Whitepaper

Advancing kidney transplant outcomes through dual-donor cfDNA monitoring

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The promise of cfDNA monitoring

Kidney transplantation is a life-saving procedure for patients with end-stage renal disease. A huge demand exists for donor kidney organs. In the US, approximately 100,000 people are on the waiting list to receive a kidney organ transplant. 20 individuals on this list die each day before receiving a transplant. Despite a yearly 5% increase in demand for donor kidney organs, the available quantity has remained relatively stagnant (Bastani, 2020). Kidney transplantation offers crucial, life-saving intervention, but patients are still faced with the challenge of graft rejection and failure post-transplantation. Now, more than ever before, there is a great need for successful kidney transplantations. Aside from preventing patient morbidity and mortality, the aim is also to prevent the patient being re-listed on the transplant waiting list and prevent further decreasing the number of available donor organs. Allograft failure and rejection in the case of kidney transplantation is incredibly costly on a personal and societal level, with an associated cost in the US of $80,000 within the first year alone (Sussell et al., 2020).

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Managing post-transplant complications, particularly graft rejection, is critical for long-term transplantation success. Nearly 20% of kidney transplants fail within 5 years, and many of these patients suffer further morbidity in the form of graft intolerance syndrome. They may be forced to undergo high-risk transplant nephrectomy (removal of the transplanted organ) (Hindi & Harb, 2023). Traditional monitoring methods, such as serum creatinine levels and invasive biopsies, have limitations in sensitivity and timeliness (Verhoeven et al., 2018). Around 2% of patients who receive invasive biopsies as part of post-transplantation monitoring suffer major complications (Morgan et al., 2016). Invasive biopsies are uncomfortable for patients and, due to their cost and risks, cannot be performed regularly. In recent years, cell-free DNA (cfDNA) has emerged as a new and promising biomarker and tool for detecting and monitoring organ damage and graft rejection. cfDNA are fragments of DNA released into the bloodstream from cells undergoing apoptosis and can be detected in a blood sample.

As discussed, traditional monitoring methods often lack sensitivity, have significant turnaround times, can be invasive, and contain additional risks. Monitoring donor-derived cell-free DNA (dd-cfDNA) released from the donor allograft represents a non-invasive biomarker that could provide early indications of graft health. Based on recent research, this whitepaper explores the innovative concept of dd-cfDNA monitoring and its potential to revolutionize post-transplantation care. One major challenge for effective dd-cfDNA monitoring is the ability to distinguish between sources of dd-cfDNA in a single individual. This whitepaper will explore promising new technology that can tackle this obstacle.

Understanding cfDNA

cfDNA originates from the breakdown of cells, releasing small DNA fragments into the bloodstream. The exact mechanism by which cfDNA is released is not known. However, it is suspected that cell death, necrosis, and the immune response are all involved in cfDNA release

(Moreira et al., 2009). In organ transplantation, dd-cfDNA has been demonstrated as a more sensitive tool for monitoring organ health post-transplantation for several years (Huang et al., 2019). dd-cfDNA monitoring can detect graft damage at subclinical levels, allowing for possible early intervention and a chance to prevent graft failure and rejection (Oellerich et al., 2019, 2021).

One of the most significant challenges with post-transplantation monitoring is detecting graft injury early enough to instigate meaningful clinical interventions. dd-cfDNA monitoring has greater sensitivity than serum creatinine monitoring. It has greater predictive power for antibody-mediated rejection (ABMR) than the detection of donor-specific anti-HLA antibodies (DSA) (Halloran et al., 2023). Therefore, it promises early intervention and treatment, saving the organ from rejection and preventing injury. The diagnostic information provided by dd-cfDNA monitoring offers a more personalized approach to treatment, preventing unnecessary graft biopsies or nephrectomies (Oellerich et al., 2019). cfDNA circulates in the blood and requires only a blood sample for testing, allowing for more frequent monitoring than a biopsy.

