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### Feasibility of Day 4 G-CSF Mobilized Peripheral Blood Stem Collection from HLA-Matched Sibling Donors

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**Background:** Guidelines from the NMDP and ASBMT recommend allogeneic donor mobilization with 4-5 days of G-CSF followed by peripheral blood stem cell (PBSC) collection beginning the 5$^{th}$ day. While our institutional standard has been 5-day mobilization and collection, given observations that some autologous transplant patients are adequately mobilized by day 4, and due to concern that a subset of allogeneic donors may be maximally mobilized earlier than day 5, we performed a feasibility study evaluating day 4 collection of allogeneic PBSC.

**Methods:** Beginning 7/1/2013, HLA-matched sibling donors were collected on day 4 after G-CSF if the peripheral blood (PB) CD34 count was $\geq$0.04x10$^6$/ml. Collected PBSC were held overnight at 4ºC until transplant. Donors with day 4 PB CD34 counts of $\leq$0.04x10$^6$/ml were collected on day 5, and those with inadequate CD34 cells/kg recipient weight in the PBSC product underwent repeat collection over two days.

**Results:** 38 patients with matched-sibling donors were eligible for inclusion in the study, with a median follow-up of 217 days (range 45-458). Of 38 donors evaluated, 22 (57.9%) had a PB CD34 count $\geq$0.04x10$^6$/ml on day 4. Eighteen of the 22 (81.8%) were adequately collected on day 4; 3 required 2-day PBSC collections on days 4 and 5. One donor with an adequate PB CD34 count was collected on day 5 due to line issues. Of the 16 donors with a PB CD34 count $<$0.04x10$^6$/ml, 7 underwent single day collection on day 5, and 9 required two-day collections. Of all donors eligible for inclusion, 18 (47.4%) were adequately collected on day 4, 8 (21.1%) were collected on day 5, and 31.6% required two-day collections. There was no significant difference in the median time to ANC and platelet engraftment based on day of PBSC collection.

**Conclusions:** In our pilot study of HLA-matched sibling donors undergoing G-CSF mobilized PBSC collection, we found that 47.4% of donors were adequately mobilized to allow for a single PBSC collection on day 4. Using a PB CD34 cell threshold of $\geq$0.04x10$^6$/ml on day 4 identified donors with high likelihood of adequate PBSC collection (81.8%). Our preliminary data suggest day 4 may be the optimal day of collection for a population of healthy donors, reducing donor G-CSF exposure leading to enhanced safety for donor and recipient, and expected cost savings. Ongoing analyses include financial and resource utilization review, detailed comparison of day 4 versus 5 PBSC product composition, and matched cohort analysis of day 4 versus 5 collection and correlation with transplant outcomes.

| Median | Day 4 n=18 | Day 5 n=8 | Two-Days n=12 |
|---|---|---|---|
| **Donor/Recipient age (years)** | 51/51 | 51.5/51 | 56/58 |
| **Donor/Recipient weight (kg)** | 87.1/86.5 | 75.5/79.0 | 83.5/94.5 |
| **Day 4 PB CD34×10$^6$/mL** | 0.07 | 0.028 | 0.024 |
| **PB CD34×l0$^6$/mL at collection** | n/a | 0.057 | 0.038 |
| **PBSC CD34×l0$^6$/kg** | 7.43 | 5.38 | 2.72 |
| **TNC×10$^8$/kg** | 6.99 | 8.24 | 12.0 |
| **MNC×10$^8$/kg** | 5.84 | 7.51 | 10.7 |
| **CD3×l0$^8$/kg** | 2.17 | 2.52 | 3.92 |
| **Day ANC >500/μl** | 13 | 14 | 15 |
| **Day platelets >20×l0$^9$/L** | 17.5 | 17.5 | 18 |

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### Placental Growth Factor (PlGF) in Allogeneic Hematopoietic Cell Transplantation (HCT): Clinical, Immunologic, and Pathologic Correlates

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**Background:** Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family, with a role in inflammation and monocyte chemotaxis. Pathologically high levels of PlGF have been described in the setting of inflammatory diseases, and upregulation of the PlGF receptor (VEGFR1) can be induced by vascular injury and inflammation. In a pilot study, we identified that circulating levels of PlGF are significantly elevated in recipients of allogeneic HCT. Thus, we hypothesized that PlGF may play an immunomodulatory role post-HCT, and sought to quantify differences in circulating PlGF levels between HCT donors and recipients, characterize peripheral blood mononuclear cell (PBMC) expression of VEGFR1, and determine GVHD target organ expression of PlGF.

