2018 ATS BEAR Cage Winning Proposal: Cell-Free DNA to Improve Lung Transplant Outcomes

The first lung transplantation in 1963 brought hope to patients with end-stage lung disease. In the two decades that followed, short-term survival remained dismal, primarily because of early surgical complications such as anastomosis dehiscence and early post-transplant allograft rejection. With advances in surgical techniques and new immunosuppression regimens, 1-year survival improved to greater than 90%. Unfortunately, long-term survival still remains low, with only 50% of patients surviving 5–6 years after transplant. Chronic lung allograft dysfunction (CLAD), an umbrella term used to describe recognized phenotypes of chronic lung rejection (1), is a leading contributor to poor long-term survival. With no available therapies for CLAD, current management strategies are focused on early detection and treatment of CLAD risk factors, which include primary graft dysfunction, acute cellular rejection (ACR), antibody-mediated rejection (AMR), and community-acquired respiratory viral infections (1–4). The introduction of genomic, proteomic, and other "omic" approaches have reenergized a new wave of innovative research that promises to improve long-term outcomes via early detection of CLAD and its risk factors. One such innovative project focused on assessing the benefit of early detection and treatment of AMR by means of a noninvasive genomic technology—donor-derived cell-free DNA (%ddcfDNA; Figure 1)—and received the 2018 American Thoracic Society (ATS) Building Education to Advance Research (BEAR) Cage Innovation Award.

AMR: Is Diagnosis the Problem?

The improved ability to detect donor-specific antibodies (DSAs) has peeled back a layer exposing AMR as a major reason for the unacceptably high attrition that typically accompanies lung transplantation. In our cohort, even with aggressive treatment, 75% of patients progressed to CLAD or died within 2 years of AMR diagnosis, similar to what was observed in another cohort (5, 6). Modifying the course of AMR may therefore reduce the high rate of CLAD and early death in lung transplant patients. Unfortunately, diagnosis remains a major challenge. AMR is linked to the development of DSAs. On their own, DSAs are nonspecific, as only a fraction of patients with DSAs develop AMR (3, 7). Diagnosis therefore relies on additional confirmatory evidence of associated allograft injury detectable by histopathology or spirometry. Unfortunately, these modalities are unsophisticated at best and limited by poorly defined standards (8), low sensitivities, and/or high interoperator variability (9). These limitations potentially delay AMR diagnosis and expose patients to invasive procedures and excessive costs without a clear benefit. Noninvasive tools with better sensitivities could predict or detect AMR earlier, prompt early treatment, and potentially lead to better outcomes.

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Originally Published in Press as DOI: 10.1164/rccm.201902-0458ED on March 18, 2019

Proposed Solution to Improve AMR Outcomes through Early Detection

We propose that %ddcfDNA provides a noninvasive, reliable, and sensitive blood screening test that can detect allograft injury from AMR earlier than histopathology or spirometry. %ddcfDNA also detects injury from ACR and some infections (10). On its own, %ddcfDNA showed a high sensitivity (100%) but low specificity (∼35%) for detecting AMR. However, when combined with the presence or absence of DSAs, the specificity increased to 90%. The test distinguished DSAs based on the risk of progression to AMR (5). Levels of %ddcfDNA were also 5× higher in AMR than in ACR and 20× higher than at nonrejection time points.

Interestingly, elevations in %ddcfDNA from baseline were detected at a median of 2–3 months before the patients developed any histopathological, spirometric, or clinical manifestations (5). Data for a prototypical patient are shown in Figure 2.

cfDNA is released from dying cells into the circulation and other biological fluids. It is abundant in plasma, with over 10 billion fragments per milliliter. In the transplantation setting, the vastly different genomes of the donor and recipient provide large numbers of distinguishing SNPs, which we leveraged to identify and reliably quantify %ddcfDNA with a great degree of reproducibility (11). The assay has broad applicability across organ transplantation (12), as well as for diagnosing prenatal complications such as trisomies (13) and cancer (14).

Our data indicate that an approach that combines %ddcfDNA and DSA testing can detect impending AMR and provide an earlier time point in the decision tree to intervene. Will early intervention halt AMR-associated injury and progression to CLAD? We intend to address this question via the eDATA (Early Detection and Treatment of AMR) study proposed for the 2018 Bear Cage Innovation Award Competition. The study proposes to compare the rates of CLAD in patients treated early for impending AMR via the use of DSA and %ddcfDNA testing, and in patients treated for clinical AMR diagnosed via usual care (DSA, histopathology, and spirometry). Early detection of AMR via the proposed approach is only effective in circumstances where DSA detection precedes AMR diagnosis. This approach may be ineffective in two circumstances:

1. In ∼10–20% of the patients in our cohorts who received a diagnosis of AMR, DSAs were first detected at AMR diagnosis and not before, making early detection via our approach impossible. Surprisingly, these patients still showed elevated %ddcfDNA for several weeks to months before circulating DSAs and AMR become detectable. The elevated %ddcfDNA without detectable circulating DSAs indicates ongoing allograft injury, which we suspect could be from DSAs adsorbed in the allograft (15). The rising %ddcfDNA levels without identifiable causes may prompt a physician to perform biopsy, DSA testing, and additional testing to guide management.
2. DSAs directed against non-HLA or self-antigens have been associated with AMR (16). Our approach is focused on HLA