Dual-donor dd-cfDNA monitoring

Whilst dd-cfDNA monitoring offers significant promises in improving transplant patient outcomes, it does come with challenges. One of the most significant is in the case of dual-donor transplantation patients. The proportion of patients who receive more than one kidney transplant has been increasing (Magee et al., 2007). In 2021, in the US, 10% of all kidney transplant recipients had received a transplant previously (Lentine et al., 2023). In most of these patients, the first kidney will not be explanted. The challenge is determining the correct source of the dd-cfDNA, as the biomarker could persist for years following transplantation. dd-cfDNA could be released persistently or intermittently following transplantation and be incorrectly attributed to a new allograft.

A new NGS-based method for dd-cfDNA monitoring

A recent study demonstrated that One Lambda Devyser Accept cfDNA has the capability to distinguish between two donor sources of dd-cfDNA (Pettersson et al., 2024). The NGS (next-generation sequencing)-based method utilizes a pre-step of genomic DNA screening of donors and the recipient. The screening of 50 indel markers allows for accurately determining the source of dd-cfDNA without overestimating the fraction of cfDNA that originates from either donor and lowering the background, which results in a highly sensitive assay.

The study, "Dual-Donor Cell-Free DNA Monitoring in Kidney Transplant Patients," was conducted by a collaboration between Dr. Jakob Nilsson, Head of Transplant Immunology at the University of Zurich, and the R&D team at Devyser. It aimed to evaluate the effectiveness of dual-donor dd-cfDNA monitoring in patients undergoing two sequential kidney transplants. A prospective cohort design was used, with the addition of artificial in vitro-generated mixed samples.

Blood samples were collected at predefined intervals post-transplantation. cfDNA was extracted and analyzed using next-generation sequencing to quantify donor-derived cfDNA levels. Fastq files were analyzed with the Advyser Solid Organs software to obtain the dd-cfDNA values fractions from each patient. The data were further analyzed using statistical models to assess the correlation between cfDNA levels and clinical outcomes, including biopsy-confirmed rejection episodes.

Key findings

The study demonstrated that dual-donor dd-cfDNA monitoring provided superior sensitivity and specificity in detecting graft rejection compared to traditional methods. Key findings from the study included:

- The assay was highly accurate in identifying the correct source of the dd-cfDNA.
- In 20% of the clinical samples, dd-cfDNA was present from the unsuccessful organ transplant.
- The quantity of dd-cfDNA from the previous kidney transplant fluctuated between 0.1-0.6%.
- One clinical sample contained detectable amounts of dd-cfDNA from a failed graft that was removed 8 years before the study.

A single participant had detectable dd-cfDNA from a graft that was explanted 8 years prior. In 13 other participants where the first transplant was explanted, dd-cfDNA was not detected from the removed allograft.

Dual-donor dd-cfDNA monitoring

dd-cfDNA monitoring has been proven to have greater sensitivity than standard methods of post-transplantation monitoring. This greater sensitivity may allow organ rejection and damage to be detected earlier. The study demonstrated that One Lambda Devyser cfDNA Accept has the capability of offering increased sensitivity in addition to the ability to distinguish between sources of dd-cfDNA.

Challenges and considerations

Most immunological laboratories store patient and donor DNA. However, the complexity of dd-cfDNA analysis requires advanced laboratory infrastructure and expertise, which may limit its immediate widespread adoption. The cost of dd-cfDNA testing could be a barrier, although further studies will be needed to compare this monitoring method to current post-transplantation monitoring methods. cfDNA Developing standardized protocols and guidelines will be crucial for consistent and accurate dd-cfDNA monitoring across clinical settings. Other studies have demonstrated that cfDNA is produced from other allograft surgeries, such as liver transplantation (Schütz et al., 2017).

Determining the source of dd-cfDNA is vital in using as a tool for monitoring dd-cfDNA for allograft health monitoring in individuals with several different transplanted organs. By the same measure, further studies are needed to determine if such variables affect the detection of dd-cfDNA. Other factors such as infection, inflammation, and patient-specific variables may affect detected cfDNA levels, necessitating careful interpretation of results. Large-scale, multicenter, longitudinal studies are essential to assess the long-term impact of dd-cfDNA monitoring on graft survival and patient quality of life.

