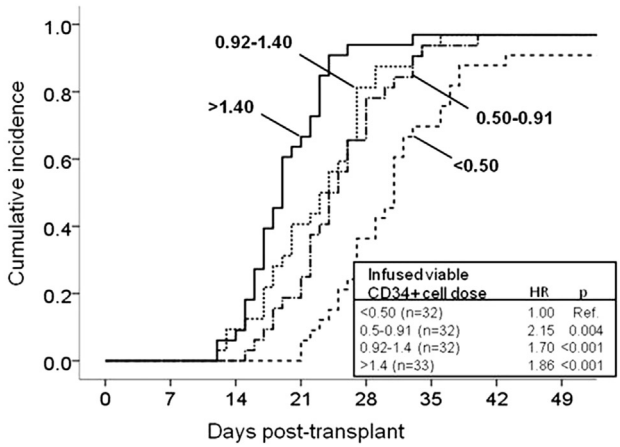

Figure. Neutrophil engraftment by dominant unit infused viable CD34+ cell dose $\times 10^5$/kg

IMMUNE RECONSTITUTION

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Immune Reconstitution after Autologous Stem Cell Transplantation for Multiple Myeloma

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Background: High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) for multiple myeloma (MM) offers a unique opportunity for the early introduction of consolidative immunotherapy to improve patient outcomes. Post-transplant reconstitution of immune cell subsets, however, occurs with disparate kinetics that can affect efficacy. A comprehensive understanding of the immunologic milieu is therefore essential to the rational development of immunotherapeutic interventions after ASCT, where relapse remains the primary cause of treatment failure.

Methods: Immune reconstitution in 40 MM patients undergoing ASCT was evaluated for one year. Peripheral blood

