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THE ROLE OF CYTOTOXIC T CELL ANTIGEN-2 (CTLA2) IN MOUSE HEMATOPOIETIC STEM CELL (HSC) TRANSPLANT ENGRAFTMENT AND RECONSTITUTION EXAMINED BY LENTIVIRAL VECTOR TRANSDUCTION

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HSCs are rare cells resident in the bone marrow. HSCs must be capable of robust proliferation and self-renewal. The regulation of HSC proliferation occurs at least partly through interactions with the microenvironment, or niche. We found that in contrast to other bone marrow cells, HSCs constitutively express high levels of CTLA2, and that after treatment with 5-FU, CTLA2 is down-regulated in HSCs. We hypothesize that interaction between cathepsin L and the endogenous cathepsin L inhibitor CTLA2 regulates interaction of HSCs with the quiescent niche. In this study, bone marrow cells from 5-FU treated mice were transduced with lentiviral vectors encoding five different transgenes (CTLA2α and β expression constructs, siRNA CTLA2α and α/β knockdown constructs, and empty construct) and were transplanted into lethally irradiated mice with unmanipulated competitors. The short-term, long-term, and lineage engraftment of transduced HSCs was studied. Peripheral blood analysis at 4 weeks after transplant demonstrated that 90 to 100% of the animals transplanted with empty and CTLA2 knockdown constructs engrafted as oppose to 0 to 25% of the mice transplanted with CTLA2 expression transgenes. The mean percentage of peripheral blood leukocytes derived from the lentivirally transduced cells at 4 weeks post-transplant was 17% for empty construct, 19% for CTLA2α/β knokdown construct, 6% for CTLA2α knockdown construct, 1.5% for CTLA2α expression construct, and 0% for CTLA2β expression construct. The differences between empty and knockdown versus expression constructs were statistically significant. When the analysis was repeated at 8 weeks post-transplant, the same result was observed. Next, we did lineage distribution analysis of engrafted populations at >12 weeks post-transplant. We did not find significant difference between the distribution of granulocytes, T cells, and B cells within peripheral blood leukocytes derived from engrafted populations when compared to untransduced cells. Thus, our preliminary findings demonstrated that HSCs transduced with CTLA2 over expression lentiviral constructs have inferior ability to engraft (or may be impaired multilineage reconstitution?) compared to empty and CTLA2 knockdown constructs. In the future, we plan to study biological responses of modified HSCs to cytotoxic and mobilization stimuli in vivo.

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EXPANSION OF UMBILICAL CORD BLOOD DERIVED OLIGODENDROCYTES

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Transplantation of children with lysosomal storage diseases (LSD) with unrelated donor-umbilical cord blood (UCB) is effective in preventing onset and progression of severe neurologic symptoms if performed early in the course of the disease. Unfortunately, many children are not diagnosed before moderate to severe neurologic damage has occurred. These symptomatic children experience disease stabilization after transplant but do not regain lost function. This may be due to the fact that irreversible damage has occurred or, conversely, that stem cells transit to the brain too slowly to effect neural cell repair. To address this problem, we have developed in vitro methods to isolate and characterize oligodendrocytes derived from human UCB. We previously described these methods and the initial characterization of these cells (Hall et al). Continuing to advance this work, we now are focusing on further characterization and expansion of this population of cells in preparation for a phase I human clinical trial in patients with symptomatic LSD. Human UCB is red cell depleted with hetastarch, mononuclear cells are isolated with ficol density separation, and plated at a density of 5×10^5 cells/ml in media containing neurotropin 3, vascular endothelial growth factor, and platelet derived growth factor. The adherent cells are washed 2× per week for 2 weeks, then passaged and replated at a density of 5×10^3 cells/ml with media changes twice weekly, once with the original oligodendrocyte media and the second with NeuroCult media (StemCell Technologies, Vancouver). Cells expand with an approximate doubling time of 6.5 days. After 4 and 6 week periods, a 16- and 64-fold expansion can be seen, respectively. We anticipate that using a 20% fraction (200×10^6 cells) of a cord blood unit we can obtain 33×10^8 cells in 6 weeks. Oligodendrocyte lineage identity of these cells, post expansion, was confirmed by PCR based methods with expression of myelin basic protein, nestin, PLP, and Neurogenin 3. We conclude that oligodendrocytes can be isolated and expanded from human UCB yielding sufficient numbers of cells for testing in phase I human trials to facilitate neural cell repair in patients with advanced LSD.

