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Relevance of the Banff Human Organ Transplant Consensus Gene Panel for Detecting Antibody and T-Cell–Mediated Rejection of Kidney Allografts

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Received 10 November 2023; revised 23 April 2024; accepted 25 April 2024; published online 4 May 2024

Kidney Int Rep (2024) **9,** 2290–2294; https://doi.org/10.1016/j.ekir.2024.04.054
KEYWORDS: antibody-mediated rejection; kidney transplantation; T-cell mediated rejection
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INTRODUCTION

Tissue-based gene expression profiling can detect disease before it is visible from histology; more precisely capture disease activity, stage, and degree of injury; and has been shown to improve histology-based diagnosis of antibody-mediated rejection (AMR) and T-cell-mediated rejection (TCMR) in kidney allografts.[1-3]

Microarray studies have defined the molecular phenotypes of allograft injury, rejection, and immune responses; however, this approach has technical limitations that hinder its applicability in a clinical setting (e.g., sampling of extra biopsy core, increased technical variation, large data processing times, and long sample turn-around). Hence, whole transcriptome-based molecular testing is not yet used in routine practice and has not been thoroughly validated in a multicentric prospective randomized trial.

The NanoString nCounter platform could overcome these issues, as it presents good sensitivity and reproducibility across different experimental settings and platforms, and it does not require an extra-core biopsy due to its applicability on formalin-fixed paraffin-embedded biopsy samples collected in routine practice.[4]

To accelerate the development of reproducible, and cost-effective molecular diagnostics applicable in transplant care and clinical trials, a consensus panel of 770 genes (Banff Human Organ Transplant Panel, B-HOT) usable on the NanoString nCounter platform was developed by the Banff Molecular Diagnostics Working Group to capture key gene expression signatures associated with innate and adaptive immune responses, as well as injury and rejection in solid organ transplants.[5] However, comprehensive validation of this targeted gene panel as a reliable proxy for whole transcriptome-based approaches to efficiently detect kidney allograft rejection has yet to be demonstrated.

The aim of this study was to perform an *in silico* evaluation of the B-HOT gene panel using whole transcriptome array-based data in order to: (i) assess the ability of this targeted panel to detect the signatures and functional pathways associated with AMR and TCMR compared to the whole transcriptome, and (ii)

evaluate the performance of B-HOT-based predictive models to detect AMR and TCMR, compared to whole transcriptome-based models.

RESULTS

This study relies on a cohort of 547 kidney transplant recipients from 11 international centers previously published.[1,6,7] Baseline population demographics are shown in Supplementary Table S1, Supplementary Methods. Among the 547 kidney transplant biopsies included, 124 were AMR cases (68 active and 56 chronic active) and 77 were TCMR cases. B-HOT panel genes were projected on whole-transcriptome array-generated data. Differential expression analysis from whole-transcriptome showed a high enrichment of B-HOT-related genes in the top-ranked (Supplementary Table S2). Of the top 30 genes, 22 (73%) were in the B-HOT panel and captured immune response and cell-type specific injury: endothelial cells (ADGRL4, CDH5, COL13A1, PLA1A, ROBO4, RAPGEF5, and RASIP1); NK cell-related (CCL4, FGFBP2, GNLY, ICAM2, KLRF1, PRF1, and SH2D1B); interferon-gamma inducible genes (GBP4, KLF4, and WARS); TGF-beta and chemokine signaling (ACVRL1, CCL3, and CXCL11); cellular injury (KLF2 and PPM1F) (Figure 1). The majority of enriched pathways,[S12] derived from differential expression analysis of all array genes, were associated with immune regulation and injury were also enriched in the B-HOT panel analysis, notably, AMR- related pathways: antigen processing cross-presentation ($q = 7.22E-13$), lymphoid and nonlymphoid immune interaction ($q = 1.07E-22$), chemokine binding ($q = 5.25E-16$) and interleukin activation ($q = 5.38E–26$) (Figure 1c). Pathway analysis of whole-transcriptome differentially expressed genes showed enrichment for fatty acid metabolism ($q = 4.13E-05$), vitamin and cofactor metabolism ($q = 6.36E-05$), and endosome cascade ($q = 1.5E-04$), processes that are not specific to the immune response.

