Table 3

		DonorDBY			DonorUTY			DonorZFY			DonorEIF1AY			DonorRPS4Y		
		-	+	P	-	+	P	-	+	P	-	+	P	-	+	P
Recipient	-	38	7	0.2	31	13	0.28	55	4	0.29	56	1	>0.99	44	5	0.72
post-HCT	+	32	13		27	19		31	0		33	0		38	3	

adoptive transfer of HY seropositivity from female donors to male recipients (Table 3).

Conclusion: Half of female donors were HY-seropositive, but there were no enough evidence to suggest that the HY sensitization can predict clinical outcome. In fact, we provided little evidence of adoptive HY B-cell immunity transfer. On-going studies will relate female HY sensitization to parity and age. The absence of adoptive immune transfer might raise a concern for the efficacy of donor vaccination strategies to augment GVL benefit.

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Immunoregulatory B Cells Are Enriched within Transitional and IgM Memory B Cell Subsets in Healthy Donors but Are Reduced and Functionally Impaired in Patients with Chronic Graft-Versus-Host Disease

Anushruti Sarvaria [1,2], Ahmad Khoder [2], Abdullah Alsuliman [2], Claude Chew [3], Takuya Sekine [2,3], Nichola Cooper [2], Hugues de Lavallade [2], Muharrem Muftuoglu [3], Eric Yvon [3], Amir Hamdi [3], Amin M. Alousi [4], Lisa St. John [3], David Marin [2], Kate Stringaris [2], Enli Liu [3], Jeffrey Molldrem [3], Nina Shah [3], Simrit Parmar [5], Ian McNiece [3], Richard E. Champlin [3], Elizabeth J. Shpall [4], Katy Rezvani [4]. [1]Stem Cell Transplantation and Cellular Therapy, M.D. Anderson Cancer Center, Houston, TX; [2]Haematology, Imperial College London, London, United Kingdom; [3]Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, Houston, TX; [4]Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX; [5]Stem Cell Transplantation and Cellular Therapy, UT M.D. Anderson Cancer Center, Houston, TX

The immunosuppressive function of IL10 producing regulatory B cells (Bregs) has been shown in several murine models of inflammation and autoimmune disease. However, there is a paucity of data regarding the existence of an equivalent regulatory B cell subset in humans and their relevance in the pathogenesis of chronic graft-versus-host disease (cGVHD) remains unknown. Here, we explored the regulatory properties of peripheral blood (PB)-derived human B cell subsets and their role in cGVHD. Using intracellular cytokine staining following in vitro stimulation with CD40 ligand, we showed that the majority of IL-10 producing B cells in healthy donors are found within the $CD24^{hi}CD38^{hi}$ transitional and $CD19^{+}IgM^{+}CD27^{+}$ memory B cell subsets. Sort-purified IgM memory and transitional B cells suppressed the proliferation, as well as the release of IFN-γ by CD3/CD28 stimulated $CD4^{+}$ T cells. The inhibitory effect of IgM memory and transitional B cells on $CD4^{+}$ T cell proliferation was cell dose dependent with the highest suppression was observed at a ratio of 1:1. These data suggest that human PB transitional and IgM memory B cells are endowed with suppressive function. This suppression was mediated partially via the provision of IL-10, but not TGF-ß, which we assessed by antibody blockade experiments. Additionally, the suppressive capacity of the B cell subsets was reversed by the addition of CD80 and CD86 mAbs. Using transwell experiments, we further determined that the suppressive function of Bregs is also partly dependent on direct T cell/B cell contact. Although blockade of IL-10 and IL-10R, CD80 and CD86 and separation of B cells and T cells by a transwell membrane individually did not completely reverse the suppressive ability of transitional and IgM memory B cells, a combination of these factors sufficiently reversed the ability of Breg subsets to suppress CD4+ T cell proliferation. Thus, analogous to murine experimental models the suppressive effect of human Breg cells involves both the release of IL-10 and co-receptor interaction. Additionally, Breg cells isolated from patients with cGVHD were refractory to CD40 stimulation and produced less IL-10 when compared to patients without cGVHD post-SCT and healthy controls. Likewise, the absolute number of IL-10 producing B cells was significantly lower in cGvHD patients compared to patients without cGVHD and healthy controls ($p=0.007$), supporting the existence of both a qualitative and quantitative defect in IL-10 producing B cells in cGvHD.

Our combined studies provide important new data defining the phenotype of B cell populations enriched in regulatory B cells in healthy humans and provide evidence of altered cellular function within such cells that may impact a broad range of deficiencies in immune regulatory cell function in cGvHD post transplant patients.

