endotoxin-free, synthetic TLR4 agonists, may allow exploitation of both IL-12 and regulatory DC to suppress GVHD.

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**Trametinib Selectively Inhibits Alloreactivity While Sparing Virus-Specific T Cells**

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We have previously shown that inhibition of the MEK pathway with selumetinib greatly reduced T cell proliferation induced by allo-DC stimulation with minimal loss in virus-specific cytokine production in EBV and CMV-stimulated T cells in vitro, and that selumetinib significantly reduced GVHD and increased survival in a GVHD mouse model. Thus, MEK inhibitors represent a novel class of immunosuppressive agents that potentially target GVHD initiation while sparing beneficial immunity in the allo-SCT setting. We sought to determine if trametinib, a recently FDA-approved MEK inhibitor based on clinical responses in the melanoma setting, would also demonstrate selective inhibition of alloreactivity while preserving virus-specific immune function. In 6 allo-DC/responder PBMC pairs, we assessed the dose response of inhibition of proliferation by trametinib, and found similar dose response patterns to selumetinib with ~ 50% inhibition of alloreactivity at the 1 μM dose and nearly complete inhibition at 10 μM (Figure 1). As previously reported with selumetinib, the percentage of polyfunctional CMV and EBV-stimulated PBMC capable of producing IL-2, IFN-γ, TNF-α or MIP1-β was unchanged by MEK inhibition (n=4, CMV pp65 peptide responses shown in Figure 2). While the overall fraction of functional responding cells to virus did not change in response to MEK inhibition, TNF-α production was reduced at higher doses of both trametinib and selumetinib. Further experiments are planned, both in vitro and in animal models, to confirm the potential utility of this class of drugs to reduce GVHD while preserving virus-specific T cell function in the allo-SCT setting.

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**Cannabidiol – an Innovative Strategy for Graft Versus Host Disease Prevention – an Update of a Phase I/II Study**

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Introduction: Cannabidiol (CBD), a safe and non-psycho-tropic ingredient of marijuana, has been shown to exhibit potent immune-modulatory and anti-inflammatory properties in animal models of various inflammatory diseases. We have recently presented data suggesting that the combination of CBD with standard GVHD prophylaxis is a safe and promising strategy to reduce the incidence of GVHD. We herein update the data in a larger cohort, analyzing both short and long term transplantation outcomes.

Methods: We conducted a phase I/II trial. All patients were given standard GVHD prophylaxis consisting of cyclosporine and short course methotrexate. CBD was orally administered at a dose of 150 mg twice daily from starting of conditioning up to day +30. We sequentially monitored a panel of 4 serum cytokines (soluble TNF receptor 1 (sTNFRI), soluble IL-2 receptor alpha (sIL2R-alpha), hepatocyte growth factor (HGF), and IL8)). Blood samples were taken at days -7(A), 0(B), +14(C), and +28(D). We assessed the difference in blood levels between the various time points (B-A, C-B and D-C).

Results: Between 9/2012 and 10/2013, 34 consecutive patients with hematological malignancies undergoing allogeneic transplantation were enrolled (median age 52, range, 22-71 years). Most patients had acute leukemia/MDS (n=28). Most (73%) were in CR/PR at transplantation. Majority (n=26) of patients were given a myeloablative conditioning. The donor was either a HLA identical sibling (n=17), a 10/10 matched unrelated donor (n=14) or 1 antigen mismatched unrelated donor (n=3). All patients were given G-CSF mobilized peripheral blood stem cell grafts. Median follow-up was 8 months (range, 3-13). There were no grade 3-4 toxicities attributed to CBD. There were no cases of graft rejection. Cumulative incidences of grade 2-4 and 3-4 acute GVHD by day +100 were 15% and 7%, respectively. Cumulative incidences of overall and extensive chronic GVHD at 12 months were 41% and 12%, respectively. Cumulative incidences of relapse, NRM and OS at 12 months were 27%, 16.5% and 71%, respectively. Patients with increased D-C serum levels of IL8 and sIL2R-alpha had a relative risk of 3.8 (95% CI 1.1-7.5, p=0.05) and 2.8 (95% CI 1.1-7.5, p=0.05), respectively, for developing chronic GVHD.

Conclusion: Combination of CBD with standard GVHD prophylaxis is a safe and promising strategy to reduce the incidence of GVHD. Further studies comparing this novel approach with standard GVHD prophylaxis are warranted.

Silencing of the beta7 Chain Using Antisense Technology to Ameliorate Gvhd

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Acute graft-versus-host disease (GVHD), one of the major complications associated with allogeneic blood and marrow transplantation (BMT), develops primarily in the skin, liver, gastrointestinal tract and lymphoid tissues of patients who receive this therapy for various hematological malignancies. The homing of T cells to GVHD target organs and their regulation via integrins, selectins and chemokine receptors have been recognized as potential novel realms for intervention to ameliorate or prevent GVHD while still allowing the beneficial graft-versus-tumor effects. A number of studies have shown that transplantation of donor T cells lacking beta7, a chain of the intestinal homing receptor alpha4beta7 integrin, was effective at ameliorating GVHD in BMT murine models. Likewise the use of monoclonal antibody against the alpha4 or the beta7 subunits have been alluded to as potential treatments for intestinal bowel disease and GVHD, and trials, at least for the former, have been initiated in the clinic. Here we investigated an alternative method to block alpha4beta7 expression on donor T cells in order to impair their capacity to infiltrate gut tissue in a murine BMT model, using antisense morpholino oligonucleotide (AMO) technology (Gene Tools, LLC). Electroporation of donor T cells permitted a greater than 90% transfection of AMO and an average 82.26±5.78% knockdown on alpha4-beta7 expression, just 24 h after treatment with translational-blocking AMO targeting the mouse beta7 chain. Beta7 expression = 84.35±5.30% in electroporation control cells vs. 41.65±6.77% in AMO treated cells (50.62±7.85% decrease), and alpha4B7 expression = 23.0±7.40% in electroporation controls cells vs. 4.1±1.91% after AMO treatment. In the MHC-haploidentical C57BL/6 (B6) into (B6xDBA/2)F1 [H2b; B6D2] BMT murine model, B6D2 mice challenged with 3x105 AMO-treated (50 mM) B6 T cells had a MST of 20 days vs. a control group given untreated/electroporated donor T cells (MST 14 days; p<0.01). This significant prolongation of survival correlated with a significant decreased infiltration of donor eGFP+B6 T cells into intestinal tissues as measured by histological analysis (p = 0.024). This data strongly suggested that silencing beta7 on donor T cells with AMO technology, albeit transiently as done here, could be...