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Advancements in noninvasive techniques for transplant rejection: from biomarker detection to molecular imaging

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Abstract

Transplant rejection remains a significant barrier to the long-term success of organ transplantation. Biopsy, although considered the gold standard, is invasive, costly, and unsuitable for routine monitoring. Traditional biomarkers, such as creatinine and troponin, offer limited predictive value owing to their low specificity, and conventional imaging techniques often fail to detect early organ damage, increasing the risk of undiagnosed rejection episodes. Considering these limitations, emerging noninvasive biomarkers and molecular imaging techniques hold promise for the early and accurate detection of transplant rejection, enabling personalized management strategies. This review highlights noninvasive biomarkers that predict, diagnose, and assess transplant prognosis by reflecting graft injury, inflammation, and immune responses. For example, donor-derived cell-free DNA (dd-cfDNA) is highly sensitive in detecting early graft injury, whereas gene expression profiling effectively excludes moderate-to-severe acute rejection (AR). Additionally, microRNA (miRNA) profiling enhances the diagnostic specificity for precise AR detection. Advanced molecular imaging techniques further augment the monitoring of rejection. Fluorescence imaging provides a high spatiotemporal resolution for AR grading, ultrasound offers real-time and portable monitoring, and magnetic resonance delivers high tissue contrast for anatomical assessments. Nuclear imaging modalities such as single photon emission computed tomography and positron emission tomography, enable dynamic visualization of immune responses within transplanted organs. Notably, dd-cfDNA and nuclear medicine imaging have already been integrated into clinical practice, thereby demonstrating the translational potential of these techniques. Unlike previous reviews, this work uniquely synthesizes advancements in both noninvasive biomarkers and molecular imaging, emphasizing their complementary strengths. Biomarkers deliver molecular-level insights, whereas imaging provides spatial and temporal resolution. Together, they create a synergistic framework for comprehensive and precise transplant monitoring. By bridging these domains, this review underscores their individual contributions and collective potential to enhance diagnostic accuracy, improve patient outcomes, and guide future research and clinical applications in transplant medicine.

Keywords Transplant rejection, Noninvasive monitoring, Biomarkers, Molecular imaging

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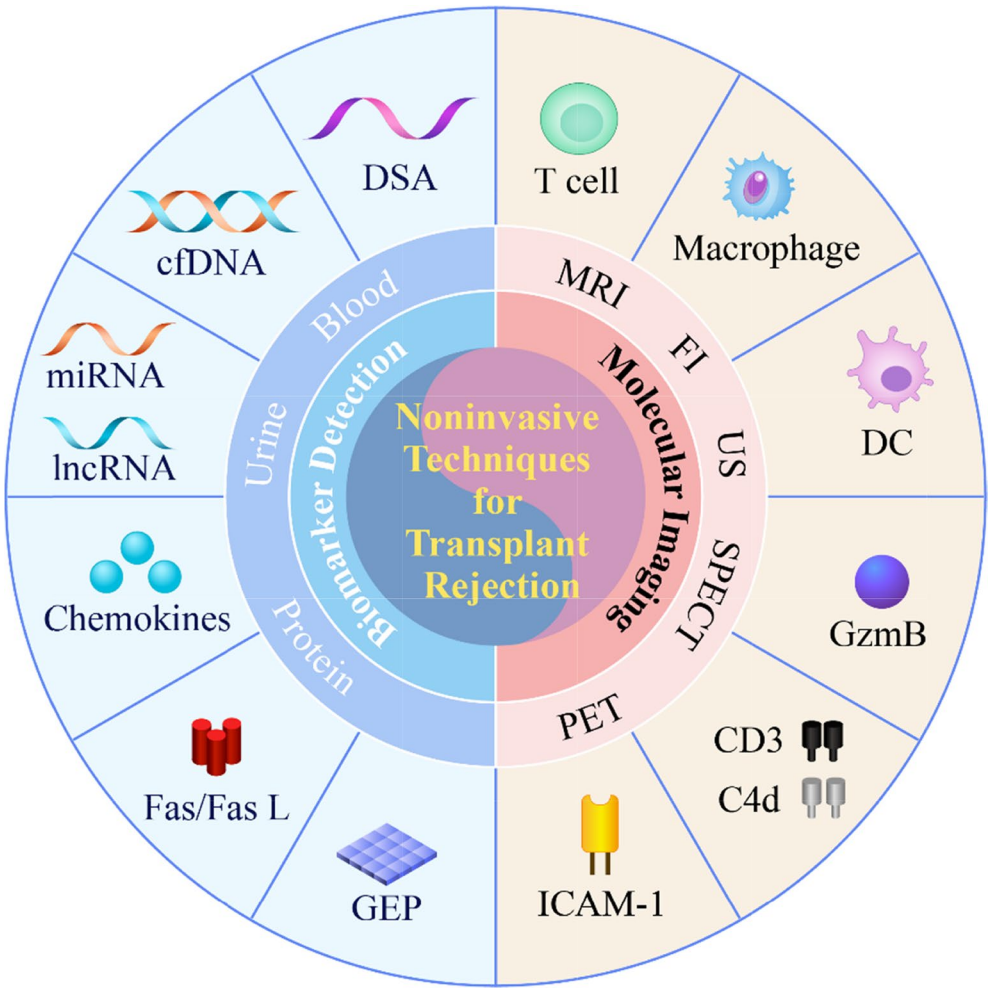
Introduction

Organ transplantation remains the definitive therapeutic option for end-stage organ failure. However, post-transplant grafts are susceptible to immune-mediated injuries, collectively referred to as transplant rejection [1, 2]. This process is initiated when the recipient's immune system recognizes the allograft as foreign, triggering a series of immune responses that can compromise graft function and survival [3]. Transplant rejection is generally classified into three primary types: hyperacute, acute, and chronic rejection [4]. Hyperacute rejection occurs within minutes to hours after transplantation due to pre-existing antibodies in the recipient's bloodstream, often directed against donor human leukocyte antigen (HLA) [5]. These antibodies activate the complement cascade, leading to endothelial damage, thrombosis, and ischemia, resulting in immediate graft failure. Acute rejection typically develops within days to weeks after transplantation and is primarily mediated by T lymphocytes [6]. $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells recognize donor antigens presented by the recipient's major histocompatibility complex (MHC), initiating inflammatory and cytotoxic responses that can damage the graft. Chronic rejection is a slow, progressive process that develops over months or years and is characterized by ongoing inflammation, vascular damage, and fibrosis within the graft [7]. This form of rejection typically leads to irreversible damage to the graft's microvasculature and parenchymal cells, eventually resulting in graft failure. Additionally, some studies have identified subacute rejection, a less common form that exhibits a mild yet progressive inflammatory response, lying between acute and chronic rejection [8]. Understanding the pathophysiology and immunology of these rejection types is critical for the development of effective monitoring strategies and therapeutic interventions to improve long-term graft survival and patient outcomes.

For the past five decades, monitoring transplant rejection has become a central focus in the field of transplantation, with the aim of accurately diagnosing various types of rejection and enabling timely and effective intervention [9]. The classic diagnosis of transplant rejection relies on clinical assessment and biopsy, while biomarkers and imaging techniques are adjunctive tools for identifying graft damage. Organ biopsy remains the gold standard for diagnosing transplant rejection, as it enables direct tissue examination. However, this invasive procedure is uncomfortable and inconvenient for patients and carries the risk of complications, such as a 3.5% incidence of gross hematuria following kidney biopsy [10]. Furthermore, biopsies are expensive, time-consuming, and subject to bias and subjectivity [11]. Traditional biomarkers such as creatinine (Cr) and blood urea nitrogen are commonly used to assess graft function, particularly for kidney transplants. While these biomarkers provide some information on graft function, they lack specificity for transplant rejection and may be influenced by factors unrelated to immune-mediated injury, such as dehydration or drug toxicity [12]. Conventional imaging techniques can help identify structural changes in the graft and blood flow alterations [13]. However, these methods often fail to detect early rejection events or subtle changes in graft function and may not accurately reflect the underlying immunological processes. Some imaging examinations are invasive, such as angiography, which is often required to assess the prognosis after heart transplantation and provides limited information on arterial wall thickness. [14] Moreover, current therapeutic approaches, such as immunosuppressive drugs, carry risks of side effects and long-term complications [15]. These treatments help reduce rejection, but diagnostic limitations may delay intervention, emphasizing the need for more precise and less invasive monitoring tools. Therefore, it is crucial to continuously develop and validate methods for monitoring rejection responses to effectively assess graft status and improve patient prognosis. In particular, noninvasive techniques that are safer and more efficient than biopsies, hold the potential to offer patients less harm and greater benefits, demonstrating significant promise in the monitoring of rejection responses.

In recent years, significant advancements have been made in noninvasive monitoring of transplant rejection. An increasing number of biomarkers have undergone clinical validation, providing methods to monitor dynamic immune changes and quantify graft injury, thereby facilitating timely clinical intervention [16]. An ideal biomarker would serve as a surrogate endpoint, accurately reflecting disease progression or transplant rejection, and should be readily accessible [17]. Biomarkers in transplant monitoring are often categorized as predictive, diagnostic, or prognostic. Predictive biomarkers help predict a patient's response to specific interventions, diagnostic biomarkers are used to detect or confirm the presence of disease, and prognostic biomarkers provide insights into long-term outcomes, such as organ survival and function. In the context of monitoring transplant rejection, emerging biomarkers hold significant value in multiple aspects [18]. For example, cell-free DNA (cfDNA) can be used both to predict rejection and to diagnose acute cellular rejection (ACR) and antibody-mediated rejection (AMR) at an early stage [19]. microRNA (miRNA) is valuable in all aspects of predicting transplant rejection, diagnosing rejection, and assessing graft prognosis. The development of reliable, multi-purpose biomarkers holds great promise for



Scheme 1 Strategies for monitoring rejection of organ transplantation

improving the ability of clinicians to monitor transplant rejection and predict patient outcomes more accurately.

On the other hand, breakthroughs in molecular imaging technologies have transformed traditional imaging methods, enabling early detection of rejection events and real-time monitoring of pathological changes in transplanted organs. Molecular imaging techniques enable noninvasive, quantitative assessments of physiological or pathological processes using specific molecular probes, such as targeted fluorescent dyes, antibody- or peptide-functionalized microbubbles, and antibody-modified iron oxide nanoparticles. These probes offer high specificity and sensitivity for transplant targets, making them invaluable tools in precision medicine for tailoring personalized therapies to individual patient and disease profiles [20]. While biomarkers have been widely used in clinical practice to assess transplant injury, and the application of molecular imaging-primarily involving animal models-remains largely preclinical.

This review focuses on the clinical applications of biomarkers and the preclinical development of molecular imaging, emphasizing their potential for future clinical translation as noninvasive monitoring tools for transplant rejection. We aimed to provide a thorough analysis of the latest advancements in noninvasive monitoring of transplant rejection, concentrating on two main areas: (1) recent biomarkers identified through clinical studies and (2) immune monitoring strategies utilizing intravital imaging modalities in preclinical research (Scheme 1). We also discuss their respective strengths, limitations, and challenges in clinical translation.

Noninvasive biomarkers for monitoring organ transplant rejection

According to the National Institutes of Health (NIH), a biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or

pharmacological responses to a therapeutic intervention" [21]. In the context of organ transplantation, although there are numerous potential biomarkers available, non-invasive monitoring typically relies on two primary substrates: urine and blood, rather than direct analysis of the transplant tissue itself [22]. These substrates provide a practical and less invasive means of evaluating the status of transplanted organs.

Modern biomarker research is increasingly incorporating data from multiple platforms, such as genotype analysis of single nucleotide polymorphisms (SNPs), epigenetic studies, miRNA profiling, and assessments of proteins, peptides, antibodies and metabolites [23]. Among these, metabolite analysis, such as blood or urine Cr levels post-transplant, is well-studied but often lacks the necessary sensitivity and specificity for accurate rejection detection. On the other hand, genetic and proteomic biomarkers, including nucleic acids and cytokines, have shown great promise due to their ability to provide comprehensive genetic information and reflect the dynamic immune processes involved in transplant rejection [24]. Despite this potential, most biomarkers are far from clinical use and lack robust studies on their performance for monitoring transplant rejection [25]. Therefore, rather than including all candidate biomarkers, our review focuses on those biomarkers that have gained recognition in clinical practice or are nearing clinical application, particularly highlighting cutting-edge research from the past five years. These biomarkers are particularly valuable because of their potential to detect early signs of rejection and guide clinical decision-making. To differentiate the complex biomarkers, we categorized them as pre-transplant biomarkers, post-transplant biomarkers reflecting graft injury, and post-transplant biomarkers indicating inflammation and immune processes (Fig. 1).

Pre-transplant biomarkers

Pre-transplant risk assessment is a crucial step in evaluating candidates for solid organ transplantation. The primary objective is to identify potential risk factors that may affect the patient's post-transplant outcomes, including the risks of rejection, infection, and other complications. The primary task at this stage is to assess immunological compatibility prior to transplantation, and the biomarkers directly related to this assessment are human leukocyte antigen (HLA) and preformed donor-specific antibodies (DSA). The human major histocompatibility complex (MHC) is a gene region located on the short arm of chromosome 6 (6p21) that spans approximately 3.6 million base pairs. MHC encodes a family of genes known as HLA, which play a crucial role in immune defense, particularly in recognizing and combating foreign pathogens and in tumor surveillance. Pre-transplant HLA matching between donor and recipient remains one of the most reliable biomarkers for predicting transplant outcomes [26]. Enhanced HLA compatibility is associated with lower rejection rates, improved transplantation success, and longer graft survival [27]. Preformed donor-specific antibodies (DSA) are antibodies present in the recipient's immune system prior to organ transplantation, typically formed through prior sensitization events such as previous transplants,

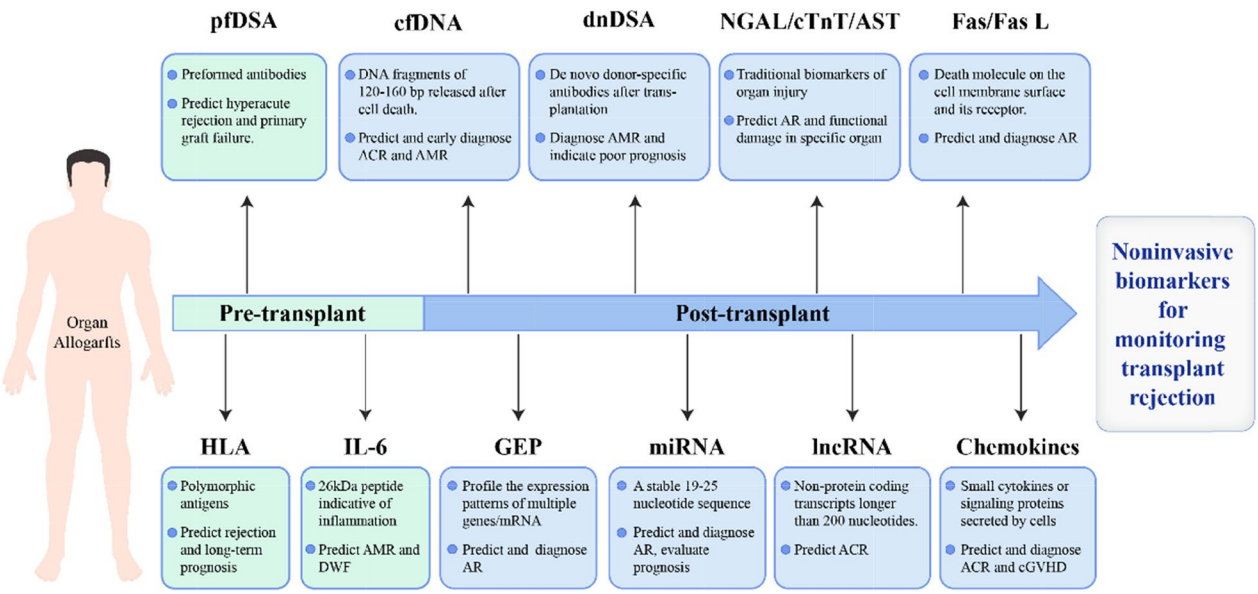


Fig. 1 Illustrative overview of noninvasive molecular biomarkers in organ transplant monitoring

blood transfusions, or pregnancies. Hyperacute rejection occurs rapidly, and if preformed DSA is present, it often leads to immediate and irreversible graft loss. Therefore, screening for DSA, such as crossmatch testing, is essential before transplantation.