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The Role of Gut Microbiota in the Development of Intestinal GVHD

Tiffany Simms-Waldrip [1,2], Michal Meir [1], Di Fan [1], Laura Coughlin [1], Milan Savani [1], Tanya Watt [1,2], Victor Aquino [1,2], Andrew Young Koh [2,3]. [1]Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX; [2]Hematology/Oncology, Children's Medical Center, Dallas, TX; [3]Pediatrics and Microbiology, University of Texas Southwestern Medical Center, Dallas, TX

Background: Commensal gut microbiota have been implicated in initiating and perpetuating intestinal graft versus host disease (iGVHD), but its role remains controversial. Recent murine studies have shown that iGVHD results in destruction of intestinal mucosal immune defenses resulting in expansion of pro-inflammatory bacteria (Enterobacteriaceae, ENTERO), and by prophylactically treating mice with oral antibiotics that suppress growth of ENTERO, iGVHD was significantly improved[1]. Furthermore, small-chain fatty acid producing (SCFA) Clostridia have been shown to induce colonic Treg cells, to dampen gut inflammation, and to cure IBD in mice[2]. But these findings have not been observed or replicated in humans.

Methods: Stool samples were collected on a weekly basis from pediatric allogeneic BMT patients from 7/26/11 – 9/30/13. Bacterial and fungal gDNA was isolated from fecal specimens. Amplicons for 16S rRNA V4 variable region and fungal ITS region were generated and sequenced on Roche 454 GS-FLX sequencer and Illumina HiSeq2000 respectively. Sequencing data was analyzed using QIIME software. The abundance of specific intestinal bacterial groups was determined by qPCR using group-specific 16S rRNA gene primers. For patients undergoing intestinal biopsy for suspicion of iGVHD (abdominal pain, diarrhea), additional pieces of intestine were obtained for transcription profiling experiments using Illumina Human HT12 V4 Expression BeadChips. Clinical characteristics (i.e. conditioning regimens, specific antibiotic use, immunosuppression, etc) were recorded.

Results: Complete bacterial/fungal pyrosequencing and bacterial group qPCR was performed on 10 patients (4 iGVHD, 6 no GVHD). As evidenced by 16S rRNA sequencing, only patients with iGVHD developed significant expansion of ENTERO and a significant decrease in SCFA Clostridia prior to the diagnosis of iGVHD. Bacterial group qPCR confirmed these findings: patients with GVHD had significantly higher ENTERO ($p< 0.01$, Mann Whitney) and significantly lower EREC and CLEPT (subgroups of the SCFA Clostridia) ($P<0.01$) than non-GVHD counterparts. Of the clinical characteristics recorded, clindamycin treatment, which is effective against *Clostrida spp*, was the most strongly associated with the development of iGVHD
Conclusion: Expansion of pro-inflammatory ENTERO and decreases in anti-inflammatory Clostridia (CLEPT and EREC) are associated with iGVHD in pediatric BMT patients. Medical therapies such as chemotherapy and/or antibiotics may disturb the baseline gut microbiota and make certain patients predisposed to the development of iGVHD. Real-time monitoring of the gut microbiota (bacterial group qPCR) has great potential as a biomarker for iGVHD.

1. Eriguchi et al. *Blood.* Jul 5 2012;120(1):223-231.
2. Atarashi et al. *Nature.* Aug 8 2013;500(7461):232-236

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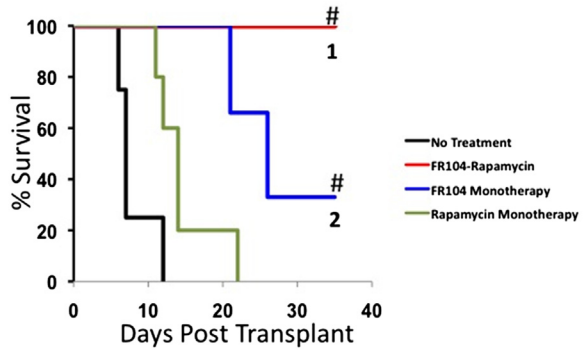
Preventing Primate Gvhd Using a Novel Antagonistic Anti-CD28 Antibody Plus Rapamycin: Downregulation of CD8 Proliferation Predicts Gvhd-Free Survival

Benjamin K. Watkins [1], Nicolas Poirier [2], Caroline Mary [3], Gilles Blancho [2], Karnail Singh [4], Aneesah Garrett [4], Kelly Hamby [4], Taylor Deane [4], Bruce R. Blazar [5], Bernard Vanhove [2], Leslie S. Kean [6]. [1]Aflac Cancer Center, Emory University, Atlanta, GA; [2]Nantes University, Nantes, France; [3]Effimune, Nantes, France; [4]Emory University, Atlanta, GA; [5]Pediatric Blood and Marrow Transplantation, University of Minnesota, Minneapolis, MN; [6]Ben Towne Center for Childhood Cancer Research, Seattle Childrens Research Institute, Seattle, WA