As significant differentially expressed genes that are not in the B-HOT panel may reflect redundant molecular and cellular functions or those not specifically related to rejection, we performed functional enrichment analysis. Network analysis (Figure 2a) showed high interconnectivity for top-ranked networks based on both B-HOT and whole-transcriptome analysis, showing top categories enriched for interferon signaling, antigen cross-presentation, interleukin signaling, toll-like receptor cascade, and immunoregulatory interaction (Supplementary Table S4).

Of the top 30 genes associated with TCMR (Figure 1), 23 (77%) were in the B-HOT panel and shared highly similar functions, including B and T lymphocyte-associated immune response (ADAMDEC1, AIM2, BATF, BTLA, CTLA4, CD3G, CD8A, CD8B, CD72, CD96, ICOS, IFNG, LAG3, SH2D1A, SELL, SIRPG, and TIGIT), cell proliferation and cytokine production (CD28), chemokine signaling (CCR7, CXCL13, and CXCR6), and transcriptional regulation (MYB and SP140) (Supplementary Table S3). Pathway analysis of significant differentially expressed genes ($FDR < 0.05$) derived from B-HOT genes were enriched for specific cell-mediated responses involved in inflammatory processes, IL-1 ($q = 4.85E-13$), IL-2 ($q = 7.72E-15$) and IL-35 ($q = 3.48E-11$) family signaling (Figure 1). Whereas TCMR-associated pathways based on whole-transcriptome genes (Figure 1d) were mostly enriched in Rho GTPase signaling ($q = 6.03E-10$) or categories associated with aberrant modification of mitotic cycle ($q = 3.01E-4$) and G1/S transition (RB1-mutants, $q = 4.02E-6$). Network analysis confirmed a large overlap of pathways detected ($FDR < 0.05$) (Figure 2b) between B-HOT and whole-transcriptome (Supplementary Table S5). B-HOT gene-based networks showed additional TCMR-related functions: PD-1 signal transduction, TLR signaling cascade, chemokine receptors binding, and phosphorylation of CD3 (subunits CD3E, CD3D, CD3G).

Finally, B-HOT-based AMR models had a higher precision recall area under the curve than models fit using array genes and a comparable receiver operating characteristic (Supplementary Tables S6 and S7). TCMR models from B-HOT and all-array genes showed comparable precision recall area under the curve and area under the receiver operating characteristic curve (Supplementary Tables S8 and S9). Overall, model performance was highly similar between using whole-transcriptome genes or B-HOT panel, demonstrating the robustness of the B-HOT panel to capture the key significant genes and pathways enriched to reliably detect AMR and TCMR rejection.

DISCUSSION

In the current study, we demonstrate *in silico* that the B-HOT panel captures key rejection-associated genes in kidney allografts by projecting the genes included in this targeted panel on whole-transcriptome array data. Enriched genes and pathways were highly similar when comparing whole-transcriptome to the B-HOT panel, capturing pathophysiological processes pertinent to AMR and TCMR. In addition, model performance metrics based on the whole-transcriptome and B-HOT panel were highly comparable, demonstrating that the genes on the targeted panel are expected to be sufficient and sensitive to serve as a surrogate to whole transcriptome-based analysis.

