# Lymphocyte Subset Recovery and Outcome after Autologous Hematopoietic Stem Cell Transplantation for Plasma Cell Myeloma

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Rapid immune reconstitution—particularly of natural killer cells (NK cells)—after allogeneic hematopoietic stem cell transplantation (HSCT) is associated with protection from relapse. Whether such an association also exists after autologous stem cell transplantation is less clear. We retrospectively assessed lymphocyte subsets after autologous HSCT in 114 patients and correlated lymphocyte recovery with outcome. CD8 T cell and NK cell counts recovered rapidly to pretransplantation levels, whereas B cell and CD4 T cell recovery were delayed. Compared with patients with low NK cells (<100/uL), high NK cell count at 1 month after HSCT was associated with significantly prolonged progression-free survival: for NK cells 100 to 200/uL hazard ratio [HR], .33 (95% confidence interval [CI]; .16 to .80; $P = .004$); for NK cells > 200/μL HR, .27 (95% CI, .13 to .58; $P = .001$). No significant protective effects were associated with rapid recovery of any other lymphocyte subset. None influenced overall survival (OS) or time to next treatment. Early NK cell recovery is associated with better progression-free survival after autologous HSCT. The failure to detect an effect on OS might be due to the salvage strategies available to these patients.



## INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) relies on graft-versus-tumor (GVT) effects for its curative potential. Accordingly, early recovery of donor-derived lymphocytes has been associated with improved survival [1-4]. In the setting of T cell–depleted transplantation, a protective effect has been attributed to early reconstitution of natural killer (NK) cells, the first lymphocyte subset to reconstitute after this transplantation type [5]. Whether a similar association also exists for patients who underwent transplantation with autologous stem cells is less clear. Early lymphocyte reconstitution has been associated with a favorable outcome of autologous HSCT for non-Hodgkin lymphoma [6,7] and plasma cell myeloma [8,9]. Which lymphocyte subset is responsible for this association has so far not been investigated.

As both autologous and allogeneic NK cells have been shown to be able to recognize and lyse plasma cells of myeloma patients [10,11], we hypothesized that early NK cell recovery might be associated with autologous GVT effects and improved disease control after autologous HSCT for plasma cell myeloma. To address this question, we retrospectively analyzed lymphocyte subset (ie, CD4 T cell, CD8 T cell, NK cell, and B cell) recovery in a cohort of patients undergoing autologous HSCT for plasma cell myeloma.

## PATIENTS AND METHODS

### *Transplantation Procedure*

We analyzed 114 patients undergoing autologous HSCT at Basel University Hospital between 2001 and 2011. All patients underwent autologous HSCT after conditioning with melphalan 200 mg/m$^2$ (140 mg/m$^2$ in case of renal failure). All patients were treated for plasma cell myeloma. All patients received unmanipulated grafts. Further patient and transplantation characteristics are summarized in Table 1. All patients gave written informed consent to the transplantation procedure and to analysis of outcome data. The study was performed according to the regulations of the local ethics committee.

### *Flow Cytometry*

Lymphocyte subpopulations were systematically assessed before start of the conditioning regimen, as well as 1, 3, 6, 12, and 24 months after transplantation. After 2005, lymphocytes were immunolabeled using BD Trucount beads (BD Biosciences) and BD Multitest reagents (BD Biosciences, San Jose, CA) with an antibody combination of CD3-FITC/CD16-PE + CD56-PE/CD45-peridin chlorophyl protein (PerCP)/CD19-allophycocyanin (APC) and CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC, according to manufacturer's recommendations [12]. Samples were analyzed within 2 hours on a FACSCalibur (BD Biosciences). Dot plots were analyzed using FACS Multiset software (BD Biosciences). Before 2005, lymphocyte subpopulations were assessed in whole blood by a dual-platform, 3-stage process: (1) the white blood cell count (WBC), (2) the percentage of WBCs that are lymphocytes, and (3) the percentage of lymphocytes that are T, B, or NK cells. The last stage in the process was performed by flow cytometry. Lymphocytes were hereby immunolabeled and analyzed using a similar procedure described above for the single-platform method; however, without BD Trucount beads.

### *Statistics*

The effect of lymphocyte reconstitution on transplantation outcome was analyzed in an univariate analysis by categorizing subset counts at different time points and calculating probabilities of overall survival (OS), progression-free survival (PFS), and time to next treatment using the Kaplan-Meier estimator. We set our cut offs for lymphocyte subset by patient numbers to have 3 similar cohorts. Estimated survival rates were compared by log-rank test. The correlation between lymphocyte counts and transplantation outcome was further analyzed in multivariable Cox models adjusting for covariates. Cox models were adjusted for disease stage, patient age, cytogenetics, and remission status at transplantation. Response to treatment and relapse/progression events were classified according to consensus guidelines [13].

