Improving The Efficiency And Reliability Of Post-Transplantation Monitoring And Prognosis

Donor-Derived Cell-Free DNA (dd-cfDNA)

Introduction

In 1954, the first successful living donor transplant saved the life of a 23- year-old American man who was suffering from chronic kidney failure[1]. Since then, advances in organ transplantation have expanded the capacity to save lives and revolutionized the quality of life for the recipient. Among the most important goals for the transplant community are: increasing the number of patients receiving transplants, improving access to transplantation for all, and improving long-term transplant outcomes. However, despite significant success of the transplant process, there remains opportunity for improvements in long-term allograft survival.

Improved allograft survival may be possible by modernizing laboratory methods used to monitor graft health and viability. Renal allograft function is often monitored by indirect markers of graft health, e.g., serum creatinine and urine protein.

The development of molecular diagnostic techniques has the potential to alter that paradigm and will enable significant improvements in monitoring long-term allograft function. This report is designed to provide education for laboratory professionals and clinicians interested in assessing molecular technologies to better manage their patients.

The transplantation process-like any major surgery-is not without risks. Complications of transplantation include[2]:

- Delayed function of the transplanted organ, which can last up to several weeks
- Wound infection and/or healing complications
- Illness secondary to required post-op immunosuppression
- Allograft rejection
- De novo systemic pathology, such as diabetes or hypertension in renal transplant recipients

One of the most serious complications is transplant rejection, which can lead to organ loss and even patient death[3]. Allograft donors and recipients are matched by blood group and HLA type. While an HLA-identical organ is ideal between an allograft recipient and donor, it is not always possible. When the recipient's immune system identifies non-self antigens in the allograft, an immune response is induced. Rejection occurs when inflammation and specific pathologic changes take place in the allograft and if uncontrolled, can destroy the graft. Rejection of transplanted organs can occur as early as within the first post-operative week, or months or years after.

Allograft rejection falls into two categories: acute and chronic and depending on the histopathology and immunological characteristics, is further classified by the mechanism of rejection, namely, antibody-mediated rejection (ABMR) and T-cell mediated rejection (TCMR).

Acute Rejection

The most common form of rejection is acute rejection that occurs within days or weeks of the transplantation[4]. It is characterized by a primary allogeneic and an innate response. As the innate response is triggered by ischemic injury, nearly all patients experience at least some level of acute rejection. The allogeneic response, on the other hand, is initiated by the recognition of foreign antigens present within graft tissue. Detection of acute rejection requires diligent surveillance, which should be initiated soon after transplantation.

Chronic Rejection

While acute rejection develops relatively quickly after transplantation, chronic rejection occurs months to years post-operatively[4], and is often described as accelerated organ aging. Chronic inflammation and other immune responses play roles in chronic rejection. The development of chronic rejection

is characterized by T-cell activation, cytokine production, alloantibody production, and activation of complement pathways[5]. Ultimately, chronic rejection can lead to vascular injury and graft loss.

Challenges In Post-Renal Transplantation Monitoring

Complex Protocols

• Current surveillance options for allograft injury and immune response, such as serum creatinine, proteinuria, elevated donor-specific antibody (DSA), and BK virus are informative[6] but highlight the need for earlier detection.

• These markers often indicate significant progression of graft injury potentially leading to graft loss, reduced quality of life, the need for repeat transplant, and high economic burden. Indicators such as dd-cfDNA can lead to earlier diagnosis confirmed by de novo DSA (dnDSA), biopsy, and MMDx Kidney.

Early, Frequent Monitoring

With a global shortage of organs available for donation, there is a significant benefit in using rejection detection methods that maximize post-operative success and improve patient quality of life. Implementing early and frequent monitoring ensures that rejection is identified swiftly and reliably, both improving outcomes and saving lives.

Methods of Detecting Allograft Rejection

Invasive Biopsy

This standard for diagnosis of rejection is invasive and often accompanied by patient discomfort.

Complications

Post-transplantation biopsies may lead to complications[7].

Blood Test

Blood tests are less invasive, time-consuming, and costly than biopsies. The use of blood tests to measure dd-cfDNA to detect injury with the suspicion of rejection earlier than biopsies can help guide clinicians on further investigative and diagnostic treatment[8].

cfDNA: A Closer Look

During cell injury and cell death, intracellular DNA is released into the bloodstream at which point it is called cell-free (cf) DNA[3]. cfDNA has been used as an effective analyte in maternal-fetal medicine and oncology. In organ transplant recipients, cell injury and death within the transplanted organ produces donor-derived (dd) cfDNA, a highly sensitive biomarker which can detect allograph injury. dd-cfDNA is cleared from circulation within 15-90 minutes of release from the cell; thus, it is a virtually immediate read-out of graft status.