In addition to the HLA system, recent studies have indicated that pre-transplant inflammatory biomarkers have a predictive value for graft damage. In kidney transplantation, elevated levels of growth differentiation factor-15, IL-6, and MIG/CXCL9 prior to transplantation can predict post-transplant death with a functioning allograft [28]. This finding offers significant potential for the risk stratification and management of kidney transplant patients. A higher pre-transplant count of $CD16^+$ monocytes count was significantly associated with an increased risk of acute rejection following kidney transplantation. Furthermore, the monocyte/macrophage lineage cells play a crucial role in the pathogenesis of rejection. A higher pre-transplant count of $CD16^+$ monocytes is significantly associated with an increased risk of acute rejection following kidney transplantation [29]. The pre-transplant critical threshold of 23.5/μL for $CD16^+$ monocytes may achieve 90% sensitivity in identifying individuals at risk of rejection.

Another source of pre-transplant biomarkers is the perfusion fluid, which can serve as predictive factors for assessing graft quality and prognosis. These include proteins released upon cell lysis, such as lactate dehydrogenases, glutathione S-Transferases, extracellular histones, and fatty acid binding proteins, as well as proteins secreted during hypothermic machine perfusion ischemia, like neutrophil gelatinase-associated lipocalins, matrix metalloproteinases, kidney injury molecule-1, and Interleukin-18 (IL-18) [30]. However, these biomarkers often lack tissue specificity and are influenced by storage temperature, raising questions regarding their value as predictive factors for short- or long-term transplant outcomes.

Post-transplant biomarkers reflecting graft injury

Post-transplant biomarkers that reflect graft injury play a crucial role in monitoring the health of transplanted organs. These biomarkers provide insights into the physiological status of the graft, indicating whether it is experiencing damage owing to rejection, ischemia, or other complications. Commonly studied biomarkers include cfDNA, de novo donor-specific antibodies (dnDSAs) and biomarkers of organ/tissue-specific injuries.

cfDNA

cfDNA was first identified by Mandel and Metais in 1948 as DNA fragments released into the bloodstream from cells undergoing apoptosis or necrosis [31]. Initially, cfDNA was initially utilized in prenatal diagnostics and cancer detection because of its presence in the blood following cell death [32]. A key advancement in the field of transplantation is the ability to distinguish donor-derived cfDNA (dd-cfDNA) from recipient-derived cfDNA. Donor cells in the allograft degrade and release dd-cfDNA into the bloodstream. These fragments typically range from 120 to 160 bp. Under normal conditions, dd-cfDNA constitutes a minor fraction of the total cfDNA in the recipient's blood but rises significantly during episodes of allograft injury.

Currently, dd-cfDNA is one of the most extensively researched biomarkers for monitoring solid organ transplantation [33]. De Vlaminck et al. reported that dd-cfDNA levels peaked at 3.8% one day after heart transplantation and gradually decreased to less than 1% by day seven [34]. A landmark study by Aubert et al. analyzed cfDNA levels in over 2000 kidney transplant recipients across 14 centers in Europe and America, revealing a strong correlation between increased dd-cfDNA levels and the presence, activity, and severity of kidney transplant rejection [35]. Another longitudinal study assessing the clinical outcomes of dd-cfDNA in kidney allograft transplantation demonstrated that for every 1% increase in dd-cfDNA levels, the rejection risk increased by 3.3 times, with an overall rejection risk ratio of 1.89 [36]. This suggests that dd-cfDNA can enhance rejection detection accuracy compared to standard monitoring practices.

In comparison to patients with non-rejection biopsies, dd-cfDNA levels were elevated up to five months prior to biopsy-confirmed AMR and two months prior to biopsy-confirmed ACR [37]. Moreover, due to its short half-life, cfDNA quickly returns to baseline levels following successful rejection treatment, making it a valuable tool for evaluating post-transplant therapeutic efficacy [38]. The baseline level of dd-cfDNA in the recipient's blood represents a stable value following the initial peak caused by surgical trauma after transplantation. Due to differences in cell quantity within the graft tissue, baseline levels of dd-cfDNA are higher after liver and lung transplants than after kidney and heart transplants [39]. Generally, dd-cfDNA returns to baseline within 1 to 2 weeks for liver, kidney, and heart transplants, while the recovery for lung and pancreas transplants is slower [40].

Notably, dd-cfDNA levels correlated with the types and severity of graft injury. dd-cfDNA levels were higher in AMR (median 2.9%) than in T-cell mediated rejection (TCMR, median 1.2%) and non-rejection cases (median 0.3%), likely due to more extensive cellular necrosis associated with AMR [41]. Furthermore, differences in dd-cfDNA levels were observed among patients with varying severities of AR. For instance, after liver transplantation, patients with mild,

moderate, and severe acute rejection exhibited dd-cfDNA levels of 9.1%, 12.1%, and 28.6%, respectively, highlighting the significant potential of dd-cfDNA as a biomarker [42].

Standardized testing for dd-cfDNA is now used to monitor rejection in heart and kidney transplant recipients [43]. One of the most advanced techniques for analyzing cfDNA is next-generation sequencing (NGS), which offers a comprehensive analysis of genetic information, including SNPs, from blood samples. In 2013, the American College of Medical Genetics and Genomics (ACMG) first released guidelines for NGS testing laboratories. Since 2017, NGS-based commercial diagnostic methods such as AlloSure and Prospera have been launched in the United States, marking the growing maturity and availability of advanced NGS technologies in developed countries. These tests have been extensively supported by clinical research, which has demonstrated their utility in detecting graft injury and rejection in a variety of organ transplant settings [44]. A 2019 multicenter study by Khush et al. validated the use of dd-cfDNA for detecting ACR and AMR in heart transplant patients [45]. This study, using targeted NGS, reported an area under the curve (AUC) of 0.64, with a negative predictive value (NPV) of 97.1% and a positive predictive value (PPV) of 8.9%.

However, while NGS is widely used, its high costs and lengthy analysis times present challenges for broader applications [46]. NGS testing requires expensive sequencing equipment and reagents. Additionally, the large volume of data generated by NGS requires significant computational resources for storage and analysis. To overcome these limitations, Sorbini et al. developed a digital droplet PCR (ddPCR) method based on HLA-DRB1 donor-recipient mismatches [47]. ddPCR is a high-throughput, specific, and sensitive detection method that is ideal for cfDNA, which has a short half-life, low concentration, and complex background [48]. Particularly, droplet microfluidics technology has enabled efficient and monodisperse droplet generation, making ddPCR devices more compact, user-friendly, and potentially cost-effective. This method demonstrated a specificity of 70.8% and a sensitivity of 64.2% in differentiating acute rejection from non-rejection, while also reducing costs and improving patient acceptance. Additionally, ddPCR can quantify cfDNA with a sensitivity ranging from 0.001 to 0.05%, highlighting its potential for "personalized immunosuppression." Not all institutions can afford standard ddPCR systems, so that developing a simplified nucleic acid extraction protocol is crucial. When integrated into ddPCR devices, this protocol eliminates the need for separate instruments, making low-cost, automated cfDNA detection more feasible. The clinical value of ddPCR and other cfDNA detection methods remains underexplored and requires further validation [49].

Despite its widespread use as a "liquid biopsy" for monitoring transplant rejection, dd-cfDNA has certain limitations. It is not a specific biomarker for allograft rejection but rather a marker of severe tissue damage, with allograft rejection being the most common cause of such damage [50]. While dd-cfDNA monitoring demonstrates high AUC and NPV for AR, its PPV is relatively low. Additionally, although dd-cfDNA is effective in distinguishing AMR, some studies have suggested that it is less effective in differentiating ACR from non-rejection in transplant recipients [51]. The fractional abundance of dd-cfDNA may also be influenced by fluctuations in recipient cfDNA levels due to factors such as infection or physical activity.

Overall, dd-cfDNA demonstrates strong diagnostic performance in detecting rejection reactions, with a high NPV [52]. Its most notable advantages are early detection and a quick return to baseline levels, while a drawback is its susceptibility to false positives from other biological factors. Thus, the continuous quantification of dd-cfDNA is essential for cost-effective and personalized transplant management. Future research should focus on establishing precise dd-cfDNA thresholds for AR and developing clinical guidelines for optimal monitoring.

dnDSA

Generally, dnDSAs arise more than three months after transplantation in the context of insufficient immunosuppression [53]. Since current organ transplantation practices usually mitigate the risk of pre-existing DSAs through sensitive preoperative DSA testing, dnDSAs are commonly associated with AMR after transplantation, often manifesting as graft dysfunction and sometimes occurring alongside or after TCMR [54].

In 2005, Tambur et al. demonstrated that the presence of DSAs is linked to adverse outcomes following heart transplantation [55]. Subsequently, it became increasingly recognized that dnDSA are a major cause of chronic graft deterioration in allo-transplantation. More than half of long-term kidney transplant failures can be attributed to dnDSA-mediated chronic rejection [56]. Furthermore, approximately 30% of non-sensitized kidney transplant recipients develop dnDSA within 10 years post-transplant. Among those who develop dnDSA, around 40% will experience graft failure within 5 years [57].

The presence of HLA antigen mismatches is a significant risk factor for the development of dnDSAs, with the most frequently detected antibodies targeting HLA-DQ antigens. These antibodies are associated with poorer graft survival and an increased sensitization post-transplant. Recent advancements in detection technologies

have significantly improved the accuracy of HLA typing and the detection of DSA, thereby enhancing the sensitivity and negative predictive value for diagnosing AMR [58, 59]. Among these technologies, solid-phase monoclonal antibody bead assay, such as Luminex, offers several key advantages over traditional ELISA methods. It requires smaller volumes of both serum and reagents, which reduces detection costs and increases efficiency. Additionally, the high-throughput capability of the Luminex technology makes it especially cost-effective for large-scale applications, further strengthening its clinical utility. This advancement enables early identification of patients at risk for poor transplant outcomes, thereby improving the potential for timely intervention and better management [60]. The measurement of DSA is routinely conducted in clinical practice, with a well-established association with transplant outcomes, particularly in monitoring AMR. Currently, DSA testing is a standard component of post-transplant care [61]. Regular DSA testing not only aids in diagnosing rejection but also serves as a tool for predicting the long-term success of the transplant and patient survival.

Biomarkers of organ/tissue-specific injury

Biomarkers of organ/tissue-specific injury are molecular indicators that reflect the response of specific organs or tissues to damage or disease. These biomarkers can be proteins, lipids, or metabolites, and their levels or patterns change in response to changes in organ function or tissue injury.

For example, in kidney transplantation, serum Cr levels and specific urinary proteins (such as albumin, β2-microglobulin, and N-acetyl-β-D-glucosaminidase) are used as biomarkers to assess kidney injury [62]. Additionally, other urinary biomarkers, such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), have been extensively studied for their potential to predict DGF and AR following kidney transplantation [63]. Similar to kidney transplantation, in heart transplantation, cardiac-specific troponins (such as cTnI and cTnT) increase in cases of myocardial injury and rejection. In liver transplantation, when rejection leads to liver damage, liver function indicators such as AST and ALT in the blood increase significantly.

These biomarkers can partially reflect the damage caused to grafts by various types of transplant rejection. However, the wide range of conditions affecting their variability, such as ischemia–reperfusion injury associated with organ transplantation, plays a significant role in the production of various injury molecules [64]. This complicates their use as highly specific indicators for monitoring rejection. Focusing solely on individual biomarkers of organ injury to monitor transplant rejection is almost meaningless, and a comprehensive assessment and the development of a scoring system are essential.

Post-transplant biomarkers indicating inflammation and immune processes

Post-transplant biomarkers that indicate inflammation and immune processes are vital for understanding the body's response to transplanted organs. These biomarkers help assess the activity of the immune system and degree of inflammation, which can influence graft survival. Key indicators include gene expression profiling (GEP), circulating miRNAs, chemokines and Fas ligand (FasL).

GEP

GEP provides a broad overview of the immune response following transplantation by simultaneously evaluating the expression levels of multiple genes [65]. Its value in monitoring transplant rejection has been well established. This noninvasive method was used to rule out moderate to severe ACR in stable heart transplant recipients. According to the guidelines of the International Society for Heart and Lung Transplantation (ISHLT), blood-based GEP is recommended for the screening of ACR in heart transplant recipients [66]. The test scores range from 0 to 40, with a score below 34 indicating a NPV exceeding 97% for ACR of grade 2R or higher.

The FDA-approved AlloMap test, which analyzes gene expression in peripheral blood mononuclear cells, is included in evidence-based guidelines along with cardiac echocardiography for heart transplant monitoring. This test has been validated in multiple clinical studies and is reimbursed by some commercial insurance companies [67]. However, it may lack sufficient sensitivity to detect early or mild rejection episodes. GEP also hardly effectively differentiate between different types of rejection, as standard blood-based GEP scores do not show significant differences between AMR and non-AMR samples [68, 69]. Additionally, the cost, availability, and need for specialized equipment and expertise may limit the widespread adoption of GEP testing.

miRNA

miRNAs are small, noncoding RNA molecules, 19–25 nucleotides long, crucial for regulating gene expression after transcription [70]. These miRNAs are remarkably stable in human serum, largely due to their encapsulation within exosomes, microvesicles, or apoptotic bodies, which protect them from degradation and enhance their potential as biomarkers for assessing organ viability and detecting immune rejection [71, 72]. The groundbreaking work by Lawrie et al. in 2008 demonstrated that miRNAs are released into the serum from damaged or apoptotic

cells, highlighting their utility as diagnostic tools [73]. Their stability, conferred by these protective membrane structures, facilitates their amplification and quantification through PCR techniques. Moreover, the organ-specific expression patterns of circulating miRNAs make them highly valuable indicators of organ damage following transplantation [74].

Over the past two decades, the role of miRNAs in monitoring transplant outcomes has gained increasing attention. For instance, Wang et al. identified miR-122a as being uniquely expressed in liver transplant recipients [75]. In 2013, a significant study demonstrated that miRNA analysis can distinguish rejection types in kidney transplants, with specific signatures correlating with ACR, AMR, and DGF [76]. Similarly, Zepeda-Quiroz et al. reported that miR-150-5p levels were significantly elevated in patients with AMR and correlated with microvascular inflammation, showing a sensitivity of 63.2%, specificity of 89.5%, and AUC of 0.87 [77].

While individual miRNA markers show promise, effective diagnosis of transplant rejection often requires monitoring dynamic changes across multiple miRNAs to capture a more comprehensive picture of the immune response [78, 79]. For example, urinary miR-10a, miR-10b, and miR-210 have been found to be differentially expressed in transplant recipients experiencing acute TCMR compared to those without rejection [80]. Seo et al. identified a three-miRNA signature—miR-21, miR-31, and miR-4532—that effectively distinguishes kidney transplant recipients with acute rejection from those with stable graft function [81]. In cases of liver damage related to rejection, increased levels of miR-155, miR-122, and miR-181a, along with decreased levels of miR-133a and miR-191, have been observed [82, 83].

The quantification of miRNAs remains a critical focus of current research, and the development of cost-effective and portable detection methods is a key direction. Studies have shown that reverse transcription quantitative PCR (RT-qPCR) provides superior diagnostic performance for miRNAs compared to digital PCR (dPCR) [84]. Although RT-qPCR offers high sensitivity and specificity, its accessibility and portability are limited due to the need for sophisticated, large-scale thermal cyclers [85]. This makes RT-qPCR unsuitable for point-of-care (POC) settings. Additionally, the short length of miRNA sequences, typically corresponding to just one primer length, presents challenges for conventional primer design, necessitating specialized expertise in both primer design and experimental execution. NGS technology has also been proven to be effective for the quantitative analysis of miRNAs. For instance, Kennel et al. used NGS to quantify three different miRNAs upregulated in heart transplant rejection, reporting outstanding AUCs of 0.938 for miR-29c-3p in association with AMR and 0.986 for miR-486-5p related to ACR [86]. Notably, the study reported impressive AUCs, with miR-29c-3p showing an AUC of 0.938 associated with AMR, and miR-486-5p achieving an even higher AUC of 0.986 in relation to ACR.

miRNAs are promising biomarkers for predicting graft outcomes and identifying patients at high risk of complications. They also offer prognostic value and can aid in monitoring the success of treatment. However, the complexity of transplant rejection and diversity of miRNAs present significant challenges in this field. Monitoring transplant rejection using miRNAs has not been widely applied in clinical practice and remains in the research and validation phase. Additionally, the lack of standardized molecular markers across different solid organs necessitates further investigation into organ-specific miRNA profiles. Future studies are needed to further validate previous findings, establish standardized diagnostic tests, and integrate them into clinical care.

lncRNA

Long non-coding RNAs (lncRNAs) are a class of non-protein-coding transcripts longer than 200 nucleotides that have been implicated in various biological and pathological processes, including transplantation [87]. They are known to participate in the regulation of gene expression at multiple levels, such as transcription, post-transcription, and translation. In the context of organ transplantation, lncRNAs have been found to be differentially expressed in biopsies, blood, plasma, urine, and specific cells of patients undergo transplant rejection [88]. These dysregulated lncRNAs can affect cellular functions and differentiation of the immune system, making them potential biomarkers for the diagnosis and management of post-transplant rejection.

