

Age, malignancy, and cell dose had no impact on OI. The median values for major leukocytes were; WBC:  $0.7 \times 10^6/\mu\text{l}$ , T cells:  $63/\mu\text{l}$ , NK cells:  $47/\mu\text{l}$ , B cells:  $0/\mu\text{l}$ , CD4+T cells:  $42/\mu\text{l}$ , %CD4+ T cells: 66%. Both the OI+ and OI- group had comparable WBC, CD3+, CD4+ T cells, NK lymphocytes, or DC1, DC2 subsets. Strikingly, 44% of circulating T cells were in cell cycle (KI-67+) and ~10% were entering apoptosis (activated Caspase-3+), regardless of OI status. Only ~16% preserved the CD45RA+/CD62L+ phenotype of the infused graft. We conclude that in lymphopenic UCBT recipients even undetectable viral infections may induce T cell maturation towards effector CD8+ Tc1 cells as soon as 2-3 weeks after UCBT allowing early identification of those at risk for clinical OI (Table1).

*Differences in Lymphocyte Reconstitution Between Those Who Will Develop Opportunistic Infections (OI) or Not*

| Variable                      | OI+ Median Value | OI- Median Value | P-Value |
|-------------------------------|------------------|------------------|---------|
| % CD8+ T cells                | 39               | 28               | .04     |
| % CCR-5+ T cells              | 85               | 56               | .005    |
| % CD8+/CD57+/CD28-            | 6                | 2.8              | .027    |
| <b>Abs</b>                    |                  |                  |         |
| CD8+/CD57+/CD28-              | 1.3              | 0.4              | .017    |
| % IFN $\gamma$ + T cells      | 35.1             | 12.2             | .006    |
| % CD4+/IFN $\gamma$ + T cells | 14               | 10               | .017    |
| % CD8+/Perforin+ T cells      | 48               | 26               | .019    |
| MFI of BCL-2 in T cells       | 76               | 54               | .036    |

Absolute values in microliter.

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**FACTORS AFFECTING IMMUNOLOGIC RECOVERY AFTER NONMYELOABLATIVE CONDITIONING**

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**Background/Methods:** We investigated factors affecting immune recovery after nonmyeloablative (NM) conditioning in 94 pts given PBSC from HLA-matched related (MRD, n = 51) or unrelated (URD, n = 43) donors after 2 Gy TBI +/- fludarabine. Postgrafting immunosuppression (IS) consisted of mycophenolate mofetil (MMF, given TID for 40 days followed by a 56 day taper in URD recipients, and BID for 28 days in MRD recipients) and cyclosporin. Univariate and multivariate analyses were performed to determine factors affecting counts of CD4 T cells, naive CD4 T cells, CD8 T cells, B cells, and frequency of CMV-specific CD4 T-helper cells (among CMV seropositive pts or CMV-seronegative pts with CMV-seropositive donors; determined by lymphoproliferation (CMV- $\Delta$ CPM)) on days 30, 80, 180, and 365 after HCT. **Results:** In multivariate analyses, URD recipients had lower counts of CD4 T cells, naive CD4 T cells, CD8 T cells, and CMV- $\Delta$ CPM than MRD on day 30 after HCT. This delay in CMV-specific immune reconstitution was accompanied by increased frequency of CMV-reactivation (and increased use of pre-emptive antiviral therapy [PET]) among CMV-seropositive pts or CMV-seronegative pts with CMV sero-positive donors given URD grafts (cumulative incidence [CI] 61%) compared with MRD (33%) recipients the first 100 days after HCT. This did not lead to increased CMV disease among URD recipients (1 episode) compared with MRD recipients (1 episode), demonstrating that PET was similarly effective in preventing CMV diseases in both groups. Higher donor age was associated with lower counts of naive CD4 T cells, suggesting that most naive CD4 T cells derived from transplanted naive CD4 T cells rather than through neo-generation. As seen in pts given myeloablative conditioning, CMV-seropositive patients had higher levels of CD8 T cells after HCT. Further, lower levels of T cells and CD34+ cells in the grafts, as well as acute GVHD, impaired immune recovery of naive CD4 T cells and B-cells (Table 1). **Conclusions:** Despite similar NM

conditioning regimens, immunologic recovery was delayed among URD recipients in comparison to MRD recipients, either because of increased/extended postgrafting IS or the greater degrees of antigenic disparities between donors and recipients. This resulted in a higher incidence of CMV-infection and increased use of PET. Other factors associated with immune recovery were donor age, patient CMV-serostatus, number of CD34 and T cells in the graft, as well as acute GVHD (Table 1).