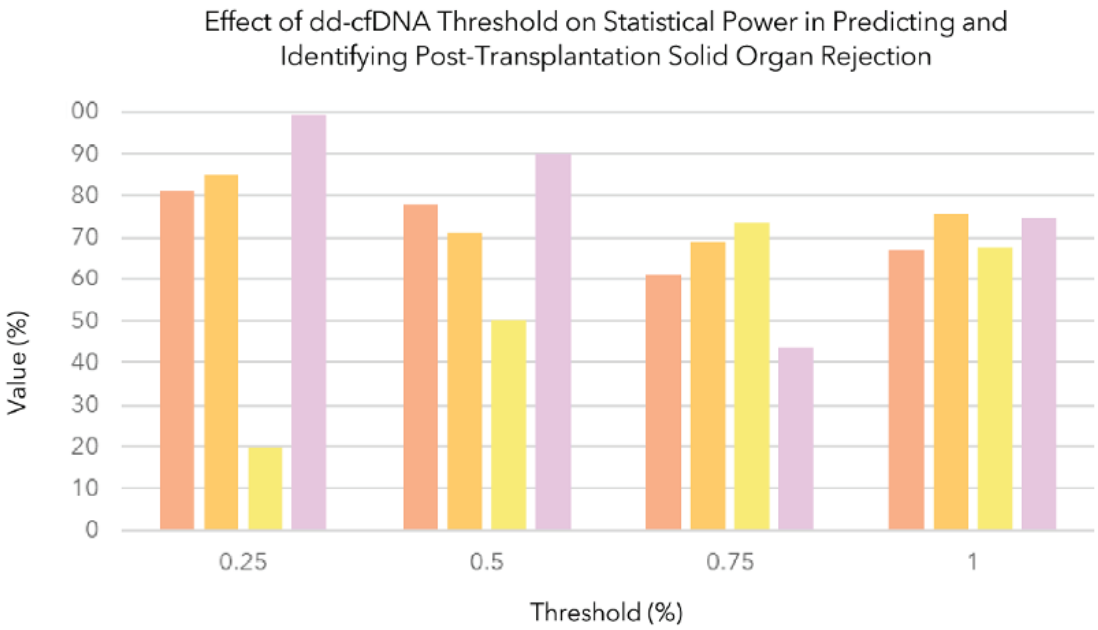
To quantify the extent of occurring cell death, dd-cfDNA must be differentiated from the recipient's own cfDNA. Methods exist to differentiate between donor and recipient DNA that exploit the genetic variation between individuals, e.g., single-nucleotide polymorphisms (SNPs) and insertions and/or deletions (indels).

Role of dd-cfDNA in Predicting Graft Injury and Rejection

Foundational Research

Study	Key Findings
Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Active Rejection in Kidney Transplant Receipients (DART)[6]	• Bloom et al. correlated cfDNA levels with allograft rejection as determined via histology. • cfDNA levels were able to discriminate between controls and biopsies showing any rejection. • PPV and NPV of cfDNA to detect active rejection were 61% and 84%, respectively.
Assessing Donor-Derived Cell-Free DNA Monitoring Insights of Kidney Allografts with Longitudinal Surveillance (ADMIRAL)[9]	• Bu et al. showed that elevations in cfDNA--0.5% or more--were significantly correlated with clinical and subclinical allograft rejection. • cfDNA elevation was associated with nearly a three-fold increase in the risk of developing de novo donor-specific antibodies (DSA) and could be detected at a median of 91 days earlier than DSA identification.
Donor-Derived Cell-Free DNA (cfDNA) for Detection of Allograft Rejection in Pediatric Kidney Transplants[10]	• Puliyanda et al. demonstrated clinical utility of dd-cfDNA in pediatric patients, a population especially sensitive to the need for repeat biopsies. • Higher dd-cfDNA levels were detected in DSA-positive recipients compared to those who were negative or had AT1R positivity alone. • dd-cfDNA >1.0% was diagnostic of rejection with a sensitivity of 86% and specificity of 100%.
Clinical Validation of a Plasma Donor-Derived Cell-Free DNA Assay to Detect Allograft Rejection and Injury in Lung Transplant[11]	• Rosenheck et al. reported median dd-cfDNA fraction was significantly higher for acute cellular rejection, antibody-mediated rejection, allograft infection, neutrophilic-responsive allograft dysfunction, and chronic lung dysfunction than in patients who did not exhibit allograft rejection.
Early Experience Using Donor-Derived Cell-Free DNA for Surveliance of Rejection Following Simultaneous Pancreas and Kidney Transplantation[12]	• Williams et al. demonstrated that dd-cfDNA differentiated rejection from graft injury in patients who received simultaneous pancreas and kidney transplants. • Among patients who did not experience rejection, 97% had dd-cfDNA below 0.5%.
Donor-Derived Cell-Free DNA Accurately Detects Acute Rejection in Lung Transplant Patients, A Multicenter Cohort Study[3]	• Jang et al. found that dd-cfDNA was 6 times higher as compared to controls. • dd-cfDNA levels correlated with severity of lung function decline and histopathological grading of rejection. • Histopathology was only able to detect one third of episodes with cfDNA levels over 1.0%, even though 90% of them were coincident to clinical complications missed by histopathology.
Noninvasive Detection of Graft Injury After Heart Transplant Using Donor-Derived Cell-Free DNA: A Prospecitve Multicenter Study[13]	• Khush et al. quantified and correlated dd-cfDNA levels to paired events of biopsy-based diagnosis of rejection. • dd-cfDNA levels were elevated three-fold in patients with antibody-mediated rejection (AMR) compared to those without AMR.
Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection[14]	• de Vlamnick et al. reported the utility of dd-cfDNA in cardiac transplant patients is a powerful and informative alternative to endomyocardial biopsy • Other potential benefits compared to biopsy included: reduction in risk, discomfort, and expense.