Pérez-Carrillo et al. investigated circulating lncRNAs in heart transplant patients to evaluate their potential as noninvasive biomarkers for the diagnosis of ACR [89]. The study found that AL359220.1 and AC025279.1 exhibited excellent diagnostic capabilities associated with the severity of rejection, serving as strong independent predictors of the presence of rejection. Zou et al. analyzed lncRNAs in kidney transplant biopsies to identify those associated with acute rejection and transplant outcomes [90]. They generated a risk score using three of these lncRNAs, which demonstrated the ability to predict transplant loss (AUC = 0.73). Notably, MIR155HG is associated with acute rejection, TCMR, and graft loss, suggesting its involvement in pathways related to immune responses.

The most widely used method for identifying novel lncRNA transcripts is RNA sequencing, which is

considered the gold standard because of its high sensitivity and specificity [91]. Additionally, reverse transcription quantitative polymerase chain reaction (RT-qPCR) and microarray analysis can also be employed for their detection and quantification [92]. Currently, the monitoring of lncRNAs in transplant rejection is still in the early stages of clinical trials. There remains significant potential for exploration in this area, necessitating further investigation of their organ specificity and the various factors influencing their expression.

Chemokines

Chemokines, a specific subset of cytokines, are crucial for mediating immune responses by interacting with G protein-coupled receptors. These interactions direct the migration of leukocytes to sites of inflammation, thereby playing a pivotal role in the inflammatory process [93]. Chemokine receptors are categorized into two main families: the CC receptor family (CCR1-CCR10) and the CXC receptor family (CXCR1-CXCR6) [94]. Among these, CXCR3 is particularly significant in organ transplantation, as it specifically binds to the ligands CXCL9, CXCL10, and CXCL11. These ligands are key in recruiting alloantigen-specific T cells to sites of inflammation and activating proinflammatory transcription factors, which are essential for mounting an immune response against transplanted tissues [95].

In lung transplant recipients, higher levels of CXCR3-associated chemokines found in bronchoalveolar lavage fluid are associated with a greater risk of developing chronic lung allograft dysfunction (CLAD) after transplantation, particularly in cases of more severe acute rejection (grade ≥ A2) [96, 97]. Moreover, significant correlations have been established between allograft injury, AR, acute lung injury, and elevated plasma chemokine levels. Monitoring CXCR3 and its ligands, particularly CXCL9 and CXCL10, can provide early diagnostic insights into rejection events, enabling timely clinical intervention.

Compared to lung and heart transplantation, chemokines are more widely utilized for monitoring rejection and detecting subclinical nephritis following kidney transplantation. Although serum Cr levels have traditionally been used to monitor kidney function, they do not accurately reflect tubulointerstitial nephritis progression. In contrast, dynamic changes in urinary CXCL9 and CXCL10 levels can precede alterations in serum Cr levels, offering a more immediate indication of tubulitis following clinical allograft rejection [98, 99]. Several studies have demonstrated that CXCL-10, both in plasma and urine, exhibits good diagnostic performance for kidney transplant rejection. Plasma CXCL-10 not only identifies high-risk patients for subclinical rejection but also serves as a valuable biomarker for the prognosis and diagnosis of TCMR and AMR [100]. Urinary CXCL9 and CXCL10 are among the most extensively studied and validated chemokines biomarkers for monitoring kidney transplantation with the PPV and NPV range from 55 to 90%, and they have been recommended by the European Society for Organ Transplantation (ESOT) for monitoring immune quiescence [101, 102]. Moreover, the detection of CXCL-10 in urine shows an AUC of 0.69 for subclinical rejection, which is superior to serum Cr, with an AUC of 0.59 [103]. However, the validation of CXCL9/CXCL10 lacks large-scale, multicenter clinical studies, and further evidence is needed, along with more reliable and cost-effective clinical testing technologies, to incorporate these biomarkers into standard clinical practice.

When using CXCL chemokines to monitor rejection, it is essential to consider the potential impact of confounding factors. Conditions such as urinary tract infections and BK virus nephropathy, which are associated with urinary inflammation, can elevate urinary chemokine levels and confound the interpretation of results [104]. To address this, Tinel et al. developed and validated a multiparameter model for diagnosing AR in kidney transplant recipients, which improves the noninvasive diagnosis of AR by accounting for, rather than excluding, confounding factors [105]. This model demonstrated high diagnostic accuracy, with an AUC of 0.85, and maintained its accuracy across various time points. Additionally, Kaminski et al. explored the use of CRISPR/Cas13 technology combined with specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) in urine to detect BKPyV mRNA and CXCL9 mRNA, significantly reducing the interference from confounding factors [106]. Despite these promising studies, further extensive research is necessary to determine the effectiveness and reliability of chemokines in clinical practice. This will ensure that chemokine-based monitoring can effectively guide treatment decisions and improve transplantation outcomes.

Fas/FasL

The Fas/FasL system is essential for maintaining immune homeostasis, self-tolerance, and immune privilege in vital organs, and it also plays a significant role in transplant rejection [107]. A study examining FasL mRNA levels in urinary cells from 35 kidney transplant patients found that those experiencing rejection had higher levels of FasL mRNA than those without rejection [108]. Heng et al. analyzed the diagnostic performance of FasL mRNA expression in relation to AR following kidney transplantation [109]. The overall sensitivity and specificity of FasL mRNA for detecting AR were found to be 0.64 and 0.90, respectively, with an area under the receiver operating characteristic curve AUC of 0.94. These results indicate

that FASL mRNA expression has significant potential as a biomarker for diagnosing acute rejection in kidney transplant patients. Overall, research on Fas/FasL as biomarkers for monitoring transplant rejection remains limited. The current evidence is primarily derived from preliminary studies, and further validation in larger and more diverse cohorts is crucial before these biomarkers can be widely adopted in clinical practice [110].

Recent studies have reported several promising biomolecules over the past five years that could serve as clinical biomarkers for organ transplant rejection (Table 1). To evaluate the effectiveness of these biomarkers, metrics such as sensitivity, specificity, and AUC are commonly used.

Molecular imaging for diagnosis of organ transplant rejection

Molecular imaging has emerged as a critical noninvasive tool for monitoring organ transplant rejection, providing the ability to detect rejection events early and potentially prevent irreversible graft damage. Studies by Christen et al. and Matar et al. have highlighted the effectiveness of various imaging modalities, including fluorescence imaging (FI), ultrasound, magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), and positron emission tomography (PET), in the early detection of transplant rejection [113, 114]. These imaging techniques often depend on specialized exogenous molecular probes tailored to specific modalities-fluorophores for fluorescence imaging, microbubbles for ultrasound, magnetic particles for MRI, and radionuclides for PET scans [115]. By integrating multimodal imaging techniques with specific molecular markers, clinicians can more accurately classify different grades of rejection, thereby enhancing diagnostic precision. Unlike traditional biomarkers, molecular imaging relies on molecular probes, which, owing to safety concerns, are often tested in preclinical models rather than directly in human subjects. As a result, aside from the limited number of clinical studies utilizing SPECT/CT and PET for monitoring human transplant rejection, other imaging modalities remain largely confined to applications in animal models. The primary focus of molecular imaging research is to identify promising molecular probes for potential clinical application as alternatives to biopsy-based assessments.

Fluorescent imaging

FI has become an important method for noninvasive monitoring of solid organ transplants owing to its rapid feedback, lack of ionizing radiation, high spatiotemporal resolution, and exceptional sensitivity [116]. This technique involves designing activity-based imaging probes that emit fluorescence signals upon cleavage by or selective binding to active forms of proteases. Granzyme B (GzmB), a serine protease secreted by cytotoxic T cells and natural killer cells, is a particularly promising target for such probes [117, 118]. In kidney transplantation, elevated levels of $GzmB^+$ lymphocytes are observed during the IA and IB stages, compared to control biopsies, and can predict rapid progression to severe ACR (TCMR grade II or higher) [119].

While conventional fluorescence probes are typically limited to superficial tissue imaging owing to tissue scattering, GzmB-targeted imaging can be extended to deeper tissues. For instance, Mac et al. developed nanosensors for the early, noninvasive detection of acute transplant rejection [120]. The activity nanosensors are engineered by attaching GzmB peptide substrates to nanoparticle scaffold, enhancing their stability and circulation time in the bloodstream (Fig. 2a). In preclinical skin graft models, the GzmB activity nanosensor allows for the noninvasive identification of early ACR and reflects recipient graft function (Fig. 2b). Compared to the isograft controls, significant accumulation of nanosensors was observed as early as postoperative day (POD) 3 in allografts (Fig. 2c). These nanoparticles increase tissue accumulation via passive diffusion. Upon GzmB cleavage, the nanosensors release peptide fragments locally, which were then detectable in urine, facilitating noninvasive monitoring (Fig. 2d).

Further advancing this field, Cheng et al. developed artificial molecular probes (AMPros) for diagnosing acute allograft rejection in renal transplantation [121]. These probes, including AMProN and AMProT, which are designed for high renal clearance and specifically target neutrophils and cytotoxic T lymphocytes (CTLs) infiltrating the allografts (Fig. 2e, f). Systemically, AMPros selectively accumulate in the kidneys, where they interact with precursor immunological biomarkers. Activation by enzymes such as γ-glutamyl transferase (GGT) and GzmB triggered near-infrared fluorescence signals (Fig. 2g). This enables easy optical analysis of urine, allowing for early detection of acute rejection before significant tissue damage occurs. This noninvasive and sensitive urine testing method offers great potential for continuous monitoring of kidney transplant status, partially overcoming the limitations of traditional optical imaging. By analyzing ex vivo urine samples, direct exposure to the patient can be avoided, allowing for precise quantification of GzmB activity, which reflects the immune status of the transplanted organ and enables timely clinical intervention.

Although FI has shown promising results in kidney transplantation, further studies are needed to determine whether this technique can be effectively used for imaging other solid organs in vivo without relying on urine

Table 1 Overview of key biomarkers in human organ transplant research

Author, year, reference	Biomarkers	Study type	No.	Organ	Detection methods	Performance	Diagnosis
Oellerich et al., 2019 [24]	dd-cfDNA	Prospective, singer center	189	Kidney	ddPCR	PPV 22 ~ 39%; Se 38%; Sp 85%; AUC 0.64	AR
Aubert et al.,2024 [35]	dd-cfDNA	Multicenter	2882	Kidney	NGS	PPV 36.1%; NPV 91.1%; Se 59.7%; Sp 79.6%; AUC 0.73	Allograft rejection
Khush et al., 2019 [45]	dd-cfDNA	Prospective, multi-center	740	Heart	NGS	PPV 8.9%; NPV 97.1%; Se 44%; Sp 80%; AUC 0.64 (AR) PPV 4.8%; NPV 98.6% (ACR); PPV 4.2%; NPV 98.6% (AMR)	AR, ACR and AMR
Khush et al.,2021 [50]	dd-cfDNA	Singer center	38	Lung	NGS	PPV 44.3%; NPV 83.6%; Se 55.6%; Sp 75.8%; AUC 0.67	Allograft rejection
Huang et al., 2019 [51]	dd-cfDNA	Retrospective, singer center	63	Kidney	NGS	PPV 77.1%; NPV 75.0%; Se 79.4%; Sp 72.4%; AUC 0.71 (AR) PPV 68.6%; NPV 100%; Se 100%; Sp 71.8%; AUC 0.82 (AMR)	AR and AMR
Kim et al., 2022 [52]	dd-cfDNA	Multicenter	223	Heart	mPCR-NGS	PPV 25.1%; NPV 97.3%; Se 78.5%; Sp 76.9%; AUC 0.86	AR
Bertrand et al., 2019 [59]	dnDSA	Retrospective, singer center	100	Kidney	Single HLA-antigen bead	Se 78%; Sp 90%; AUC 0.844 (iDSA); Se 80%; Sp 94%; AUC 0.892 (sDSA)	AMR
Zepeda-Quiroz et al., 2024 [77]	miR-150-5p	Cross-section, single center	27	Kidney	RT-PCR	Se 63.2%; Sp 89.5%; AUC 0.87	AMR
Seo et al., 2023 [81]	miR-21-5p, 31-5p, 4532	Prospective, multi-center	108	Kidney	Quantitative RT-PCR	PPV 63%; NPV 95%; Se 99%; Sp 26%; AUC 0.77	AR
Ruiz et al., 2020 [83]	miR-155, 122, 181a	Prospective, singer center	49	Liver	Quantitative RT-PCR	AUC 0.87 (miR-155-5p); AUC 0.91 (miR-122-5p); AUC 0.89 (miR-181a-5p)	ACR
Crespo-Leiro et al., 2023 [84]	miR-181	Retrospective, multicenter	97	Heart	RT-qPCR	AUC 0.65	ACR
Kennel et al., 2021 [86]	miR-29c-3p, 486-5p	Cross-section, single center	33	Heart	NGS	AUC 0.938 (miR-29c-3p); AUC 0.986 (miR-486-5p)	ACR and AMR
Pérez-Carrillo et al., 2024 [89]	lncRNA	Singer center	40	Heart	ncRNA-seq	AUC ≥ 0.75 (1R); AUC > 0.85 (2R)	ACR
Shino et al., 2020 [97]	CXCL9/CXCL10	Prospective, multi-center	184	Lung	Luminex assays	/	AR and CLAD
Van Loon et al., 2024 [98]	CXCL9/CXCL10	Prospective, singer center	622	Kidney	Ella immunoassay	PPV 22.2%; NPV 96.3%; Se 75.2%; Sp 71.4%; AUC 0.813	AR
Blydt-Hansen et al., 2020 [99]	CXCL10/Cr	Prospective, multi-center	97	Kidney	Electrochemilumi-nescence	Se 46%; Sp 90%; AUC 0.76 (High CXCL10/Cr level); Se 90%; Sp 38%; AUC 0.76 (Low CXCL10/Cr level)	TCMR
Tinel et al., 2020 [105]	CXCL9/CXCL10	Multicenter	329	Kidney	ELISA	PPV 46.6%; NPV 93.3%; Se 84.4%; Sp 69.3%; AUC 0.85	AR

Table 1 (continued)

Author, year, reference	Biomarkers	Study type	No.	Organ	Detection methods	Performance	Diagnosis
Kaminski et al., 2020 [106]	CXCL9 mRNA	Prospective, singer center	31	Kidney	CRISPR-based detection	Se 93%; Sp 76%; AUC 0.91	TCMR
Gielis et al., 2019 [111]	dd-cfDNA	Prospective, multi-center	107	Kidney	mPCR-NGS	PPV 22 ~ 39%; Se 38%; Sp 85%; AUC 0.64	AR
Millán et al., 2023 [112]	miR155,181a, CXCL10	Prospective, single center	79	Liver	PCR/ELISA	PPV 90.7%; NPV 97.1%; Se 79.6%; Sp 99.1%; AUC 0.975	TCMR

cfDNA cell-free DNA, *NGS* next-generation sequencing, *PPV* positive predictive value, *NPV* negative predictive value, *Se* sensitivity, *Sp* specificity, *AUC* area under curve, *AR* acute rejection, *ACR* acute cellular rejection, *AMR* antibody mediated rejection, *ddPCR* droplet digital polymerase chain reaction, *SNP* single nucleotide polymorphism, *miR* microRNA, *RT* reverse transcription, *TCMR* T cell mediated rejection, *CXCL* chemokine (C-X-C motif) ligand, *DSA* donor specific antibodies

analysis. Imaging in the second near-infrared window (NIR-II, 1000–1700 nm) enhanced tissue penetration and imaging sensitivity, enabling clearer visualization of rejection processes [122]. Chen et al. introduced ErGZ, a granzyme B responsive nano-sensor with NIR-II emission, aimed at the early detection of allograft rejection [123]. The sensor is composed of Erbium-doped nanoparticles (ErNPs) that emit in the NIR-II range, a particle enzyme B-cleavable peptide, and a cleavable fluorophore ZW800, with enhanced stability provided by polyethylene glycol (PEG) coating (Fig. 3a). When the sensor reaches the rejection site, the linker peptide can be cleaved by granzyme B protease, allowing the fluorescent portion of the ErNPs to accumulate in the graft, whereas the dissociated ZW800 is filtered and excreted by the kidneys. The ErGZ sensor can detect allograft rejection as early as five days after skin transplantation (Fig. 3b), and its application allows for highly sensitive differentiation of early rejection in mouse models of pancreatic islet transplantation.

