Evaluation of donor-derived cell-free DNA and peripheral blood gene-expression profiling as non-invasive biomarkers in lung transplant recipients

- Our preliminary data indicate that integrating **dd-cfDNA** and **GEP** might detect allograft injury and characterize underlying immune pathways, even when biopsies remain non-diagnostic.
- This **dual-biomarker strategy might offer a more comprehensive view of the immune landscape**, identifying signatures like reduced B-cell associated gene expression in the presence of DSA, that may reflect localized humoral activity, potentially optimizing rejection management.

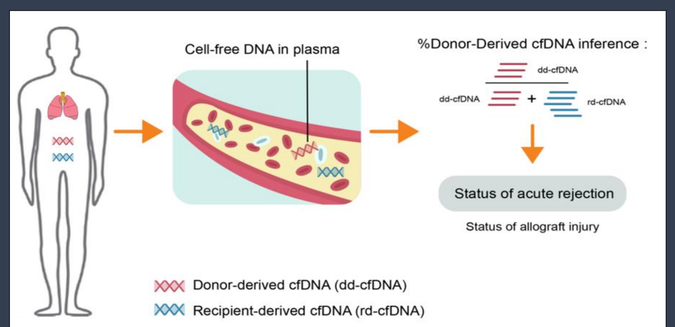
INTRODUCTION

Long-term survival after lung transplantation (LT) is challenged by allograft rejection. Given the limitations of invasive transbronchial biopsies, there is a critical need for sensitive, non-invasive diagnostics. We aimed to evaluate the clinical utility of combining donor-derived cell-free DNA (dd-cfDNA) and peripheral blood gene-expression profiling (GEP) to enhance rejection surveillance and identify subclinical allograft injury.

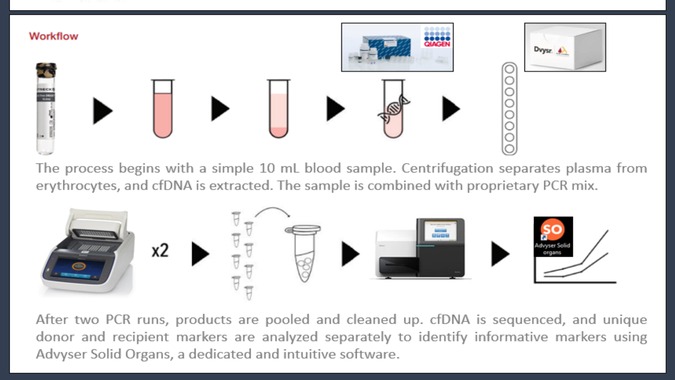
METHODS

We evaluated the clinical utility of these biomarkers in samples collected from lung transplant recipients between December 2023 and December 2025. Serial samples were obtained at multiple time points post-transplantation (24-72h, week one, and monthly post-transplantation). Plasma dd-cfDNA was quantified using the **One Lambda Devyser Accept cfDNA assay**. For GEP, a custom **AmpliSeq PLUS Illumina RNA panel** was utilized to target 17 candidate genes associated with immune activation and rejection (*IL10, NLRP3, CTLA4, CD80, PTX3, BLK, ANXA1, ITGB1, TOLLIP, POU2AF1, CD27, TCL1A, HSP90AA1, CD68, IFI30, FOXP3,* and *CD40LG*). Data were normalized against 4 housekeeping genes (*HPRT1, B2M, ACTB, RPLP0*). Differential expression analysis was performed using **DESeq2** within the **BaseSpace RNA Amplicon v2.0** environment to identify molecular signatures associated with allograft injury.

One Lambda Devyser Accept cfDNA assay



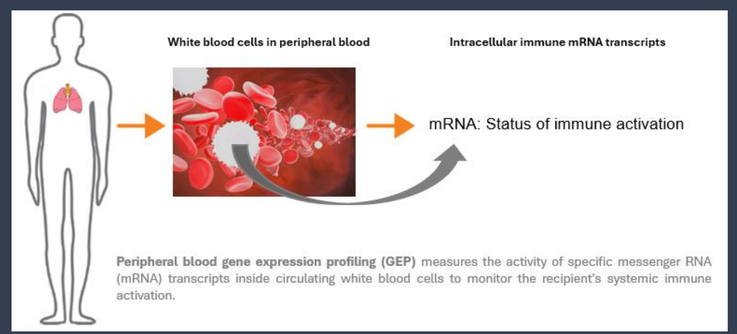
Dd-cfDNA represents fragmented DNA that is continuously released into the bloodstream from the allograft undergoing injury.



