Pre-Transplant T-cell Receptor Network Analysis Can Risk Stratify and May Predict Kidney Allograft Rejection

Jes M Sanders[1], Nick Borcherding[2], Greg Martens[3], Naoka Murakmai[4], Natty Diolicho[3], Barb Banbury[5], Jie He[1], Joseph R Leventhal[1,6], James M Mathew[1,6,7]

[1]Comprehensive Transplant Center, Department of Surgery, Northwestern University, [2]Department of Pathology and Immunology, Washington University School of Medicine, [3]Section of Abdominal Transplantation, Division of General Surgery, Washington University in St. Louis, [4]Divisiion of Nephrology, Washington University in St. Louis, [5]Adaptive Biotechnologies, [6]Simpson-Querrey Institute of BioNanotechnology, [7]Department of Microbiology and Immunology, Northwestern University

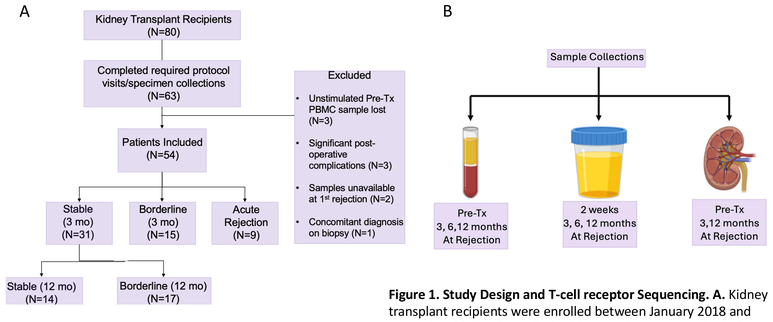
Background

Despite pretransplant serological screening and HLA matching, 10–15% of kidney allografts experience acute rejection within the first year. Currently, risk stratification for transplantation relies primarily on antibody reactivity to HLA molecules, with no assessment of the T cell compartment before or after transplantation. We have previously used bulk T-cell receptor (TCR) sequencing to identify alloreactive clones pre-transplant and to monitor for these clones over time in a cohort of kidney transplant recipients. The results identified a subset of $CD8^+$ donor reactive clones that expanded in patients with rejection. Using this same cohort, we applied network analysis to TCR repertoires in order to establish a prediction model for rejection.

Research Objectives

- Apply sequence similarity network analysis to T-cell receptor sequencing data obtained from a cohort of kidney transplant recipients
- Evaluate the ability of network analysis to classify and predict subjects at risk for rejection events

Patient Selection, Sample Collection, TCR Sequencing



Adaptive Immunosequencing. Adaptive Biotechnologies.

Figure 1. Study Design and T-cell receptor Sequencing. A. Kidney transplant recipients were enrolled between January 2018 and December 2019. All enrolled subjects underwent standard of care (SOC) kidney transplantation (living donor or deceased donor) including SOC induction and immunosuppressive regimens. Subjects were followed for one year to assess clinical outcomes. Subjects were classified as "Stable", "Borderline changes" or "Acute rejection" using allograft biopsy specimens and Banff scoring criteria. **B.** Blood, urine, and allograft samples were obtained from recipients and donors (blood samples only) at varying time points including pre-transplant and at post-transplant intervals. Samples were also obtained at any potential rejection episode. PBMCs and cellular components were isolated from samples, flash frozen, and stored at -80 degrees Celsius until batch T-cell receptor sequencing could be performed. **C.** Adaptive Immunosequencing is a multiplex PCR-based method that amplifies the CDR3 region of the TCRβ chain using high-throughput sequencing technology with a PCR amplification bias-control process, which ensures a quantitative read-out of the immune repertoire. Genomic DNA was extracted from samples using the QIAamp DNA blood Mini kit (Qiagen) and amplified using forward and reverse primers specific to CDR3 Vβ and Jβ region. The CDR3 region of rearranged TCRβ, which was defined based on IMGT/Junction Analysis was then sequenced.

