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## ⌘ Transforming Diagnostics in Lung Transplantation: From Bronchoscopy to an Artificial Intelligence–driven Approach

Transbronchial biopsies to obtain lung tissue specimens remain the gold standard to identify acute lung allograft rejection. However, bronchoscopic biopsy practices, including biopsy schedule, frequency, follow-up after abnormal results or results suggesting rejection, and the role and composition of a biopsy review panel, differ between transplant centers (1). Because of this heterogeneity, there have been few studies for at least a decade analyzing posttransplant biopsy data collected prospectively from multiple centers. A study by Todd and colleagues (pp. 576–585) published in this issue of the *Journal* is a welcome exception (2).

In this multicenter study, Todd and colleagues present a careful and extensive analysis of 2,026 lung biopsies obtained during surveillance (83.4%) and for-cause (16.6%) transbronchial biopsies from 400 lung transplant recipients to determine the incidence and severity of acute rejection within the first year after transplant with a focus on identifying potential risk factors for acute rejection. Results were obtained from five high-volume transplant centers in North America that used nonidentical but congruous biopsy schedules, which increases the priority of this study. Todd and colleagues report an incidence of acute rejection of 53.3%, with the majority of patients experiencing mild A1 rejection. High-level HLA mismatch between donor and recipient was associated with an increased risk for acute rejection. Double lung transplantation and the use of induction immunosuppression were associated with a decreased risk for acute rejection during the first year after transplantation. When Todd and colleagues normalized for number of biopsies performed during the first year after transplant and analyzed time-independent variables associated with acute rejection, they found that patients with double lung transplantation and patients with fewer than four HLA mismatches continued to have a decreased risk for acute rejection (2).

These results are consistent with previous findings, highly reproducible, and clinically useful based on the solid study design

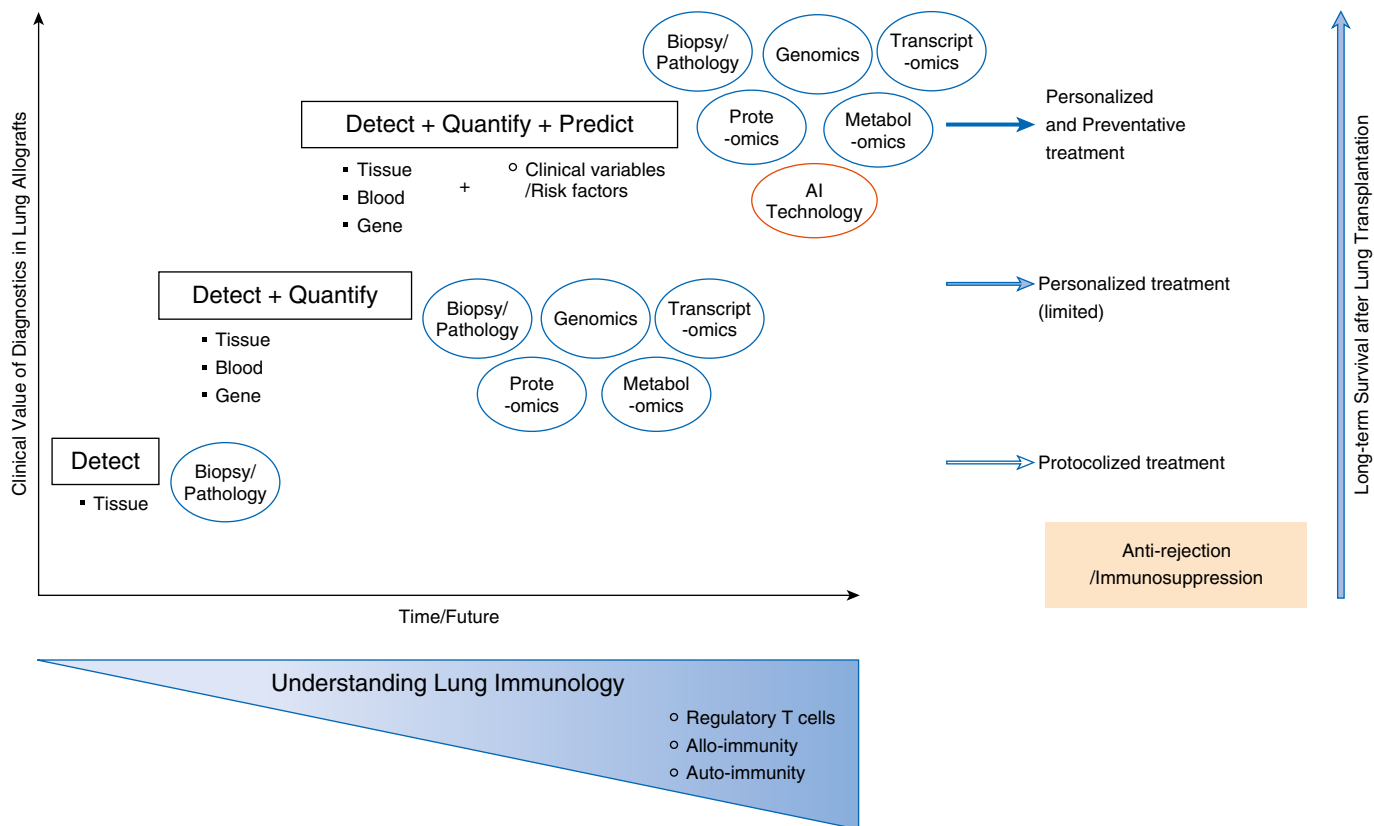
with prospective data collection from multiple centers. However, surveillance transbronchial biopsy has inherent limitations. It is invasive and costly, is subject to sampling errors, and is not capable of anticipating alloimmune events (3). Therefore, new diagnostic venues that can be combined with available pathological data should be explored.