**Patients and Methods:** Plasma and PBMCs from a cohort of 14 adult patients undergoing allogeneic HCT were collected at 3 months post-HCT and compared to samples obtained from their matched sibling donors (MSD). Plasma PlGF levels were quantified by ELISA; PBMC expression of VEGFR1 was determined by flow cytometry. Immunohistochemical (IHC) staining for PlGF was performed on biopsies of aGVHD target organs.

**Results:** PlGF levels and VEGFR1 expression: PlGF levels were 3.5-fold higher (25.1 versus 7.1 pg/mL, p=0.006) in recipients at 3 months post-HCT compared to their MSD and negatively associated with steroid dose (r=-0.6, p=0.02). Allogeneic HCT recipients had significantly higher levels of circulating monocytes (15.4 vs 8.1%, p=0.05) and approximately double the percentage of both CD8$^+$ T-cell (1.5 vs 0.6%, p=0.009) and CD3$^-$CD11b$^+$DR(lo) myeloid-derived suppressor cell (6.0 vs 3.3%, p=0.01) subsets expressing VEGFR1, compared to MSDs. PlGF tissue expression: Seven of 9 skin biopsies were consistent with aGVHD by pathology review. PlGF was present at 2-3+ staining intensity in all 7 cases of skin aGVHD, while the 2 cases without aGVHD, as well as normal skin, had little to no PlGF staining (0-1+). In contrast, control GI tissues showed higher PlGF staining (2-3+). Fourteen of 15 GI biopsies were consistent with aGVHD. PlGF expression in patients with GI aGVHD was less intense than control GI biopsies in 11/15 cases, with lowest scores observed in two patients who died of steroid refractory GI aGVHD.

**Conclusion:** Elevated PlGF in HCT recipients is associated with an increase in monocytes and VEGFR1-expressing

lymphoid and myeloid cells compared to their MSD. Additionally, increased tissue expression of PlGF by IHC was seen in skin biopsies of aGVHD, while GI aGVHD tissue demonstrated pathologically low PlGF expression. To our knowledge, these results provide the first evidence of altered circulating and tissue expression of PlGF occurring in the HCT setting. Studies are ongoing to determine the role of PlGF in neovascularization and tissue repair in aGVHD, and on the direct angiogenic and immune regulatory effect of PlGF in HCT.

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### Viral Infections after Umbilical Cord Blood Transplant: A Retrospective Analysis of 156 Children Transplanted at a Single Institution

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Umbilical cord blood (UCB) is an alternative option for hematopoietic stem cell transplantation and it has been successfully used to treat children with malignant and non malignant diseases. The slower rate of neutrophil engraftment and delayed immune reconstitution impose a substantial risk for infections and mortality. Furthermore, the use of anti-thymocyte globulin (ATG) has been linked to a higher risk of viral infections. In order to assess the occurrence of viral infections we retrospectively analyzed the outcomes of 156 children who underwent a UCB transplant (UCBT) between January 1996 and October 2012 in a single institution. Diagnostic methods varied over time: initially antigenemia, immunofluorescence and immunoenzymatic tests were used and since 2005 Polymerase Chain Reaction (PCR) was employed for vigilance and diagnosis of viral reactivation. Fifty-one girls and 105 boys with a median age of 60 months (range 2 – 168 months) were transplanted for malignant (48 pts) and non-malignant diseases: BM failure syndromes: 55 patients (pts), primary immunodeficiencies (PID): 41 pts and inborn errors of metabolism (IEM): 12 pts. HLA compatibility: 6/6 or 5/6: 98 pts and 4/6: 58 pts. Conditioning Regimen was based on chemotherapy in 119 pts and TBI based in 37 pts. The median number of Total Nucleated Cell was 5.2 x $10^7$/Kg (range: 1.4 - 36.4 x $10^7$/Kg). One hundred and forty-three pts were evaluable for engraftment with a median time for neutrophil recovery of 26 days and for platelet recovery of 42 days. Viral infection occurred in 99/156 pts. In the univariate analysis it was more frequent in pts who received ATG: 87/122 (p 0.0001), in those who developed acute GVHD: 65/80 (p 0.0001) or chronic GVHD: 49/59 (p 0.0001) and in 4/6 HLA mismatched UCBT (p 0.001). The most frequent virus detected in this cohort was Cytomegalovirus (CMV): 74 pts, followed by Epstein-Barr virus (EBV): 14 pts and adenovirus: 8pts. CMV status pre-transplant correlated with higher incidence of CMV infection post transplant. Moreover, viral infections were associated with bacterial infection in 79/127 pts (p 0.01). Death occurred in 81 pts and the main cause was bacterial infection. In this analysis the presence of viral infection per se was not associated with a high mortality.

