(43.2 ± 13 nmol/mg proteins) declined by ~33% (p = 0.05) relative to Syn group (68 ± 16 nmol/mg proteins), indicating impaired GSH synthesis. Additionally, a significant twofold (p = 0.0007) rise in hepatic homocysteine and ophthalmic acid levels were detected in Allo mice, linking both SAA and redox metabolic imbalance to GVHD.

To investigate mechanism(s) of GSH redox loss in GVHD, hepatic mRNA and protein levels of gamma-glutamylcysteine ligase [GCLC (rate-limiting enzyme)], were quantified. The hepatic GCLC transcript and protein declined in Allo compared to Syn mice [GCLC fold change: day +4 = 0.4 vs 1.03±0.15; day +10 = 0.3± 0.05 vs 1.0± 0.05 (p = 0.0004), respectively]. Target organs like lungs and gut also revealed depletion of intracellular thiol in Allo mice that directly correlated with the degree of T cell infiltration. Together, these data show that the GSH synthetic pathway is specifically affected in GVHD. The tight transcriptional deregulation of GCLC with depletion of GSH reserve is an early event and precedes histopathological changes of GVHD. Identification of modifiers of GCLC/GSH/GSSG system may provide a novel target for treatment of GVHD.

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RETROSPECTIVE ANALYSIS OF STANDARD VERSUS MINI-DOSE METHOTREXATE IN THE PREVENTION OF ACUTE GRAFT-VERSUS-HOST DISEASE IN ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

Michael, A.G.[1], Adel, N.[1], Chou, J.F.[2], Heller, G.[2], Boulad, F.[3], Castro-Malaspina, H.R.[3], Jakubowski, A.A.[3], O'Reilly, R.J.[3], Papadopoulos, E.B.[3], Perales, M.[3], Giralt, S.A.[3] [1]Memorial Sloan-Kettering Cancer Center, New York, NY; [2]Memorial Sloan-Kettering Cancer Center, New York, NY; [3]Memorial Sloan-Kettering Cancer Center, New York, NY

Background: Graft-versus-host disease (GVHD) is one of the primary contributing factors to morbidity and mortality following allogeneic stem cell transplantation. Methotrexate (MTX) has been an integral component of prophylaxis regimens against acute GVHD when combined with calcineurin inhibitors. Recent data suggests that lower doses of MTX are effective in preventing aGVHD and may be associated with less toxicity. However, few comparative studies have been performed between so called mini-MTX and standard MTX dosing schemas. The primary objective of this analysis was to compare the incidence of acute GVHD with standard versus mini-dose MTX prophylaxis. Secondary objectives evaluated the safety and the toxicity associated with MTX administration.

Methods: This retrospective review included allogeneic stem cell transplant recipients at Memorial Sloan-Kettering Cancer Center (MSKCC) between 8/1/2003-8/16/2010 that received standard or mini-MTX as part of post-transplant GVHD prophylaxis. Standard MTX dosing was defined as 15 mg/m^2 on day 1 with subsequent doses of 10 mg/m^2 administered on days 3, 6 and 11. The mini-MTX group included patients who never received a dose of 15 mg/m^2.

Results: 226 patients (138 standard and 88 mini-MTX) were included in the analysis. Based on clinical practice at MSKCC, the standard dose arm had a higher frequency of leukemia patients while the mini-dose group had a higher frequency of lymphoma patients. Median age at transplant was 27 years and 50 years for standard and mini-dose groups, respectively (p<0.001). The median follow up for patients alive without acute GVHD was 33 months (range 10-90 months). Cumulative incidence of GVHD at 3 months was 28.9% [95% CI: (21.4-36.6%)] and 28.4% [95% CI (19.0-37.9%)] for the standard and mini-dose treatment arms (p = 0.91). The adult subgroup also revealed no difference in aGVHD at 3 months (p = 0.56). A univariate analysis showed that CMV seropositivity was positively associated with higher rates of GVHD (HR: 1.81 [95% CI: 1.14-2.85, p = 0.01). Dose adjustments or delay of MTX were significantly different between groups (43.5% in standard arm vs. 15.9% mini-dose arm, p<0.0001) suggesting an improved toxicity profile, however this may have been impacted by the primary conditioning regimen.

Conclusion: Prophylaxis with mini-MTX may be associated with a similar incidence of aGVHD and an improved toxicity profile when compared to a standard dose schema.

