between GVHD and the magnitude of mHA in donor-recipient pairs (DRP) is not known. Whole exome sequencing (WES) was performed to identify nonsynonymous single nucleotide polymorphisms (SNPs) that would result in peptide antigens that may be presented to donor T cells. Previously cryopreserved DNA from SCT DRP was obtained, n = 77; 27 related donors (MRD) & 50 unrelated (URD). An average 2,490 SNPs were identified in MRD and 4,287 in URD DRP ($P < .01$). Flanking DNA sequences of the nsSNP were determined and all resulting alloreactive 9-mer peptide (AP) sequences, putative mHA, derived *in silico*; binding affinity of the AP to HLA-A, B & C in each DRP was calculated (NetMHCpan ver2.0); the tissue expression of proteins that AP were derived from was determined as well (GTex). MRD DRP had an average 3,626 HLA-AP complexes with an IC50 of <500 nM and URD had 5,386 ($P < .01$). The array of HLA-AP complexes in each patient was considered as an *operator* matrix modifying a hypothetical cytotoxic T cell clonal *vector* matrix in which each responding T cell clone's proliferation is quantified by the logistic equation of growth. This allowed simulation of a steady state T cell clonal response to the AP-HLA complexes in each DRP accounting for the HLA binding affinity and expression of each AP. The resulting *simulated* organ-specific alloreactive T cell clonal growth revealed marked variability in different donors and recipients (Figure 1). The sum of all T cell clones for common GVHD target organs was: MRD median 188,821 cytotoxic T cells at steady state (n = 26), MUD, 201,176 (n = 35), single locus HLA-mismatch MUD: 56,229 (n = 10). Despite an estimated uniform set of constants used in the model for all DRP and a heterogeneously treated group of patients, overall there was a non-significant trend for higher organ specific T cell counts in patients with GVHD compared to those without. Within individuals the variability between organ-specific T-cell estimates was associated (HR 1.08 $P = .03$) with cumulative as well as chronic GVHD (HR 1.08 $P = .04$), as was the maximum organ-specific T-cell estimate (HR = 1.01 $P = .04$ & HR 1.01 $P = .06$). T cell estimate for the liver was weakly associated with cumulative chronic ($P = .04$) and overall GVHD ($P = .05$); salivary gland T cell estimate was associated with chronic oral GVHD ($P = .01$). Exome wide sequence differences and the variable AP binding affinity of the HLA in each DRP yields a large range of possible alloreactive donor T cell responses possibly contributing to the random nature of alloimmune responses. In the future such simulations may provide a quantitative basis for optimizing donor selection and titration of immunosuppression.

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Biomarkers Predict Graft-Vs-Host Disease Outcomes Better Than Clinical Response after One Week of Treatment

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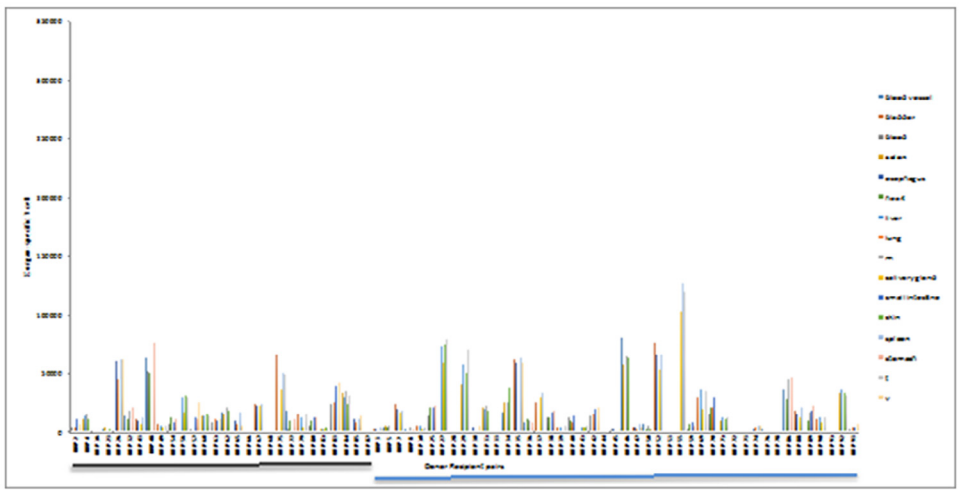

Figure 1. Simulated organ specific T cell responses in MRD (black) and URD (blue) DRP.