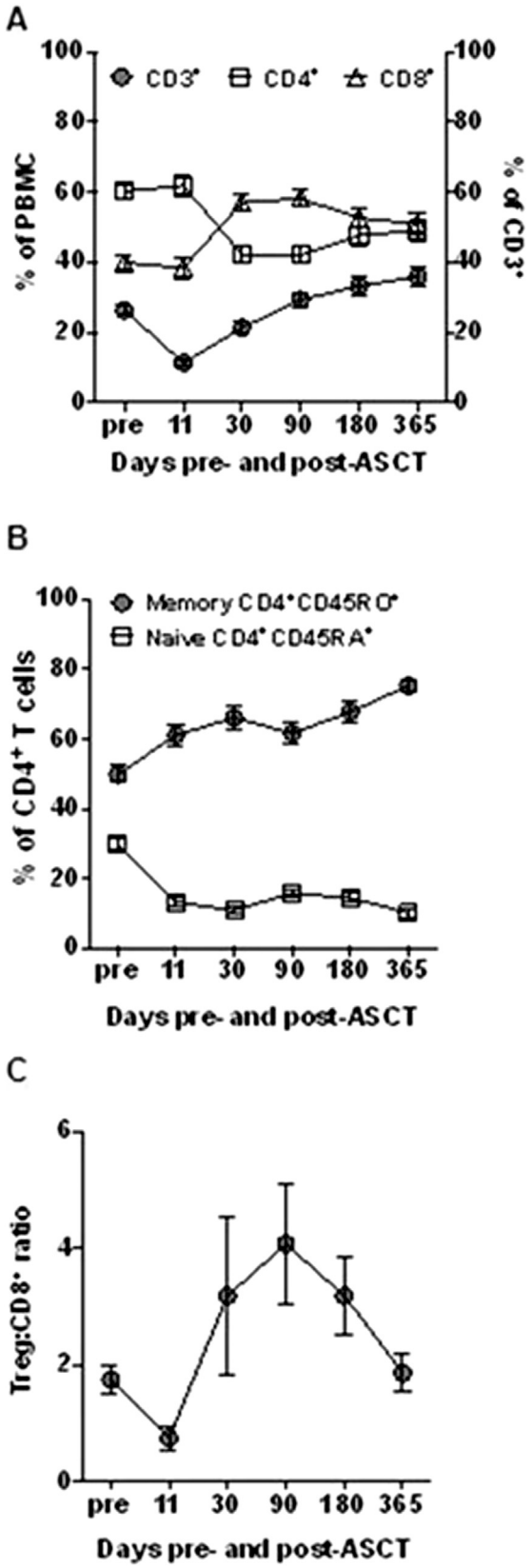

Figure 1. T cell reconstitution in MM patients after high-dose chemotherapy and autologous stem cell transplantations (ASCT).
(A) T cell composition: Peripheral blood was analyzed by flow cytometry for total $CD3^+$, $CD3^+$, $CD4^+$ and $CD3^+$ $CD8^+$ T cells at the indicated time points (d + 11, 30, 90, 180, and 365) after ASCT and compared with pre-ASCT values (pre), Total $CD3^+$ cells are plotted against the LEFT Y axis. $CD4^+$ and $CD8^+$ cells are plotted against the RIGHT Y axis.
(B) Naïve and memory $CD4^+$ T cells: Peripheral blood was analyzed by flow cytometry for naïve ($CD4^+$ $CD45RA^+$) and memory ($CD4^+$ $CD45RO^+$) T cells at the indicated time points (d +11, 30, 90, 180, 365) after ASCT and compared with pre-ASCT values (pre). Pooled data (mean ± SD) from 40 pts are shown.
(C) Treg:CD8+ effector ratio: Peripheral blood was analyzed by flow cytometry for Tregs ($CD3^+$ $CD4^+$ $CD25^{bright}$ $CD127^{neg}$) and $CD8^+$ T cells at the indicated time points before (pre) and after (d+11, 30, 90, 180, and 365) ASCT to calculate Treg/$CD8^+$ affector ratios. Pooled data (mean ± SD) from 40 pts are shown.

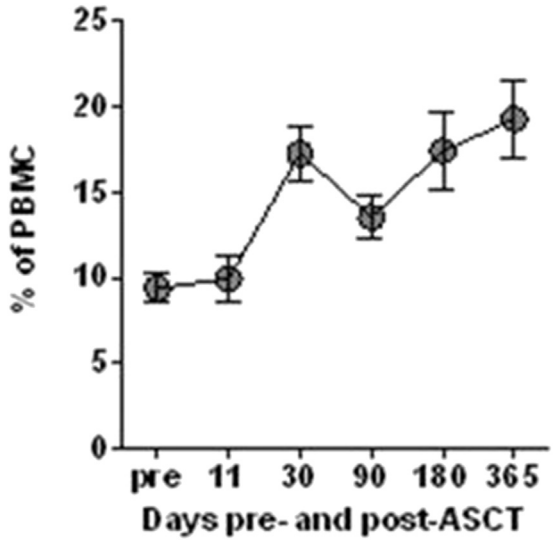

Figure 2. Natural killer (NK) cell reconstitution in MM patients after high-dose chemotherapy and autologous stem cell transplantation (ASCT). Peripheral blood was analyzed by flow cytometry for NK cells ($CD3^{neg}CD56^+$) at the indicated time points (d +11, 30, 90, 180, and 365) after ASCT and compared with pre-ASCT values (pre). Pooled data (mean ± SD) from 40 pts are shown.

from patients was obtained before ASCT and on d +11, +30, +90, +180, and +365 after ASCT. Mononuclear cells were analyzed by flow cytometry for phenotypic assessment of lymphocyte subset composition. Functional assessment of T cell responsiveness was assayed as follows: Monocyte-derived dendritic cells (moDCs) generated from peripheral blood were electroporated with WT1 mRNA and then added in serial doses to triplicate microwells containing autologous T cells obtained pre- and post-ASCT (d +11, 30, 90), supplemented with IL15 x 7d. Antigen-specific target cell lysis by cytotoxic T lymphocytes (CTLs) induced by moDCs was evaluated using a flow cytometry-based assay.

Results: Total $CD3^+$ T cell levels return to normal by d +30 (Fig 1A). The recovery of $CD8^+$ T cells, however, precedes the recovery $CD4^+$ T cells, resulting in an inverted CD4/CD8 ratio (Fig 1A). $CD4^+CD45RO^+$ memory T cell recovery occurs by d +11, whereas $CD4^+CD45RA^+$ naïve T cells remain low at one year (Fig 1B). Immune-suppressive $CD3^+CD4^+CD25^{bright}$ $CD127^{neg}$ regulatory T cells (Tregs) are reduced early post-nadir as $CD8^+$ T cell recovery occurs, resulting in a markedly reduced Treg:$CD8^+$ effector T cell ratio (Fig 1C). T cells uniformly express the negative regulatory molecules, CTLA4 and PD1, after ASCT (data not shown). $CD3^{neg}CD56^+$ natural killer cells exhibit rapid and sustained recovery after ASCT (Fig 2). Robust antigen-specific CTLs are induced by autologous mRNA-electroporated moDCs after only 7 days' stimulation of T cells *in vitro,* demonstrating that active cellular immune responses can be elicited as early as d +11 (Fig 3). Subgroup analysis of patients based on pre- and post-ASCT disease status (i.e., PR vs VGPR vs CR) revealed no significant