**Table 1. Multivariate Analyses of Factors Affecting Immune Recovery After NM Conditioning\***

| Cell Subset        | Day After HCT | Factor(s) Associated With Lower Cell Subset Counts  |
|--------------------|---------------|---|
| CD4 T-cell         | 30            | URD vs MRD (P = .06)  |
| CD4 T-cell         | 80            | URD vs MRD (P = .003); High donor age** (P = .006)  |
| CD4 T-cell         | 180 & 365     | MRD vs URD (P = .035)   |
| Naive CD4 T-cell   | 30            | URD vs MRD (P < .001); High donor age** (P = .001)  |
| Naive CD4 T-cell   | 80            | Low # of CD34 cells transplanted (P = .006); Grade II-IV acute GVHD (P = .007)              |
| Naive CD4 T-cell   | 180 & 365     | High donor age** (P = .003); Pt CMV seropositive (P = .03)                                  |
| CD8 T-cell         | 30            | URD vs MRD (P < .001)   |
| CD8 T-cell         | 80            | Pt CMV seronegative (P = .018)  |
| CD8 T-cell         | 180 & 365     | Pt CMV seronegative (P = .06)   |
| B-cell             | 30            | Low no. of CD34 cells transplanted** (P = .022); Low # of T-cells transplanted** (P = .039) |
| B-cell             | 80            | Low no. of T-cells transplanted** (P = .002); Grade II-IV acute GVHD (P = .08)              |
| B-cell             | 180 & 365     | Grade II-IV acute GVHD (P = .031)   |
| CMV- $\Delta$ CPM† | 30            | URD vs MRD (P = .007)   |
| CMV- $\Delta$ CPM† | 80            | URD vs MRD (P = .008); Low no. of T-cells transplanted** (P = .02)                          |
| CMV- $\Delta$ CPM† | 180 & 365     | Low no. of T-cells transplanted** (P = .01)   |

\*Other factors assessed were pt age, prior chemotherapy or not, day 28 T-cell chimerism, extensive chronic GVHD; \*\*continuous linear variable; †analyses restricted to CMV seropositive pt or donor.

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**SIGNIFICANCE OF LYMPHOCYTE CONTRIBUTION POST PROCESSING IN CORD BLOOD TRANSPLANTATION**

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In cord blood transplantation, engrafting cell populations include hematopoietic stem/progenitor cells. Naive and antigen-specific T and B cells mediate protective immune responses as well as graft-versus-host reactions. Total Nucleated Cell Dose (TNC) has consistently been shown to correlate with recipient outcome. A major complication, including death, in post transplant recovery is infection. In this preliminary analysis, we attempt to determine the significance of the lymphocyte contribution with regard to infection control post cord blood infusion. The outcomes of 318 single cord blood unit transplants have been evaluated. Recipients were assigned to groups based on the percentage of lymphocytes post processing. The overall mean was 30%. Group 1 consists of recipi-

ients with a lymphocyte percentage  $\leq 30\%$  ( $n = 153$ ), and group 2 consists of recipients with a lymphocyte percentage  $>30\%$  ( $n = 165$ ). As this is a preliminary analysis, the population has not been limited by disease, disease status, and other potential factors influencing outcome. Total Nucleated Cell Dose ( $P = .77$ ), HLA Matching ( $P = .14$ ), and Recipient Age ( $P = .03$ ), have been evaluated to eliminate bias between the two groups. The purpose of this analysis is to determine if a relationship exists between lymphocyte percentage and the incidence of infection and cause of death by infection. There was not a significant difference in the incidence of infection (bacterial, viral, fungal) post transplant between the two groups. Group 1 had an incidence of 131 (86%) patients with infection, and Group 2 had an incidence of 134 (81%) ( $P = .36$ ). There was however, an extremely significant difference in the number of patients with a primary cause of death by infection. Group 1 experienced 73 deaths with 31 (43%) by infection, while Group 2 experienced 75 deaths with 10 (13%) by infection ( $P \leq .0001$ ). With median TNC dose/kg being nearly equal between the two groups (4.0;4.5  $P = .77$ ), this preliminary analysis indicates that there may be characteristics within the nucleated cell populations that are critical to transplant outcome.

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### ASSESSING IMMUNE FUNCTION IN ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANT RECIPIENTS USING THE Cylex<sup>®</sup> ImmuKnow<sup>™</sup> ASSAY

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The Cylex Immune Cell Function Assay (ImmuKnow) is cleared by the FDA for the detection of cell-mediated immunity in an immunosuppressed population. The assay measures the immune function of CD4+ T-helper cells from a small sample of whole blood by stimulating the sample with phytohemagglutinin, selection of CD4+ cells with antibody coated magnetic particles, and detection of ATP by bioluminescence. CD4+ T-helper cells that respond to stimulating increase their production of ATP that provide an objective measure of immune responsiveness. This test has demonstrated clinical utility for managing immunosuppressive therapies in solid-organ transplantation (SOT). Outcome data from SOT recipients have identified risk zones for infection or allograft rejection based on immune function values determined by the ImmuKnow assay. Observational studies conducted at several cancer centers and Universities in the United States involving over 200 patients have shown that this test provides an independent measurement of engraftment, immune reconstitution, and immune function in allogeneic and autologous bone marrow transplant recipients. By assessing immune function in BMT recipients it is possible to avoid infectious complications, manage immunosuppressive therapy, and scheduling of chemotherapy.