To monitor deeper cardiac transplant rejection, Gao et al. developed an activatable fluorescent probe, CYGB, for GzmB imaging [124]. GzmB cleaves the peptide sequence of CYGB, inducing a cascade reaction that generates CyOH-Cl that activates a near-infrared fluorescence signal (Fig. 3c). This method utilizes activation of the NIRF signal by endogenous GzmB in $CD8^+$ T cells, allowing specific imaging of allograft rejection. In vivo imaging in mouse heart graft models showed that CYGB responded rapidly to GzmB within 2 h post-injection, enabling early diagnosis of rejection (Fig. 3d). Additionally, Gao et al. reported the use of bionic dextran particles (HBTTPEP/GPs) with aggregation-induced emission (AIE) properties, which can target transplant sites through oral administration via macrophage-mediated delivery [125]. To enable AIEgens to emit fluorescence actively in GPs, aggregation and activation of fluorescence emission were achieved through a one-step incubation process (Fig. 3e). Mice orally administered HBTTPEP/GPs showed consistent results, with strong fluorescence observed in the transplanted skin of allograft mice, whereas the fluorescence in isograft mice skin was weak (Fig. 3f). This method allows for accurate assessment of immunosuppressive therapy effectiveness after a single oral dose (Fig. 3g). This noninvasive administration route not only improves patient compliance but also reduces monitoring costs, offering the potential for clinical translation as an immune monitoring tool for solid organ transplantation.

Despite progress in fluorescence imaging, several challenges remain in its clinical application. First, high-performance fluorophores or nanoparticles must undergo rigorous clinical validation to ensure their safety and favorable pharmacokinetics in humans. Second, although near-infrared optical imaging is effective in small animals, its limited imaging depth may pose challenges for its use in transplant patients. Finally, standardization of fluorescence imaging systems is essential and requires extensive validation before clinical implementation. While fluorescence imaging is not yet ready for widespread clinical use, it holds substantial promise and is rapidly advancing as a tool for monitoring transplant rejection.

Ultrasound imaging

Ultrasound molecular imaging is increasingly valued for its ability to deliver immediate, noninvasive, and safe results, which are critical for AR [126]. Weller et al. were the first to successfully image intra-graft T cells and intercellular adhesion molecule-1 (ICAM-1) expression using targeted ultrasound, demonstrating the potential of this modality in transplant monitoring [127]. Similarly, Jin et al. developed microbubbles containing either anti-granzyme B antibodies (MBGzb) or isotype control antibodies (MBcon) to evaluate acute rejection in a murine cardiac transplant model (Fig. 4a) [128]. In the allogeneic MBGzb group, a significant reduction in contrast signals was observed in the myocardium, followed by a gradual refill of microbubbles seconds after the flash pulse (Fig. 4b). This group also showed a markedly greater decrease in peak intensity on postoperative days 2 and 5 compared with both the allogeneic and syngeneic MBcon group. This approach allows for dynamic, repeatable quantification of granzyme B (GzB) expression in vivo, providing a useful measure of rejection responses. However, the potential effects of microbubble destruction can compromise the accuracy of the data.

Recent advancements in ultrasound molecular imaging also offer promising noninvasive approaches for

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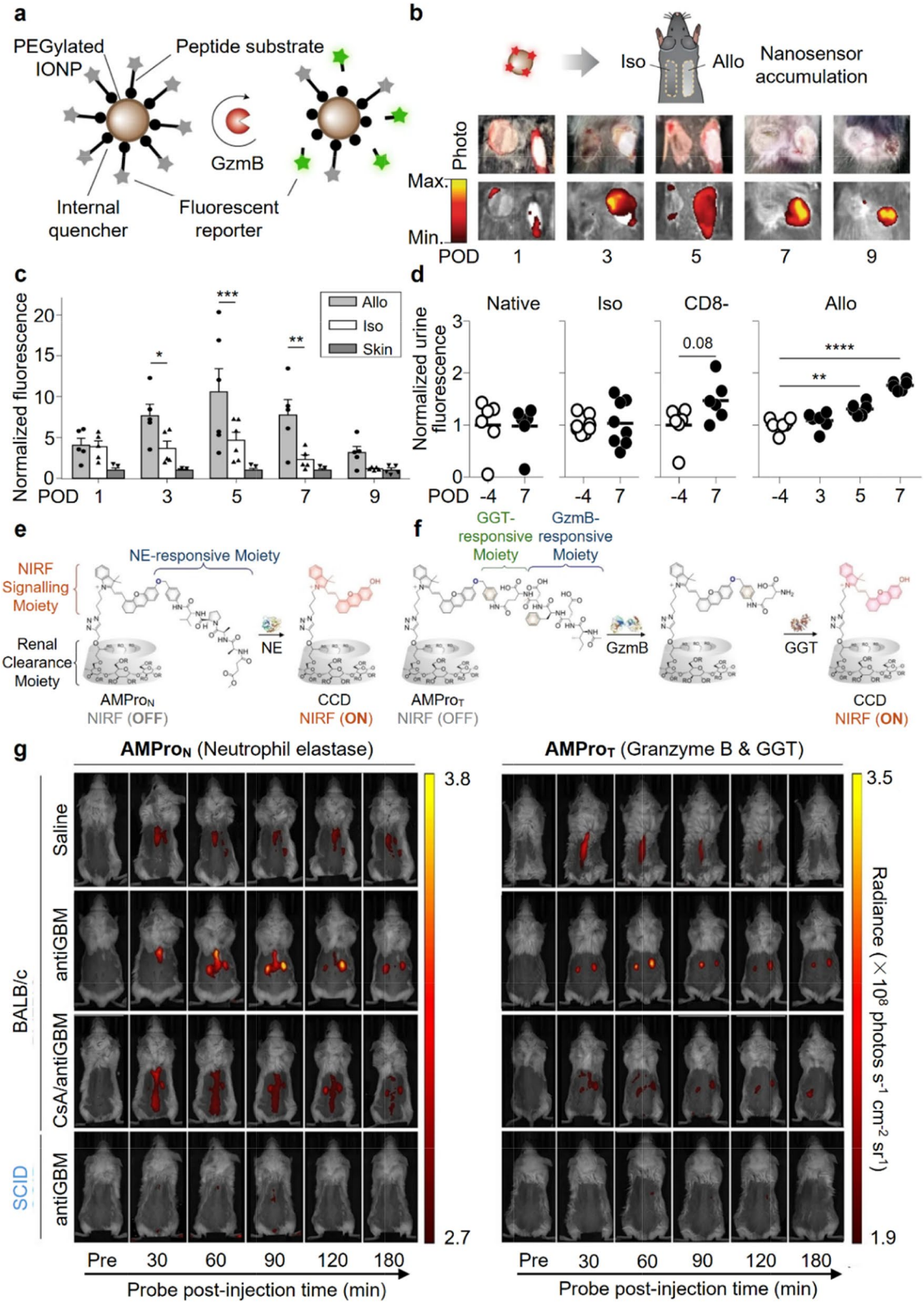
Fig. 2 **a** Response mechanism of the PEGylated-IONPs to granzyme B. **b** Construction and in vivo NIR fluorescence images of mice bearing dual skin grafts. **c** Quantified fluorescent intensities of excised skin grafts. **d** Normalized urine fluorescence in naive mice, isograft mice, CD8-depleted allograft mice, and allograft mice pre- and post-transplant surgery. Reproduced with permission from ref. [67] Copyright 2019 nature biomedical engineering. **e**, **f** The chemical structures and activation forms of AMProN (**e**) and AMProT (**f**). **g** Representative NIRF images of live mice following intravenous injection of AMProN and AMProT 4 days after drug administration. Reproduced with permission from ref. [68] Copyright 2023 Wiley–VCH



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diagnosing AMR following cardiac transplantation. Liu et al. developed nanobubbles containing CD3 antibodies (NBCD3) to detect acute rejection in heart transplantation models (Fig. 4c) [129]. Ex vivo imaging results showed that the signal intensity of nanobubbles increased with increasing concentration and demonstrated excellent stability (Fig. 4d). Key observations included enhanced adhesion and signal amplification of anti-CD3 antibody-coated nanobubbles to T lymphocytes, particularly in rats with allografts, indicating increased infiltration of T lymphocytes (Fig. 4e). This technique holds promise for noninvasive rejection detection, although further research is required to confirm its clinical relevance.

Liao et al. explored the use of C4d, a specific biomarker for AMR, in targeted ultrasound imaging to quantitatively assess rejection levels after heart transplantation [130]. Although C4d is strongly recommended for AMR surveillance, its application has been constrained by the need for invasive biopsy. Serial dilutions of C4d-targeted microbubbles (MB_{C4d}) was designed using a streptavidin–biotin conjugation method and rat cardiac transplantation models were established. The high sensitivity of targeted ultrasound and significant C4d expression during AMR episodes demonstrated real-time C4d distribution. Crucially, MBC4d injections did not cause additional damage, indicating the safety of this method and significant potential for its rapid clinical application as a noninvasive method to evaluate AMR. Importantly, MBC4d injections did not affect the survival of the patients or cause additional injury, confirming its safety. Given the widespread use of contrast-enhanced ultrasound, this safe and quantitative evaluation method for C4d shows significant potential for rapid clinical application.

Ultrasound molecular imaging provides dynamic and continuous imaging, making it a promising approach for monitoring transplant rejection. Targeted microbubbles can detect key molecules such as GzB and C4d, offering high sensitivity for detecting allograft rejection. However, there are some limitations to this study. The accuracy of ultrasound molecular imaging can be affected by the physical destruction of microbubbles. Furthermore, ultrasound imaging is constrained by its medium and has a lower contrast resolution than computed tomography (CT) and MRI. These factors suggest that ultrasound is best utilized in combination with other imaging techniques in a multimodal approach to enhance the diagnostic accuracy.

MRI

MRI is a powerful imaging technique known for its ability to provide detailed anatomical views with high resolution and excellent tissue contrast [131]. Its uses T1 and T2 contrast agents to enhance imaging quality, making it particularly useful for monitoring transplant rejection and investigating various molecular processes [132]. Recent studies focusing on immune cell-based molecular imaging have demonstrated the potential of MRI to deliver detailed insights into immune rejection mechanisms. The sensitivity of MRI for tracking the movement and function of immune cells is especially important for the early detection of ACR.

Guo et al. demonstrated the effective delivery of plasmid DNA (pDNA) and superparamagnetic iron oxide nanoparticles (SPIO) to primary T cells in a rat heart transplantation model. This was achieved using SPIOs loaded with a CD3 single-chain antibody/pDNA polymer complex [133]. The complex consisted of PEG-g-PEI functionalized with a CD3 single-chain antibody (scAbD3) and SPIONs (Fig. 5a). From 0 to 10-day period post-transplantation, rats that received targeted polyplexes containing null plasmid showed prominent low-signal regions on MRI-balanced turbo field echo images of the transplanted hearts, indicating the accumulation of targeted polymers in the allografts (Fig. 5b, c). Additionally, this material facilitated gene transfection in T cells, and the immune response in the transplanted rats was significantly suppressed following gene therapy. This combined strategy enables noninvasive monitoring of the entire treatment process using MRI and provides real-time feedback on treatment effectiveness.

Macrophages, owing to their ability to readily phagocytose contrast agents, can be conveniently labeled in situ with iron oxide particles, making this approach a promising method for noninvasive in vivo imaging of immune

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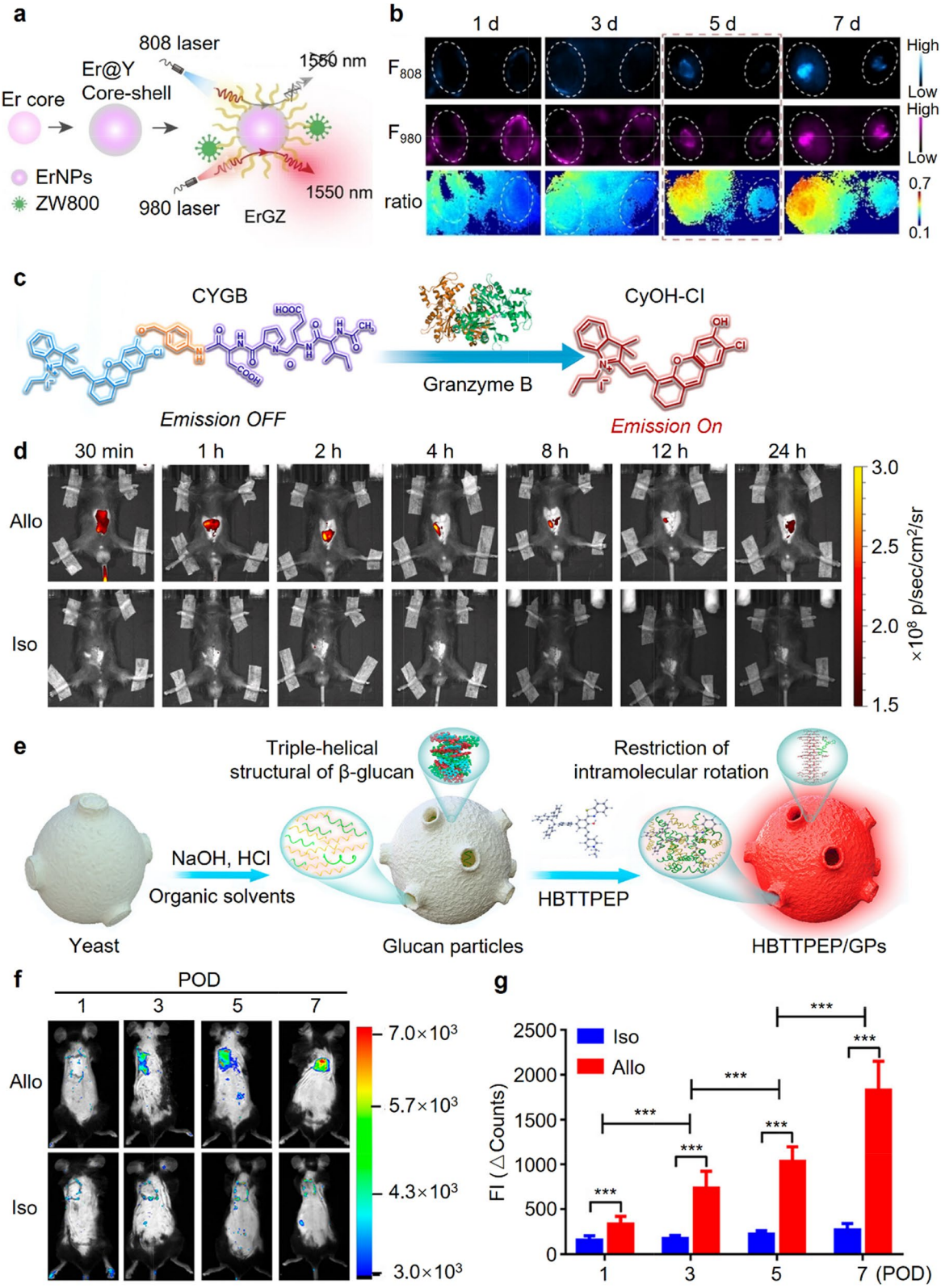
Fig. 3 **a** The design of ErGZ sensor and the absorption competition-induced emission process. **b** Representative F_{808} and F_{980} fluorescence and ratio images mice receiving isograft and allograft skins on postoperative day (POD) 1, 3, 5, and 7. Reproduced with permission from ref. [69] Copyright 2023 Wiley–VCH. c The structure and response mechanism of the GzmB-activatable probe CYGB. d In vivo fluorescent imaging at different time points post-injection of CYGB, 3 days after heart transplantation in mice. Reproduced with permission from ref. [70] Copyright 2023 Elsevier. **e** Schematic diagram of fluorescence emission activation by HBTTPEP aggregation within glucan particles. **f**, **g** In vivo fluorescent images (**f**) and quantification (**g**) of mice after oral administration of HBTTPEP/GPs on POD 1, 3, 5, and 7. Reproduced with permission from ref. [71] Copyright 2021 American Chemical Society



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cells. Wu et al. reported the noninvasive detection of organ rejection and in vivo imaging of macrophages using cellular MRI with iron oxide particles [134]. Macrophages were selected as the target because they play key roles in organ rejection, inflammation, and autoimmune diseases [135, 136]. Furthermore, the potential for MRI tracking of T cells and lymphocytes has also been demonstrated [137, 138]. Liu et al. augmented the uptake of SPIO nanoparticles by T cells through nanoparticle modification with amination [139]. The researchers synthesized IOPC-NH2 particles from IOPC particles through EDAC-coupling reactions (Fig. 5d). T cells that were labeled aggregated within allogeneic heart and lung grafts after reinfusion, facilitating the early detection of transplant rejection using MRI (Fig. 5e, f). Immune cells that internalize nanoscale SPIO particles become magnetic and can be concentrated in large numbers at sites of inflammation, which can then be monitored using MRI. These labeled immune cells demonstrated good adhesion properties and biosafety.

