414 EFFECTIVE GRAFT-VERSUS-LEUKEMIA RESPONSES TO DONOR LYMPHOCYTE INFUSION ARE ASSOCIATED WITH PREEXISTING CD8+ T CELL MARROW INFILTRATES

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Donor lymphocyte infusion is a highly effective treatment for relapsed CML after allogeneic hematopoietic stem cell transplant (HSCT), but predictors of response are unknown. In CML, marrow is the primary site of disease as well as a reservoir of high-avidity antigen-specific memory B and T cells that can recognize tumor antigens. We therefore examined serial bone marrow biopsies obtained before and after DLI for evidence of immune cell response from 31 patients with relapsed CML following allogeneic HSCT at our center between 1994-2001. All patients received CD4+ DLI (3-10 x 10^9 CD4+ cells/kg) from the original stem cell donor. Twenty-four patients achieved complete cytogenetic remission following DLI, while 7 patients were non-responders. Marrow biopsies, collected prior to DLI, within the first 2-3 months following DLI and approximately 1 year after DLI were examined for cells expressing CD3, CD8, CD20 and CD138 by immunohistochemistry, and were also scored for cellularity. DLI responders demonstrated an increase in CD3+ T cells following DLI compared to pre DLI (p = 0.07), while no increase was observed among DLI non-responders. This infiltrate, appearing following DLI, consisted almost entirely of CD8+ T cells. Significant changes in cells expressing CD20, CD138 were not observed. Examination of the pre-treatment marrow revealed that infiltration by leukemia-reactive T cells was closely associated with response to DLI. We detected a significantly increased percentage of marrow-infiltrative CD3+ CD8+ T cells in pre-treatment marrow biopsies of responders (median 8%, range 2-20) compared to non-responders (2.5%, range 1-2.5), (p = 0.013). Degree of marrow cellularity prior to DLI was also highly predictive of outcome. While responders demonstrated a 50% median cellularity prior to DLI, non-responders demonstrated more advanced relapse with median cellularity at the time of DLI of 93% (p = 0.064). Of the responders, we observed that 3 patients had 70% or greater marrow cellularity, but also had 5% CD3+ CD8+ T cell infiltration, while all non-responders demonstrated <5% T cells, suggesting that T cells can overcome advanced tumor cellularity in some cases. These studies suggest that the presence of T cell infiltrates in the marrow microenvironment is a significant predictor of DLI response. This also implies that the effectiveness of DLI may depend in part on the enhancement of pre-existing CD8+ T cell response. This also implies that the effectiveness of DLI may depend in part on the enhancement of pre-existing CD8+ T cell response.

415 ROMEDEPESIN (RM), A HADA, SIGNIFICANTLY INCREASES THE EXPRESSION OF NKGD2 LIGANDS, MIC A/B, IN LEUKEMIALYMPHOMA CELLS (LL), IN PART THROUGH THE GLYCOCEN SYTHASE KINASE-3 (GSK-3) PATHWAY, RESULTING IN ENHANCED NK CYTOTOXICITY: TRANSLATIONAL APPROACH FOR ADOPTIVE NK CELL IMMUNOTHERAPY

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Introduction: Natural killer (NK) cells recognize malignant cells through the tumor-associated expression of NKGD-2 ligands, including MIC A/B. However, tumor cells may shed MIC A/B and escape immuno-surveillance. Glycogen synthase kinase-3 (GSK-3), a constitutively active serine-threonine kinase with numerous functions including regulation of cellular differentiation, stress and apoptosis, also an important regulatory enzyme in the expression of MIC A/B in response to RM (Skov et al, Cancer Res, 2005).

Objective: To determine the expression of MIC A/B in response to RM in various leukemia and lymphoma cells (LL), its influence on NK cell mediated cytotoxicity and to investigate the role of the GSK-3 pathway in the regulation of expression of MIC A/B in response to RM.

Methods: LL cells (10^6/ml), RS 4:11 [MLL-ALL], REH [pre-B cell ALL], Jurkat [T-cell ALL], Toledo [DLBCL], Ramos [Burkitt’s Lymphoma]) were exposed to RM (10 ng/ml) for 24 hours, followed by FACs staining with PE-conjugated anti-MIC A/B. Peripheral blood NK cells were isolated via magnetic separation followed by IL-2 activation. Cytotoxicity assays ( europium assay) were performed at effector:target (E:T) ratio of 5:10. LL cells were also pre-treated for 1 hour with 100 mM lithium chloride (LiCl), a potent inhibitor of GSK-3 activity. Blocking studies were also performed with anti-NKG2D antibodies.

