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

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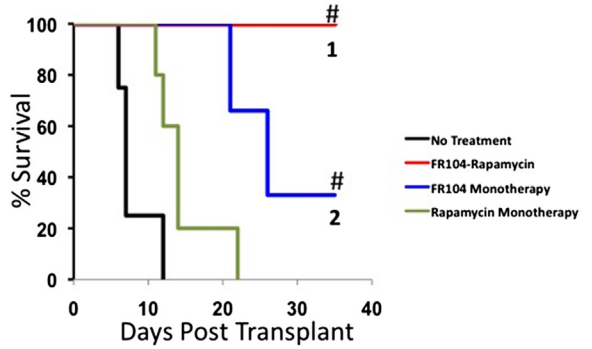
Introduction: We have previously shown, using a non-human primate (NHP) model, that inhibition of CD28:CD80/86 costimulation with CTLA4-Ig could prevent GVHD, and this strategy is now being tested in a Phase 2 clinical trial. Despite the potential efficacy of CTLA4-Ig, there is concern that it may have 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). GHVD 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.

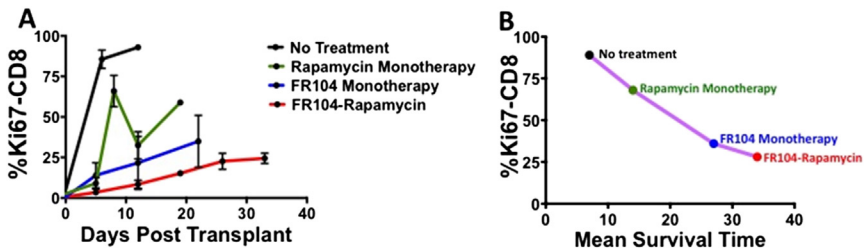


Figure 2. CD28-blockade-mediated Inhibition of GVHD Correlates with Control of CD8+ T cell Proliferation. **A.** % of Proliferating CD8+ T cells Measured Longitudinally Post-transplant. **B.** Inhibition of CD8+ proliferation correlates with GVHD-free survival.