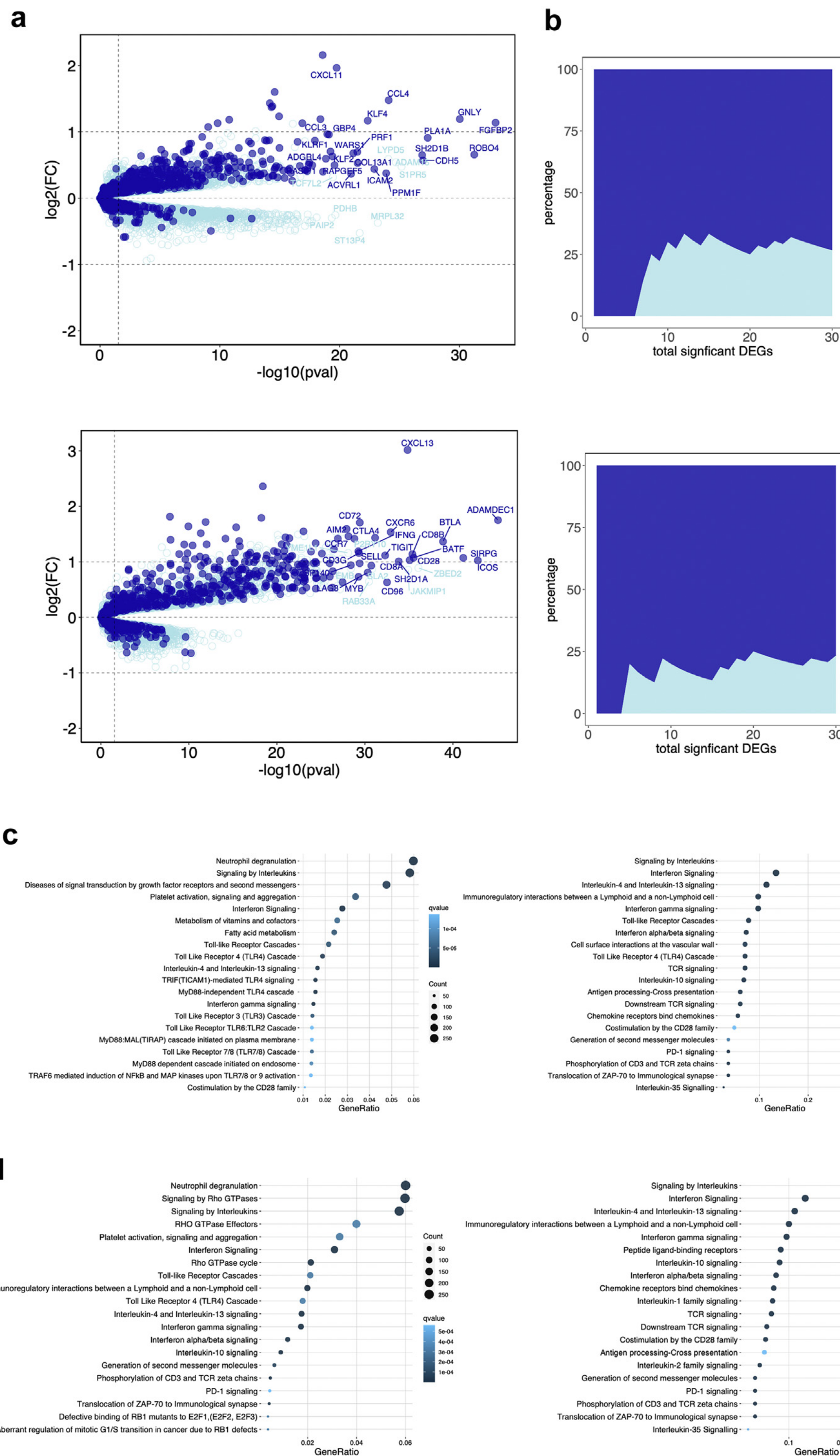

Figure 1. Differential expression analysis and top ranked pathways associated with AMR and TCMR genes based on the B-HOT panel or whole-transcriptome genes. (a) Volcano plots of differentially expressed genes associated with AMR (top panel) and TCMR (bottom panel) in kidney allografts. Dots represent individual genes. Dark blue points indicate genes targeted in the B-HOT panel and light blue points represent all additional genes included on the microarray. The top 30 differentially expressed gene symbols are shown after applying a threshold of 0.05 to false discovery rate corrected *P* values. (b) Stacked area plots of the percentage of the 30 top-ranked differentially expressed genes in AMR (top panel) and TCMR (bottom panel) included in the B-HOT panel (dark blue) or not (light blue). Dot plots show the top 20 enriched pathways based on significant differentially expressed genes (FDR < 0.05) associated with (c) AMR and (d) TCMR in kidney allografts (left panel: whole transcriptome; right panel: B-HOT only). The top 20 significant pathways are plotted in order of gene ratio (number of genes associated with the given pathway divided by the total number of genes analyzed). The size of the dots represents the number of genes in the significant differentially expressed gene list associated with the pathway and the color intensity represents the *P* value adjusted for false discovery rate. AMR, antibody-mediated rejection; B-HOT, Banff Human Organ Transplant panel; TCMR, T-cell mediated rejection.