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Graft—versus—host disease (GVHD) does not always respond to high dose systemic steroid treatment. We recently showed that a 3 biomarker (TNFR1, ST2, and REG3α) algorithm applied at onset of GVHD predicts day 28 response to treatment and 6month NRM (*Levine, Lancet Haem, 2015*). We sought to determine if the same GVHD algorithm also predicts treatment response and mortality when applied after one week of systemic steroid treatment. We measured the level of the 3 biomarkers after 1 week of systemic steroid treatment in 378 patients (pts) with acute GVHD from 11 centers in the Mount Sinai Acute GVHD International Consortium. Patients were divided into test (n = 236) and validation (n = 142) cohorts. We then applied the GVHD algorithm to the biomarker levels to calculate the predicted probability of 6month NRM in the form of a value between 0 and 1, which we term the treatment score (TS). We employed unsupervised kmedoid clustering to partition TS values from the test cohort into two groups (high and low). This unbiased approach identified a group of 25% of pts (n = 58) with high scores in the test cohort. Pts with high scores in the test cohort had a significantly lower day 28 response rate (CR + PR) compared to low scores (Figure 1A). Pts in the validation cohort showed similarly

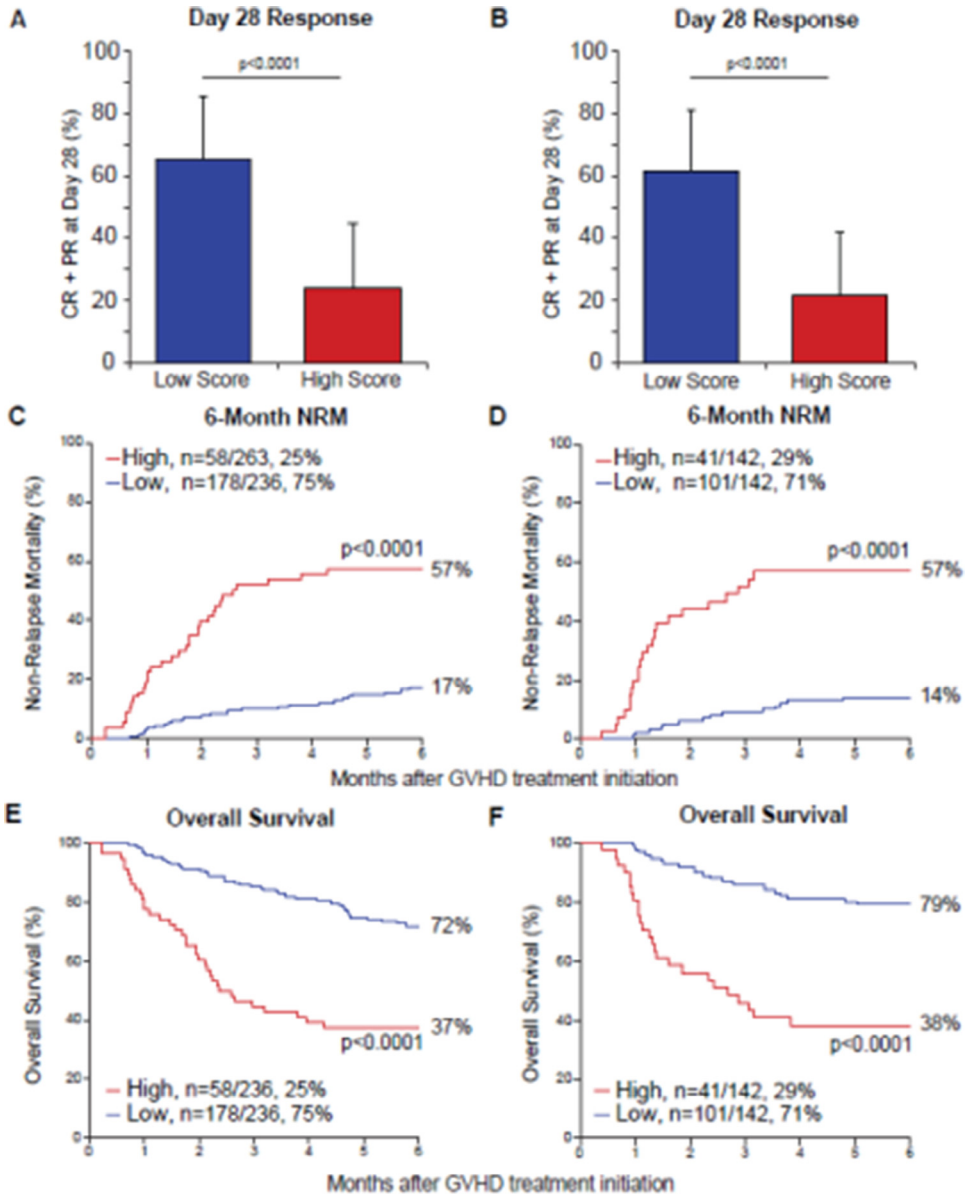

Figure 1. Test Cohort, n = 236 (left), Validation Cohort, n = 142 (right).

different response rates (Figure 1B). Furthermore, NRM was nearly 4 time greater in pts with high scores versus low scores (Figure 1C/D). Thus pts with high TS experienced significantly worse overall survival in both cohorts (Figure 1E/F). As expected, the NRM in pts treated for GVHD was primarily due to GVHD (test: 95%; validation: 89%).

