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clones in peripheral blood could provide a way to risk stratify patients.

We collected endoscopic GI tract biopsy samples and matched blood for fourteen patients undergoing myeloblative HCT with suspected GI GVHD within one day of corticosteroid therapy. We also collected peripheral blood approximately thirty days after biopsy. Seven of these patients were negative for GVHD or had mild steroid responsive GI GVHD and seven had severe steroid refractory GI GVHD. We extracted genomic DNA and performed TCR beta CDR3 repertoire sequencing at Stanford and with Gigagen GigaMune Rep-Seq, using the Illumina next generation sequencing MiSeq and HiSeq platforms.

For each patient, between 1,000-5,000 unique T cell sequences were identified in the endoscopic tissue. We bracketed the most frequent clones in the GI tissue samples by rank order cut-offs and a floating measure of skewness and followed these clones in the blood. Both methods showed that, on average, the GI identified clones increased in frequency more in severe patients (51.2 +/- 39.2) when compared to mild or negative patients (3.08 +/- 2.8; see Figure 1). Additionally, patients in both groups who received more steroids (mg/kg/day) showed a correlated reduction in GI identified T cell expansion in the blood over time. These results support the use of T cell repertoire sequencing and associated approaches in human patients to both clarify the pathophysiology of GVHD and may provide an independent immune biomarker that could guide GVHD therapy.

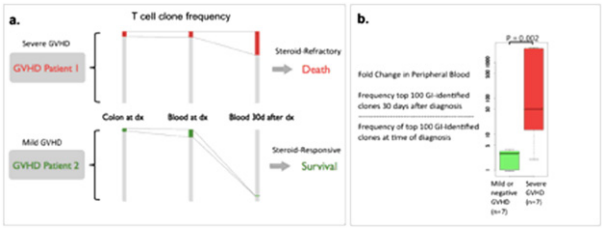

Figure 1. GI identified TCR sequences become much more frequent in the blood in an analysis of patients with severe steroid refractory GI GVHD as compared to a patients with steroid responsive GVHD or without GVHD. (a) Graphical depiction of the most frequent TCR sequence identified in the colon and blood. Top panel shows the increasing frequency of the topped rank clone (red), in contrast to the lower panel which shows the decreasing frequency of the top ranked clone (green). (b) The mean fold change of TCR sequences identified in the GI tract of patients tracked in the blood at day 30 to day of diagnosis of 7 patients with severe GVHD and 7 patients with mild or no GVHD (Wilcoxin Test, $P < .002$). GI sequences were identified as the top 100 by rank order.

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Identification of Y-Chromosomally Encoded Minor Histocompatibility Antigens Using a Reverse Immunology Approach

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Introduction: In allogeneic hematopoietic cell transplantation (HCT), minor histocompatibility antigens (mHags) are known to play an important role in generating immune responses leading to graft-versus-leukaemia (GVL) effects and graft-versus-host-disease (GVHD). mHags are results of polymorphisms in the recipients genome, which cause expression of peptides that can be recognised by donor T-cells. Y-chromosomally encoded proteins constitute a constant source of mHags relevant in allogeneic HCTs with female donor and male recipient due to the disparities between these and their homologue X-chromosomally encoded counterparts.

Methods: A panel containing 8-11 mer peptides encompassing multiple putative and known mHags encoded by the Y-chromosome was designed using a bioinformatics predictor of peptide-HLA binding, NetMHCpan. These peptides were synthesized and used to screen for peptide-specific T-cell responses in peripheral blood mononuclear cells (PBMCs) obtained post-HCT from male recipients of female donor grafts. Following in vitro stimulation, PBMCs were analysed with an inteferon-γ ELISpot assay. When a response was found, the T-cells were further analyzed with intracellular cytokine staining (ICS) and flow cytometry to determine whether it was a CD4- or a CD8-response. The optimal epitope and the HLA-restriction was determined by tetramer staining.

Results: In one male recipient of a female donor graft a T-cell response was observed with ELISpot against the peptides YFYYNAFHWAI and RESEEESVSL. ICS and flow cytometry revealed that both were CD8 responses. Both peptides were earlier described mHags restricted by HLA-A*24:02 and HLA-B60, respectively. Tetramer staining confirmed that the optimal epitopes were YYNAFHWAI and RESEEESVSL presented on HLA-A*24:02 and HLA-B*40:01 (a member of the previously designated HLA-B60 specificity), respectively. PBMCs obtained post HCT from five other male recipients of female donor grafts have been analysed for T-cell responses with ELISpot. Responses have been observed and further analysis is ongoing.

Conclusion: Using a HLA-tetramer approach to identify the optimal epitopes of two known mHags encoded by the Y-chromosome as well as the presenting HLA restriction elements at high resolution, we have demonstrated the feasibility of a reverse immunology approach in mHag discovery.

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Decreased Pulmonary Function in Asymptomatic Long Term Survivors After Busulfan-Based Myeloablative Allogeneic Hematopoietic Stem Cell Transplant

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Pulmonary function post allogeneic hematopoietic stem cell transplant (HSCT) can be impaired by previous exposure to chemotherapy, infection, or graft versus host disease (GVHD). In this retrospective study, we analyzed 21 patients with hematologic malignancies who are long term transplant survivors with a median follow-up of 48 months who had received a related (60%) or unrelated (40%) HSCT conditioned with a busulfan-based regimen. Of 21 patients, 7 are of Caucasian and 14 non-Caucasian ethnicity. All patients had routine pulmonary function tests (PFTs) repeated within two years from transplant and none had underlying lung disease. To eliminate inter-lab variability, all PFTs were