Results: 156 patients, median age 5 (range 0-22) years, were included; 50 for a malignancy; 22 AML, 22 ALL, and 6 other. 127 (81%) patients received Bu-based and 29 (19%) TBI-based conditioning. In multivariate analyses, above median number PIRCHE-I were associated with lower probability of relapse (HR 0.139, p=0.006, Fig 1), and a trend for improved disease-free survival (HR 0.411, p=0.086). This effect was not present for PIRCHE-II. In the whole cohort, PIRCHE-I or −II were neither associated with acute-GVHD grade II-IV (HR 0.747, p=0.466, HR 1.263, p=0.554) nor extensive chronic GVHD (HR 1.502, p=0.671, HR 3.420, p=0.293), nor TRM (HR 1.753, p=0.198, HR 0.991, p=0.983, for PIRCHE-I and −II respectively).
Conclusion: High numbers of PIRCHE-I lead to improved GVL effects after UCBT, while GVHD was not impacted by the number of PIRCHE. PIRCHE may provide an additional tool for individualized donor selection to improve survival chances. The effects observed in this study need to be validated in other cohorts.

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An Increasing Severity of Chronic GvHD Is Associated to an Activated and Cytotoxic T Cell Mediated Immune-Phenotype

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Introduction: Chronic graft-versus-host-disease (cGvHD) is a late complication (>90 days) after allogeneic hematopoietic stem cell transplantation (HSCT). Symptoms might occur in multiple organs and vary from mild to severe. In this study, the NIH criteria were used to group the patients. We performed an extensive immune-phenotypic analysis using cells and plasma from patients diagnosed with and without cGvHD. The aim of the study is to gain information on possible markers that could be used by clinicians to aid in the diagnosis of cGvHD.
Methods: Peripheral blood samples were obtained from patients ≥ 1 year post-HSCT with an acute GvHD no higher than grade I. Patients were divided into 4 groups: no (N=10), mild (N=6), moderate (N=5) and severe cGvHD (N=8). Cytokine levels were measured by Luminex and an immune-phenotypic analysis was done by multicolor flow cytometry. Univariate analysis comparing the different patient groups was done with the Mann-Whitney U test.
Results: Due to usage of immunosuppressive treatments in the moderate and severe cGvHD groups, we chose to compare patients with no versus mild cGvHD and moderate versus severe cGvHD. Expression of the cell activation marker CD38 was higher on total T cells and CD8+ T cells (p=0.005 for both subsets) in mild cGvHD patients compared to patients without cGVHD. Furthermore, severe cGvHD patients had higher levels of CD8+ T cells (p=0.03) than patients with moderate cGvHD. Additionally, several Th2-associated cytokines were down regulated in severe cGvHD patients, compared to the other patient groups. An analysis was also done based on the presence of cGvHD in specific organs (lung, mouth and skin) within the moderate and severe cGvHD group. In patients with lung cGvHD; both the expression of degranulation marker CD107a (p=0.003) on T cells and the expression of KIR receptor CD158b on NK cells negative for the Fc receptor CD16 (p=0.035) were found to be lower. Anti-inflammatory cytokine IL-10 was decreased (p=0.018) in patients with mouth cGvHD. Lastly, CD158b+ CD16+ NK cells and total CD158b+ NK cells were found to be less frequent in patients with skin cGvHD (p=0.017 for both subsets).
Discussion: Mild and severe cGvHD patients appear to have a more activated and cytotoxic immune-phenotype compared to patients with no and moderate cGvHD, respectively. Additionally, patients with specific organ-oriented cGvHD seem to have a lower percentage of NK cell subsets. In conclusion, it appears that patients with an increasing severity of cGvHD have a more activated cytotoxic phenotype, which seems to mostly depend on CD8+ T cells and not on NK cells. By studying the phenotype of the immune system in these cGvHD patients, we can gain new insights in the pathogenesis behind cGvHD. Our findings, i.e. distorted immune cell phenotypes, could potentially be used as biomarkers in the diagnostics of cGvHD.

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Response and Survival Following Second-Line Therapy in 113 Patients with Steroid-Refractory Acute Graft-Versus-Host Disease

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Background: Steroid-refractory acute graft-versus-host disease (SR-aGVHD) remains the major, non-relapse obstacle to successful hematopoietic cell transplantation (HCT). Evolving donor and cell sources, conditioning regimens and GVHD prophylaxis are altering the field of HCT. We evaluated

Table
Factors associated with day 28 CR/PR and 6-month NRM

Factor	N	OR of CR/PR (95% CI)	*P*	RR of NRM (95% CI)	*P*
Second Line Rx					
ATG*	50	1.0		1.0	
MMF	28	2.1 (0.7-6.8)	0.20	0.5 (0.2-1.1)	0.07
Boost	25	1.9 (0.5-7.6)	0.36	0.2 (0.1-0.6)	**<0.01**
Other	10	0.2 (0.02-2.2)	0.19	0.4 (0.1-1.1)	0.08
GVHD Risk Score (at 2nd Rx)					
Standard*	55	1.0		1.0	
High	58	0.3 (0.1-0.9)	**0.04**	1.0 (0.5-1.7)	0.89
Time from Onset to 2nd Rx					
²14 days*	63	1.0		1.0	
>14	50	0.3 (0.1-1.0)	**0.05**	1.2 (0.6-2.6)	0.57

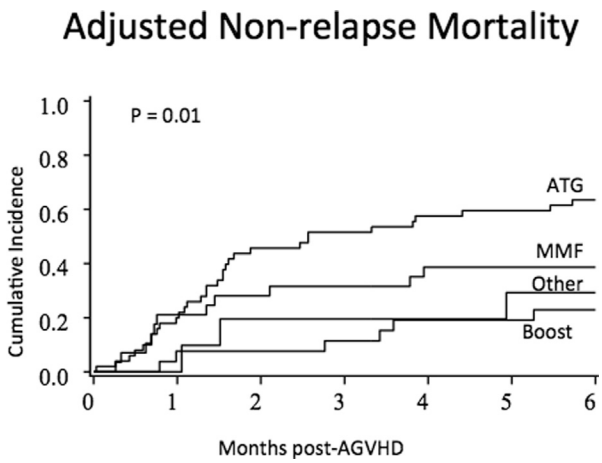

Figure 1. Six-month NRM is lower among steroid boost recipients compared to ATG

response rates following second-line therapy for SR-aGVHD in this modern era.