Introduction: We have previously shown, using a non-human primate (NHP) model, that inhibition of CD28:CD80/86 costimulation with CTLA4-Ig could prevent GVHD, and this strategy is now being tested in a Phase 2 clinical trial. Despite the potential efficacy of CTLA4-Ig, there is concern that it may have off-target effects, given its inhibition of both (+) CD28 signaling and (-) CTLA4 signaling. In order to specifically target CD28, we have developed an antagonistic humanized anti-CD28 monovalent Fab' antibody (FR104). Here we investigate the efficacy and mechanism of action of FR104 in NHP, to potentially facilitate its most rapid clinical translation.
Methods: NHP underwent MHC-mismatched HCT after myeloablative TBI. They were transplanted with GCSF-mobilized PBSCs ($4 \pm 1x10^8$ TNC/kg and $2 \pm 0.5x\ 10^7$ CD3+ T cells/kg). GVHD prophylaxis was with FR104 monotherapy



 # Timed Terminal Analysis
[1] p=0.017 versus untreated controls, p=0.013 versus rapamycin monotherapy
[2] p=0.02 versus untreated controls, p=0.05 versus rapamycin monotherapy

Figure 1. Improved GHVD-free Survival by Blocking CD28

(5mg/kg/wk IV, resulting in <1% CD28 expression) or with FR104 + mTOR inhibition with rapamycin, and were compared to two controls groups (untreated recipients and rapamycin monotherapy). Clinical and histologic GVHD was monitored and longitudinal immunologic analysis performed.
Results: Untreated controls ($n = 5$) developed rapid, severe AGVHD (MST=7 d). Rapamycin alone ($n = 6$) partially protected recipients, with GI-predominant AGVHD (MST = 14 d, Fig. 1). FR104 monotherapy showed statistically significant prolongation in survival (MST = 27 days, $n = 3$) compared to rapamycin ($p = 0.05$) and untreated controls ($p=0.02$), with breakthrough GVHD occurring in two animals (liver and skin). In contrast, FR104 + rapamycin ($n = 3$) controlled GVHD, with all recipients reaching the timed terminal analysis at day +33-35 without clinical disease ($p = 0.017$ vs. untreated, $p = 0.013$ versus rapamycin alone).

Our previous work has documented a major role for T cell proliferation (by Ki-67 expression) in GVHD, with untreated controls demonstrating rampant CD8+ proliferation ($89 \pm 5\%$ Ki-67+ CD8+ T cells at terminal analysis (d +6) vs. 4% pre-transplant, Fig. 2A). Rapamycin and FR104 monotherapy both partially controlled proliferation ($9\% \pm 4\%$ and $14\% \pm 8\%$ Ki-67+ CD8+ on d +6) with rapamycin + FR104 combination therapy resulting in further control ($3.5\% \pm 0.3\%$ Ki-67+ CD8+ on d +6). Moreover, the degree of CD8+ proliferation correlated closely with GVHD-free survival (Fig. 2B), suggesting that Ki-67 expression may be a predictive biomarker of this disease.
Implications: These results show that specific blockade of CD28 can inhibit NHP GVHD and when combined with rapamycin, can effectively control this disease for the length of dual therapy. Importantly, clinical control of GVHD with FR104 correlated with normalization of T cell proliferation. Our results suggest that selective CD28 blockade may be a safe and effective adjunctive strategy to inhibit GVHD-associated T cell activation, and is deserving of clinical evaluation in patients undergoing HCT.

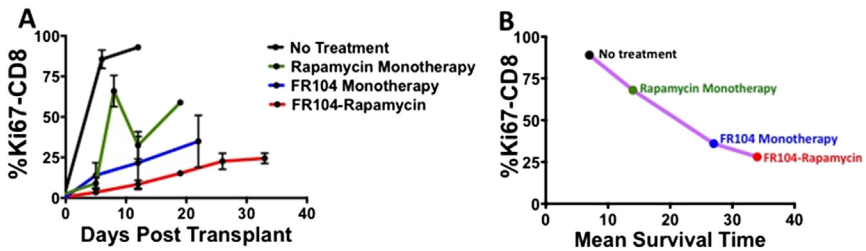


Figure 2. CD28-blockade-mediated Inhibition of GVHD Correlates with Control of CD8+ T cell Proliferation. **A.** % of Proliferating CD8+ T cells Measured Longitudinally Post-transplant. **B.** Inhibition of CD8+ proliferation correlates with GVHD-free survival.