Validation of a Peripheral Blood Gene Expression Profile for Sub-Clinical Acute Rejection in Kidney Transplant Recipients – Findings from the CTOT 08 Study

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Background
Sub-clinical acute rejection (subAR) is defined as histologic rejection with normal serum creatinine and is associated with worse long term graft survival. Protocol kidney biopsies (PBx) are done at some centers to detect subAR. We have previously identified a pattern of peripheral blood gene expression that can distinguish subAR on a protocol kidney biopsy from normal biopsies (TX) and biopsies done for cause that show clinical acute rejection (cAR). We have now validated this discovery signature using different technologies against an independent, multicenter cohort from the CTOT 08 trial.

Hypotheses
A unique molecular profile in the peripheral blood exists for subAR. This gene expression signature can be used for minimally-invasive serial monitoring in kidney transplant recipients.

Methods
Discovery Cohort: 154 pts enrolled at 5 centers had serial blood samples and PBx. The discovery cohort consisted of: 1) cAR, n=31; subAR, n=33; TX, n=29. Validation cohort: Northwestern University Biorepository: 1) cAR, n=21; 2) subAR, n=23; and 3) TX, n=29. Gene expression analysis was performed with Hu133 PM Peg Arrays & further validated on a next-gen sequencing platform. 3-way ANOVA analysis of cAR vs. subAR vs. TX (p < 0.001; FDR < 10%) identified potential candidates for biomarker discovery. Locked signatures were tested on the validation cohort.

Results
From over 2,500 significantly differentially expressed probesets (FDR<10%) we derived a 61 (FDR<5%) probeset classifier to create a diagnostic signature on the discovery set (CTOT08). This signature gave optimism corrected AUCs ranging from 0.86-0.89. The 3-way classifier validated on the Northwestern samples, with AUCs ranging from 0.83-0.90. Our molecular scoring system from the classifier genes revealed clear separations between each key phenotypes and demonstrated an increase from TX to subAR to cAR. Further validation was performed using orthogonal Next-gen generation sequencing technology with similar results.

Discussion
We have discovered and clinically validated a gene expression profile in the peripheral blood of patients with subAR. This gene expression profile (subAR) differs from a previously validated peripheral blood signature for patients with clinical acute cellular rejection found on a “for cause” biopsy (cAR). We also developed a molecular scoring system to help determine clinical phenotypes based on peripheral blood gene expression profiles. Our study expands the repertoire of clinical phenotypes that can be identified through non-invasive genomic monitoring, and demonstrates the potential of integrating predictive molecular biomarkers into clinical practice to serially monitor and improve long-term outcomes for kidney transplant patients.

References

Numerical Score for Peripheral Blood Gene Expression Profile

Discovery Set Using 200 Genes

Blood Expression Profiling of Kidney Transplant Patients – Discovery & Validation Cohort Using 61 Genes – 3-Way Classifier cAR vs. subAR vs. TX

Validation Cohort (Northwestern Biorepository; n=69) - Orthogonal Validation with Next-Generation Sequencing