

**Official Title:** Bridging Pediatric and Adult Biomarkers in Graft-Versus-Host Disease

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## Study population

The study accrued 415 HCT recipients between 2013 and 2018; 170 children  $\leq 10$  years and 245 subjects  $> 10$  years, including both children and adults. All subjects were followed for at least 1 year. This study registered at clinicaltrials.gov under NCT02194439 was approved by the respective Institutional Review Boards at six adult and pediatric centers: Children's National Medical Center, Texas Children's Hospital, Fred Hutchinson Cancer Research Center, Boston Children's/Dana Farber Cancer Institute, Johns Hopkins, and Indiana University. Adult patients were solely from two centers. Informed consent was obtained from all patients or their legal guardians.

## Samples preparation and ELISA

All plasma samples were prospectively collected and stored per institutional guidelines. Frozen samples were shipped to the Paczesny Laboratory at Indiana University for analysis. ST2, IL6, REG3 $\alpha$ , and TNFR1 were measured pre-HCT (day -7) and on days +7, +14, +21 post-HCT as previously examined in the aGVHD setting. All of these biomarkers were measured using sequential enzyme-linked immunosorbent assay (ELISA), as previously reported.

## Statistical analysis

Descriptive statistics of demographic variables and NRM including frequencies and percentages were summarized by age groups ( $\leq 10$  years vs.  $> 10$  years). Chi-square or nonparametric Mann-Whitney tests were performed to assess potential differences in demographic variables between age categories. Cumulative incidences of NRM and aGVHD were analyzed with relapse as a competing risk. The Aalen-Johansen estimator was used to nonparametrically estimate the cumulative incidence function (CIF) of NRM and aGVHD. In order to account for the time-dependent biomarker values (pre-HCT, and at 7, 14 and 21 days post-HCT) we employed landmark analyses. Separate CIFs were estimated by biomarker category (above and below the median at day 21 for the whole cohort which is also a clinically relevant threshold based on previous studies) for each landmark time point, the biomarker value at this landmark time point was considered and, also, patients who survived until that landmark. To statistically evaluate the difference of the CIFs of NRM and GVHD between the two biomarker groups, while accounting for the left truncation at each landmark point, we used two-sample nonparametric linear tests. To perform a multivariable competing risks analysis, the biomarker values for all landmarks points were stacked into a single variable and a semiparametric proportional cause-specific hazards model was fitted to the data. Hazard ratios with confidence intervals were reported to demonstrate effect size. To account for the potential association across the repeated records for each patient we used a proper sandwich variance estimator in a population-averaged estimation framework. For each model, the interaction between the biomarker and the landmark quantified the change of the association between each biomarker and the outcome over time. If the interaction was not statistically significant then there was no evidence that the association between the biomarker and the outcome changed over time. The models also included an interaction term between the biomarker and patient's age. A significant biomarker by age interaction indicated that the association between the biomarker and the outcome differed between age groups. Multivariable analyses were conducted using the following covariates: race/ethnicity, malignant disease, graft source, and aGVHD prophylaxis. A cause-specific analysis that included type of malignant disease type, disease risk index, and disease status at HCT was also performed in the subgroup of patients with malignant diseases. We sought to determine if the combination of biomarkers improves the predictive accuracy for NRM.

To compare the predictive accuracy across different baseline biomarkers we used the time-dependent prediction error estimate proposed by Schoop et al. This measure, estimates predictive accuracy in situations with time-to-event outcomes and competing risks. A lower value of the estimated prediction error indicates a better predictive performance. A p-value of  $<0.05$  was considered significant.