## LEUKEMIA

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### BUSULFAN AND MELPHALAN AS PREPARATIVE THERAPY FOR STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA (AML) AND MYELODYSPLASIA (MDS)

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Fifty-two pediatric patients (ages 10 months to 23 years) with primary AML ( $n = 39$ ), secondary AML ( $n = 5$ ), and MDS ( $n = 8$ ) received busulfan (600 mg/m<sup>2</sup>) and melphalan (140–180 mg/m<sup>2</sup>) with equine antithymocyte globulin (60 mg/kg) for unrelated donor stem cell recipients as preparative therapy for stem cell transplant. Stem cell sources consisted of allogeneic marrow (sibling  $n = 16$ , parent  $n = 2$ ), unrelated marrow ( $n = 3$ ), unrelated cord blood ( $n = 30$ ), and syngeneic marrow ( $n = 1$ ). Regimen-related toxicities included grade 3 or 4 mucositis ( $n = 52$ ) and venoocclusive disease ( $n = 3$ ). All patients surviving 30 days demonstrated myeloid engraftment. One hundred day survival was 77% (40/52);

100% for matched family and syngeneic donor and 64% (21/33) for unrelated donor recipients. Causes of deaths within 100 days were: infection +/- GVHD ( $n = 6$ ), multiorgan failure ( $n = 2$ ), CNS hemorrhage ( $n = 2$ ), alveolar proteinosis ( $n = 1$ ), and transfusion reaction ( $n = 1$ ). Of 18 patients transplanted from allogeneic family donors, 15 survived event-free a median of 84+ months post transplant; 2 of the 3 who relapsed underwent second transplants and survived event-free 108+ and 13+ months. Of 33 patients who underwent unrelated donor transplants, 16 survived event-free a median of 46+ months post transplant. Four of the 33 patients relapsed and a total of 13 died of transplant-related complications. Of the 33 patients who received unrelated donor transplants, 9 of 15 with AML in CR1 or CR2 survived event-free a median of 60+ months, 3 of 10 in relapse survived event-free 114+, 107+, and 8+ months, and 4 of 8 with MDS survived event-free 35+, 35+, 23+, and 5+ months. The combination of busulfan and melphalan is an effective preparative regimen for pediatric patients with acute myelogenous leukemia and myelodysplasia and, in combination with equine antithymocyte globulin, provides adequate immunosuppression for unrelated donor transplants.

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### IMPACT OF TIMING OF ALLOGENEIC STEM CELL TRANSPLANTATION IN CML PATIENTS IN THE IMATINIB ERA

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Imatinib mesylate (IM), a small molecule targeting the BCR-ABL kinase, is now considered to be the standard first line treatment of chronic myeloid leukemia (CML). Although IM induces a high rate of cytogenetic remissions and may prolong progression-free survival in CML patients (pts), complete molecular remissions remain a rare event. Therefore, it remains questionable whether IM represents a curative treatment. By contrast, the curative potential of allogeneic stem cell transplantation (SCT) has been proven. However, because of its treatment-related mortality (TRM) nowadays SCT often is being offered to pts only after failure of IM and thereby also delayed beyond one year after diagnosis. The best timing for SCT for CML pts on IM remains to be defined. Therefore, we retrospectively analyzed the outcome of all pts transplanted at our center who had received IM prior to SCT. From the 27 consecutive CML pts included in this analysis, 11 had proven to be resistant to IM therapy. Resistance was defined by primary cytogenetic unresponsiveness or increasing percentage of Ph+ metaphases or hematological progression. Fifteen pts were transplanted in first chronic phase and 12 in advanced disease (CP2  $n = 10$ ; BC  $n = 2$ ). There were no significant differences with respect to age, donor (sibling vs unrelated), and stage of disease by means of  $\chi$ -square tests between the IM resistant and responding groups. However, significantly more pts were transplanted within the first year after diagnosis in the IM responding group. No differences could be observed concerning the incidences of acute GvHD grades II–IV, or extensive chronic GvHD between the two groups. IM resistance was associated with a higher TRM at 100 days (36 vs 0%,  $P = .008$ ) and an inferior projected overall survival at 2 years (39 vs 70%,  $P = .028$ ). The poorer outcome of the IM resistant group may be due to a longer time interval between diagnosis and transplantation or to a transformation of the CML clone into a more resistant leukemia requiring more aggressive chemotherapy prior to transplantation and thus increasing the risk of TRM. In summary, these data provide some evidence that either IM resistance may be an adverse prognostic factor for CML pts undergoing allogeneic SCT or that the better prognosis for pts transplanted early during the disease also holds true in the IM era. However, larger patient numbers and prospective trials are mandatory to define the best timing for SCT for CML pts on IM therapy.