GVHD specific histopathology but similar levels of DAMPs such as HMGB1. Donor T cell analyses demonstrated significantly greater expansion of activated CD69^{+}, Th1 (IFN-g^{+}) and Th17 (IL-17A^{+}) cells in the spleen and in the GVHD target organs, namely liver and gut ($P<0.05$). Enhanced GVHD mortality and severity was observed in multiple irradiated BMT models (MHC-matched multiple minor antigen mismatched C3H.sw->B6 and haploidentical B6->F1 models). We performed detailed phenotypic analysis of various T cell subsets in naïve Siglec-G$^{-/-}$ and WT B6 animals and found similar distribution of naïve, memory, effector and regulatory T cells suggesting that alteration in T-cell subsets in the donor T-cell inoculum was not the cause for increase in GVHD. Functional analyses of naïve Siglec-G$^{-/-}$ T cells, showed similar proliferation *in vitro* after stimulation with allogeneic DCs or CD3/CD28 antibody stimulation when compared to WT T cells. Furthermore Siglec-G$^{-/-}$ Tregs as well as WT Tregs were equally suppressive in repressing either WT or Siglec-G$^{-/-}$ naïve T-cells demonstrating that Siglec-G expression on Tregs is not critical for their suppressive function or on naïve T-cells to be regulated by Tregs. By contrast Siglec-G$^{-/-}$ T cells showed higher proliferation after TCR stimulation (CD3/CD28) only upon with addition of DAMP (HMGB-1) demonstrating that direct regulation of T-cell response to DAMP by Siglec-G regulates its TCR driven proliferative responses. Finally, in order to explore the Siglec-G^{+}T cells interaction with its ligand in hosts, we next used CD24$^{-/-}$ BALB/c animals as hosts. CD24$^{-/-}$ animals also demonstrated enhanced GVHD mortality when compared to WT animals. Collectively, our data suggest, for the first time to our knowledge that Siglec-G mediated responses to DAMPs has T-cell autonomous effects and through its engagement with its ligand CD24 in the hosts is critical for mitigating GVHD.

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Encouraging Results of a Phase II Trial of Inhaled Fluticasone Propionate, Azithromycin, and Montelukast (FAM) May Maintain Lung Function in Bronchiolitis Obliterans Syndrome (BOS) after Hematopoietic Cell Transplantation

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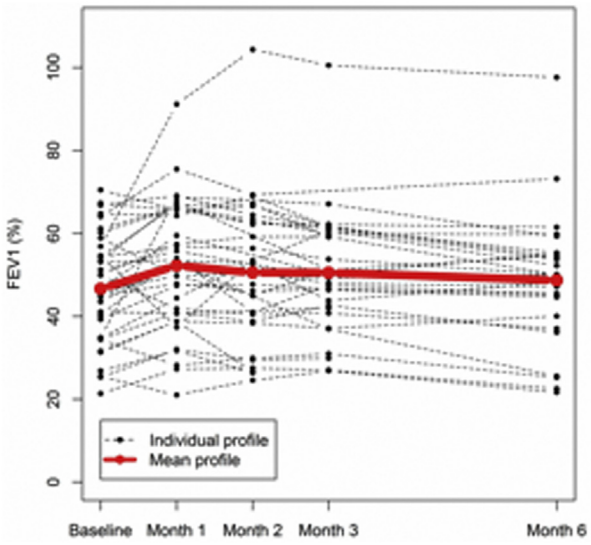

Figure. Change in individual and mean percentage FEV1% over time.

Background: Bronchiolitis obliterans syndrome (BOS) after allogeneic hematopoietic cell transplant (HCT) is associated with high mortality. Recently, we showed efficacy for montelukast to treat BOS. We hypothesized that FAM (**F**luticasone propionate (440 mcg inhaled bid, provided by GlaxoSmithKline), **A**zithromycin (250 mg 3x/week), and **M**ontelukast (10mg QD, provided by Merck) for up to 6 months, with brief steroid pulse could avert progression of new-onset BOS and tested this in a phase II, single-arm, open label, multicenter study (NCT01307462).

Results: Thirty-six patients from 10 institutions were enrolled within 6 months of BOS diagnosis (NIH modified criteria: FEV1<75% predicted, FEV1/VC <0.7, and >10% FEV1 decline from pre-HSCT, absence of infection). The median age was 57 years (range 23-72), 47% were females, with moderate obstruction (median FEV1 46%; FEV1/FVC 0.5) at enrollment. The primary endpoint was treatment success, defined as < 10% FEV1 decline at 3 months, with 60% treatment success seen in historical controls (published data). FAM was well tolerated with only 1 grade 4 SAE possibly related to FAM (infection), and no patient stopped FAM prior to 3 months. 3 month results: Eighty-three percent (n=30/36) had treatment success (vs. 60% in historical controls, p=0.004) at 3 months; 5 lacked pulmonary function tests (PFTs), and 1 had decline. Assuming patients without PFTs at 3 months were treatment failures, 36% (n=13/36) had increased FEV1 by 5% or more, and 33% (n=12/36) had less than 5% decline in FEV1. Steroid exposure was reduced significantly from a median of 0.65 mg/kg/day at enrollment to 0.31 mg/kg/day at 3 months in evaluable patients, with only 19% receiving added immunosuppression during that period (associated with <20% of successes). Based on NIH calculated overall cGVHD response, 19% had a CR or PR, while 22% had stable disease at 3 months. Patient-reported outcomes (n=24) were better from baseline to 3 months for SF-36 social functioning score (p=0.03) and mental component score (p=0.02), FACT emotional well-being (p=0.03), and Lee symptom scores in lung (p=0.01), skin (p=0.03), mouth (p=0.03), and overall summary score (p=0.001). Six minute walk test improved by a median of 127 feet (p=0.02). 6 month results: 10 patients could not be evaluated at 6 months: 1 died, 1 withdrew, 5 did not undergo PFTs and 3 were on study < 6 months; 20/26

(77%) evaluable patients had treatment success at 6 months. Overall survival at 6 months was 97% (n=35/36).