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**Table 1**
Patient Characteristics

| Characteristic | Value |
|---|---|
| No. of patients | 114 |
| Year, median (range) | 2008 (2001-2011) |
| Age, median (range), yr | 61 (27-76) |
| Sex (male/female) | 78/36 |
| Types | |
| IgG | 58 |
| IgA | 29 |
| IgM | 2 |
| IgD | 1 |
| Light chain | 21 |
| Nonsecretory | 3 |
| Cytogenetics | |
| Normal | 37 |
| Del13.q14 | 22 |
| Del13.q14 and del17 | 9 |
| Del13.q14 and IgH rearrangement | 6 |
| IgH rearrangement | 4 |
| NA | 36 |
| ISS | |
| I | 34 |
| II | 45 |
| III | 10 |
| NA | 25 |
| Durie-Salmon | |
| I | 16 |
| II | 35 |
| III | 63 |
| Renal function | |
| Creatinine $<$ 2 mg/dL | 88 |
| Creatinine $>$ 2 mg/dL | 15 |
| NA | 11 |
| Lines of treatment before treatment, median (range), n | 1 (0-5) |
| No. of transplantations | |
| First | 84 |
| Not first | 30 |
| Remission status at transplantation | |
| sCR/CR | 46 |
| VGPR/PR | 54 |
| SD/PD | 12 |
| NA | 2 |
| Novel agents before HSCT | |
| Yes | 59 |
| No | 55 |

ISS indicates International Staging System; sCR, stringent complete remission; CR, complete remission; VGPR, very good partial response; PR, partial response; SD, stable disease; PD, progressive disease; NA, not available; HSCT, hematopoietic stem cell transplantation.

## RESULTS

Of the 114 patients, 85 were alive at last contact with a median follow-up of 38 months. Median OS, PFS, and time to next treatment were 81 months, 11 months, and 40 months, respectively. The majority of patients had stage II or higher disease and were in partial or better remission at the time of transplantation (Table 1).

Mean lymphocyte counts for $CD3^+/CD8^+$ T cells, $CD3^+/CD4^+$ T cells, $CD3^-/CD56^+$ NK cells, and $CD19^+$ B cells at the time points analyzed are shown in Figure 1. CD8 T cell and NK cells achieved healthy donor levels at 1 month, whereas $CD4^+$ T cells and B cells showed delayed recovery.

### *Lymphocyte Subset Reconstitution and Disease Relapse*

Correlation of lymphocyte subset recovery and transplantation outcome revealed a significant effect of NK cell recovery at 1 month on PFS. Median PFS was 2.2 months for patients with NK cell count below 100 cells/μL and 11.6 months both for patients with an NK cell count between 100 and 200 or above 200 cells/μL ($P = .001$) (Figure 2A). A similar difference in PFS was detected if the analysis was restricted to patients receiving their first transplantation (PFS for NK cells $<$ 100/μL, 2.7 months; for NK cells 100 to 200/μL, 15.8 months; and for NK cells $>$ 200/μL, 10.8 months; $P < .001$). Multivariable analysis confirmed the 1-month NK cell count as an independent predictor of PFS (Table 2), along with remission status at transplantation and cytogenetic profile. No similar association was detected at this time point for numbers of CD8 T cells (PFS at 6, 16, and 11 months for 1-month CD8 counts of $<$ 400, 400 to 800, and $>$ 800/μL), the number of CD4 T cells (PFS at 10, 12, and 11 months for CD4 counts of $<$200, 200 to 300, and $>$300/μL), or the number of B cells (PFS at 10, 9, and 12 months for B cell counts of $<$ 10, 10 to 50, and $>$ 50/μL).

No significant association with PFS was detected for any of the other time points analyzed. In contrast to PFS, 1-month NK cell count had no significant influence on either time to next treatment ($P = .93$) (Figure 2B) or OS ($P = .52$) (Figure 2C).

## DISCUSSION

The aim of our study was to analyze whether rapid lymphocyte subset recovery after autologous HSCT in plasma cell myeloma had an impact on outcome. The role of immune recovery subsequent to allogeneic HSCT has been widely analyzed. Several studies demonstrated that rapid immune reconstitution has a significant positive effect on outcome. It was shown that rapid recovery of lymphocytes, measured as absolute lymphocyte count (ALC) at different time points, was a strong and independent positive predictive factor on OS and PFS. Savani et al. [5] showed that high ALC on day 30 after T cell–depleted allogeneic HSCT was associated with increased survival, less relapse, lower nonrelapse mortality, and less acute GVHD. These results were confirmed in multiple studies [3,4,6,8,9,14-16]. Breaking down ALC into the various subsets, it was observed that NK cells were the first lymphocytes to recover after transplantation and correlated with ALC as a prognostic marker [17]. Having the ability to perform direct cytotoxicity without prior sensitization, NK cells play a significant role in the innate immunity and in antitumor response [18,19].

Immune reconstitution after autologous HSCT has also recently been studied and correlated with outcome. Several studies indicated a similar association between rapid immune recovery and favorable outcome as in allogeneic transplantation. Porrata et al. [15] reported that ALC at day 15 with a cut off of $>$ 500 cells/μL had significant association with prolonged survival in AML postautologous HSCT. Similar results were described in the setting of T cell non-Hodgkin's lymphoma using 1000 cells/μL as a discriminator [14] within 25 days postautologous HSCT. Results showed significant positive effects on OS and PFS.

In plasma cell myeloma, Porrata et al. [9] were the first to demonstrate a positive association between high ALC on day 15 (cut off of $>$ 500 cells/μL) and outcome. Kim et al. [8] confirmed these findings, observing ALC $\geq$ 1000/mm$^3$ at day 23 as positive prognostic factor for OS and PFS. Hiwase et al. [20] showed similar results, using ALC at day 30 as a prognostic cut off. Hiwase et al. analyzed additionally the kinetics of the lymphocyte recovery, measuring ALC at day 30, 60, 100, 180, and 365. The benefit on survival could only be confirmed for high ALC at day 30. This reinforces the hypothesis that a fast immune recovery is essential for a beneficial effect on outcome.

To investigate which lymphocytes were responsible for the beneficial impact, we conducted a retrospective analysis