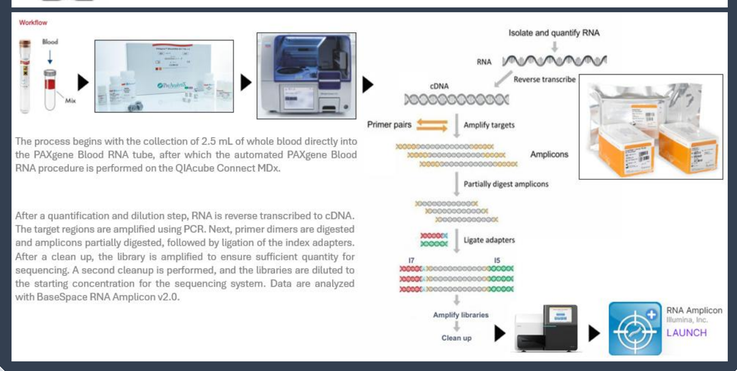
The process begins with a simple 10 mL blood sample. Centrifugation separates plasma from erythrocytes, and cfDNA is extracted. The sample is combined with proprietary PCR mix.

After two PCR runs, products are pooled and cleaned up. cfDNA is sequenced, and unique donor and recipient markers are analyzed separately to identify informative markers using Advyser Solid Organs, a dedicated and intuitive software.

Peripheral blood gene-expression profiling Custom AmpliSeq PLUS Illumina RNA panel



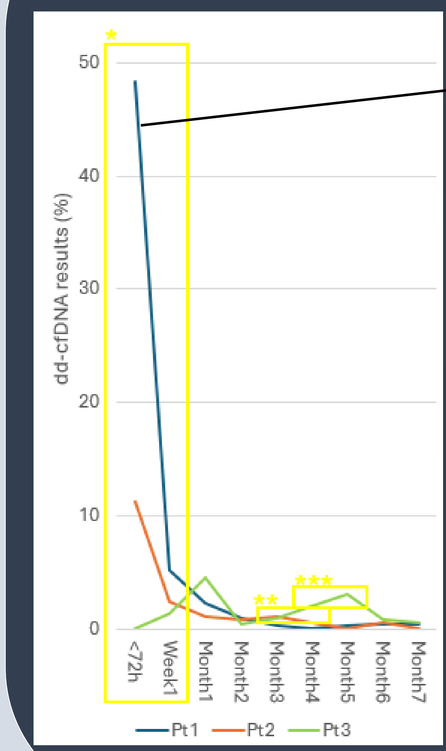
Peripheral blood gene expression profiling (GEP) measures the activity of specific messenger RNA (mRNA) transcripts inside circulating white blood cells to monitor the recipient's systemic immune activation.



The process begins with the collection of 2.5 mL of whole blood directly into the PAXgene Blood RNA tube, after which the automated PAXgene Blood RNA procedure is performed on the QIAcube Connect MDx.

After a quantification and dilution step, RNA is reverse transcribed to cDNA. The target regions are amplified using PCR. Next, primer dimers are digested and amplicons partially digested, followed by ligation of the index adapters. After a clean up, the library is amplified to ensure sufficient quantity for sequencing. A second cleanup is performed, and the libraries are diluted to the starting concentration for the sequencing system. Data are analyzed with BaseSpace RNA Amplicon v2.0.

%dd-cfDNA and GEP results of 3 representative cases at different time points after LT



*** < 1 week after LT: IL-10 downregulation**

→ Pt1: Pneumonia: Elevation of CD27, FOXP3, and CD40LG

**** Pt2**

Month 1	Month 2	Month 3	Month 4
		Suspected clinical rejection with inconclusive biopsies	
Elevated %dd-cfDNA			
			CTLA4 overexpression (cellular activation)

***** Pt3**

Month 1	Month 2	Month 3	Month 4	Month 5
			Suspected clinical rejection with inconclusive biopsies	
Elevated %dd-cfDNA				
Donor-specific anti-HLA antibodies (DSA) detectable				
	POU2AF1 and BLK downregulation			

This B-cell related gene signature aligns with previously identified predictors of chronic lung allograft dysfunction (Danger et al., 2018) and may reflect lymphocyte recruitment from the periphery to the allograft.

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