TCR Sequence Similarity Network Construction

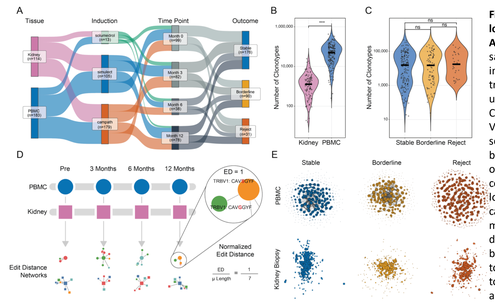


Figure 2. Application of edit-distance networks to a longitudinal TCRβ sequencing renal transplant cohort. A. Sankey diagram summarizing the cohort of 297 samples from 54 patients by tissue, immunosuppression induction, time point, and transplant outcome. **B**. Violin plot of the number of unique clonotypes per sequencing run by tissue. Central black bar indicates the mean. *** P < 0.001. **C**. Violin plot of the number of unique clonotypes per sequencing run by transplant outcome. Central black bar indicates the mean. ns, not significant. **D**. Schematic of normalized edit-distance-based network construction from paired tissue samples across longitudinal time points. Normalized edit distance is calculated as the edit distance (ED) divided by the mean CDR3 sequence length. **E**. Representative edit-distance-based networks from pre-transplant samples by tissue and transplant outcome. Node size is relative to the number of edges with each network subsampled to the equal number of clones to enable comparisons across groups.

Network Correlations with Transplant Factors

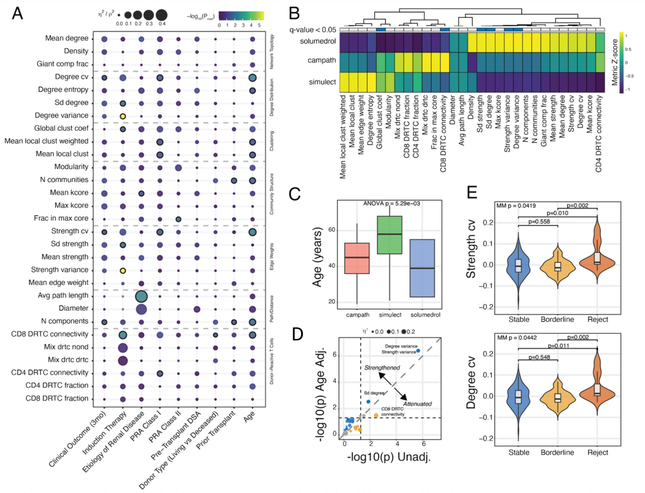


Figure 3. Network metric associations with clinical transplant parameters in peripheral blood. A. Bubble plot summarizing associations between nine clinical variables and size-corrected (residualized) TCR network metrics in PBMC samples. Bubble size encodes effect size (η^2 from Kruskal-Wallis for categorical variables; ρ^2 from Spearman correlation for age). Bubble color encodes statistical significance as $-\log_{10}$(raw mixed-effects model p-value), with timepoint and log-transformed repertoire size as fixed-effect covariates and patient as a random intercept. **B**. Summary heatmap of scaled group means across all residualized network metrics, stratified by induction therapy regimen (Campath, Simulect, Solumedrol). Rows (metrics) and columns (groups) are hierarchically clustered. **C**. Boxplot of recipient age at transplant stratified by induction regimen. **D**. Scatterplot of the comparison of induction therapy associations before and after adjusting for recipient age. Left: unadjusted mixed-model effect sizes; right: age-adjusted effect sizes. **E**. Violin plots of selected network metrics stratified by 3-month clinical outcome (Stable, Borderline, Reject) in PBMC samples. Overlaid boxplots show medians and interquartile ranges; individual observations are jittered. Overall p-values are from mixed-effects models (timepoint and log-transformed repertoire size as covariates, patient as a random effect). Pairwise comparisons use the Wilcoxon rank-sum test with the Benjamini-Hochberg correction.