MRI molecular imaging, which is closely tied to immune cells, utilizes specific contrast agents, such as SPIOs, to track cellular pathologies. MRI provides significant advantages owing to its high resolution and excellent soft tissue contrast. However, there are several challenges in its practical clinical implementation. Compared with other molecular imaging techniques, MRI often requires higher concentrations of probes, which could pose potential toxicity concerns. Additionally, longer examination times and higher costs associated with MRI limit its application in certain patient populations. Despite these challenges, rapid advancements in high-contrast probes and hyperpolarization techniques have enhanced the detection of lower concentrations of biological molecules. These developments are crucial for improving the monitoring of transplant rejection, particularly for the early detection of ACR.

Nuclear medicine imaging

Nuclear medicine imaging techniques, primarily SPECT and PET, have become alternative tools for the noninvasive monitoring of organ transplant rejection, utilizing radioactive tracers to provide detailed imaging [140]. SPECT has proven valuable in monitoring immune responses post-transplantation, with several successful applications in preclinical models. For example, targeted macrophage imaging using ^{68}Ga-labeled CD163 and optically labeled CD206 has demonstrated the feasibility and effectiveness of these approaches [141, 142]. O'Neil et al. used an anti-Sn monoclonal antibody (SER-4) radiolabeled with ^{99m}Tc pertechnetate to visualize sialoadhesin (Sn, Siglec 1, or CD169) to effectively track macrophages to monitor transplant rejection responses [143].

SPECT imaging also offers the capability to monitor the dynamics of transplant rejection by visualizing T-cell infiltration. Li et al. developed a novel radiolabeled probe, ^{99m}Tc-HYNIC-mAb$_{CD4}$, to detect CD4$^+$ T lymphocyte infiltration in transplanted hearts (Fig. 6a) [144]. In allograft recipients, transplanted hearts showed significant radiotracer uptake as early as 1 h post-injection, with peak uptake at 6 h (Fig. 6b). The uptake of ^{99m}Tc-HYNIC-mAbCD4 in allograft hearts was notably higher than in both treatment and autograft groups (Fig. 6c). SPECT/CT imaging, with its ability to distinguish allografts with higher uptake of ^{99m}Tc-HYNIC-mAb$_{CD4}$, represents a promising advancement for the noninvasive monitoring and diagnosis of acute cardiac rejection. Furthermore, Sharif-Paghaleh et al. successfully targeted complement molecule C3 using ^{99m}Tc-rCR2, validated through histological and autoradiography evidence, underscoring the potential of SPECT/CT imaging as a valuable tool for monitoring transplant injury [145].

PET imaging is another revolutionary tool in medical diagnostics and plays a critical role in detecting early stages of ACR in transplants [146–150]. In a notable study using a murine cardiac rejection model, the utility of PET imaging was evaluated using two radiotracers: ^{18}F-labeled fluorodeoxyglucose ([^{18}F]-FDG) and ^{13}N-labeled ammonia ([^{13}N]-NH$_3$).[151] In this study, which involved heterotopic heart transplants in mice with minor mismatches in the MHC, serial [^{18}F]FDG PET imaging showed a significant increase in radiotracer uptake in allografts, particularly between 14 and 28 days post-transplant, strongly correlating with increasing rejection grades. Conversely, [^{13}N-]NH$_3$ imaging indicated a significant decrease in myocardial perfusion in allografts with chronic vasculopathy compared with controls. These findings highlight the potential of combined [^{18}F]-FDG and [^{13}N]-NH$_3$ PET imaging for noninvasive,

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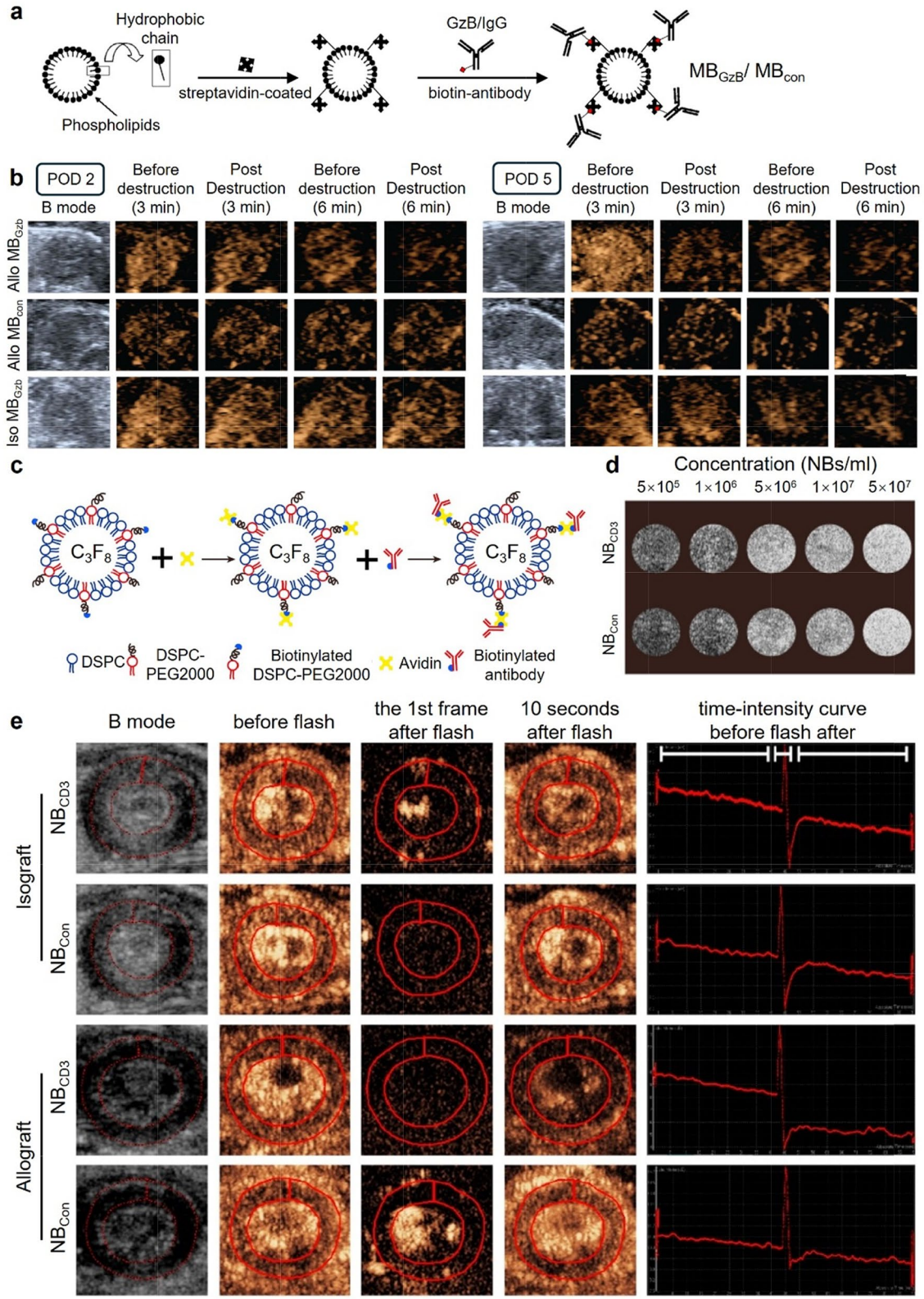
Fig. 4 **a** Synthesis schematic of anti-granzyme B antibodies (MB$_{Gzb}$) and isotype antibodies (MB$_{con}$). **b** After injecting MB$_{Gzb}$ or MB$_{con}$ into mice, contrast-enhanced ultrasound images were obtained on POD 2 and 5. Reproduced with permission from ref. [75] Copyright 2023 Jin et al. c Biotin-avidin–biotin linking scheme for the fabrication of CD3-targeted nanobubbles (NBCD3). **d** Representative in vitro imaging images of NBCD3 and NBcon at different concentrations. **e** B-mode ultrasound images of isograft and allograft rats after injection with NBCD3 or NBcon. Reproduced with permission from ref. [76] Copyright 2018 Elsevier



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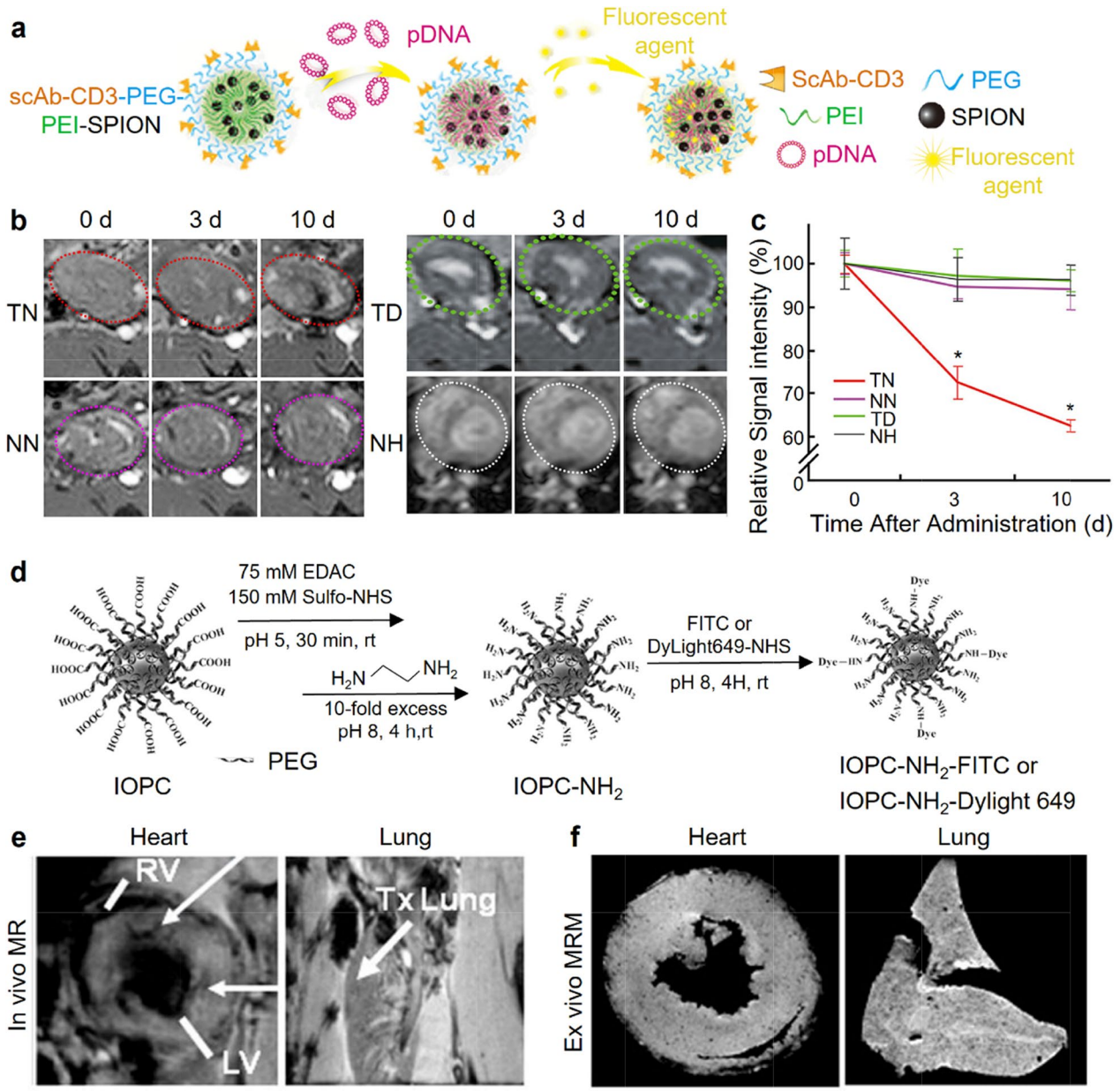

Fig. 5 **a** Schematic of magnetic targeting activation polyplex scAbCD3-PEG-g-PEI-SPION. **b** Magnetic resonance images (MRI) of rat model on POD 0, 3 and 10 following heart transplantation. **c** MRI signal intensity variations in the transplanted heart from POD 0 to 10 after transplantation. Reproduced with permission from ref. [80] Copyright 2019 American Chemical Society. **d** Synthetic scheme of IOPC-NH2 series particles; **e** In vivo MR images of allograft heart and lung on POD 3 before T-cell infusion. **f** Ex vivo MR microscopy images of allograft heart and allograft lung on POD 6. Reproduced with permission from ref. [86] Copyright 2018 Elsevier

quantitative monitoring of allograft rejection, suggesting its potential in human transplantations.

Advancements in PET imaging have led to the development of probes that target specific immune cell markers. For instance, Ueno et al. demonstrated an innovative approach to detect cardiac transplant rejection by using PET-CT to image macrophage activity in allografts [152]. Similarly, Hirai et al. identified OX40 as an imaging target for activated T cells and developed an immune PET tracer targeting the OX40 receptor (Fig. 6d) [153]. OX40 ImmunoPET provides quantitative data on the temporal dynamics of reactive T cell expansion and infiltration, which aligns with findings from flow cytometry studies (Fig. 6e).

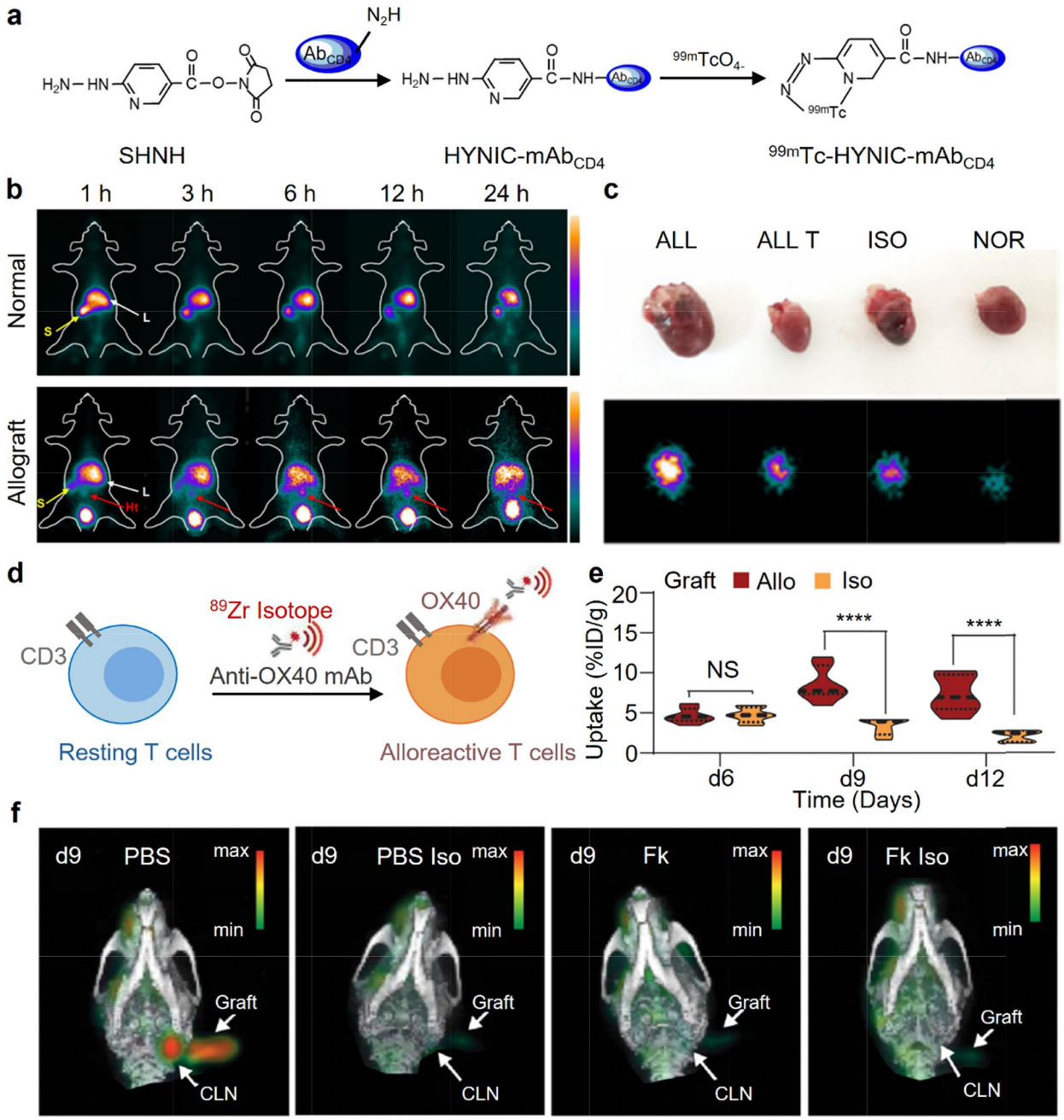

Fig. 6 **a** Synthetic scheme of nuclide molecular probe (^{99m}Tc-HYNIC-mAb$_{CD4}$). **b** In vivo SPECT imaging of rats at various time points (1, 3, 6, 12, 24 h) post-injection of ^{99m}Tc-HYNIC-mAb$_{CD4}$. **c** Ex vivo normal images and representative SPECT images of graft hearts and native hearts. Reproduced with permission from ref. [91] Copyright 2021 American Chemical Society. d Schematic diagram of activation of T cells by ^{89}Zr isotope. e Comparison of radioactive isotope uptake calculated from image ROIs for allo- and isografts. f Representative OX40 or Iso control PET/CT images of mice bearing a single allograft at d9 after transplantation. Reproduced with permission from ref. [101] Copyright 2021 Hirai et al.