**Conclusion:** We observed a high prevalence of viral infections among UCB recipients and a positive association of its occurrence with HLA-mismatch, development of acute or chronic GVHD and the use of ATG. Viral infections were usually associated with other infectious agents such bacteria or fungi and this could be an important factor for morbidity and mortality. Currently, close PCR surveillance allows early detection of viremia and aggressive treatment.

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### Paradoxical Effect of Donor Cytomegalovirus (CMV) Status on CMV Reactivation after T-Cell Depleted (TCD) Stem Cell Transplantation (SCT)

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**Background:** CMV recipient seropositivity (R+) is a predictor for CMV viremia after SCT. Cytotoxic T-lymphocytes (CTL) are critical for CMV immunity. Conventional allografts from CMV seropositive donors (D+) confer some CMV immunity though transfer of donor CTLs to the recipient. Thus for CMV R+, CMV D+ are preferentially chosen over CMV D-. In contrast, ex-vivo T-cell depleted (TCD) allografts are deficient in CMV CTLs. The effect of donor serostatus on CMV reactivation has not been examined after TCD SCT. We studied the effect of CMV donor serostatus on the incidence of CMV viremia in a cohort of adult CMV R+, TCD SCT monitored by CMV PCR and treated preemptively.

**Methods:** CMV R+ adults with hematologic malignancies who received peripheral blood CD34+ selected allografts after myeloablative conditioning regimens from March 1, 2010 through May 2013 were monitored prospectively by CMV PCR at least weekly from day (d) +14 through +100 and at least once every 2 weeks through d +180. Preemptive therapy was initiated for CMV viremia, defined as $\geq$ 1 positive PCR value. There was no CMV prophylaxis during the study period. Follow up was through May 31, 2014. Time-dependent Cox-proportional hazard model of univariate and multivariate stepwise selection analyses were performed to identify predictors of CMV viremia.

**Results:** Of 113 CMV R+ (median age 57.6 years, 22.5-73.0), 60 (53%) had acute leukemia, 23 (20%) myelodysplastic syndrome, 18 (16%) multiple myeloma and 12 (11%) other. Donors were 48 (42%) matched-related (MRD), 45 (40%) matched unrelated (MUD), or 20 (18%) mismatched; 68 (60%) CMV D+ or 45 (40%) D-. The cumulative incidence of CMV viremia at 6 months was 79%. Time to onset of viremia was median 26 days (13-83) after SCT, and time to resolution of viremia a median 30.5 days from onset of viremia. The incidence of CMV viremia was higher in R+/D+ compared to R+/D- (90.5% versus 62.2%; $P<0.0001$). Median time to onset and to resolution for R+/D+ vs R+/D- were not significantly different. In multivariable analysis, R+/D- pts were at lower risk for CMV viremia compared to R+/D+ (HR: 0.57, 95% CI: 0.36-0.90; $P=0.017$). In contrast, multiple myeloma (HR: 2.22, 95% CI: 1.24-3.98; $P=0.008$) was associated with increased risk compared with acute leukemia. Having a mismatched donor was also associated with increased risk