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OPTIMUM TEMPERATURE FOR MAINTAINING THE VIABILITY OF CD34+ CELLS DURING STORAGE AND TRANSPORT OF FRESH HAEMATOPOIETIC PROGENITOR CELLS

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The optimum conditions for storage and transport of freshly harvested hemopoietic progenitor cells (HPC) in the liquid state is uncertain. It is not specified in commonly applied standards for stem cell transplantation. We used a viable CD34 assay to determine the optimum temperature for maintaining progenitor cell viability in freshly harvested bone marrow and peripheral blood stem cells. Our aim was to identify standardized conditions for storage and transport of marrow or peripheral blood products that would optimize CD34 recovery leading to better transplant outcomes. Samples were aseptically removed from 46 fresh HPC harvests (34 PBSC and 12 BM) and stored at refrigerated temperature (2°–8° C), room temperature (18°–24° C) and 37° C, for up to 72 hours. Samples were analysed for viable CD34+ cells/ml at 0, 24, 48, and 72 hours.

The mean viable CD34+ yield prior to storage was 7.7×10^6/Kg (range 0.7–30.3). The mean loss of viable CD34+ cells in HPC products at refrigerated temperature was 9.4%, 19.4%, and 28% at 24, 48, and 72 hours, respectively. In contrast, the mean loss of viable CD34+ cells at room temperature was 21.9%, 30.7%, and 43.3% at 24, 48, and 72 hours, respectively. No viable CD34+ cells remained after storage at 37° C for 24 hours. Only PBSC products and not BM showed temperature related loss of CD34 viability. Greater loss of viable CD34+ cells was observed for allogeneic PBSC compared to autologous PBSC. These results demonstrate that the optimum temperature to maintain the viability of CD34+ cells during overnight storage and transport of freshly harvested HPC is 2°–8° C. These findings allow for the development of standard guidelines for HPC storage and transport.

SUPPORTIVE CARE

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DIFFUSE ALVEOLAR HEMORRHAGE (DAH) AND INFECTION ASSOCIATED ALVEOLAR HEMORRHAGE (IAH) FOLLOWING HEMATOPOIETIC STEM-CELL TRANSPLANTATION: RELATED AND HIGH RISK CLINICAL SYNDROMES WITH POOR RESPONSE TO HIGH-DOSE CORTICOSTEROIDS

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Diffuse alveolar hemorrhage (DAH) is a non-infectious pulmonary complication of hematopoietic stem-cell transplantation (HSCT) and is associated with significant mortality. The pathogenesis and treatment of DAH is unclear. We reviewed

prospectively collected data on 1958 consecutive HSCTs performed between 1995 and 2004 and compared patients with DAH and infection associated alveolar hemorrhage (IAH) who presented with similar hypoxemia, pulmonary infiltrates, and progressively bloody alveolar lavage but also had microorganisms (excluding *Aspergillus* spp) isolated from blood, bronchoalveolar lavage (BAL), or tracheal aspirate within 1 week of alveolar hemorrhage. Overall, 116 (5.9%) patients had alveolar hemorrhage diagnosed by BAL (DAH = 45, IAH = 71). Sixty-day mortality from the onset of alveolar hemorrhage was 74% (95% confidence-interval (CI), 66–82%). For the whole cohort, presence of infection ($P = .04$) and hemorrhage within 5 days of engraftment ($P < .01$) were predictive of survival beyond 60 days from onset of alveolar hemorrhage. The DAH and IAH groups were comparable except for greater use of total body irradiation containing conditioning regimen in the DAH group ($P = .04$) and umbilical cord blood donor source in the IAH group ($P = .04$). Survival at 60 days from the onset of alveolar hemorrhage was 16% (95% CI, 5–27%) for the DAH group and 32% (95% CI, 21%–43%) for the IAH group ($P = .18$). All except 20 patients (DAH = 4, IAH = 16) were treated with a standard regimen of high-dose corticosteroids. The steroid and no-steroid groups were comparable except for a trend towards greater use of corticosteroids in the DAH group ($P = .06$). Sixty day survival from the onset of alveolar hemorrhage was 26% (95% CI, 17–35%) in patients receiving corticosteroids compared to 25% (95% CI, 6–44%) in those not receiving corticosteroids ($P = .17$). The pathogenesis of alveolar hemorrhage following HSCT is multifactorial and we propose that IAH and DAH are related clinical syndromes with similar clinical presentation, risks, and associated high mortality. Furthermore, our results show no benefit of high-dose corticosteroids in the management of this disorder. Ongoing study to identify the pathophysiologic mechanisms of alveolar injury and to define new therapy is still needed.