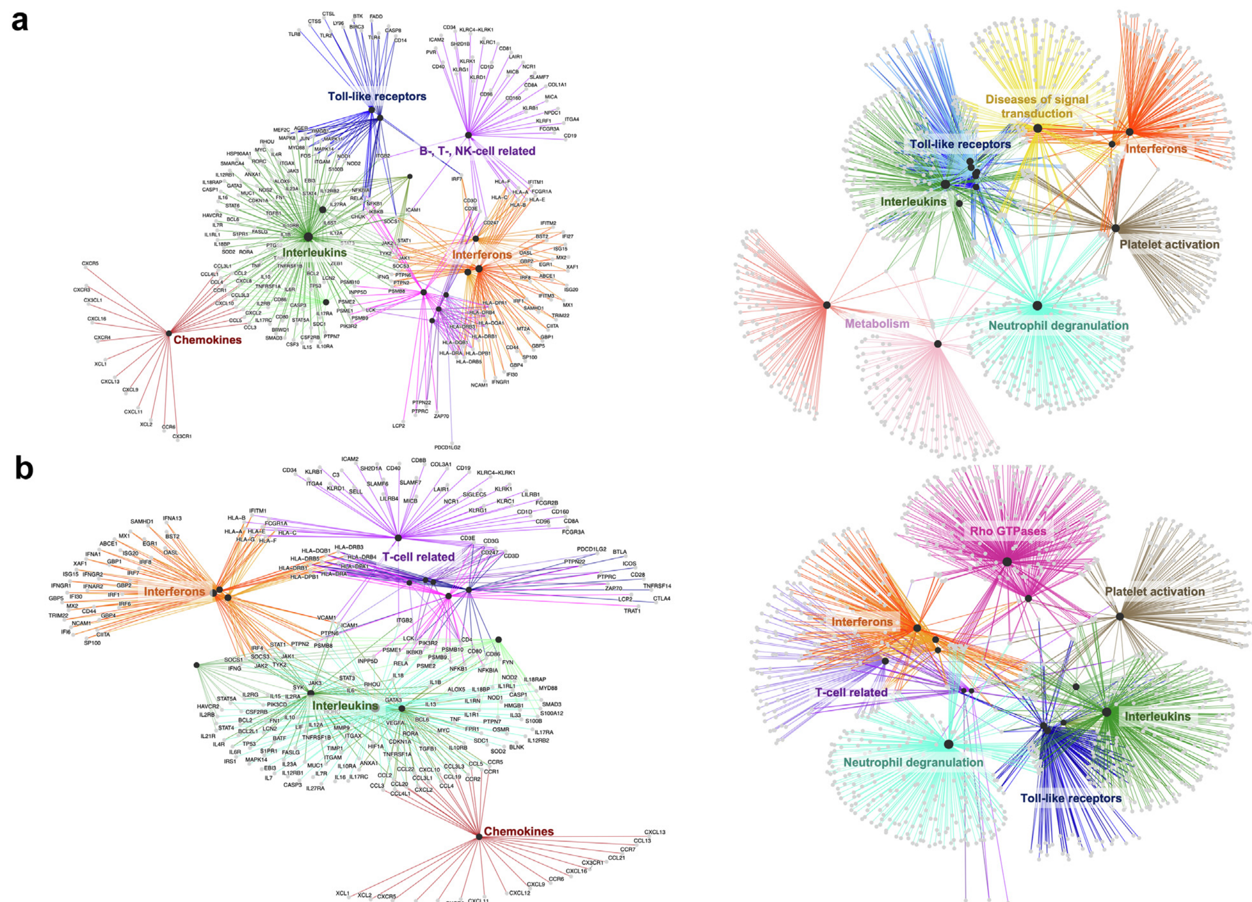

Figure 2. Network analysis of significant differentially expressed genes associated with antibody and T-cell mediated rejection using B-HOT panel or all microarray genes. The interaction networks contextualize the genes involved in the significant pathways. The top 15 pathways are shown due to space constraints. Node size represents the number of differentially expressed genes in the enriched pathway. A complete list of the top 30 signaling pathway with the general class of pathophysiological event involved for AMR and TCMR derived from B-HOT and whole-transcriptome genes is displayed in Supplementary Tables S4 and S5. (a) Network plot of AMR-associated genes (left panel: B-HOT genes; right panel: whole transcriptome genes). For AMR, several pathways were shared between B-HOT and whole transcriptome genes, especially related to interferon and interleukin signaling and toll-like receptor cascade. The network derived from B-HOT genes included pathways more specific to AMR pathophysiology associated with NK and B cells, endothelial activation/dysfunction, T helper cell recruitment. Networks based on whole transcriptome genes showed categories with less specificity for AMR, including disease of signal transduction and metabolism. (b) Network plot of TCMR associated genes (right panel: B-HOT genes; left panel: whole transcriptome genes). For TCMR, B-HOT and whole transcriptome genes shared networks related to T cells, interleukin, and interferon signaling. For B-HOT panel, pathways were more specific to TCMR including chemokine activation. Networks based on whole transcriptome genes showed additional categories less specific to TCMR including those related to neutrophil degranulation and adhesion molecules. AMR, antibody-mediated rejection; B-HOT, Banff Human Organ Transplant panel; TCMR, T-cell mediated rejection.