Since OX40 is mostly present on activated CD4$^+$ T cells, ^{89Zr}OX40mAb effectively identified these cells in allograft hearts early in the rejection process (Fig. 6f). These results suggest that nanoparticle-enhanced macrophage PET-CT not only efficiently detects heart transplant rejection but also serves as a predictive tool for organ survival.

Among the imaging modalities in nuclear medicine, several imaging agents have been thoroughly validated for safety and clinical applications, particularly in patients with cancer. As a result, nuclear medicine

imaging, compared to other molecular imaging techniques, has advanced further toward clinical translation for monitoring transplant rejection. Dar et al. reported a single-center study using [^{18}F]FDG PET-CT to evaluate cardiac transplant rejection, demonstrating the potential role of PET in diagnosing cardiac transplant rejection in preliminary clinical practice [154]. Despite ongoing concerns regarding the cost and safety of SPECT and PET, nuclear medicine is expected to become one of the first molecular imaging techniques to be widely adopted for clinical monitoring of transplant rejection. In conclusion, the rapid development of molecular imaging technologies has opened up new possibilities for monitoring transplant rejection (Table 2). Future research is expected to explore more advanced imaging agents and valuable targets, thereby providing safer and more effective methods for post-transplant care.

Discussion

Although biopsy remains the gold standard for diagnosing transplant rejection, it has significant limitations. As an invasive and expensive procedure, biopsy is associated with sampling bias due to the small size of tissue specimens and observer bias during tissue grading [155]. These drawbacks highlight the need for noninvasive monitoring methods that can reduce reliance on biopsies. For patients at higher risk of acute rejection (AR), invasive testing and more aggressive immunosuppressive strategies may still be required. Conversely, for stable patients, minimizing repeated biopsies and prolonged immunosuppressive therapy can help reduce complications and adverse effects.

Noninvasive monitoring methods are broadly categorized into biomarker testing and imaging techniques. Advances in molecular biology have enabled biomarkers to evolve into "liquid biopsies," which eliminate the need for tissue sampling and provide dynamic insights

Table 2 The application of imaging monitoring

Imaging modality	Targting strategy	Imaging agent	Tracer	Application	Refs
NIR fluorescent imaging	Identify ACR by GzmB activity	IONPs	GzmB	Skin, mouse	[120]
	Detection of allograft-infiltrating neutrophils and CTLs	AMPro	NE and CTLs	Kidney, mouse	[121]
	NIR-II ratiometric FI of GzB protease activity	ErGZ	GzmB	Skin and islets, mouse	[123]
	Reacting with GzmB secreted by cytotoxic T cells	CYGB	GzmB	Skin and heart, mouse	[124]
	Monitoring of macrophage infiltration	HBTTPEP/GPs	Macrophages	Skin, mouse	[125]
Ultrasound	Microbubbles targeting the endothelial cell inflammatory marker	MB_{ICAM}	ICAM-1	Heart, rat	[127]
	Antigen–antibody reaction	MB_{Gzb}	GzmB	Heart, mouse	[128]
	T lymphocyte-targeted nanobubbles	NBCD3	T cells	Heart, rat	[129]
	Streptavidin–biotin conjugation system	MB_{C4d}	C4d	Heart, rat	[130]
MRI	Multifunctional polymeric nanocarriers for T-cell targeting	scAbCD3-PEG-g-PEI-SPION	CD3	Heart, rat	[133]
	Receptor mediated endocytosis	MPIO	Macrophages	Heart, rat	[134]
	Magnetic particles of IOPC-NH_2 labeled with rat and human T cells	IOPC-NH_2	T cells	Heart and lung, rat	[139]
SPECT/CT	Targeting pro-inflammatory macrophages	^{99m}Tc-SER-4	SER-4	Heart, mouse	[143]
	Infiltration of $CD4^+$T lymphocytes	^{99m}Tc-HYNIC-mAb_{CD4}	T cells	Heart, mouse	[144]
	Complement activation post-IRI	^{99m}Tc-rCR2	C3d	Heart, mouse	[145]
PET	Fluorine-18-FDG and [^{13}N]NH_3 can provides a measure of local inflammatory activity and microblood flow	[^{18}F]FDG/[^{13}N]NH_3	Glycolysis and microhematology	Heart, mouse	[151]
	Dextran nanoparticles were derivatized with the PET isotope copper-64	^{64}Cu-CLIO-VT680	Macrophages	Heart, mouse	[152]
	Tracking T cells by targeting OX40	^{89}Zr radiolabeled immunoPET probe	T cells	Heart, mouse	[153]

NIR near infrared, *ACR* acute cellular rejection, *GzmB* Granzyme B, *GPs* glucan particles, *IONPs* iron oxide nanoparticles, *ICAM* intercellular cell adhesion molecule, *C4d* complement 4d, *MRI* magnetic resonance imaging, *MPIO* micrometer-sized paramagnetic iron-oxide, *SPECT* single photon emission computed tomography, *IRI* ischemia–reperfusion injury, *PET* positron emission tomography

into graft status [156]. Simultaneously, molecular imaging has emerged as "transparent pathology," offering noninvasive visualization of tissue and cellular processes in real time [157]. Together, these approaches hold significant promise for improving the detection and management of transplant rejection, though several clinical challenges persist.

For biomarkers, key challenges include their low concentrations in blood and urine, complicating accurate detection. Establishing appropriate threshold values is critical, as inappropriate thresholds can compromise sensitivity and specificity, leading to false negatives or positives [158]. Additionally, biomarkers are highly influenced by external factors, such as demographic and environmental variability, necessitating validation through multicenter studies to establish standardized protocols for routine clinical use.

In molecular imaging, the safety and efficacy of imaging probes require rigorous validation. Each imaging modality has unique advantages and limitations. Ultrasound is cost-effective, real-time, and noninvasive but has lower resolution compared to other techniques. MRI provides multi-sequence, multi-plane, and multi-parameter imaging capabilities. However, its longer examination times and difficulty in detecting small lesions present challenges [159]. Fluorescence imaging, while promising in preclinical models, has limited imaging depth for certain human applications. Nuclear medicine, including PET and SPECT, has been a cornerstone of clinical transplant monitoring but faces constraints due to high costs and radiation exposure [160]. To overcome these limitations, future advancements will likely involve the development of multimodal imaging approaches that integrate the strengths of different modalities for comprehensive graft assessment.

Molecular imaging plays a pivotal role in visualizing biomarkers that are challenging to detect through conventional methods and enables the imaging of highly specific, artificially designed biomarkers. For example, Cheng et al. developed AMPros to quantify granzyme B (GzmB) activity for monitoring kidney transplant rejection through ex vivo urine analysis [121]. Similarly, Weller et al. employed ultrasound molecular imaging to target T cells and ICAM-1 within the graft, allowing dynamic, in vivo quantification [127]. These innovations demonstrate the potential of molecular imaging to serve as a noninvasive alternative to traditional procedures for detecting tissue biomarkers.

Furthermore, the synergy between biomarkers and molecular imaging is pivotal in monitoring transplant rejection. This combination provides a comprehensive, rapid, and effective approach to assessing immune responses following transplantation. Molecular imaging techniques, such as PET, offer real-time visualization of metabolic and molecular processes within the graft, enabling early detection of inflammatory activity associated with rejection. For instance, PET imaging can identify areas of increased glucose metabolism indicative of immune cell infiltration [161]. However, while PET provides spatial localization of rejection, it may lack specificity in distinguishing between different types of graft injury. On the other hand, biomarkers like dd-cfDNA serve as a sensitive biomarker for detecting graft injury at the molecular level. Elevated levels of dd-cfDNA in the bloodstream correlate with cellular damage and have been associated with both ACR and AMR. Studies have demonstrated that dd-cfDNA levels rise prior to histopathological evidence of rejection, offering a lead time for potential intervention [37].

Recent advancements in imaging agents targeting specific immune processes have enhanced the specificity of molecular imaging in detecting transplant rejection. Wiwat et al. demonstrated that combining molecular immunomonitoring with a scoring system provides a more robust approach to predicting rejection compared to using a single indicator [162]. While biomarkers enable early and sensitive detection of graft injury, molecular imaging precisely localizes and characterizes the extent of the injury [163]. This integrated approach enables timely and targeted interventions, potentially improving patient outcomes. For instance, when dd-cfDNA levels are elevated, subsequent PET imaging can help determine the specific location and severity of rejection, thereby guiding biopsy decisions and therapeutic strategies. Overall, the complementary strengths of molecular imaging and biomarkers detection offer significant potential for advancing noninvasive monitoring and personalized management of transplant recipients. By combining these technologies, we can achieve more accurate, timely, and effective monitoring of transplant rejection, ultimately improving patient care and outcomes.

Conclusion and future directions

The noninvasive monitoring of transplant rejection has advanced significantly, providing safer and more effective approaches to surveillance for transplant recipients. Biomarkers such as donor-derived cell-free DNA (dd-cfDNA), miRNAs, and chemokines offer valuable molecular insights into graft status and immune responses. Complementing these, molecular imaging techniques-including fluorescence imaging, ultrasound, MRI, SPECT, and PET-enable precise, real-time visualization of rejection processes. Despite these advancements, challenges remain regarding sensitivity, specificity, and the need for standardized protocols across diverse patient populations. Integrating molecular imaging with

biomarker-based assays offers a promising pathway to enhance diagnostic accuracy and improve clinical outcomes. Future progress in noninvasive monitoring will hinge on technological and translational innovations. This outlook is anchored on two key aspects: (1) the role of intelligent technologies in advancing noninvasive monitoring, and (2) the feasibility of integrating these advanced technologies into routine transplant care.

Artificial intelligence (AI) is poised to drive precision diagnostics by managing and interpreting complex datasets from biomarker assays and molecular imaging. The development of advanced imaging modalities, alongside safer and more effective contrast agents, will further enhance diagnostic capabilities. The integration of biomarkers with imaging technologies provides complementary information, improving diagnostic accuracy and enabling earlier detection of rejection episodes. However, the analytical complexity of extensive biomarker and imaging datasets demands sophisticated solutions, highlighting the pivotal role of AI. By uncovering patterns missed by traditional methods, AI can significantly improve diagnostic accuracy and facilitate personalized patient care.

Efforts focused on developing advanced algorithms and collecting bioinformatics data are essential to advance AI technology into routine clinical practice [164]. The development of comprehensive scoring systems, such as the iBox system for kidney transplant patients, demonstrates the ability of AI to integrate multiple indicators to predict long-term allograft function. The endorsement of such systems by regulatory bodies such as the U.S. Food and Drug Administration's Biomarker Qualification Program not only validates their effectiveness but also confirms their applicability across diverse patient demographics and healthcare settings [165]. Additionally, AI's integration with medical imaging holds significant potential for improving the assessment of risks associated with transplant-related complications [166]. AI algorithms have achieved high accuracy in detecting ACR following heart transplants, representing a major advancement in pathology-based diagnostics [167]. Although the application of AI is still in the early stages of being used for noninvasive monitoring in organ transplant recipients, further preclinical studies are needed to adapt AI technologies to the specific diagnostic needs of patients undergoing different types of allogeneic transplants [168, 169].

Traditional transplant care often emphasizes infection and complication management, potentially overlooking early rejection episodes that are asymptomatic and challenging to detect with conventional methods [170]. Repeated biopsies for monitoring rejection are not entirely satisfactory, and some heart transplant recipients may undergo up to 14 EMBs in the first-year post-transplant. Moreover, biopsies are associated with high costs and inherent risks. The cost-effectiveness of noninvasive testing has not been comprehensively analyzed and varies across countries, influenced by healthcare development and insurance policies. In the United States, reimbursement for GEP and dd-cfDNA testing is primarily limited to outpatient settings, and it may be even more restricted in other countries. While there is currently no unified study assessing the cost of various testing technologies, the accessibility and cost-effectiveness of noninvasive techniques hold greater promise for the future [171]. Advancements in technology are anticipated to reduce the costs of both biopsy and noninvasive monitoring. However, biopsies still require specialized personnel, presenting additional cost challenges.

It is foreseeable that noninvasive technologies capable of predicting graft injury due to rejection and providing prognostic value will play a pivotal role in the future of transplantation. For instance, patients exhibiting a sustained decline in donor fraction and/or elevated cfDNA within one-week post-transplant may have a poorer prognosis, indicating the need for timely immunological intervention [172]. Early post-transplant and longitudinal assessments using PET can help identify high-risk patients, enabling intensified monitoring [173]. Integrating these noninvasive approaches into standard transplant protocols presents challenges, including issues of accessibility and the need for standardized result interpretation. Furthermore, education and training for the transplant team are crucial for the effective utilization of these technologies. Emerging research supports the feasibility of such integration, ultimately allowing for personalized care tailored to the unique needs of each transplant recipient, leading to improved overall outcomes.

Overall, the integration of noninvasive monitoring techniques, driven by advances in biomarker research and molecular imaging, represents a significant paradigm shift in the treatment and care of organ transplant recipients. These advancements are expected to lead to more effective and individualized treatment strategies that not only enhance patient outcomes but also improve overall quality of life. As these technologies continue to evolve and gain wider acceptance, they are likely to transform the landscape of transplant medicine, making it more efficient and patient-centered.

