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The pathophysiology of chronic Graft-versus-Host Disease (cGVHD) is complex and poorly understood. Storage of peripheral blood mononucleated cells (PBMC) for research in patient cohorts is vital. However, conventional peripheral blood draws are often insufficient for larger scale immunology studies; in particular, cGVHD patients are frequently lymphopenic, which further limits an ability to study the role of relatively rare populations such as Treg cells. To address this limitation, we evaluated the safety of collecting large quantities of lymphocytes in cGVHD patients by steady-state peripheral blood leukapheresis. In total, 132 patients with cGVHD underwent PBMC collection for research purposes via leukapheresis performed from 2004 to 2014 on NCI cGVHD Natural History Protocol, NCT00092235. Various apheresis machines were used, namely: Baxter CS3000 (n=63, 47%), Haemonetics MCS-P (n=18, 14%), and Terumo SPECTRA (n=52, 39%). Median whole blood/anticoagulant (ACDA) ratio was 12:1; median whole blood flow rate (WBFR) was 60 ml/min (35-85); and median product volume was 147 ml (59-450). Median age was 50 years (18-68). NIH cGVHD Global scores were moderate (n=46, 35%) or severe (n=82, 62%). Mean absolute circulating PBMC level pre-leukapheresis was 2.4 K/μL (0.3-11.6). 22 patients (17%) had an absolute lymphocyte count <1.0 K/μl. A subset of patients (17/132) had sufficiently low pre-leukapheresis peripheral counts that required special approval for the procedure (Hb <9.0 g/dL, n=3; WBC < 3.0 K/μL, n=5; platelets < 120 K/μL, n=9). A two-arm continuous flow apheresis procedure was used in 108 patients (81%) whereas a one-arm procedure was used in 25 patients (8%). Only nine patients underwent leukapheresis via central venous catheter (the remainder were pheresed using peripheral access). 94 (71%) achieved the goal collection of 2 x 109 PBMCs, with a mean volume processed of 4.6 L. Median total run time was 88 minutes. Mean number of cells collected and efficiencies were: (1) lymphocytes, 3.7 x 109, 66.5%; (2) monocytes, 1.1 x 109, 51.1%; and (3) PBMCs: 4.8 x 109, 59.7%. Mild decreases in absolute peripheral counts were observed, including (median change 1 hour post-leukapheresis; each value significant, p<0.0001): Hgb: -8%; Plt: -22%; lymphocytes: -26%; and monocytes: -17%. Grade 1 AEs (CTCAE v4.03) were experienced by 21 patients (16%): hypotension (n=1, 1%), oral dysesthesia (n=10, 8%), paresthesias (n=6, 5%), anxiety (n=1, 1%), localized bleeding (n=2, 2%), and nausea (n=1, 1%). In conclusion, we found that steady-state PBMC leukapheresis represents a safe and effective method for collecting high numbers of immune cells in patients with moderate-to-severe cGVHD. Wider utilization of leukapheresis in the cGVHD setting should accelerate immunology research into the pathogenesis of cGVHD, particularly relating to the role of relatively rare cell populations.

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Immune Reconstitution Analysis of Patients Undergoing Extracorporeal Photopheresis for the Treatment of Chronic Graft-Versus-Host Disease

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Allogeneic blood and marrow transplantation for the treatment of hematologic malignancies continues to be plagued by complications such as chronic graft-versus-host disease (cGVHD), which can occur in upwards of 50% of patients. Standard therapy involves corticosteroid administration, which is not always effective and impedes the patient's return to a completely competent immune system, thus increasing the risk of infectious complications. Extracorporeal photopheresis (ECP) has been successfully used to abate cGVHD symptoms in many patients. The effect this therapy has on lymphocyte subsets in the reconstituting immune compartment has yet to be fully ascertained. In the present study, we used flow cytometric analysis to examine lymphocyte subsets in patients undergoing ECP for the treatment of cGVHD. Peripheral blood was collected from 5 patients prior to starting ECP and after 90 days of treatment. $CD4^+$T cell populations were analyzed for expression of CD45RA and CCR7 to classify naïve (RA^+, $CCR7^+$), central memory (T^{CM}; RA^-, $CCR7^+$), effector memory (T^{EM}; RA^-, $CCR7^-$), and terminally differentiated effector memory (T^{EMRA}; RA^+, $CCR7^-$) T cells. The data reported supports previous studies which show that all $CD4^+$ T cell populations analyzed are altered in cGVHD patients (n=11) compared to healthy controls (n=5). In particular, $CD4^+$ T^{CM} and naïve cells were decreased in cGVHD patients, while $CD4^+$ T^{EM} and T^{EMRA} were elevated in these patients. Analysis of the same subsets in cGVHD patients after 90 days of ECP treatment revealed that the percentage of $CD4^+$ T^{CM}increased above baseline levels in the 5 cGVHD patients examined, while $CD4^+$ T^{EMRA} were decreased during the same time frame. The increase in $CD4^+$ T^{CM} and the decrease in $CD4^+$ T^{EMRA} were statistically significant as determined by the paired Student's t test. Additionally, the ratio of $CD4^+$ T^{CM} to T^{EM} and $CD4^+$ T^{CM} to T^{EMRA} was calculated in patients before and after ECP treatment. Results indicate significantly increased ratios of both T^{CM}:$T^{EM}$and $CD4^+$ T^{CM}:T^{EMRA} in these patients as well. This data strongly supports the role of ECP in the normalization of $CD4^+$ T cell populations in cGVHD patients.

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T-Cell Transcriptome Analysis Reveals the Mechanisms Controlling Synergy Between Costimulation Blockade and mTOR Inhibition during GVHD Prevention

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While the first-in-disease trials of GvHD prevention with costimulation blockade add CTLA4-Ig to standard tacrolimus/methotrexate, previous work has suggested that mTOR inhibition with sirolimus is more pro-tolerogenic when paired with costimulation blockade. We have now investigated combination CTLA4-Ig + sirolimus to prevent GvHD in the NHP model, and have interrogated the outcomes using a systems-based approach. Our experiments provide strong clinical, immunologic and trancriptomic evidence for potent synergy when CTLA4-Ig and sirolimus are combined for GvHD prevention.

We determined the impact of the following treatments on GvHD after haplo-identical HSCT: 1) no therapy (n=4) 2) CTLA4-Ig monotherapy (using belatacept, n=3) 3) sirolimus monotherapy (n=4) 4) combination belatacept and sirolimus (n=3). To determine the relative impact of each therapeutic approach, we monitored clinical GvHD, GvHD-free survival, and flow cytometric signs of immune activation