Whole-transcriptome expression studies in transplantation have described the molecular profile of rejection and demonstrated good performance of predictive models. However, this method typically requires sampling of an invasive extra biopsy core, specially processed, which prevents direct comparison with the histology.[1,S3,S4] An alternative transcriptome approach, that is reproducible on different platforms and based on a smaller set of genes is the B-HOT panel combined with the NanoString nCounter technology. Different from array-generated data, the NanoString nCounter technology with the B-HOT panel does not require an extra-core biopsy due to its applicability on formalin-fixed paraffin embedded biopsy samples. In addition, the technology is reproducible, does not require cDNA conversion, and generates significantly smaller data files. These advantages can potentially facilitate the application of molecular diagnostics in routine transplant care and clinical trials. In our comparative study, the pathophysiological contextualization of rejection-associated genes demonstrates the clinical relevance of the B-HOT panel compared to the whole-transcriptome. Moreover, we demonstrate the reliability of predictive models for AMR and TCMR based on the B-HOT panel with performance metrics highly similar to the whole transcriptome-based and in accordance with previous studies on transcriptomic analysis in kidney allografts.[8,9,S14,S15]

Our study has some limitations. First, our cohort does not include chronic active TCMR cases, thus limiting the generalizability of the findings. Second, our study is retrospective, using selective available material and microarray data. Future prospective multicenter cohorts and randomized controlled trials are necessary to confirm the clinical value and reproducibility of B-HOT-based molecular diagnostics.

In conclusion, this comparative analysis demonstrates that the B-HOT gene panel correctly captures AMR and T-cell-mediated rejection in kidney allografts. Our findings support the use of the B-HOT panel as a proxy to whole transcriptome profiling to develop robust and clinically implementable precision diagnostic systems for kidney transplant recipients.

DISCLOSURE

MM is the chairman of the Board of Trustees of the Banff Foundation for Allograft Pathology and receives consultancy honoraria from CSL Behring and Novartis as a central review pathologist for clinical trials. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

This study was funded by the French government financial support managed by the National Research Agency (ANR) under the program “Investissements d’avenir” KTD-Innov (Kidney Transplantation Diagnostics INNOVation), with the grant agreement ANR-17-RHUS-0010, by OrganX Non-Profit Health Organization, and by the European Union’s Horizon 2020 research and innovation program EU-TRAIN (EUropean TRAnsplantation and INnovation Consortium EU-TRAIN), under grant agreement no. 754995. VG received grants from the French Foundation for Medical Research and the French-speaking Society of Transplantation.

AUTHOR CONTRIBUTIONS

AG, DZ, and AL conception and design of the study. AG, VG, OA, DZ, and AL data analysis and interpretation. AG, VG, DZ, and AL writing, original draft preparation. OA, CL, and AL data acquisition. AG, VG, BR, AA, OA, MS, IR, RS, CR, BA, MH, RC, CL, MM, DZ, and AL data interpretation and critical review of the manuscript. All authors revised the manuscript for important intellectual content.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Table S1. Characteristics of kidney transplant recipients and donors.

Table S2. Top 30 significant genes associated with antibody-mediated rejection in kidney allografts.

Table S3. Top 30 significant genes associated with T-cell mediated rejection in kidney allografts.

Table S4. Top 30 significant pathways associated with antibody-mediate rejection based on the B-HOT panel or whole-transcriptome genes.

Table S5. Top 30 significant pathways associated with T-cell mediated rejection on the B-HOT panel or whole-transcriptome genes.

Table S6. Cross-validated AMR model performance metrics using B-HOT genes.

Table S7. Cross-validated AMR model performance metrics using all genes.

Table S8. Cross-validated TCMR model performance metrics using B-HOT genes.

Table S9. Cross-validated TCMR model performance metrics using all genes.

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