Abbreviations

HLA	Human leukocyte antigen
cfDNA	Cell-free DNA
NGS	Next-generation sequencing
PPV	Positive predictive value
NPV	Negative predictive value
Se	Sensitivity
Sp	Specificity
AUC	Area under the curve
AR	Acute rejection

ACR	Acute cellular rejection
AMR	Antibody-mediated rejection
ddPCR	Droplet digital polymerase chain reaction
SNP	Single nucleotide polymorphism
miR	MicroRNA
RT	Reverse transcription
TCMR	T cell mediated rejection
CXCL	Chemokine (C-X-C motif) ligand
DSA	Donor specific antibodies
NIR	Near infrared
GzmB	Granzyme B
GPs	Glucan particles
IONPs	Iron oxide nanoparticles
ICAM	Intercellular cell adhesion molecule
C4d	Complement 4d
MRI	Magnetic resonance imaging
MPIO	Micrometer-sized paramagnetic iron-oxide
SPECT	Single photon emission computed tomography
IRI	Ischemia-reperfusion injury
PET	Positron emission tomography

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References

1. Lenschow DJ, Zeng Y, Thistlethwaite JR, et al. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4lg. Science. 1992;257(5071):789–92.
2. Perez VL, Van Parijs L, Biuckians A, et al. Induction of peripheral T cell tolerance in vivo requires CTLA-4 engagement. Immunity. 1997;6(4):411–7.
3. Kowalski RJ, Post DR, Mannon RB, et al. Assessing relative risks of infection and rejection: a meta-analysis using an immune function assay. Transplantation. 2006;82(5):663–8.
4. Montano-Loza AJ, Rodríguez-Perálvarez ML, Pageaux GP, et al. Liver transplantation immunology: Immunosuppression, rejection, and immunomodulation. J Hepatol. 2023;78(6):1199–215.
5. Mehra NK, Baranwal AK. Clinical and immunological relevance of antibodies in solid organ transplantation. Int J Immunogenet. 2016;43(6):351–68.
6. Fuehner T, Benden C, Gottlieb J. Initial immunosuppression and managing rejection. Intensive Care Med. 2019;45:388–90.
7. Gauthier JM, Ruiz-Pérez D, Li W, et al. Diagnosis, pathophysiology and experimental models of chronic lung allograft rejection. Transplantation. 2018;102(9):1459–66.
8. Volkan-Salanci B, Erbas B. Imaging in renal transplants: an update. Semin Nucl Med. 2021;51(4):364–79.
9. Cooper JE. Evaluation and treatment of acute rejection in kidney allografts. Clin J Am Soc Nephrol. 2020;15(3):430–8.
10. Knight SR, Thorne A, Faro MLL. Donor-specific cell-free DNA as a biomarker in solid organ transplantation. A systematic review. Transplantation. 2019;103(2):273–83.
11. From AM, Maleszewski JJ, Rihal CS. Current status of endomyocardial biopsy. Mayo Clin Proc. 2011;86(11):1095–102.
12. Parajuli S, Zhong W, Pantha M, et al. The trend of serum creatinine does not predict follow-up biopsy findings among kidney transplant recipients with antibody-mediated rejection. Transplant Direct. 2023;9(6): e1489.
13. Hanssen O, Erpicum P, Lovinfosse P, et al. Non-invasive approaches in the diagnosis of acute rejection in kidney transplant recipients. Part I. In vivo imaging methods. Clin Kidney J. 2017;10(1):97–105.
14. Olymbios M, Kwiecinski J, Berman DS, et al. Imaging in heart transplant patients. JACC-Cardiovasc Imag. 2018;11(10):1514–30.
15. Alasfar S, Kodali L, Schinstock CA. Current therapies in kidney transplant rejection. J Clin Med. 2023;12(15):4927.
16. Menon MC, Murphy B, Heeger PS. Moving biomarkers toward clinical implementation in kidney transplantation. J Am Soc Nephrol. 2017;28(3):735–47.
17. Naesens M, Anglicheau D. Precision transplant medicine: biomarkers to the rescue. J Am Soc Nephrol. 2018;29(1):24–34.
18. Fernando JJ, Biswas R, Biswas L. Non-invasive molecular biomarkers for monitoring solid organ transplantation: a comprehensive overview. Int J Immunogenet. 2024;51(2):47–62.
19. Khachatoorian Y, Khachadourian V, Chang E, et al. Noninvasive biomarkers for prediction and diagnosis of heart transplantation rejection. Transplant Rev-Orlan. 2021;35(1): 100590.
20. Klinkhammer BM, Lammers T, Mottaghy FM, et al. Non-invasive molecular imaging of kidney diseases. Nat Rev Nephrol. 2021;17(10):688–703.
21. Working GBD. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89–95.
22. Lo DJ, Kaplan B, Kirk AD. Biomarkers for kidney transplant rejection. Nat Rev Nephrol. 2014;10(4):215–25.
23. Roedder S, Vitalone M, Khatri P, et al. Biomarkers in solid organ transplantation: establishing personalized transplantation medicine. Genome Med. 2011;3:37.
24. Oellerich M, Shipkova M, Asendorf T, et al. Absolute quantification of donor-derived cell-free DNA as a marker of rejection and graft injury in kidney transplantation: results from a prospective observational study. Am J Transplant. 2019;19(11):3087–99.
25. Coutance G, Desiré E, Duong Van Huyen JP. A review of biomarkers of cardiac allograft rejection: toward an integrated diagnosis of rejection. Biomolecules. 2022;12(8):1135.
26. Duquesnoy RJ. Are we ready for rpitope-based HLA matching in clinical organ transplantation? Transplantation. 2017;101(8):1755–65.
27. Joher N, Matignon M, Grimbert P. HLA desensitization in solid organ transplantation: anti-CD38 to across the immunological barriers. Front Immunol. 2021;12: 688301.

28. Lorenz EC, Smith BH, Liang Y, et al. Increased pretransplant inflammatory biomarkers predict death with function after kidney transplantation. Transplantation. 2024;108(12):2434–45.
29. Van den Bosch TPP, Hilbrands LB, Kraaijeveld R, et al. Pretransplant numbers of $CD16^+$ monocytes as a novel biomarker to predict acute rejection after kidney transplantation: a pilot study. Am J Transplant. 2017;17(10):2659–67.
30. Baryła M, Skrzycki M, Danielewicz R, et al. Protein biomarkers in assessing kidney quality before transplantation-current status and future perspectives. Int J Mol Med. 2024;54(6):107.
31. Mandel P, Metais P. Nucleic acids of human blood plasma. Compte Rendu des Seances de la Societe de Biologie. 1948;142:241–3.
32. Andrade-Campos MM, Salar A, Sanchez-Gonzalez B, et al. Assessment of cell-free DNA (cfDNA) in 221 patients with lymphoproliferative malignancies at diagnosis and during follow-up. Blood. 2019;134(Suppl 1):492.
33. De Vlaminck I. The proportion of donor-specific cell-free DNA in blood as a marker of transplant rejection: not an absolute. Clin Chem. 2020;66(10):1257–8.
34. De Vlaminck I, Valantine HA, Snyder TM, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci Transl Med. 2014;6(241):241ra77.
35. Aubert O, Ursule-Dufait C, Brousse R, et al. Cell-free DNA for the detection of kidney allograft rejection. Nat Med. 2024;30:2320–7.
36. Bu L, Gupta G, Pai A, et al. Clinical outcomes from the assessing donor-derived cell-free DNA monitoring insights of kidney allografts with longitudinal surveillance (ADMIRAL) study. Kidney Int. 2022;101(4):793–803.
37. Bromberg JS, Bunnapradist S, Samaniego-Picota M, et al. Elevation of donor-derived cell-free DNA before biopsy-proven rejection in kidney transplant. Transplantation. 2024;108(9):1994–2004.
38. Casas S, Tangprasertchai NS, Oikonomaki K, et al. Multi-centre analytical performance verification of an IVD assay to quantify donor-derived cell-free DNA in solid organ transplant recipients. HLA. 2024;103(5): e15518.
39. Beck J, Bierau S, Balzer S, et al. Digital droplet PCR for rapid quantification of donor DNA in the circulation of transplant recipients as a potential universal biomarker of graft injury. Clin Chem. 2013;59(12):1732–41.
40. Zhang W, Liu B, Jia D, et al. Application of graft-derived cell-free DNA for solid organ transplantation. Front Immunol. 2024;15:1461480.
41. Bloom RD, Bromberg JS, Poggio ED, et al. Cell-free DNA and active rejection in kidney allografts. J Am Soc Nephrol. 2017;28(7):2221–32.
42. Cox DRA, Low N, Goh SK, et al. Low levels of hepatocyte-specific methylation in cell-free DNA are a strong negative predictor for acute T cell-mediated rejection requiring treatment following liver transplantation. Liver Transpl. 2022;28(6):1024–38.
43. Wijtvliet VPWM, Plaeke P, Abrams S, et al. Donor-derived cell-free DNA as a biomarker for rejection after kidney transplantation: a systematic review and meta-analysis. Transpl Int. 2020;33(12):1626–42.
44. Garg N, Mandelbrot DA, Parajuli S, et al. The clinical value of donor-derived cell-free DNA measurements in kidney transplantation. Transplant Rev-Orlan. 2021;35(4): 100649.
45. Khush KK, Patel J, Pinney S, et al. Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: a prospective multicenter study. Am J Transplant. 2019;19(10):2889–99.
46. Dengu F. Next-generation sequencing methods to detect donor-derived cell-free DNA after transplantation. Transplant Rev-Orlan. 2020;34(3): 100542.
47. Sorbini M, Togliatto GM, Simonato E, et al. HLA-DRB1 mismatch-based identification of donor-derived cell free DNA (dd-cfDNA) as a marker of rejection in heart transplant recipients: a single-institution pilot study. J Heart Lung Transplant. 2021;40(8):794–804.
48. Hou Y, Chen S, Zheng Y, et al. Droplet-based digital PCR (ddPCR) and its applications. Trac-Trend Anal Chem. 2023;158: 116897.
49. Huo Q, Zhou M, Cooper DKC, et al. Circulating miRNA or circulating DNA-Potential biomarkers for organ transplant rejection. Xenotransplantation. 2019;26(1): e12444.
50. Khush KK, De Vlaminck I, Luikart H, et al. Donor-derived, cell-free DNA levels by next-generation targeted sequencing are elevated in allograft rejection after lung transplantation. ERJ Open Res. 2021;7(1):00462–2020.
51. Huang E, Sethi S, Peng A, et al. Early clinical experience using donor-derived cell-free DNA to detect rejection in kidney transplant recipients. Am J Transplant. 2019;19(6):1663–70.
52. Kim PJ, Olymbios M, Siu A, et al. A novel donor-derived cell-free DNA assay for the detection of acute rejection in heart transplantation. J Heart Lung Transplant. 2022;41(7):919–27.
53. Schinstock CA, Mannon RB, Budde K, et al. Recommended treatment for antibody-mediated rejection after kidney transplantation: the 2019 expert consensus from the transplantion society working group. Transplantation. 2020;104(5):911–22.
54. Marco I, López-Azor García JC, González Martín J, et al. De novo donor-specific antibodies after heart transplantation: a comprehensive guide for clinicians. J Clin Med. 2023;12(23):7474.
55. Tambur AR, Pamboukian SV, Costanzo MR, et al. The presence of HLA-directed antibodies after heart transplantation is associated with poor allograft outcome. Transplantation. 2005;80(8):1019–25.
56. Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. Nat Rev Nephrol. 2012;8(6):348–57.
57. Tambur AR, Kosmoliaptsis V, Claas FHJ, et al. Significance of HLA-DQ in kidney transplantation: time to reevaluate human leukocyte antigen-matching priorities to improve transplant outcomes? An expert review and recommendations. Kidney Int. 2021;100(5):1012–22.
58. Meneghini M, Perona A, Crespo E, et al. On the clinical relevance of using complete high-resolution HLA typing for an accurate interpretation of posttransplant immune-mediated graft outcomes. Front Immunol. 2022;13: 924825.
59. Bertrand D, Farce F, Laurent C, et al. Comparison of two luminex single-antigen bead flow cytometry assays for detection of donor-specific antibodies after renal transplantation. Transplantation. 2019;103(3):597–603.
60. Ware AL, Malmberg E, Delgado JC, et al. The use of circulating donor specific antibody to predict biopsy diagnosis of antibody-mediated rejection and to provide prognostic value after heart transplantation in children. J Heart Lung Transplant. 2016;35(2):179–85.
61. Lefaucheur C, Louis K, Morris AB, et al. Clinical recommendations for posttransplant assessment of anti-HLA (Human Leukocyte Antigen) donor-specific antibodies: a sensitization in transplantation: assessment of risk consensus document. Am J Transplant. 2023;23(1):115–32.
62. Sharaby I, Alksas A, Abou El-Ghar M, et al. Biomarkers for kidney-transplant rejection: a short review study. Biomedicines. 2023;11(9):2437.
63. Tabernero G, Pescador M, Ruiz Ferreras E, et al. Evaluation of NAG, NGAL, and KIM-1 as prognostic markers of the initial evolution of kidney transplantation. Diagnostics. 2023;13(1):1843.
64. van Duijl TT, de Rooij ENM, Treep MM, et al. Urinary kidney injury biomarkers are associated with ischemia-reperfusion injury severity in kidney allograft recipients. Clin Chem. 2023;69(8):924–35.
65. Kobashigawa J, Hall S, Shah P, et al. The evolving use of biomarkers in heart transplantation: Consensus of an expert panel. Am J Transplant. 2023;23(6):727–35.
66. Velleca A, Shullo MA, Dhital K, et al. The International Society for Heart and Lung Transplantation (ISHLT) guidelines for the care of heart transplant recipients. J Heart Lung Transplant. 2023;42(5):e1-141.
67. Deng MC. The AlloMap genomic biomarker story: 10 years after. Clin Transplant. 2017;31(3): e12900.
68. Carey SA, Tecson KM, Jamil AK, et al. Gene expression profiling scores in dual organ transplant patients are similar to those in heart-only recipients. Transpl Immunol. 2018;49:28–32.
69. Lee DH, Usmani A, Ravichandran V, et al. Relationship between blood and tissue-based rejection-related transcripts in heart transplantation. J Heart Lung Transplant. 2024;43(3):359–68.
70. Großhans H, Filipowicz W. The expanding world of small RNAs. Nature. 2008;451(7177):414–6.
71. Van de Vrie M, Deegens JK, Eikmans M, et al. Urinary microRNA as biomarker in renal transplantation. Am J Transplant. 2017;17(5):1160–6.
72. Gimenez-Escamilla I, Perez-Carrillo L, Sanchez-Lazaro I, et al. Non-invasive diagnosis of cardiac rejection using small non-coding RNAs. Eur Heart J. 2022;43(Suppl 2):ehac544.2159.
73. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Brit J Haematol. 2008;141(5):672–5.