Determination Of Thresholds In dd-cfDNA Detection



Various studies have sought to determine the optimal threshold for dd cfDNA in detecting allograft rejection. The graph above and corresponding data table summarize the findings of several key publications. Results vary by study and are influenced by factors such as type of rejection and sample size. Zhang et al. (2020) report the optimal threshold to be 0.25; Bu et. al (2021) report a threshold of 0.5%; Murad et al. (2022) report 0.75%. The data corresponding to a threshold of 1.0% represent mean findings of Zhang et al., Bu et al., Murad et al., and Huang et al. (2019), and the different thresholds represent the balance between sensitivity and specificity.

dd-cfDNA Threshold {%)	0.25	0.5	0.75	1.0
Sensitivity (%)	81.0	**78**	**61.02**	**67.05**
Specificity (%)	85.0	**71.0**	**69.05**	**75.71**
PPV (%)	19.6	**50.0**	**73.47**	**67.64**
NPV (%)	99.2	**90.0**	**43.4**	**74.7**

Thermo Fisher Scientific and Devyser: Fulfilling A Demonstrated Need

The use of dd-cfDNA for early detection of signs of allograft injury or rejection has been demonstrated. For renal transplant patients in particular, there is pressing need for affordable and accessible surveillance methods. Improved long-term allograft survival is critical, and will unlock global cost savings, ultimately facilitating enhanced access to transplants for more patients.

One Lambda Devyser Accept cfDNA is a method of injury monitoring which can directly impact patient care and surgical outcomes.

One Lambda and Devyser strive to innovate in the space of transplant diagnostics and advocate for ongoing study of these tools to further clarify their utility and cost-effectiveness.

