Medical Policy

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Title: Laboratory Tests Post Transplant and for Heart Failure

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Populations	Interventions	Comparators	Outcomes
Individuals: • With chronic heart failure	Interventions of interest are: • Soluble suppression of tumorigenicity-2 assay to determine prognosis and/or to guide management	Comparators of interest are: • Standard prognostic markers, including B-type natriuretic peptide levels	Relevant outcomes include: • Overall survival • Quality of life • Hospitalizations
Individuals: • With heart transplant	Interventions of interest are: • Soluble suppression of tumorigenicity-2 assay to determine prognosis and/or to predict acute cellular rejection	Comparators of interest are: • Routine endomyocardial biopsy	Relevant outcomes include: • Overall survival • Test validity • Morbid events • Hospitalizations

Populations	Interventions	Comparators	Outcomes
Individuals: • With heart transplant	Interventions of interest are: • Measurement of volatile organic compounds to assess cardiac allograft rejection	Comparators of interest are: • Routine endomyocardial biopsy	Relevant outcomes include: • Overall survival • Test validity • Morbid events • Hospitalizations
Individuals: • With heart transplant	Interventions of interest are: • Testing donor-derived cell-free DNA in Blood to assess cardiac allograft rejection	Comparators of interest are: • Routine endomyocardial biopsy	Relevant outcomes include: • Overall survival • Test validity • Morbid events Hospitalizations
Individuals: • With heart transplant	Interventions of interest are: • Gene expression profiling to assess cardiac allograft rejection	Comparators of interest are: • Routine endomyocardial biopsy	Relevant outcomes include: • Overall survival • Test validity • Morbid events • Hospitalizations
Individuals: • With renal transplant who are undergoing surveillance or have clinical suspicion of allograft rejection	Interventions of interest are: • Testing donor-derived cell-free DNA in blood to assess renal allograft rejection	Comparators of interest are: • Renal biopsy	Relevant outcomes include: • Overall survival • Test validity • Morbid events • Hospitalizations
Individuals: • With lung transplant who are undergoing surveillance allograft rejection	Interventions of interest are: • Testing donor-derived cell-free DNA in blood to assess lung allograft rejection	Comparators of interest are: • Bronchoscopy with biopsy	Relevant outcomes include: • Overall survival • Test validity • Morbid events • Hospitalizations

DESCRIPTION

Clinical assessment and noninvasive imaging of chronic heart failure can be limited in accurately diagnosing patients with heart failure because symptoms and signs can poorly correlate with objective methods of assessing cardiac dysfunction. For management of heart failure, clinical signs and symptoms (eg, shortness of breath) are relatively crude markers of decompensation and occur late in the course of an exacerbation. Thus, circulating biomarkers have potential benefit in heart failure diagnosis and management.

In transplant recipients, despite the progress in immunosuppressant therapy, the risk of rejection remains. Diagnosis of allograft rejection continues to rely on clinical monitoring and histologic confirmation by tissue biopsy. However, due to limitations of tissue biopsy, including a high degree of interobserver variability in the grading of results and its potential complications, less

invasive alternatives have been investigated. Several laboratory-tested biomarkers of transplant rejection have been evaluated and are commercially available for use. The laboratory tests for heart transplant rejection currently evaluated in this policy include the Presage® ST2 Assay kit, which measures the soluble suppression of tumorigenicity-2 (sST2) protein biomarker; ; the Heartsbreath test, which measures breath markers of oxidative stress; the AlloSure, Prospera Heart and myTAI$_{HEART}$ tests for assessment of donor-derived cell-free DNA (dd-cfDNA); the AlloMap test, which uses gene expression profiling (GEP); and the HeartCare test, which combines AlloMap GEP testing with the AlloSure test. Also included in this policy are the AlloSure and Prospera dd-cfDNA tests for assessment of renal and lung transplant rejection.