An evolving body of recent evidence consistently supports that antibody-mediated rejection is an important contributor to acute and chronic lung allograft rejection after lung transplantation and that Foxp3<sup>+</sup> regulatory CD4<sup>+</sup> (cluster of differentiation 4–positive) T lymphocytes play a central role in recovery from acute injuries in lung allografts regardless of the cause of the injuries (4, 5). Indeed, since their discovery in 1995, regulatory T cells have been characterized as master regulatory cells with simultaneous, multidirectional functions in immune tolerance that are involved in both innate and adaptive immunity (6–8). These findings should be duly translated into clinical practice in a “bench-to-bedside manner” for assessment of regulatory T-cell function along with the routine tests currently utilized throughout the lung transplant process, including transbronchial biopsies.

Our increased understanding of the underlying immunology along with evolving analytic technologies provide the basis for new surveillance approaches with the aim of better predicting immune-mediated allograft damage that will determine whether the patient will suffer chronic lung allograft dysfunction (CLAD) or be free of CLAD. For instance, noninvasive biomarkers, including regulatory T cells circulating in the blood (9) and immune-cell–based assays that replicate antidonor alloimmune responses *ex vivo* (10), have recently been described and are associated with short-term and long-term transplant outcomes. The evaluation of key cellular events and signaling pathways underlying detectable posttransplant immunologic processes will help to more accurately quantify lung injuries associated with acute rejection in lung allografts. This includes evaluation of acute rejection with biomarkers identified with the evolving “-omics” technologies, including direct genome sequencing, genomics, transcriptomics, proteomics, and metabolomic analyses. Most notably, molecular measurement of gene expression using machine-learning–based microarray analysis has been developed over the last 3 years to overcome the limitations of conventional diagnostics used after abdominal organ transplantation (11, 12).

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**Figure 1.** Clinical value of diagnostics in lung transplant recipients and implications for care. AI = artificial intelligence.

The scientific community should be able to use this evolving artificial intelligence technology in an integrated manner for complex analyses not only of gene transcript data but also combining -omics data with clinical variables or risk factors that may impact transplant outcomes.