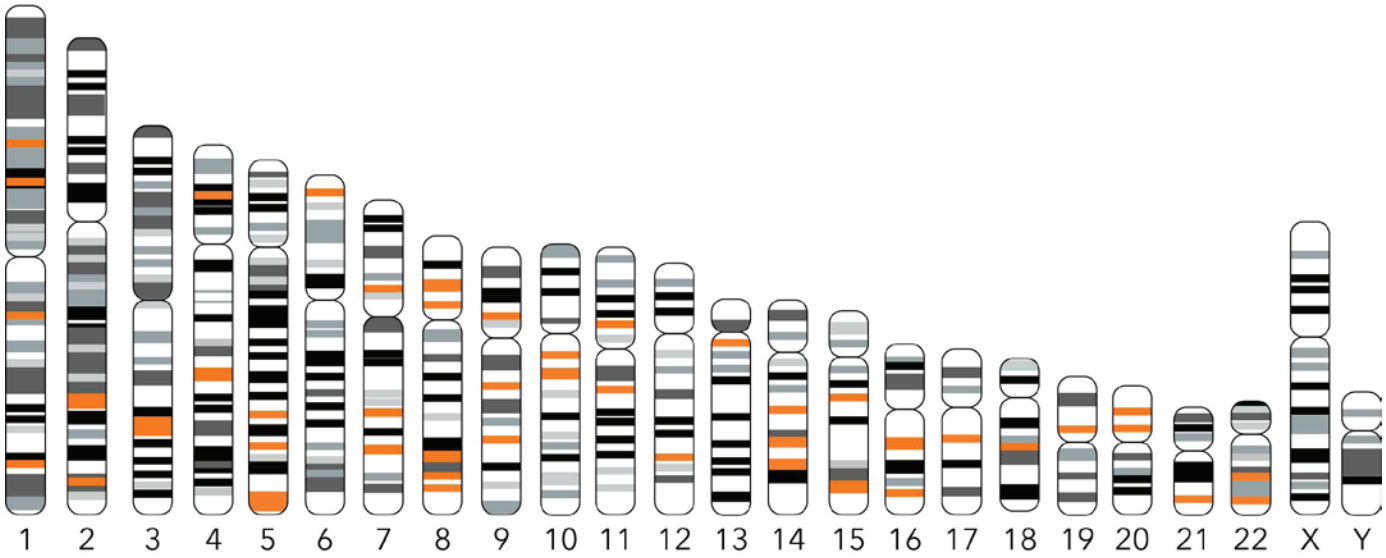
The One Lambda Devyser Solution

A single-tube NGS assay for detection of dd-cfDNA.

	One Lambda Devyser Accept cfDNA	Competitor Alternative
Samples per run	Up to 50	24
Workflow	<45 min hands-on time	1.5h hands-on time
Design	50 indels	202 SNPs

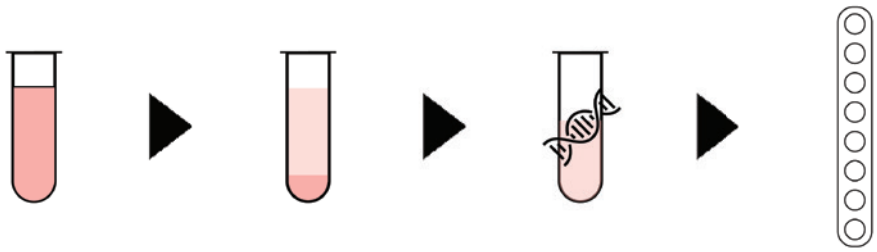
Products are CE marked but not 510(k)-cleared.

One Lambda Devyser Accept cfDNA offers a sensitive, reproducible NGS-based assay. It is a population-independent assay, developed and validated in accordance with IVDR requirements.

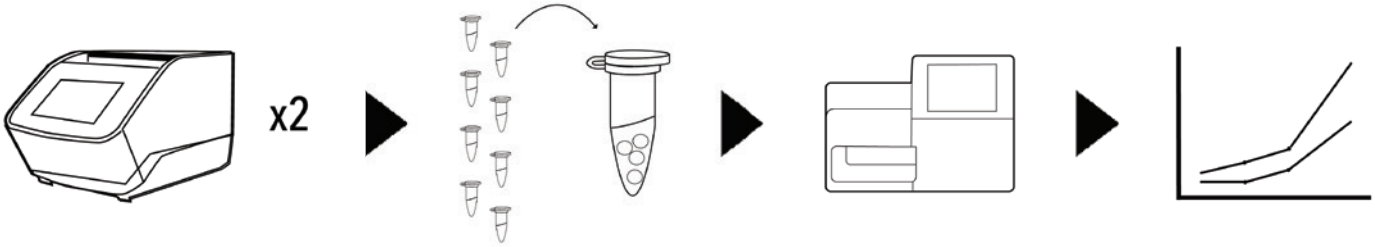


Highly discriminative, 2-6 bp indel markers on autosomal chromosomes provide robust results.

Workflow



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.

The One Lambda Devyser Accept cfDNA Assay and Advyser Software Bring a Sensitive Graft Injury Detection Method to the Market

Post-Transplantation Monitoring: Cost Considerations

Long-term outcomes for renal transplant recipients are currently suboptimal, with approximately 20% of allografts failing within 5 years post-transplantation[15]. There is strong evidence demonstrating the need to detect and treat the incidence of allograft earlier to reduce graft damage and prolong transplant survival. This benefits not only patient outcome, but also alleviates the global economic burden imposed by graft failure. Whilst being highly cost effective in terms of dialysis savings, transplantation initially incurs a heavy financial burden including transplantation cost and post-operative costs such as immunosuppressant medications which are highest in the early post-transplant period. The need for repeat transplantation compounds these costs, both for individual patients and society, and are in addition to the cost of lost labor productivity from the recipient. In the United States, failure of renal transplants incurs nearly $80,000 USD in unnecessary cost within the first year of return to dialysis[16]. In Europe, comparable costs range from approximately €19,000 to nearly €40,000, depending on the country. This cost is mainly comprised of hospitalization and medication expenses within the first year; within the first three years, dialysis is the leading cost of post-transplantation expenses. Over a lifetime, models have demonstrated that graft failure incurs a lifetime medical cost of more than $1 billion USD, and close to 30,000 quality-adjusted life-years[16]. Of note, Medicare beneficiaries who receive renal transplants are eligible for coverage of dd-cfDNA surveillance; utilizing these benefits and technology to detect early injury potentially leading to significant financial savings.

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