to guide clinical decision making in patients with acquired or congenital immunosuppression.

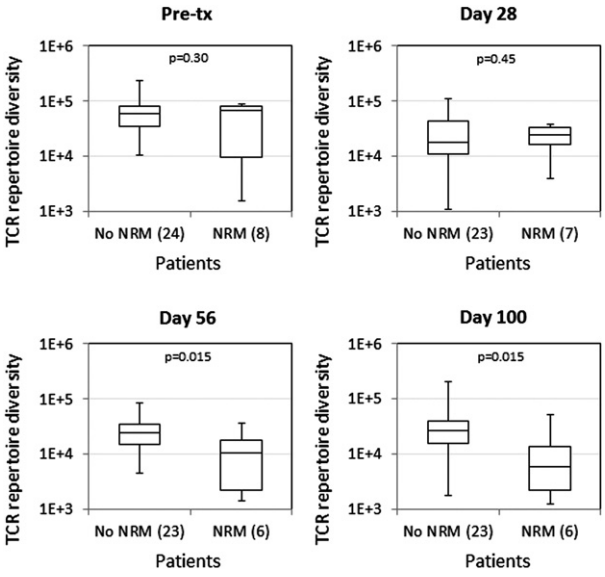

Figure 1. TCR Repertoire Size: NRM v. other patients
The figure shows a comparison of TCR repertoire size, estimated based on high-throughput sequencing of TCRβ rearrangements, for all surviving patients (N is indicated below each panel) with and without eventual non-relapse mortality (NRM). TCR repertoire size values are given as quartiles for both populations, with whiskers representing the maximum and minimum values. Significance is assessed using a one-tailed Mann-Whitney U test; patients who went on to suffer from non-relapse mortality had significantly lower estimated repertoire sizes at 56 and 100 days post-transplant.

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Impact of Atorvastatin On Cellular Immunome of Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation (AHSCT)

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Introduction: Acute graft-versus-host-disease (aGVHD) is a frequent and lethal complication of HSCT despite current prophylaxis. Release of pro-inflammatory cytokines lead to damage of host tissues. Statins have been shown to reduce pro-inflammatory cytokines while increasing the migration of anti-inflammatory cytokines, especially when donor and recipient are exposed to it, thus reducing aGVHD. We present data on 14 patients enrolled in an ongoing phase II study evaluating the efficacy of atorvastatin as aGVHD prophylaxis in patients undergoing matched-related donor AHSCT.

Table 1
Effect of Statin on Immunomes

Statin vs. non-statin D30 (%)	*P*-value	Median(Range) Non-statin exposed	Median(Range) Statin exposed
Absolute Lymphocyte count	0.04688	850 (100-1300)	774.8 (109-2120)
DR+/14+ (absolute)	0.375	77.6 (43.1-86.3)	69.3 (19.2-92.1)
DR-/14+ (absolute)	0.1094	12.5 (2.7-34.4)	25.4 (1.8-78.6)
CD3+/56-/16-	0.04688	55 (15.4-78.2)	78.7 (52.5-95.8)
CD19+	1	1 (0.2-2.0)	0.7 (0-39.4)
CD69 +/CD3+	0.8655	3.2 (0.7-4.7)	2.7 (1.1-4.4)
CD3+/DR+	1	5 (1.4-30.1)	5.1 (0.4-13.7)
CD3+/DR -	0.1563	48 (17.5-77.1)	70.1 (52.9-85.1)
CD3-/56+/16+	0.03125	33.5 (16.5-42.2)	7.9 (2.7-21.0)
CD3-/56+/16+/314+	0.03125	18.1 (6.5-34.0)	4.6 (0.7-10)
CD3-/56+/16+/117-	0.03125	22.4 (11.8-36.4)	6.3 (0.9-12.9)

Method: In this phase II study donors receive atorvastatin 40 mg daily for at least 14 days before leukapheresis. Recipient patients receive Atorvastatin 40mg daily starting at least 7 days before initiation of transplant conditioning regimen in addition to standard approved GVHD prophylaxis with tacrolimus and methotrexate. Atorvastatin is continued until development of grade II or higher aGVHD, cessation of aGVHD prophylaxis and or adverse event. We compared the immune reconstitution pattern of patients on statin to those of historic control patients not on statin matched to age, sex, type of AHSCT and intensity of regimen.
Result: Median age of patients is 47 (range 27-67) and of donor 46.5 (29-64). Ten patients received reduced intensity AHSCT. Median neutrophil and platelets engraftment were 18.9 and 12.9 days. With a median follow up of 120 days (23-287), two patients have relapsed one of whom died due to disease. The median day 30 CD3 and CD34 chimerism were 81% and 100%. Two patients (14%) have developed grade II aGVHD with no patients developing grade III or IV aGVHD. Two patients developed chronic GVHD both mild and limited. At day 30 post HSCT, compared to historic control, patients exposed to statin had no difference in B cells(CD19+) or total T cells (CD3+/DR+; CD3+/DR-), but a significant decline in NK cells, including total NK cells and NK cells positive for CD314 (Table 1).
Conclusion: While this is a small sample, and no conclusion can be deduced, it is interesting to note the significant difference in the number of NK cells in patients exposed to statin as compared to historic control.

