# Outfoxing Rejection: Urinary FOXP3 mRNA, TCMR and the Fate of Allografts

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Histopathologic examination of allograft biopsy specimens remains the gold standard to diagnose etiologies of allograft injury including acute T-cell mediated rejection (TCMR). Allograft needle biopsies suffer from sampling errors from patchy nature of pathology, as well as inter- and intra-observer variability. Additionally, the invasive nature of biopsies discourages serial sampling to assess response to therapy after TCMR. Several lines of research have thus pursued novel noninvasive biomarkers for diagnosis of rejection or prognostication of allograft fate. These have involved quantitation of gene expression profiles or cellular products of activated immune cells in blood, tissue or urine. Translational progress in the field of noninvasive rejection diagnostics is exemplified by the adoption in clinic of gene expression profiling of peripheral mononuclear cells for immune surveillance of low-risk heart transplants as a substitute for surveillance endomyocardial biopsies.[1] Several assays are also in various stages of development for use in the care of kidney transplant recipients.[2]

In this issue of *Transplantation*, Luan et al.[3] add to their existing body of work on the utility of urinary cell mRNA profiling in diagnosing, or predicting the reversibility of TCMR.[4–6] They compared 18s rRNA-normalized log-transformed urinary cell mRNA levels of FOXP3, CD25, CD3E, and perforin in 3 subgroups of patients who participated in the Clinical Trials in Organ Transplantation-04 (CTOT-04): patients who developed acute TCMR; patients who had no pathologic evidence of TCMR (No Rejection group); and patients with average serum creatinine 2 mg/dL who had not undergone allograft biopsy and not required treatment for acute rejection, or cytomegaloviral or BK infection within 12 months of

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Authorship Contribution

1. FMT: Design of the manuscript and interpretation of data. Main role in the drafting of the manuscript, and approval of the final version.

2. LSR: Participated in the summarizing of available data in regard to urinary and tissue FOXP3 mRNA and outcomes on T-Cell mediated rejection. Participated in the drafting of the manuscript and approval of the final version.

3. MCM: Corresponding author. Design of the manuscript and interpretation. Design, Critical revision of the initial draft. In charge of approving the final version.

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transplant (Stable group). Patients with borderline rejection, antibody mediated rejection or BK virus nephropathy were excluded. Median urinary levels of all mRNAs were higher in the TCMR group than the No Rejection or Stable groups. Among 33 patients in the TCMR group, median urinary cell FOXP3 mRNA level (but not other mRNAs) was higher in those with reversible TCMR (i.e. 21 patients with 4-week creatinine within 15% of baseline) than in nonreversible TCMR. An 18s rRNA-normalized log-transformed urinary cell FOXP3 mRNA of −1.33 predicted TCMR reversal with a sensitivity of 75% and specificity of 67%. Interestingly, prospective urinary mRNA trajectory analyses showed that patients with TCMR-reversibility had higher FOXP3 mRNA but a lower CTOT-4 signature score after TCMR treatment, whereas those with nonreversible TCMR had lower urinary FOXP3 mRNA and higher CTOT-4 signature scores (CTOT-04 signature is a composite of urinary 18s rRNA, and CD3E and interferon-inducible protein 10 [IP-10] mRNA previously associated with TCMR[6]). Multiple logistic regression showed urinary cell FOXP3 mRNA level predicted TCMR reversal independent of clinical variables. A predictive model for TCMR-reversibility that combined clinical variables and urinary cell FOXP3 mRNA level outperformed a model with clinical variables or FOXP3 mRNA alone. Importantly, FOXP3 mRNA levels independently associated with improved death-censored graft survival, an association that the authors reported to be mediated through TCMR-reversibility in their adjusted analyses.

The findings by Luan et al. elegantly validate in an independent multicenter prospective cohort their previous report of the association between FOXP3 mRNA and TCMR-reversibility.[5] These findings could have significant potential clinical utility in tailoring the intensity of anti-TCMR therapy, identifying the need for repeat biopsies, and overall prognostication. However, as the authors themselves acknowledge some limitations need to be considered. Patient populations and causes of graft dysfunction in clinical practice are likely to be more heterogeneous than the scenarios examined in the study. Additionally, work with urinary mRNA requires considerable rigor and expertise that the authors' group has repeatedly demonstrated. Hence, the scalability and performance of urinary mRNA profiling in clinical practice will need to be examined. Next, in the predictive model for graft loss, the urinary FOXP3 mRNA was no longer significant when TCMR-reversibility was added in as a covariate in the adjusted models, suggesting that clinically defined TCMR-reversibility is the important determinant of graft survival. However, when applied as a test, the urine FOXP3mRNA data would be potentially available at the time of initial biopsy, without needing to wait 4 weeks to assess clinical TCMR-reversibility. It must also be noted that Banff component scores and DSA data were not used for predictive modelling in this study vs other reports.[7]

Mechanistically, the report by Luan et al. provides further translational clues regarding the role of Regulatory CD4 T-cells (Tregs) in the alloimmune response and TCMR.[8] Though not directly tested here, urinary FOXP3 is a surrogate for intragraft FOXP3 expression and Treg infiltration. Human and animal data have identified the expansion of donor-specific Tregs in immunologic allograft tolerance,[9] and several trials are already testing the utility/safety of ex vivo expanded T-regs in allo-transplantation. The association between urinary cell FOXP3 mRNA and graft outcomes identified here raises fundamental biologic questions that require further inquiry: 1) the reason/s why some recipients vs others, mount more robust Treg

responses during inflammation associated with TCMR,[8] 2) whether improved graft outcome in patients who have reversible TCMR is mediated through regulation of alloimmune injury or facilitation of tissue repair or both by Tregs[10] and 3) the relative role played by the induction of prolonged tolerance to donor antigens during TCMR episodes that extend into the post-TCMR mileu, and its impact on graft outcomes. For instance, the trajectory analyses shown in the current study may imply that within the allograft in cases where TCMR-reversibility was seen, Tregs could preferentially persist beyond the acute TCMR episode while T-effector cells (represented by the CTOT-04 signature in urine) are reduced following the episode. This inference could be tested in future studies that include post-TCMR biopsies and serial Treg assessments. However, as is reflected by the best survival curve in the "Stable" patients in this study as well as in prior work,[4] the absence of inflammation or indeed the need for a biopsy in this patient group, is still better for the allograft than the presence of any inflammatory infiltrate, even if associated with Tregs.

The current report by Luan et al. is an extension of their prior work on urine transcriptomics in kidney transplantation and advances the field of biomarker discovery. Studies that build on their current body of work hold the promise of answering both relevant mechanistic and clinical questions, an exciting future in transplantation medicine.

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