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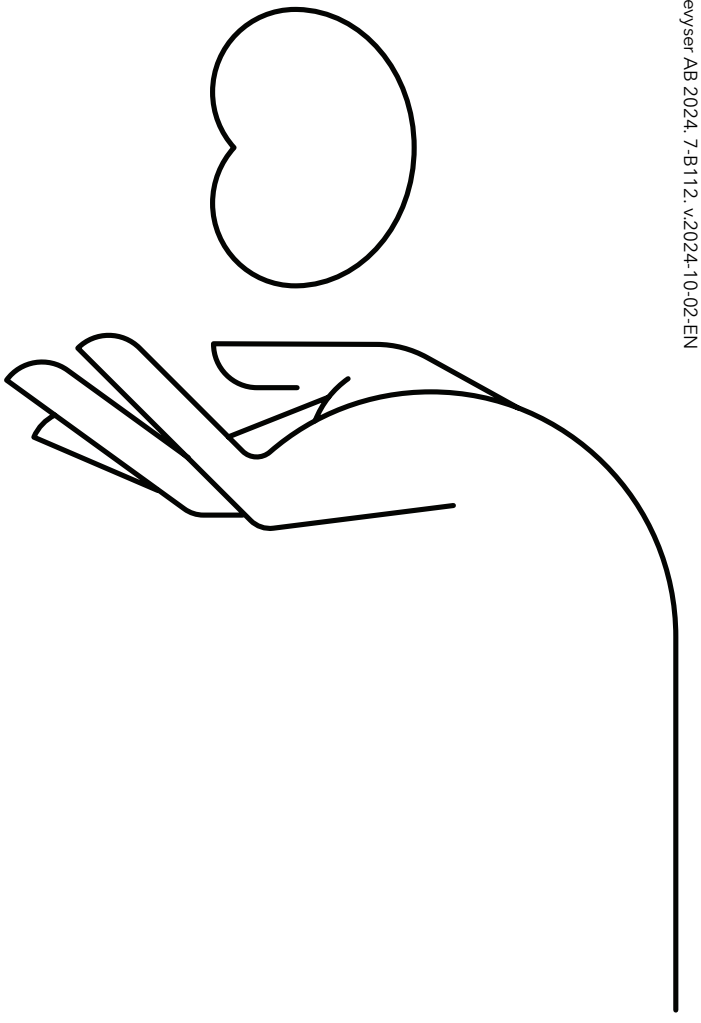
Conclusion

dd-cfDNA monitoring represents a significant advancement in the field of kidney transplantation. Offering a non-invasive and sensitive method for early detection of graft rejection can potentially improve patient outcomes and transform post-transplant care. With its high sensitivity, this method could reduce the reliance on invasive biopsies, minimizing patient discomfort and associated risks. Tailored immunosuppressive therapy adjustments based on dd-cfDNA levels could improve graft survival and patient outcomes. Implementing dd-cfDNA monitoring in routine post-transplant care could enhance current practices, though standardization and clinician training are necessary.

The study discussed in this whitepaper validates the feasibility of dd-cfDNA monitoring to differentiate between sources of dd-cfDNA (Pettersson et al., 2024). The study even highlighted the challenge faced by dd-cfDNA monitoring, as the assay detected dd-cfDNA from an organ explanted 8 years previously. The NGS-based methodology allows for scalable deployment of the technology, with the ability to analyze multiple samples with a turnaround time acceptable for clinical applications.

cfDNA release has been detected from liver and lung transplants (Agbor-Enoh et al., 2018; Schütz et al., 2017). Therefore, the capability of discerning different sources of dd-cfDNA also offers interesting possibilities for using this technology in patients with multiple transplanted organs. Exploring the utility of dd-cfDNA monitoring for these organs would further expand the clinical utility of this technology, although further research is needed in this field.



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