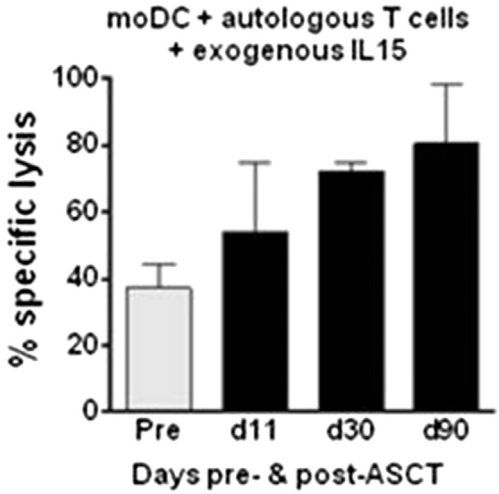

Figure 3. mRNA-electroporated, CDs from MM patients induce autologous antigen-specific CTLs after autologous stem cell transplantation (ASCT). Cytokine-generated, moDCs derived form peripheral blood were eclectroporated with WT1 mRNA, terminally matured by a combination of inflammatory cytokines, and then added in serial doses to triplicate micorwells containing 1 $\times$ 10^2 autologous T cells obtained pre- and post-ASCT (d +11, 30, and 90) and cultured with IL 15 for 7 days, Antigen-specific target cell lysis by CTLs stimulated by WT1 mRNA-electroporated moDCs was evaluated using a flow cytometry-based assay.
Target cells for were 697 cells (HLA-A*0201$^+$, WT1$^+$ cell line). Specific lysis is plotted against the y-axis comparing the lysis activity of T cells stimulated by mRNA-electroporated moDCs at the indicated time points (d +11, 30, and 90) after ASCT and compared with pre-ASCT value (pre). Data points ate the averages $\pm$ SEM of triplicate means from one experiment.

differences in the pattern of immune reconstitution between groups.

Conclusion: In addition to decreased disease burden, a favorable shift in Treg to effector T cell ratios characterizes the early post-transplant period. This provides a critical window for immunotherapeutic modalities like vaccines and checkpoint blockade agents to induce antitumor immunity.

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A 2-Step Approach to Haploidentical Versus Matched Related Hematopoietic Stem Cell Transplantation (HSCT): Equivalent Early Immune Reconstitution with Comparable Outcomes

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Haploidentical (HI) HSCT enfranchises patients (pts) who have no HLA matched donor. In the 2-step approach to HI HSCT, a large *fixed* dose of allogeneic T cells are infused after conditioning, followed 2 days later by cyclophosphamide (CY) to induce bidirectional tolerance. A CD34 selected stem cell product is infused after CY. CY eradicates only activated T cells, supporting post HSCT immune reconstitution (IR) and low rates of GVHD. Given the favorable results with this approach in HI HSCT, we applied the regimen to MR HSCT. Delivering a fixed dose of donor T cells within the same 2 step regimen provides a level baseline from which to evaluate whether HI HSCT is inherently inferior to MR HSCT. Thus, we compared IR and clinical outcomes of patients undergoing HI HSCT versus MR HSCT using this approach.

	HI HSCT (%)	MR HSCT (%)
Number	29	23
Age (range)	49 (21-65)	49 (27-64)
Sex (M/F)	19/10	17/6
Median CD34 cells (x 10^6/kg)	4.7	4.32
Disease		
AML/MDS	16 (55)	11 (48)
ALL	11 (38)	5 (22)
NHL	2 (7)	7 (30)
Conditioning		
MA	29 (100)	21 (91)
RIC	0	2 (9)
Outcomes:		
Median ANC recovery -days	11	11
Median Platelet recovery- days	17	18
aGVHD III-IV	3 (10)	1 (4)
cGVHD	5 (12)	1 (4)
CMV Reactivation	10 (35)	4 (17)
Deaths	5 (17)	5 (22)
Cause of Death:		
Infection	0	0
Relapse	4 (14)	4 (17)
Toxicity	1 (3)	1 (4)
GVHD	0	0

Flow cytometric assessment of T-cell subsets was used to measure IR at day (D) +28 and +90 in pts undergoing HI and MR HSCT. Pts were evaluable for IR analysis if they had full donor chimerism, had no evidence of disease, and were fully