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FIRST ANALYSIS OF HUMAN HERPESVIRUS 6 T-CELL RESPONSES: SPECIFIC BOOSTING AFTER HHV6 REACTIVATION IN STEM CELL TRANSPLANTATION RECIPIENTS

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Background: Human herpes virus 6 (HHV6) reactivation after hematopoietic stem cell transplantation (HSCT) is associated with acute-Graft-versus-host-disease (aGvHD) and poor survival. Characterization of HHV6 immune responses is essential to develop HHV6-specific (immuno) therapy and to elucidate the association with aGvHD.

Methods: Prospectively, HHV6 DNA-load was weekly measured by realtime-PCR. HHV6-reactivation was defined as DNA-load > 1000cp/mL. Alloreactive disease was defined as aGvHD and/or idiopathic pulmonary syndrome. T-cell reconstitution was measured by immunophenotyping. Numbers of IFNγ-producing HHV6-T-cells in peripheral blood mononuclear cells (PBMC) 2 months after HSCT were retrospectively determined by enzyme-linked immunospot (ELIspot) assay. HHV6-specific T-cell proliferative capacity after HSCT was analyzed using a newly developed assay using autologous HHV6-infected PBMC.

Results: Fifty-six patients were included (median age 4.6 years; range 0.2-21.2 yrs) with a median followup of 30 months (range 0-30). All patients received myeloablative conditioning and standardized graft-versus-host disease prophylaxis. During follow-up, 29/56 (52%) patients developed HHV6-reactivation with a median time of reactivation of 14 (range 1-41) days. In total, 13/56 (23%) of patients developed alloreactive disease during followup. In all cases, HHV6 reactivation preceded the alloreactive disease. No difference in overall T-cell numbers was observed between patients with or without HHV6-reactivation. The median number of Interferon-γ producing HHV6-specific T-cells at 2 months and the HHV6-specific CD8+T-cell proliferative capacity at 6 months after HSCT were increased after HHV6-reactivation compared to non-reactivating patients ($P = 0.006$ and $p = 0.036$, respectively).

Conclusion: HHV6-specific T-cell responses were specifically increased after HHV6-reactivation, despite comparable numbers of total T-cells. Although overall T-cell numbers are extremely low early after HSCT, these analyses show a clear anti-viral response. This identification of HHV6-specific T-cell responses early after HSCT is essential to develop antiviral immunotherapy, to booster these responses to prevent reactivation after HSCT. This first analysis of HHV6-specific T-cells may contribute to unravel the role of HHV6-reactivation in the development of aGvHD.

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EARLY LYMPHOCYTE RECOVERY POST UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANT (UHSCT) IS ASSOCIATED WITH BETTER SURVIVAL AND LESS ACUTE GRAFT VERSUS HOST DISEASE (aGVHD)

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Background: UHSCT is associated with significant morbidity and mortality. Graft versus host disease and infections are significant contributing factors. Early lymphocyte recovery has been associated with improved outcomes in matched sibling transplants; however the same association is unclear in UHSCT.

Methods: We retrospectively evaluated a cohort of consecutive UHSCT patients for transplant outcomes as well as absolute lymphocyte recovery at day 30 post transplant (ALC 30). Patients were transplanted between January 2006 and June 2010 at Karmanos Cancer Center, and received Mycophenolate Mofetil and Tacrolimus for GVHD prophylaxis (N = 154). We performed a multivariate analysis to determine predictors of ALC 30 as well as impact of ALC 30 (≥ or < than 400×10^6/L) on the incidence of aGVHD, cGVHD, CMV reactivation, relapse and overall survival.