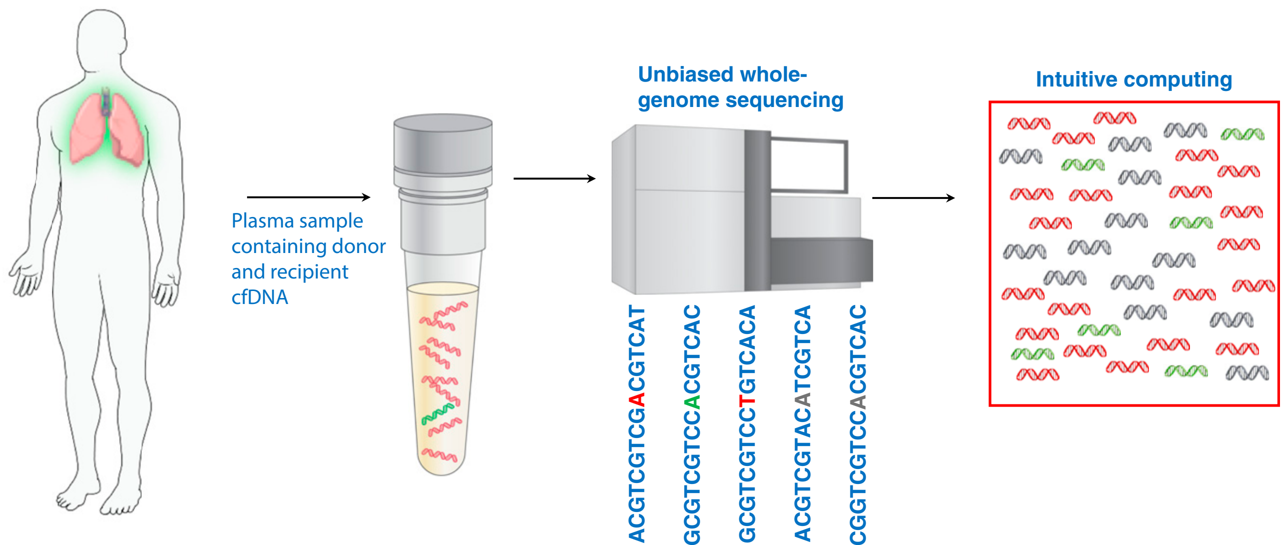

Figure 1. Quantification of donor-derived cell-free DNA (%ddcfDNA). The donor and recipient are genotyped to identify SNPs. After transplantation, recipient plasma is obtained for cfDNA isolation, library construction, and shotgun sequencing. Recipient plasma contains donor (green) and recipient (red) cfDNA, as well as common cfDNA that is indistinguishable between the donor and recipient (gray). Sequence reads are surveyed to identify donor and recipient SNPs. Reads that overlap with these SNPs are used to compute %ddcfDNA. SNPs that overlap with cfDNA reads are used to compute the error rate.

DSAs and can be extended to include non-HLA DSAs as the test becomes clinically available.

The ATS Bear Cage Innovation Award

The ATS BEAR Cage Innovation Award is sponsored by the ATS Drug/Device Discovery and Development Committee. The committee's aim is to spur innovative thinking in basic and translational research, with an emphasis on technology development and application. For this award, early-stage career applicants are asked to submit proposals, and three finalists are selected to make an oral presentation to a committee of experts from industry and academia at the ATS annual meeting. By bringing expertise from industry and academia, the ATS BEAR Cage Innovation Award Committee provides a rare opportunity for early-stage investigators to engage different and often contrasting approaches to the same scientific problem—an interaction that is rare in academia. This encounter has broadened my scope and provided practical insights that have reshaped my thinking and clinical study design. Winning this award has given a significant boost to my career and attracted essential collaborations. Furthermore, in my view, the selection of this proposal by a panel of academic and industrial leaders validates the potential of this novel genomic approach to reshape clinical practice and studies even in conditions beyond transplantation. ■

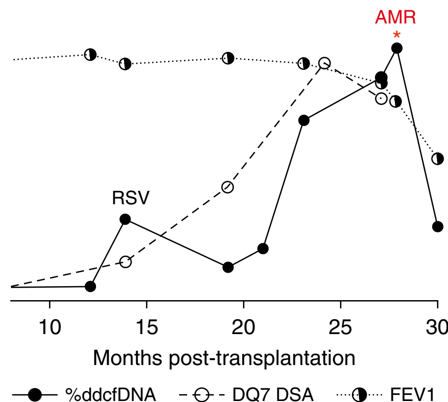

Figure 2. Donor-derived cell-free DNA (%ddcfDNA) elevation precedes antibody-mediated rejection (AMR) diagnosis. Data for a prototypical patient with a diagnosis of AMR (*) are shown. Trends of %ddcfDNA (solid line), donor-specific antibodies (DSAs; dashed line), and FEV_1 (dotted line) are shown. An episode of asymptomatic respiratory syncytial virus (RSV) detected via routine BAL sampling is also shown.

Author disclosures are available with the text of this article at www.atsjournals.org.

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