

Fibrosis after Ischemic Injury by Decreased Macrophage Recruitment and Activation," in Vol. 32, Iss. 5, on pages 1037–1052.

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## Authors' Reply

We appreciate Liu and Zhang's interest in our recent study. We agree that, given previous studies by Lassen *et al.*<sup>1</sup> and Lorenz *et al.*,<sup>2</sup> our findings that selective myeloid deletion of interferon regulatory factor 4 (IRF4) decreased development of tubulointerstitial fibrosis<sup>3</sup> after ischemic kidney injury were somewhat unexpected. However, there are two crucial differences between our study and the two previous studies. Whereas we only deleted IRF4 expression in myeloid cells, both Lassen *et al.*<sup>1</sup> and Lorenz *et al.*<sup>2</sup> used mice with global IRF4 deletion, and IRF4 is also expressed in cells of nonmyeloid lineage.<sup>4</sup> In addition, we used a model of moderate kidney ischemia, whereas the ischemic injury in the previous studies was more severe. Therefore, we agree with Liu and Zhang that global IRF4 deletion and a more proinflammatory milieu may overcome the migratory defect in IRF4<sup>−/−</sup> myeloid cells and lead to persistent renal macrophage activation and subsequent fibrosis. Whether or not myeloid IRF4 deletion is deleterious in other models of CKD is an area of ongoing study in our laboratory.

## DISCLOSURES

R.C. Harris reports having consultancy agreements with, and ownership interest in, Bayer; receiving research funding from Bayer; serving on the Bayer Scientific Advisory Board; and having patents and inventions relating to the eNOS db/db mouse.

## FUNDING

None.

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See related letter to the editor, "Heterogenous Role of IRF4 in Kidney Fibrosis," on pages 2971–2972, and original article, "Deletion of Myeloid Interferon Regulatory Factor 4 (Irf4) in Mouse Model Protects against Kidney Fibrosis after Ischemic Injury by Decreased Macrophage Recruitment and Activation," in Vol. 32, Iss. 5, on pages 1037–1052.

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## Need for a Validation Study before Using the Two-Step Algorithm for dd-cfDNA to Screen for Acute Rejection

The study by Bunnapradist *et al.*<sup>1</sup> proposes using a two-step algorithm threshold for donor derived cell free DNA (dd-cfDNA) to increase sensitivity for detection of acute rejection. Although this hypothesis is both tenable and biologically plausible, we have concerns if this study allows for any rigorously derived conclusions. Of the 41 patients in the study, 16 had (for cause) biopsies and 9 had biopsy-proven rejections. The new algorithm detected all nine acute rejections. Even though pre-ordained separate cutoffs were utilized, this is the first study to test this algorithm and thus must be considered as discovery and merely the first of many steps in biomarker assessment and ultimately utilization.<sup>2</sup> In addition, the improved test performance was accompanied by large confidence intervals and thus has a high risk of type 1 error due to the small sample size.<sup>3</sup> Given that many of the rejections were severe, it is unclear if this algorithm would retain this performance in the general transplant population.

The main challenge now will be conducting an adequately powered validation study upholding these results.<sup>2</sup> This can be difficult given the low prevalence of acute

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rejection (especially severe rejections) potentially lowering the positive predictive value. Previous studies assessing the performance of dd-cfDNA may have been underpowered.<sup>3</sup> Other design limitations, including use of serum creatinine alone for comparison and absence of kidney biopsies in all participants could also inflate the specificity.<sup>1,4</sup> A clinically useful test should have a high sensitivity or specificity depending on the goal (rule-in or rule-out rejection). As lowering thresholds to increase sensitivity compromises specificity (and *vice versa*), this goal may be elusive with dd-cfDNA. As long as a true positive or negative is arbitrated by a kidney biopsy, absence of biopsy should not be imputed as no acute rejection. Thus, using a sample size of 16, the new algorithm increases the sensitivity from 78% to 100% but decreases the specificity from 57% to 42%. Dd-cfDNA may be a more accurate measure of kidney injury rather than acute rejection, hence the difficulty in detecting acute rejection.

Thus, despite these promising results, much work remains before advocating using dd-cfDNA as standard of care. Further validation would need to demonstrate greater accuracy than current standards for detecting rejection, keeping in consideration the unique conditions of each patient. In the absence of a well powered validation study using this two-step algorithm, extreme caution is needed before using these methods clinically.

## DISCLOSURES

All authors have nothing to disclose.

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See related, reply on pages 2973–2974, and original article, “Using both the Fraction and Quantity of Donor-Derived Cell-Free DNA to Detect Kidney Allograft Rejection,” in Vol. 32, Iss. 10, on pages 2439–2441.

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## Authors' Reply

We thank Drs. Gupta and Kaplan for their insightful comments. As we noted in our Research Letter, this preliminary report of the novel two-threshold algorithm had a small cohort size and limited confirmatory biopsy specimen data.<sup>1</sup> We are currently in the process of applying this two-threshold algorithm to a larger cohort as part of a prospective, >30-site study with full biopsy matching. This study will contain more cases of biopsy specimen-proven acute rejection than the combined initial validation studies of the two clinically available donor-derived cell-free DNA (dd-cfDNA) fraction assays,<sup>2,3</sup> addressing concerns of inadequate sample size.

We agree that the clinical utilization of diagnostic testing should be evidence driven. The two-threshold algorithm disclosed in our Research Letter did identify several rejections with elevated cfDNA and borderline dd-cfDNA results, and thus may be useful in patients with borderline dd-cfDNA fractions. As such, Natera intends to include both the dd-cfDNA fraction and the absolute dd-cfDNA quantity in our clinical reports. This will allow transplant physicians and surgeons to continue using the dd-cfDNA fraction cutoff as usual, while also having access to the two-threshold algorithm, which may prove valuable in key cases.

We are excited about the promise of biomarkers, especially dd-cfDNA, in kidney transplant surveillance, and agree that the implementation and interpretation of biomarker assays are best supported by solid clinical evidence.

## DISCLOSURES

S. Bunnapradist reports consultancy agreements with CareDx, Taekeda and Natera; research funding from Astellas, CareDx, Merck, Angion, Vitae-ris and Natera, Angion; honoraria from BMS, Veloxis, Caredx, and Sanofi; and speakers bureau from Eurofins, TGI, Veloxis, Caredx, Natera, and Sanofi. H. Tabriziani reports current employment, consultancy agreements,

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