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TRAIL OVER-EXPRESSION ON DONOR T CELLS ENHANCES GVT AND SUPPRESSES GVHD BY INHIBITING ALLOREACTIVE T CELLS AND IMPAIRING HOST APC FUNCTIONS

Ghosh, A.[1], Dogan, Y.[1], Holland, A.M.[1], Young, L.F.[1], West, M.L.[1], Singer, N.V.[1], Smith, O.M.[1], Jenq, R.R.[1], Na, I.-K.[2], Penack, O.[2], Hanash, A.M.[1], Sauer, M.G.[3], Sadelain, M.[1], van den Brink, M.R.M.[1] [1]Memorial Sloan-Kettering Cancer Center, New York, NY; [2]Charité CBF, Berlin, Germany; [3]Medizinische Hochschule Hannover, Hannover, Germany

Strategies to suppress GVHD are often associated with broader suppression of the immune system leading to a compromised GVT effect. We have demonstrated that genetically engineered T cells over-expressing TNF-Related Apoptosis Inducing Ligand (TRAIL) have enhanced GVT effects and explicitly suppress GVHD. TRAIL induces apoptotic signals through death receptor (DR) 4 and 5 molecules (only DR5 in mice) expressed on certain tumors and inducible on others. TRAIL is therefore an attractive candidate for genetic engineering of donor T cells to enhance their GVT potential.

Mature T cells derived from donor B6 splenocytes were transduced with a lentiviral TRAIL expression vector. The transduced TRAIL+ T cells were adoptively transferred into lethally irradiated CBF1 recipients of T cell depleted allografts and LB27.4 tumor to assess their GVHD and GVT activity. TRAIL+ T cells displayed significantly enhanced antitumor immunity compared to mock (GFP)-transduced controls in vitro and upon transfer into tumor bearing allo-BMT recipients (p<0.01, 100% survival in TRAIL+ T cell group). Additionally, the recipients treated with TRAIL+ T cells had significantly less GVHD lethality and morbidity. This was observed across multiple GVHD models (B6 → CBF1 and B10.BR → B6). Using in vitro proliferation assays and in vivo bioluminescent imaging, we found that TRAIL+ T cells suppressed proliferation of non-transduced alloreactive T cells. To explore the factors contributing to TRAIL-mediated suppression of GVHD, we used recipients deficient in DR5 (DR5ko) and found that TRAIL+ T cells suppress GVHD by impairing host APC functions. Precursor (pre)T cells can regenerate the T cell compartment without GVHD. We generated and expanded TRAIL+ preT cells using the OP9-DL1 co-culture system. Adoptive transfer of B6 TRAIL+ preT cells into syngeneic-transplanted BALB/c mice reconstituted the T cell compartment with TRAIL+ T cells and caused enhanced antitumor activity (p<0.05) compared to controls.

Collectively, our data demonstrate that donor T cells genetically engineered to express TRAIL can enhance GVT effects and suppress lethal GVHD through impairment of host APCs in recipients of allo-HSCT. Furthermore, we demonstrated that allogeneic ex vivo generated preT cells expressing TRAIL could mediate a strong protection against tumor challenge in syngeneic HSCT recipients, representing an "off the shelf" strategy in both allogeneic and autologous recipients.

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ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION SIGNIFICANTLY INCREASES THE RISK OF CHRONIC GRAFT-VERSUS-HOST DISEASE OF LUNG COMPARED TO BONE MARROW TRANSPLANTATION

Alam, N., How, J., Gupta, V., Kuruvilla, J., Lipton, J., Messner, H., Kim, D. Princess Margaret Hospital, University of Toronto, Toronto, ON, Canada

Introduction: Chronic graft versus host disease (GVHD) of lung is associated with higher transplant related morbidity and mortality after allogeneic hematopoeitic cell transplant (HCT). Risk factors for lung GVHD are not yet fully elucidated in detail. We attempted to identify clinical risk factors for lung GVHD after allogeneic HCT based on single center experience.

Methods: 401 patients were transplanted between year 2000 and 2007 at the Princess Margaret Hospital, Toronto, Canada. 280 (70%) and 121 (30%) patients received peripheral blood stem cell (PBSC) and bone marrow (BM) as a source of stem cells, respectively. Monitoring with pulmonary function tests (PFT) were performed prior to transplant, at day 180, then annually. PFT was also