74. Enache LS, Enache EL, Ramière C, et al. Circulating RNA molecules as biomarkers in liver disease. Int J Mol Sci. 2014;15(10):17644–66.
75. Wang K, Zhang S, Marzolf B, et al. Circulating microRNAs, potential biomarkers for drug-induced liver injury. Proc Natl Acad Sci U S A. 2009;106(11):4402–7.
76. Wilflingseder J, Regele H, Kainz A, et al. miRNA profiling discriminates types of rejection and injury in human renal allografts. Transplantation. 2013;95(6):835–41.
77. Zepeda-Quiroz I, Guzmán-Martín CA, Peña-Peña M, et al. Plasma miR-150-5p in renal transplant recipients with acute antibody-mediated rejection. J Clin Med. 2024;13(6):1600.
78. Lagos-Quintana M, Rauhut R, Yalcin A, et al. Identification of tissue-specific microRNAs from mouse. Curr Biol. 2002;12(9):735–9.
79. Sarma NJ, Tiriveedhi V, Ramachandran S, et al. Modulation of immune responses following solid organ transplantation by microRNA. Exp Mol Pathol. 2012;93(3):378–85.
80. Lorenzen JM, Volkmann I, Fiedler J, et al. Urinary miR-210 as a mediator of acute T-cell mediated rejection in renal allograft recipients. Am J Transplant. 2011;11(10):2221–7.
81. Seo JW, Lee YH, Tae DH, et al. Development and validation of urinary exosomal microRNA biomarkers for the diagnosis of acute rejection in kidney transplant recipients. Front Immunol. 2023;14:1190576.
82. Farid WRR, Pan Q, van der Meer AJP, et al. Hepatocyte-derived microRNAs as serum biomarkers of hepatic injury and rejection after liver transplantation. Liver Transplant. 2012;18(3):290–7.
83. Ruiz P, Millán O, Ríos J, et al. MicroRNAs 155–5p, 122–5p, and 181a–5p identify patients with graft dysfunction due to T cell-mediated rejection after liver transplantation. Liver Transplant. 2020;26(10):1275–86.
84. Crespo-Leiro M, Constanso-Conde IP, Almenar-Bonet L, et al. Validation of miR-181A-5p as a biomarker of acute rejection after heart transplantation. Multicenter study. Eur Heart J. 2023;44(Suppl 2):ehad655.2299.
85. Dave VP, Ngo TA, Pernestig AK, et al. MicroRNA amplification and detection technologies: opportunities and challenges for point of care diagnostics. Lab Invest. 2019;99(4):452–69.
86. Kennel PJ, Yahi A, Naka Y, et al. Longitudinal profiling of circulating miRNA during cardiac allograft rejection: a proof-of-concept study. ESC Heart Fail. 2021;8(3):1840–9.
87. Salehi S, Afzali S, Shahi A, et al. Potential roles of long noncoding RNAs as therapeutic targets in organ transplantation. Front Immunol. 2022;13: 835746.
88. Mattick JS, Amaral PP, Carninci P, et al. Long non-coding RNAs: definitions, functions, challenges and recommendations. Nat Rev Mol Cell Biol. 2023;24(6):430–47.
89. Pérez-Carrillo L, Giménez-Escamilla I, González-Torrent I, et al. Circulating long non-coding RNAs detection after heart transplantation and its accuracy in the diagnosis of acute cardiac rejection. Biomark Res. 2024;12(1):49.
90. Zhang Z, Tang Y, Zhuang H, et al. Identifying 4 novel lncRNAs as potential biomarkers for acute rejection and graft loss of renal allograft. J Immunol Res. 2020;2020(1):2415374.
91. Teng Y, Huang Z, Yao L, et al. Emerging roles of long non-coding RNAs in allotransplant rejection. Transpl Immunol. 2022;70: 101408.
92. Corchete LA, Rojas EA, Alonso-López D, et al. Systematic comparison and assessment of RNA-seq procedures for gene expression quantitative analysis. Sci Rep. 2020;10(1):19737.
93. Hancock WW, Gao W, Faia KL, et al. Chemokines and their receptors in allograft rejection. Curr Opin Immunol. 2000;12(5):511–6.
94. Hancock WW, Wang L, Ye Q, et al. Chemokines and their receptors as markers of allograft rejection and targets for immunosuppression. Curr Opin Immunol. 2003;15(5):479–86.
95. Steinmetz OM, Turner JE, Paust HJ, et al. CXCR3 mediates renal Th1 and Th17 immune response in murine lupus nephritis. J Immunol. 2009;183(7):4693–704.
96. Shino MY, Weigt SS, Li N, et al. The prognostic importance of bronchoalveolar lavage fluid CXCL9 during minimal acute rejection on the risk of chronic lung allograft dysfunction. Am J Transplant. 2018;18(1):136–44.
97. Shino MY, Todd JL, Neely ML, et al. Plasma CXCL9 and CXCL10 at allograft injury predict chronic lung allograft dysfunction. Am J Transplant. 2022;22(9):2169–79.
98. Van Loon E, Tinel C, de Loor H, et al. Automated urinary chemokine assays for noninvasive detection of kidney transplant rejection: a prospective cohort study. Am J Kidney Dis. 2024;83(4):467–76.
99. Blydt-Hansen TD, Sharma A, Gibson IW, et al. Validity and utility of urinary CXCL10/Cr immune monitoring in pediatric kidney transplant recipients. Am J Transplant. 2021;21(4):1545–55.
100. Millán O, Rovira J, Guirado L, et al. Advantages of plasmatic CXCL-10 as a prognostic and diagnostic biomarker for the risk of rejection and subclinical rejection in kidney transplantation. Clin Immunol. 2021;229: 108792.
101. Schaub S, Nickerson P, Rush D, et al. Urinary CXCL9 and CXCL10 levels correlate with the extent of subclinical tubulitis. Am J Transplant. 2009;9(6):1347–53.
102. Park S, Sellares J, Tinel C, et al. European society of organ transplantation consensus statement on testing for non-invasive diagnosis of kidney allograft rejection. Transpl Int. 2024;36:12115.
103. Ho J, Schaub S, Jackson AM, et al. Multicenter validation of a urine CXCL10 assay for noninvasive monitoring of renal transplants. Transplantation. 2023;107(7):1630–41.
104. Matz M, Beyer J, Wunsch D, et al. Early post-transplant urinary IP-10 expression after kidney transplantation is predictive of short- and long-term graft function. Kidney Int. 2006;69(9):1683–90.
105. Tinel C, Devresse A, Vermorel A, et al. Development and validation of an optimized integrative model using urinary chemokines for noninvasive diagnosis of acute allograft rejection. Am J Transplant. 2020;20(12):3462–76.
106. Kaminski MM, Alcantar MA, Lape IT, et al. A CRISPR-based assay for the detection of opportunistic infections post-transplantation and for the monitoring of transplant rejection. Nat Biomed Eng. 2020;4(6):601–9.
107. Strasser A, Jost PJ, Nagata S. The many roles of FAS receptor signaling in the immune system. Immunity. 2009;30(2):180–92.
108. Aquino-Dias EC, Joelsons G, Da Silva DM, et al. Non-invasive diagnosis of acute rejection in kidney transplants with delayed graft function. Kidney Int. 2008;73(7):877–84.
109. Heng B, Ding H, Ren H, et al. Diagnostic performance of Fas ligand mRNA expression for acute rejection after kidney transplantation: a systematic review and meta-analysis. PLoS ONE. 2016;11(11): e0165628.
110. Dong G, Li X, Li J, et al. Intercellular adhesion molecular-1, Fas, and Fas ligand as diagnostic biomarkers for acute allograft rejection of pancreaticoduodenal transplantation in pigs. Digest Dis Sci. 2014;59:778–86.
111. Gielis EM, Ledeganck KJ, Dendooven A, et al. The use of plasma donor-derived, cell-free DNA to monitor acute rejection after kidney transplantation. Nephrol Dial Transplant. 2020;35(4):714–21.
112. Millán O, Ruiz P, Julian J, et al. A plasmatic score using a miRNA signature and CXCL-10 for accurate prediction and diagnosis of liver allograft rejection. Front Immunol. 2023;14:1196882.
113. Christen T, Nahrendorf M, Wildgruber M, et al. Molecular imaging of innate immune cell function in transplant rejection. Circulation. 2009;119(14):1925–32.
114. Matar AJ, Crepeau RL, Duran-Struuck R. Non-invasive imaging for the diagnosis of acute rejection in transplantation: the next frontier. Transpl Immunol. 2021;68: 101431.
115. Chen Y, Zhang L, Liu J, et al. Molecular imaging of acute cardiac transplant rejection: animal experiments and prospects. Transplantation. 2017;101(9):1977–86.
116. Huang J, Chen X, Jiang Y, et al. Renal clearable polyfluorophore nanosensors for early diagnosis of cancer and allograft rejection. Nat Mater. 2022;21(5):598–607.
117. Li B, Hartono C, Ding R, et al. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. N Engl J Med. 2001;344(13):947–54.
118. Choy JC. Granzymes and perforin in solid organ transplant rejection. Cell Death Differ. 2010;17(4):567–76.
119. Liu SJ, Ma K, Liu LS, et al. Point-of-care non-invasive enzyme-cleavable nanosensors for acute transplant rejection detection. Biosens Bioelectron. 2022;215: 114568.
120. Mac QD, Mathews DV, Kahla JA, et al. Non-invasive early detection of acute transplant rejection via nanosensors of granzyme B activity. Nat Biomed Eng. 2019;3(4):281–91.

121. Cheng P, Wang R, He S, et al. Artificial urinary biomarkers for early diagnosis of acute renal allograft rejection. Angew Chem Int Ed. 2023;62(39): e202306539.
122. Wang F, Zhong Y, Bruns O, et al. In vivo NIR-II fluorescence imaging for biology and medicine. Nat Photon. 2024;18:535–47.
123. Chen Y, Pei P, Yang Y, et al. Noninvasive early diagnosis of allograft rejection by a granzyme B protease responsive NIR-II bioimaging nanosensor. Angew Chem Int Ed. 2023;135(23): e202301696.
124. Gao T, Yi L, Wang Y, et al. Granzyme B-responsive fluorescent probe for non-invasive early diagnosis of transplant rejection. Biosens Bioelectron. 2023;232: 115303.
125. Gao T, Wu Y, Wang W, et al. Biomimetic glucan particles with aggregation-induced emission characteristics for noninvasive monitoring of transplant immune response. ACS Nano. 2021;15(7):11908–28.
126. Morawski AM, Lanza GA, Wickline SA. Targeted contrast agents for magnetic resonance imaging and ultrasound. Curr Opin Biotech. 2005;16(1):89–92.
127. Weller GER, Lu E, Csikari MM, et al. Ultrasound Imaging of acute cardiac transplant rejection with microbubbles targeted to intercellular adhesion molecule-1. Circulation. 2003;108(2):218–24.
128. Jin Y, Gao P, Liang L, et al. Noninvasive quantification of granzyme B in cardiac allograft rejection using targeted ultrasound imaging. Front Immunol. 2023;14:1164183.
129. Liu J, Chen Y, Wang G, et al. Ultrasound molecular imaging of acute cardiac transplantation rejection using nanobubbles targeted to T lymphocytes. Biomaterials. 2018;162:200–7.
130. Liao T, Liu X, Ren J, et al. Noninvasive and quantitative measurement of C4d deposition for the diagnosis of antibody-mediated cardiac allograft rejection. EBioMedicine. 2018;37:236–45.
131. Mahmood U, Josephson L. Molecular MR imaging probes. Proc IEEE. 2005;93(4):800–8.
132. Aherne T, Tscholakoff D, Finkbeiner W, et al. Magnetic resonance imaging of cardiac transplants: the evaluation of rejection of cardiac allografts with and without immunosuppression. Circulation. 1986;74(1):145–56.
133. Guo Y, Chen W, Wang W, et al. Simultaneous diagnosis and gene therapy of immuno-rejection in rat allogeneic heart transplantation model using a T-cell-targeted theranostic nanosystem. ACS Nano. 2012;6(12):10646–57.
134. Wu YL, Ye Q, Foley LM, et al. In situ labeling of immune cells with iron oxide particles: an approach to detect organ rejection by cellular MRI. Proc Natl Acad Sci U S A. 2006;103(6):1852–7.
135. Toki D, Zhang W, Hor KLM, et al. The role of macrophages in the development of human renal allograft fibrosis in the first year after transplantation. Am J Transplant. 2014;14(9):2126–36.
136. Salehi S, Reed EF. The divergent roles of macrophages in solid organ transplantation. Curr Opin Organ Tran. 2015;20(4):446–53.
137. Ye Q, Wu YL, Foley LM, et al. Longitudinal tracking of recipient macrophages in a rat chronic cardiac allograft rejection model with noninvasive magnetic resonance imaging using micrometer-sized paramagnetic iron oxide particles. Circulation. 2008;118(2):149–56.
138. Dolan RS, Rahsepar AA, Blaisdell J, et al. Multiparametric cardiac magnetic resonance imaging can detect acute cardiac allograft rejection after heart transplantation. JACC-Cardiovasc Imag. 2019;12(8 part 2):1632–41.
139. Liu L, Ye Q, Wu Y, et al. Tracking T-cells in vivo with a new nano-sized MRI contrast agent. Nanomedicine. 2012;8(8):1345–54.
140. Ge J, Zhang Q, Zeng J, et al. Radiolabeling nanomaterials for multimodality imaging: new insights into nuclear medicine and cancer diagnosis. Biomaterials. 2020;228: 119553.
141. Eichendorff S, Svendsen P, Bender D, et al. Biodistribution and PET imaging of a novel [^{68}Ga]-anti-CD163-antibody conjugate in rats with collagen-induced arthritis and in controls. Mol Imaging Biol. 2015;17:87–93.
142. Fiordelisi MF, Auletta L, Meomartino L, et al. Preclinical molecular imaging for precision medicine in breast cancer mouse models. Contrast Media Mol I. 2019;2019(1):8946729.
143. O'Neill ASG, Terry SYA, Brown K, et al. Non-invasive molecular imaging of inflammatory macrophages in allograft rejection. EJNMMI Res. 2015;5:69.
144. Li H, Chen Y, Jin Q, et al. Noninvasive radionuclide molecular imaging of the CD4-positive T lymphocytes in acute cardiac rejection. Mol Pharm. 2021;18(3):1317–26.
145. Sharif-Paghaleh E, Yap ML, Meader LL, et al. Noninvasive imaging of activated complement in ischemia-reperfusion injury post-cardiac transplant. Am J Transplant. 2015;15(9):2483–90.
146. Bhatnagar A, Narula J. Radionuclide imaging of cardiac pathology: a mechanistic perspective. Adv Drug Deliv Rev. 1999;37(1–3):213–23.
147. Grabner A, Kentrup D, Edemir B, et al. PET with ^{18}F-FDG-labeled T lymphocytes for diagnosis of acute rat renal allograft rejection. J Nucl Med. 2013;54(7):1147–53.
148. Konishi M, Erdem SS, Weissleder R, et al. Imaging granzyme B activity assesses immune-mediated myocarditis. Circ Res. 2015;117(6):502–12.
149. Larimer BM, Wehrenberg-Klee E, Dubois F, et al. Granzyme B PET imaging as a predictive biomarker of immunotherapy response. Cancer Res. 2017;77(9):2318–27.
150. Schwenck J, Sonanini D, Cotton JM, et al. Advances in PET imaging of cancer. Nat Rev Cancer. 2023;23(7):474–90.
151. Daly KP, Dearling JLJ, Seto T, et al. Use of [^{18}F]FDG positron emission tomography to monitor the development of cardiac allograft rejection. Transplantation. 2015;99(9):e132–9.
152. Ueno T, Dutta P, Keliher E, et al. Nanoparticle PET-CT detects rejection and immunomodulation in cardiac allografts. Circ-Cardiovasc Imag. 2013;6(4):568–73.
153. Hirai T, Mayer AT, Nobashi TW, et al. Imaging alloreactive T cells provides early warning of organ transplant rejection. JCI Insight. 2021;6(13): e145360.
154. Dar O, Dulay MS, Riesgo-Gil F, et al. Cardiac transplant rejection assessment with 18F-FDG PET-CT: initial single-centre experience for diagnosis and management. EJNMMI Rep. 2024;8(1):9.
155. Cusi V, Rodgers N, Wettersten N, et al. Benefit and risk of endomyocardial biopsy for heart transplant patients in the contemporary era. J Card Fail. 2024;30(1):249.
156. Loy C, Ahmann L, De Vlaminck I, et al. Liquid biopsy based on cell-free DNA and RNA. Annu Rev Biomed Eng. 2024;26:169–95.
157. Tian M, He X, Jin C, et al. Transpathology: molecular imaging-based pathology. Eur J Nucl Med Mol Imaging. 2021;48:2338–50.
158. Novacescu D, Latcu SC, Bardan R, et al. Contemporary biomarkers for renal transplantation: a narrative overview. J Pers Med. 2023;13(8):1216.
159. Gannon MP, Schaub E, Grines CL, et al. State of the art: evaluation and prognostication of myocarditis using cardiac MRI. J Magn Reson Imaging. 2019;49(7):e122–31.
160. Marengo M, Martin CJ, Rubow S, et al. Radiation safety and accidental radiation exposures in nuclear medicine. Semin Nucl Med. 2022;52(2):94–113.
161. Watson AM, Bhutiani N, Philips P, et al. The role of FDG-PET in detecting rejection after liver transplantation. Surgery. 2018;164(2):257–61.
162. Chancharoenthana W, Traitanon O, Leelahavanichkul A, et al. Molecular immune monitoring in kidney transplant rejection: a state-of-the-art review. Front Immunol. 2023;14:1206929.
163. Mehlman Y, Valledor AF, Moeller C, et al. The utilization of molecular microscope in management of heart transplant recipients in the era of noninvasive monitoring. Clin Transplant. 2023;37(12): e15131.
164. Goswami R. The current state of artificial intelligence in cardiac transplantation. Curr Opin Organ Tran. 2021;26(3):296–301.
165. Loupy A, Aubert O, Orandi BJ, et al. Prediction system for risk of allograft loss in patients receiving kidney transplants: international derivation and validation study. BMJ. 2019;366: l4923.
166. Kalfa D, Agrawal S, Goldshtrom N, et al. Wireless monitoring and artificial intelligence: a bright future in cardiothoracic surgery. J Thorac Cardiov Sur. 2020;160(3):809–12.
167. Glass C, Davis R, Xiong B, et al. The use of artificial intelligence (AI) machine learning to determine myocyte damage in cardiac transplant acute cellular rejection. J Heart Lung Transplant. 2020;39(4):S59.
168. Clement J, Maldonado AQ. Augmenting the transplant team with artificial intelligence: toward meaningful AI use in solid organ transplant. Front Immunol. 2021;12: 694222.
169. Maldonado AQ, West-Thielke P, Joyal K, et al. Advances in personalized medicine and noninvasive diagnostics in solid organ transplantation. Pharmacotherapy. 2021;41(1):132–43.
170. Brunet M, Shipkova M, Van Gelder T, et al. Barcelona consensus on biomarker-based immunosuppressive drugs management in solid organ transplantation. Ther Drug Monit. 2016;38(Suppl 1):S1-20.
171. Holzhauser L, DeFilippis EM, Nikolova A, et al. The end of endomyocardial biopsy? A practical guide for noninvasive heart transplant rejection surveillance. JACC Heart Fail. 2023;11(3):263–76.
172. Zangwill SD, Kindel SJ, Ragalie WS, et al. Early changes in cell-free DNA levels in newly transplanted heart transplant patients. Pediatr Transplant. 2020;24(1): e13622.

173. Wiefels C, Almufleh A, Yao J, et al. Prognostic utility of longitudinal quantification of PET myocardial blood flow early post heart transplantation. J Nucl Cardiol. 2022;29(2):712–23.

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