Network Analysis May Predict Rejection Independent of HLA Matching

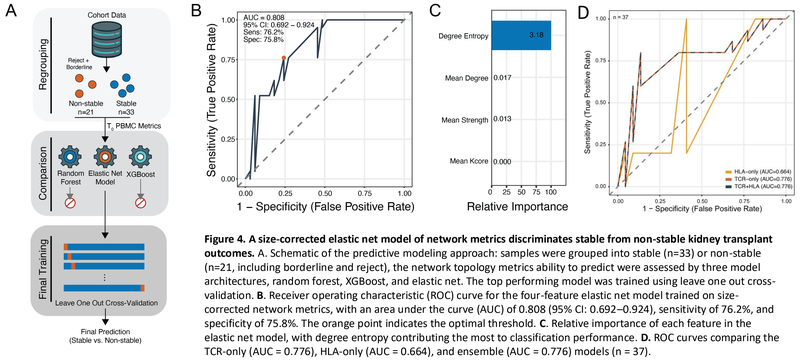


Figure 4. A size-corrected elastic net model of network metrics discriminates stable from non-stable kidney transplant outcomes. A. Schematic of the predictive modeling approach: samples were grouped into stable (n=33) or non-stable (n=21, including borderline and reject), the network topology metrics ability to predict were assessed by three model architectures, random forest, XGBoost, and elastic net. The top performing model was trained using leave one out cross-validation. **B**. Receiver operating characteristic (ROC) curve for the four-feature elastic net model trained on size-corrected network metrics, with an area under the curve (AUC) of 0.808 (95% CI: 0.692–0.924), sensitivity of 76.2%, and specificity of 75.8%. The orange point indicates the optimal threshold. **C**. Relative importance of each feature in the elastic net model, with degree entropy contributing the most to classification performance. **D.** ROC curves comparing the TCR-only (AUC = 0.776), HLA-only (AUC = 0.664), and ensemble (AUC = 0.776) models (n = 37).

Integrating Changes in TCR Networks Improves Predictive Accuracy

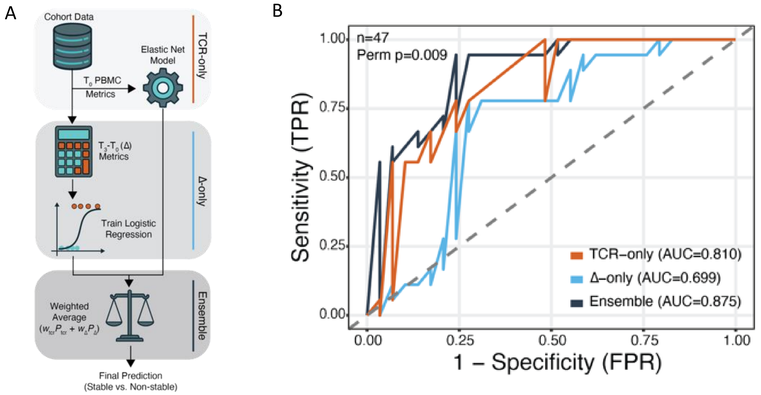


Figure 5. Longitudinal changes in network metrics improve prediction of graft outcome when ensembled with the baseline TCR network model. **A**. Schematic of the ensemble modeling approach: the baseline elastic net model from Figure 4 (TCR-only) is combined with a logistic regression model trained on Δ metrics (Δ-only), and the two are integrated via a weighted average of predicted probabilities to generate a final stability prediction. **B**. ROC curves comparing the TCR-only (AUC = 0.810), Δ-only (AUC = 0.699), and ensemble (AUC = 0.875) models (n = 47), with the ensemble achieving the highest discriminative performance (permutation p = 0.009).

Limitations

- Cohort size—Plan to expand the cohort size and perform multi-institutional studies
- Lack of phenotypic data which may allow for clustering of sequences into cell types, additionally providing functional insight (i.e., what types of cells are these?)
- Induction types grouped together—Studies with per induction analysis may hold higher predictive capacity

Conclusion

- Our results demonstrate network analysis can be applied to TCR sequencing data in the solid organ transplant setting. This is the first study of its type to thoroughly apply such analyses in a transplant cohort
- Network analysis can stratify patients and may predict patients at risk for rejection from a single pre-transplant blood draw

Disclosure

N.B. was previously employed by Santa Ana Bio, Inc and Omniscope, Inc and works as a scientific advisor to Epana Bio, Inc and as a consultant to Columbus Instruments. B.B. is a salaried employee of Adaptive Biotechnologies.

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