Results: The median age was 52 years, (Interquartile range IQR 21-64). Disease diagnoses were: AML41%, MDS 12%, ALL 13%, CML 4.5%, DLBCL 4.5%, CLL 5.2%, MCL 5.2%. Disease status at transplant was: First complete remission (CR1) 23.7%, CR2 11.8%, relapsed or progressive disease 47%, and untreated disease 12.5%. Conditioning regimens included Bu/Flu 43%, Bu/Flu/TBI 17%, R-BEAM 11%, Flu/Mel/TBI 8%, VP16/TBI 7%, and Cy/TBI 6.5%. HLA Match status was: 10/10 58%, 9/10 32%, 8/10 9%, and 7/10 1%. The median CD 34+ count was 7.4 million/Kg (IQR 5.34-9.69). Donors were 65% males with a median age of 31 yrs (IQR 19-53). CMV status of donors was positive in 43%, compared to 53% of recipients. The incidence of ALC 30 ≥ 400×10^6/L was 68% (N = 97), aGVHD grade II-IV 53% (N = 82), cGVHD 53.3% (N = 81), CMV reactivation 32% (N = 49), and relapse 13.25% (N = 20). At a median follow up of 339 (109-891) days, 48% of patients were alive. In multivariate analysis, CMV status, age, gender of donor and recipient, CD34+ cell count, degree of mismatch or disease status were not significant predictors of ALC 30. ALC 30 ≥ 400×10^6/L was significantly associated with decreased risk of mortality (HR 0.22, CI 0.11-0.44; $P = 0.0001$) and reduction in the risk of subsequent aGVHD ($P = 0.01$). ALC 30 ≥ 400×10^6/L was not found to predict less incidence of cGVHD ($P = 0.59$), CMV reactivation ($P = 0.123$), or relapse ($P = 0.93$).

Conclusion: The above results demonstrate that higher day 30 absolute lymphocyte recovery can predict better overall survival and less aGVHD in UHSCT.

Table 1. Absolut Lymphocyte Count Day 30 (ALC 30) Predicts Clinical Outcomes in UHSCT

Clinical Outcome	Hazard Ratio HR	P value	Confidence interval CI
Mortality	0.22	0.001	0.11-0.44
Acute GVHD	0.09	0.018	0.01-0.66
Chronic GVHD	1.26	0.593	0.53-3.03
CMV reactivation	0.48	0.123	0.19-1.21
Relapse	1.09	0.933	0.14-8.16

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IMMUNE RECONSTITUTION AFTER NONMYELOABLATIVE, T-CELL REPLETE, HLA-HAPLOIDENTICAL BMT WITH POST-TRANSPLANTATION CYCLOPHOSPHAMIDE

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Delayed immune reconstitution with increased risk of opportunistic infection is a major complication of HLA-haploidentical stem cell transplantation, especially in protocols employing extensive T cell depletion of the graft. Here we characterize phenotypic immune reconstitution in hematologic malignancies patients receiving non-myeloablative conditioning, T cell-replete, HLA-haploidentical bone marrow transplantation (BMT), and graft versus host disease (GVHD) prophylaxis including high-dose, post-transplantation cyclophosphamide (Cy). Patients with advanced hematologic malignancies (median age 45, range 29-69; 3 AML, 1 bilineage leukemia, 1 ALL, 1 MDS, 1 CML, 1 CLL, 1 CMML, 7 NHL, 4 Hodgkins, 1 mantle cell) received Cy 14.5 mg/kg/day IV on days -6 and -5, fludarabine 30 mg/m2/day IV on days -6 to -2, 200 cGy of TBI on day -1 and T cell replete bone marrow from donors with a median age of 44 (range 14-68). GVHD prophylaxis consisted of Cy (50 mg/kg/day) on days 3 and 4, mycophenolate mofetil for 30 days, and tacrolimus for 6 months. Grafts contained an infused median TNC/kg of $4.1e^8$ (range $3.1\text{-}5.4\ e^8$), $CD3^+$/kg $3.8e^6$ (range $1.7\text{-}8.8e^6$) and a $CD34^+$/kg of $3.8e^7$ (range $1.54\text{-}6.2e^7$). Donor engraftment occurred in 81% of patients (17/21). The median times to neutrophil (> 500/μL) and platelet recovery (> 20,000/μL) are 17 days (range, 13-92 days) and 30 days (range, 14-580 days), respectively. Post-transplantation recovery of lymphocyte subsets is shown in Table 1 and is notable for the following: 1) The median lymphocyte count at day 30 after