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Different Natural Killer (NK) Cell Subsets Elicit Unique Target Induced Immune Responses: Implications for Assessment of Posttransplant Functional Recovery of the Relevant NK Cell Subsets and Their Impact On HCT Outcomes

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Background: A normal, fully reconstituted immune system after hematopoeitc cell transplantation (HCT) is critical for the control of post-transplant infections, establishment of graft tolerance and, in some cases, mediation of graft-versus leukemia effects. Natural Killer (NK) cells, being the first in line of defense against tumors and infections, are also the earliest among lymphocyte populations to reconstitute and achieve functional maturity after transplantation. However, many HCT recipients with normal recovery of NK cells continue to suffer from complications including infections and disease relapse suggesting that different NK cell subsets may be responsible for anti-leukemic or anti-viral immune response. Here, we set out to determine in healthy individuals, whether different NK cell subsets (cytolytic or regulatory) elicit unique immune responses against different targets (leukemia cells or herpes viruses).
Methods: Peripheral Blood Mononuclear Cells (PBMNCs) from 25 healthy donors were stimulated with different targets including a leukemic cell line (K562) and herpesviral (Epstein - Barr virus, EBV) infected cell lysate. A 5-colour flow cytometry based estimation of cytotoxicity (expression of CD107a, a surrogate marker for degranulation) and cytokine (IFN-γ) production was performed for both $CD56^{bright}CD16^{neg}$ regulatory and $CD56^{dim}CD16^{pos}$ cytolytic NK cell subsets.
Results: Different NK cell subsets were immunodominant against different targets. Leukemia (K562) – specific response includes both degranuation and IFN-γ production mediated by the cytolytic and regulatory NK cell subsets. On the contrary, EBV specific NK cell response was primarily characterized by degranulation and was dominated by cytolytic NK cells. A consistent shedding of CD16 was found associated with degranulation of cytolytic NK cells in response to EBV but not to K562 cells. Cytolytic NK cells in general exhibited a bifunctional immune response against both targets while regulatory NK cells were primarily IFN-γ producers.
Conclusions: NK cell subsets elicit a unique immune response against different targets (leukemia cells or herpesviruses). Assessment of posttransplant recovery of these target specific functional NK cell subsets will be more relevant for the prediction of transplant outcomes and may have future implications for the cellular therapy/prophylaxis of herpesviral disease or leukemia relapse.

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The Restorative Effect of Flt3-Ligand and IL-7 On CD4+ T Cell Homeostatic Proliferation During Graft-Versus-Host Disease

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Allogeneic hematopoietic stem cell transplantation (SCT) is the best treatment for numerous types of hematological malignancies. However, graft-versus-host disease (GVHD) is the major cause of morbidity and mortality and its effect on T cell regeneration greatly exacerbates the immunodeficiency normally associated with this treatment. As a result, patients with GVHD are profoundly lymphopenic and T cell reconstitution typically takes several months or years.

We previously reported that $IL7Ra^{-/-}$ mice have a peripheral lymphoid niche that is highly permissive for homeostatic proliferation (HP) of $CD4^+$ T cells. Given that $CD4^+$ T cells regeneration is impaired during GVHD, we hypothesized that using $IL7Ra^{-/-}$ bone marrow (BM) might improve their recovery in GVHD hosts. To study the impact of GVHD on the peripheral niche regulating CD4 HP, we used the mouse model B6 into $B6D2F_1$. Since $IL7Ra^{-/-}$ DCs support efficiently HP of naïve $CD4^+$ T cells during lymphopenia, we used BM stem cells from $B6IL7Ra^{-/-}$ mice and induced GVHD by adding $1x10^6$ B6 T cells. Finally, to understand the impact of GVHD on HP, we transferred CFSE labelled anti-HY $CD4^+$ T cells (Marilyn) into GVHD hosts and measured their homeostatic proliferation 7 days later.

In non GVHD hosts, Marilyn T cells underwent robust HP while they completely failed in GVHD hosts. Absence of HP during GVHD was associated with a severe depletion of all DC subsets, including myeloid, lymphoid and pDCs. Low number of DCs during GVHD was in part due to their elimination by GVHD T cells but most importantly to a myelosuppression affecting DC production from BM progenitor cells. Interestingly, treatment of GVHD mice with FLT3 ligand (FL) significantly increased the number of DCs, yet it was insufficient for restoring CD4 HP. Given that stromal cells and IL7 mRNA levels were also diminished in GVHD hosts, we provided both FL and IL-7 to GVHD mice and significantly restored HP of Marilyn $CD4^+$ T cells in this setting.

Thus far, our data support a model wherein loss of CD4 HP during GVHD relates to lower numbers of DC and diminished systemic IL7.

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NK Cell Recovery and Costimulatory Molecule Profiles After Autologous Hematopoietic Cell Transplantation (HCT) in Multiple Myeloma (MM) Patients

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Background: Enhanced immune responses post autologous HCT is known to be beneficial for long term disease control in MM. Early responses are mediated by NK cells and alternate inhibitory/stimulatory pathways that include the costimulatory molecules. This pilot study assesses the expression of NK cytolytic receptor (CD16) as well as the stimulatory (OX-40, ICOS, 4-1BB, CD28, NKG2D) and inhibitory (PD-1 and CTLA-4) molecules on NK cells after auto-HCT in MM patients.
Methods: 22 patients with MM undergoing HCT, median age 59.6 years (36 - 71.4), were included in the study. Peripheral blood samples were taken 3 days prior to HCT and 14, 30, 60, 90, 180 days after HCT. At d180 post-HCT, 18/22 patients were receiving lenalidomide with d86 as median start date. NK cells and their costimulatory molecules were evaluated by flowcytometry using 2 six color panels of antibodies. One way ANOVA