In the lungs, immune regulation is more complex than in other solid organs, and the lungs possess their own secondary lymphoid tissue, bronchus-associated lymphoid tissue. Foxp3<sup>+</sup> regulatory CD4<sup>+</sup> T lymphocytes have been very recently found to regulate immune tolerance in lung allografts (4). Diagnostic approaches need to be sophisticated enough to predict lung injuries in transplanted allografts and eventually the incidence of CLAD. By keeping abreast of recent findings detailing the basic immunology in lung allografts after transplantation with a special focus on newer key players, including regulatory T cells, next-generation pulmonary diagnostics should be able to transform the surveillance paradigm from “Detect” to “Detect, Quantify, and Predict” by synchronously analyzing all the translatable data with the assistance of artificial intelligence technology (Figure 1).

We urgently need a strategic approach to validate an accurate predictive model for graft rejection in lung transplant recipients that duly incorporates the crosstalk between immune cells and lung allografts, similar to a model tested for liver transplant recipients (13). Biopsy data remains an integral part of such a model; however, partnering bronchoscopy with evolving technologies should yield diagnostic data that facilitates personalized and

preventative treatments, including immunosuppression regimens, that mitigate CLAD and optimize long-term outcomes after lung transplantation (14). Todd and colleagues should be congratulated for their thorough and important study utilizing a multicenter database with prospective collection of transbronchial biopsy data. Their results and clinical interpretation highlight the significance of acute rejection events in determining outcomes after lung transplantation. This study is one step of many that need to be taken toward overcoming the challenge of suboptimal long-term outcomes after lung transplantation. ■

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## Clarifying the Pulmonary Arterial Hypertension Molecular Landscape Using Functional Genetics

Pulmonary arterial hypertension (PAH) is a complex cardiopulmonary disease that is associated with numerous pathogenetic molecular mechanisms and results in mixed hypertrophic, plexigenic, and fibrotic vascular remodeling of distal pulmonary arterioles. Enhanced clinician awareness and early implementation of multiple PAH-specific therapies have improved the 3-year survival rate to 84% from 52% in the prior era (1). Nonetheless, PAH remains highly morbid, including impaired health-related quality of life that is akin to that of chronic obstructive pulmonary disease, particularly regarding physical inactivity and mental health burden (2). Despite widely heterogeneous pathobiology, approved PAH medical therapies (still) target only nitric oxide, prostacyclin, or endothelin receptor biology. Furthermore, treatment responsiveness to PAH pharmacotherapies is highly variable even under tightly controlled circumstances customary among randomized clinical trials, leaving no doubt that as-yet undiscovered therapeutic targets exist by which to subgroup patients and modify their clinical course.

Precision-based methods for diagnosing and prognosticating PAH have focused largely on single genetic variants. In 2001, Newman and colleagues leveraged the wider availability of gene sequencing to complete an observational cohort study spanning 20 years and reported that a thymine-to-guanine transversion at position 354 in exon 3 of the *BMPR2* gene was present in 18 families with PAH (3). This finding gave rise to the era of hereditary PAH and, ultimately, the description of 17 disease-causing variants (4) and important advances using genetics for PAH diagnosis, prognosis, and family screening (5). However, <30% of patients have single variants in causative genes, and posttranscriptional mechanisms in numerous cell types have been reported in PAH (4). Together, these findings suggest that, akin to other complex disorders, it is unlikely a single sentinel genetic event underlies the entire PAH phenotypic spectrum.

In 1995, findings from the first *bona fide* microarray technology were published by Schena and colleagues using a high-speed robotic printing of complementary DNAs on glass (6). Transcriptomic platforms have expanded greatly since then in both sophistication and availability. Greater reliance on multiplex big data platforms, however, has not necessarily been coupled with definitive progress in understanding the mechanistic basis of disease (7). Indeed, data on differentially expressed genes from array probes have been published widely in PAH, although these outputs do not in and of themselves inform the pathobiological function of specific transcripts, and numerous examples showing an uncoupling between transcript quantity and disease relevance exist.

These shortcomings in PAH science establish the following major objective for our field in the modern era: integrating genetic

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