Conclusion: These data suggest that: FAM was well tolerated and may halt pulmonary compromise in newly diagnosed BOS and permit steroid reductions, which collectively improve cGVHD outcomes and quality of life.

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A Biomarker Panel for Chronic Graft-Versus-Host Disease

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Background: Chronic GVHD (cGVHD) remains the major contributor to morbidity and mortality for survivors of allogeneic hematopoietic cell transplant (HCT), but it remains a clinical diagnosis.

Methods: We used a proteomics discovery approach comparing plasma pools from onset of de novo cGVHD (N=17), progressive cGVHD (N=18), and matched time-point samples from 19 patients without GVHD. Of 105 proteins that showed at least 1.3-fold change in the quantification ratio, we further selected 24 proteins based on their involvement in relevant pathway networks, and the availability of ELISA. In addition, two markers (CXCL9 and ST2) were measured based on previously noted associations with cGVHD or refractory acute GVHD.

Levels of these 26 proteins were measured by ELISA in plasma from an independent set of 178 patients with cGVHD, and from 33 controls without cGVHD. Logistic regression was used to evaluate the association between cGVHD and biomarkers after log transformation. All analyses were adjusted for significant clinical variables considering age, sex, stem cell source, conditioning (nonmyeloablative vs. others), donor (matched sibling vs. others), and time from HCT to sample collection. To determine the best combination model, we used forward selection with a 0.05 significance threshold, confirmed by backward selection. ROC curves were generated for the best single biomarker, and the combination model. The analysis of nonrelapse mortality (NRM) divided the panel weighted sum on the median value among cGVHD cases (N = 178), and compared cases above and below the median.

Results: Of the 26 proteins tested, 9 were associated with cGVHD with p-values < 0.05 (Table 1). Together ST2, CXCL9, MMP3, and OPN compose a biomarker panel for diagnosis of cGVHD with an AUC of 0.89 (Fig 1). We next compared the biomarker panel between groups with different cGVHD severity (none, mild/moderate, severe) (Fig 2). Severity of cGVHD was associated with the biomarker panel (p<0.0001 compared to control, and p=0.007

Table 1

	Area under ROC	P-value	Adjusted P-value	Combination
ST2	0.832	<0.0001	<0.0001	**Yes**
MMP3	0.787	<0.0001	<0.0001	**Yes**
TRAILR3	0.716	0.0002	0.0002	
OPN	0.708	<0.0001	<0.0001	**Yes**
SELP	0.661	0.008	0.005	
CKIT	0.654	0.007	0.01	
COMP	0.643	0.009	0.002	
CXCL9	0.628	0.02	0.01	**Yes**
CD146	0.616	0.03	0.04	
Combination	**0.885**	**<0.0001**	**<0.0001**	

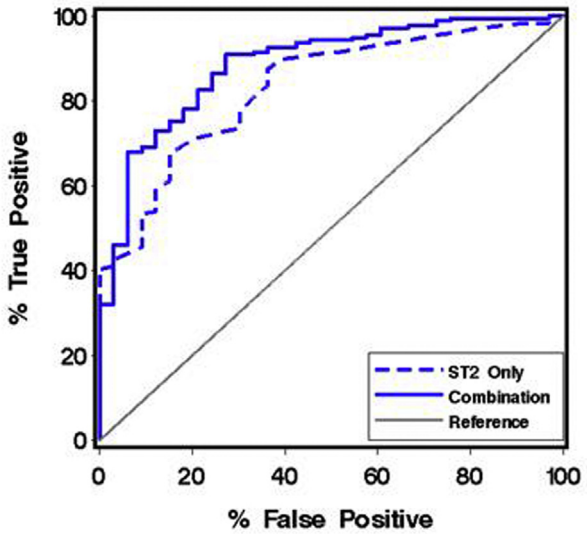

Figure 1.

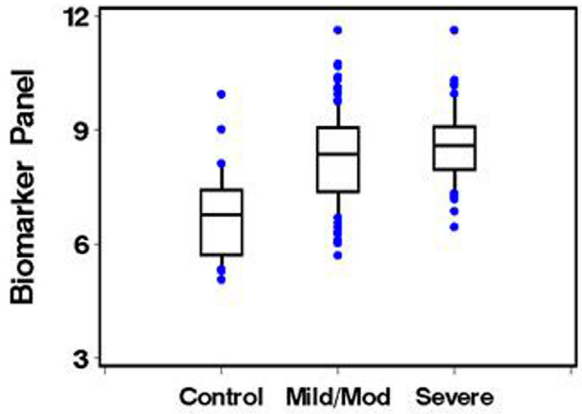

Figure 2.

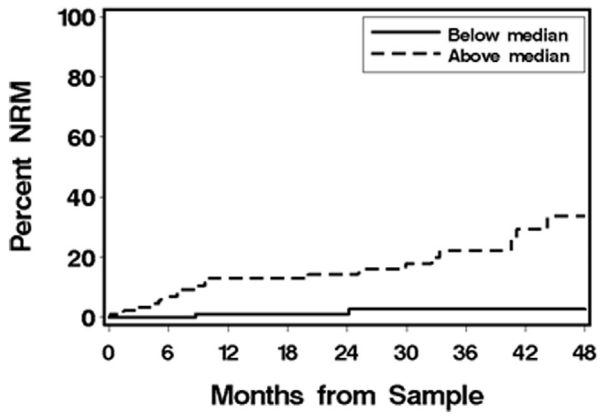

Figure 3.