Methods: We retrospectively analyzed day 28 response rates and clinical outcomes of 113 HCT patients (median age 39 years, range 1-75) who received second-line therapy for SR-aGVHD from 1998-2012 at the University of Minnesota.

Results: Patients received grafts from 43 related (38 matched, 5 mismatched), 31 unrelated (23 HLA 6-8/8, 8 HLA ^{2}5/8) and 39 cord blood donors (23 HLA 6-8/8, 16 HLA ^{2}5/8); 76 patients (67.3%) received myeloablative regimens. Second-line therapy consisted of ATG (44.2%), MMF (24.8%), steroid boost (22.1%) or other (8.8%). Patients receiving ATG had higher-risk GVHD Risk Scores (MacMillan et al, Br J Haem, 2012) at second-line initiation compared with patients receiving MMF or steroids. At day 28, overall response (complete response [CR]/partial response [PR]) was observed in 32 patients (28%). All therapies had similar responses. In multivariate analysis, factors associated with day 28 CR/PR included GVHD Risk Score and days from onset of aGVHD to second-line therapy (Table); Six-month non-relapse mortality (NRM) was lower among patients receiving steroid boosts (24% [7-41%, 95% CI]) vs. ATG (64% [48-90%, 95% CI]) with no differences vs. MMF or other therapies (P=0.01, Figure 1).

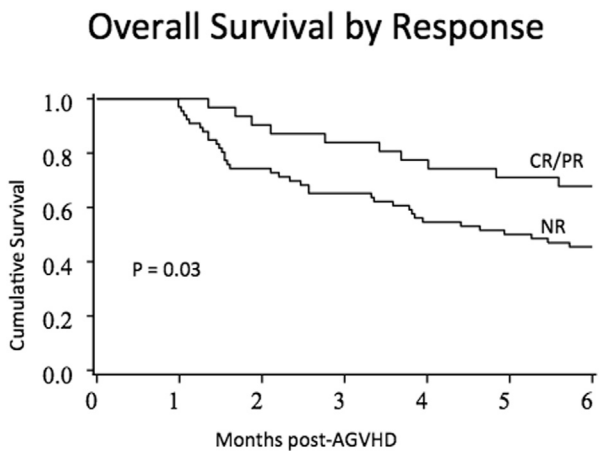

Figure 2. Six-month OS is increased among day 28 second-line responders.

In multivariate analysis, use of steroids vs. ATG was associated with reduced 6-month NRM (Table). Overall survival (OS) at 6 months was increased among day 28 responders (68% [48-81, 95% CI]) vs. non-responders (NR) (45% [33-57, 95% CI]) (P=0.03, Figure 2). Donor and cell source, conditioning regimen and age did not impact response, NRM or OS.

Conclusions: Effective therapy for SR-aGHVD remains inadequate. Day 28 CR/PR was similar among second-line therapies. ATG recipients experienced higher rates of NRM but also exhibited higher-risk GVHD by Risk Score. Identification of high-risk GVHD at initial onset using the GVHD Risk Score may identify patients likely to fail upfront steroids in need of additional or alternative agents.

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Survival without Progressive Impairment As a Novel Endpoint in Chronic Graft-Versus-Host Disease

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Background: A primary goal in the management of chronic graft-versus-host disease (cGVHD) is to prevent the progression of organ-specific and global functional impairment due to the natural history of cGVHD or its treatment. We tested a definition of progressive impairment in a multicenter cohort.

Methods: Of the measures recommended by the 2005 NIH Consensus Conference, changes in 21 items having face validity for "impairment" were selected from these categories: clinician-reported organ involvement; FEV1; and patient-reported performance status and physical function.

Results: 575 patients were evaluated, and 237 (41%) met criteria for progressive impairment during a median of 39.9 (range 3.8 to 69.2) months of follow-up. 125 (53%) had progressive impairment on the basis of clinically assessed measures, 61 (26%) on the basis of patient-reported performance status or physical function, and 51 (22%) on the basis of more than one category. The top 5 items indicating progressive impairment were: decline in FEV1 by 10% (81/237), decrease in the Human Activity Profile Adjusted Activity Score (HAP AAS) score by $\geq$0.5 standard deviation (76/237), increase in the global measure of skin sclerotic changes (70/237), increase in the fascia score (57/237), and increase in the global skin score (51/237). Rates of survival without progressive impairment or relapse were 72% at 6 months, 52% at 12 months, and 35% at 24 months. Cumulative incidences of progressive impairment were 20% at 6 months, 33% at 12 months, and 43% at 24 months. Patients with progressive impairment had significant worsening in clinician and patient-assessed cGVHD severity, symptoms and quality of life as compared to those without progressive impairment (Table).

Conclusion: Treatments that prevent unacceptable clinical deterioration in patients with chronic GVHD could be identified by using survival without progressive impairment as an endpoint in clinical trials.