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POSACONAZOLE (POS) VS FLUCONAZOLE (FLU) FOR PROPHYLAXIS OF INVASIVE FUNGAL INFECTIONS (IFIs) IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) RECIPIENTS WITH GRAFT-VERSUS-HOST DISEASE (GVHD): A MULTICENTER TRIAL

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Introduction: Allogeneic HSCT recipients are at risk for life-threatening IFI. In patients with GVHD, IFIs are mainly due to moulds, limiting the utility of prophylactic FLU. We compared POS with FLU in preventing IFI in HSCT recipients with GVHD on intensive immunosuppressives. **Methods:** Patients in this double-blind, double-dummy study received oral POS (200 mg tid) or FLU (400 mg qd) for up to 16 weeks (112 days). Incidence of IFI was determined at 16 weeks and up to 7 days after last dose by EORTC/MSG criteria adjudicated by a blinded expert panel. **Results:** 600 patients were enrolled (301 POS; 299 FLU). Mean duration of therapy was 80 days in the POS arm and 77 days in the FLU arm. Incidence of proven/probable IFIs is shown below. Mortality rate due to IFI was POS (1%) versus FLU (4%); overall rate was 25% versus 28%. Safety and tolerability were comparable. Discontinuations due to treatment failure were lower for POS versus FLU (3% vs 8%); those due to adverse events were similar (33% each). **Conclusions:** POS was superior to FLU in preventing aspergillosis and other breakthrough IFIs in HSCT recipients with GVHD. Although POS was noninferior to FLU in preventing total IFIs during the study period, the criteria for superiority were not met. This may be because of the high rate of discontinuation before day 112 (45% of POS patients and 52% of FLU patients), contributing to the small number of IFI events. Both agents were well tolerated (Table1).

Table 1.

Proven/Probable IFIs	POS, n (%)	FLU, n (%)	Odds Ratio (95% CI)	*P* Value
IFIs during study period (day 112)				
Total	16 (5)	27 (9)	0.56 (0.30–1.1)	.07
Aspergillus	7 (2)	21 (7)	0.31 (0.13–0.75)	.006
Breakthrough infections (while on treatment)				
Total	7 (2)	22 (8)	0.30 (0.12–0.71)	.004
Aspergillus	3 (1)	17 (6)	0.17 (0.05–0.57)	.001

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ACTIVE TREATMENT OF ASYMPTOMATIC RADIOLOGICALLY DOCUMENTED SINUSITIS MAY BE NECESSARY FOR PATIENTS RECEIVING TOTAL BODY IRRADIATION CONDITIONING

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Background: In hematopoietic stem cell transplantation (HSCT), careful evaluation of paranasal sinus is necessary because increased risk of sinusitis may be problematic during post-transplant neutropenic period. However, there are no criteria for diagnosis and optimal treatment of asymptomatic radiologically documented or symptomatic sinusitis. **Methods:** A retrospective review of the medical records of 256 patients who received autologous (n = 124) or allogeneic (n = 128) HSCT for hematologic malignancies at Samsung Medical Center from April 1996 to December 2003 was performed. Four patients were excluded because of tumor originated in nasal cavity. Radiologically documented sinusitis was defined as mucosal thickening, opacification and air-fluid level of sinus without symptom. Symptomatic sinusitis was defined as symptoms such as postnasal drip, rhinorrhea, nasal obstruction, cough, fever, headache with radiologic abnormalities.

Results: The median age was 38 years (range 15–68 years). One hundred thirty-three (52.8%) of 252 patients had no evidence of sinusitis and 23 (9.1%) patients had symptomatic sinusitis before transplantation. The remaining 96 patients (38.1%) had asymptomatic radiologically documented sinusitis. All symptomatic sinusitis were treated sufficiently before proceeding to HSCT. Of 96 patients with radiologically documented sinusitis, but without symptom, 54 were treated with antibiotics, 6 were operated endoscopically, and 36 were not treated.

After transplantation, sinusitis developed in 76 patients (30.2%), 40 (15.9%) were documented clinically and 36 (14.3%) were radiologically. Median day of onset of sinusitis was 52.5 days (range 1–420 days) after transplantation. There were no deaths or graft failure related to sinus complications. In patients with asymptomatic radiologically documented sinusitis, there was no difference in the incidence of post-transplant sinusitis among surgery and medical therapy and observation ($P = .479$). In patients who received total body irradiation (TBI) as conditioning regimen, radiologically documented sinusitis was related to higher risk of developing post-transplantation sinusitis ($P = .033$). **Conclusions:** Our data suggest that aggressive treatment of asymptomatic radiologically documented sinusitis at pre-transplantation may not be necessary. However, if recipient is scheduled to receive of TBI as conditioning regimen, active treatment of asymptomatic radiologically documented sinusitis is to be considered.

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ACUTE GRAFT-VERSUS-HOST DISEASE AND IMMUNOSUPPRESSIVE THERAPY IDENTIFY PATIENTS LESS LIKELY TO HAVE A DIAGNOSTIC YIELD WITH A BRONCHOALVEOLAR LAVAGE: THE ST. JUDE EXPERIENCE 1990–2002

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