Approximately half of the pts in each cohort (test: 48%; validation: 44%) showed early responses (eCR + ePR) to the first week of steroid treatment; these pts had significantly lower 6month NRM than pts with no response (eNR) (test: 17% vs 36%, $P = .0002$; validation: 13% vs 36%, $P = .0014$). Importantly, the TS continued to stratify mortality risk independently of clinical response. In the test cohort, 16% of all early responders had a high score and experienced more than twice the NRM than early responders with a low score (Figure 2A). Conversely, 67% of early non-responders in the test cohort had a low score and fared much better than those with a high score (Figure 2B). These highly significant results reproduced in the independent validation cohort in similar proportions (Figure 2C/D).

In conclusion, a treatment score based on GVHD biomarkers measured after one week of steroid treatment stratifies pts into two groups with distinct risks for treatment failure and 6month NRM. Notably, the TS identifies two subsets of pts with steroid refractory (SR) GVHD who have highly different outcomes (Figure 2B/D). The much larger group, approximately two thirds of all SR pts, may not need the same degree of treatment escalation as is traditional for clinical nonresponse, and thus overtreatment might be avoided.

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Extended Course of Maraviroc, a CCR5 Antagonist, Is Safe and Effective in Graft-Versus-Host Disease Prophylaxis. Final Results of a Phase II Study

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Background: Blocking lymphocyte migration may prevent GvHD without interfering with graft-versus-tumor activity. We previously reported that brief (up to day+30) CCR5 blockade using maraviroc (MVC) resulted in a low incidence of acute GvHD and absence of early liver and gut GvHD, although delayed GvHD still occurred. We conducted a phase II study to test prolonged administration of MVC. The primary end-point was day-180 cumulative incidence of acute GvHD grade 2-4. We hypothesized that MVC up to day+90 will decrease the rate to less than 30% from a historical rate of 52%.

Patients: We enrolled 37 patients (pts) who received allogeneic stem cell transplantation from unrelated donors using Flu/Bu2 conditioning followed by peripheral blood stem cells.

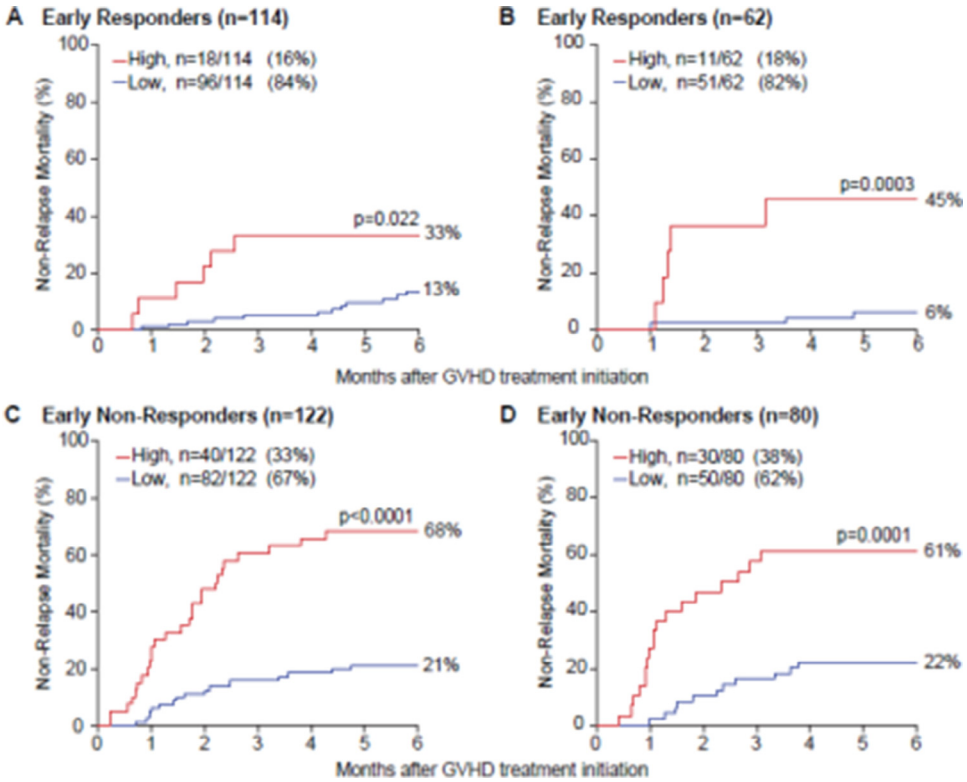

Figure 2. Test Cohort, n = 236 (left), Validation Cohort, n = 142 (right).