Results: MIC A/B expression significantly increased in LL cells in response to RM (RS4:11 0.2% vs 82%, p < 0.0001), [REH 0.2% vs 46% (p = 0.0003), [Jurkat 1.12% vs 85%, p < 0.0001], [Toledo 0.5% vs 15.8%, p = 0.0001], Ramos 0.57% vs 67%, p = 0.0003). Enhanced expression of MIC A/B in response to RM was inhibited when LL cells are pre-treated with LiCl (Jurkat [RM vs RM + LiCl] 85% vs 18%, p < 0.0001; RS 4:11 [RM vs RM + LiCl] 82% vs 5%, p < 0.0001; Ramos [RM vs RM + LiCl] 67% vs 35%, p < 0.0001). Cytotoxicity assays revealed significant increases in vitro cytotoxicity in RS 4:11, Ramos and REH cells at E:T ratio of 5:10 (Table 1). NKG2D receptor-blocking resulted in significant decrease in NK cell mediated cytotoxicity in REH (p < 0.03) and Ramos cells (p < 0.01). In-vivo experiments are underway.

Conclusion: Expression of MICA/B in LL cells is significantly increased by RM leading to enhanced susceptibility for NKG2D- MICA/B mediated cytotoxicity by NK cells. Furthermore, up-regulation of MICA/B in LL cells secondary to RM exposure is in part regulated by the GSK-3 signal transduction pathway.

416 PRELIMINARY RESULTS OF A PHASE II TRIAL OF MONTELUKAST FOR THE TREATMENT OF BRONCHIOLITIS OBLITERANS SYNDROME AFTER HSCT

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Bronchiolitis obliterans syndrome (BOS) after allogeneic HSCT is a severe manifestation of cGVHD. Current treatments yield poor and transient responses. Although the pathogenesis of BOS after HSCT is unknown, a similar disease, BOS after lung transplant is associated with elevated leukotriene levels. We present
preliminary results from an IRB-approved prospective, open label, phase II trial to test the efficacy of montelukast, a leukotriene inhibitor, for the treatment of BOS after HSCT. BOS diagnostic criteria included: FEV1 < 75%, FEV1/VC < 0.7 or air trapping on CT and RV > 120% or RV/TLC > 120% in the absence of infection and presence of another cGVHD manifestation. Eleven patients have enrolled to date. One withdrew prior to medication initiation and 9/10 are currently on study medication (10 mg montelukast po nightly). Patient characteristics include age range 15-60 years, 7/11 female, baseline FEV1 range from 33 to 71% predicted, and 3/11 patients requiring oxygen supplementation. Sixty-four % (7/11) have reached the primary endpoint (6 months of study drug). FEV1 increased by 6-10% predicted in 3 patients, remained stable in 3, and declined by less than 15% in 1. Slope of the FEV1 value generated as linear regression of FEV1 volume vs. days post-transplant revealed: 5/7 increase in slope, 2/7 decrease in slope from pre-study FEV1 values. Three patients had immunosuppression reduced during this time period with complete cessation of tacrolimus in 1, cessation of steroids in 1, and decreased tacrolimus in 1(including 2 with stable FEV1) 1 patient had an increase in steroid dose less than 1 mg/kg/day. Two patients had worsening of other cGVHD manifestations on study, including a skin flare that resolved without increasing systemic therapy (I) and gastrointestinal cGVHD flare that improved with increased steroids including local therapy (I). Montelukast was well-tolerated with one grade II attributable adverse event (insomnia) during the six-month collection period. Improvements were also noted in oral mucosa cGVHD manifestations in 3/7 and liver in 2/7. These preliminary findings suggest that montelukast may have a role in the therapy of BOS after allogeneic HSCT.

There are ongoing improvements <PR in skin, mouth, and eyes. 3 subjects have a global PR.

Conclusion: POM can be given for corticosteroid-resistant cGVHD without toxicities that limit use of thalidomide in this context. Our data suggest POM is active in moderate/severe corticosteroid-resistant cGVHD. 3 mg/d is likely a too high starting dose: we recommend 1-2 mg/d in future studies. Subject accrual continues, as do correlative laboratory studies; these data will be reported.