OBJECTIVE

The objective of this evidence review is to determine whether the measurement of various selected biomarkers improves the detection of allograft rejection in transplant patients or in the diagnosis and management of heart failure, thus improving net health outcomes.

BACKGROUND

Heart Failure

Heart failure is a major cause of morbidity and mortality worldwide. The term *heart failure* refers to a complex clinical syndrome that impairs the heart's ability to move blood through the circulatory system.[1,] The prevalence of heart failure in the U.S. between 2013 and 2016 was an estimated 6.2 million for Americans ≥20 years old, up from 5.7 million between 2009 and 2012.[2,3,] Heart failure is the leading cause of hospitalization among people older than age 65 years, with direct and indirect costs estimated at $37 billion annually in the U.S.[2,] Although survival has improved with treatment advances, absolute mortality rates of heart failure remain near 50% within 5 years of diagnosis.

Physiology

Heart failure can be caused by disorders of the pericardium, myocardium, endocardium, heart valves or great vessels, or metabolic abnormalities. Individuals with heart failure may present with a wide range of left ventricular (LV) anatomy and function. Some have normal LV size and preserved ejection fraction; others have severe LV dilatation and depressed ejection fraction. However, most patients present with key signs and symptoms secondary to congestion in the lungs from impaired LV myocardial function.[1,] They include dyspnea, orthopnea, and paroxysmal dyspnea. Other symptoms include weight gain due to fluid retention, fatigue, weakness, and exercise intolerance secondary to diminished cardiac output.

Diagnosis

Initial evaluation of a patient with suspected heart failure is typically based on clinical history, physical examination, and chest radiograph. Because people with heart failure may present with nonspecific signs and symptoms (eg, dyspnea), accurate diagnosis can be challenging. Therefore, noninvasive imaging procedures (eg, echocardiography, radionuclide angiography) are used to quantify pump function of the heart, thus identifying or excluding heart failure in patients with

characteristic signs and symptoms. These tests can also be used to assess prognosis by determining the severity of the underlying cardiac dysfunction.[1,] However, clinical assessment and noninvasive imaging can be limited in accurately evaluating patients with heart failure because symptoms and signs can poorly correlate with objective methods of assessing cardiac dysfunction.[4,5,6,] Thus, invasive procedures (eg, cardiac angiography, catheterization) are used in select patients with presumed heart failure symptoms to determine the etiology (ie, ischemic vs. nonischemic) and physiologic characteristics of the condition.

Treatment

Patients with heart failure may be treated using a number of interventions. Lifestyle factors such as the restriction of salt and fluid intake, monitoring for increased weight, and structured exercise programs are beneficial components of self-management. A variety of medications are available to treat heart failure. They include diuretics (eg, furosemide, hydrochlorothiazide, spironolactone), angiotensin-converting enzyme inhibitors (eg, captopril, enalapril, lisinopril), angiotensin receptor blockers (eg, losartan, valsartan, candesartan), b-blockers (eg, carvedilol, metoprolol succinate), and vasodilators (eg, hydralazine, isosorbide dinitrate). Numerous device-based therapies are also available. Implantable cardioverter defibrillators reduce mortality in patients with an increased risk of sudden cardiac death. Cardiac resynchronization therapy improves symptoms and reduces mortality for patients who have disordered LV conduction evidenced by a wide QRS complex on electrocardiogram. Ventricular assist devices are indicated for patients with end-stage heart failure who have failed all other therapies and are also used as a bridge to cardiac transplantation in select patients.[1,]

Heart Failure Biomarkers

Because of limitations inherent in standard clinical assessments of patients with heart failure, a number of objective disease biomarkers have been investigated to diagnose and assess heart failure patient prognosis, with the additional goal of using biomarkers to guide therapy.[7,] They include a number of proteins, peptides, or other small molecules whose production and release into circulation reflect the activation of remodeling and neurohormonal pathways that lead to LV impairment. Examples include B-type natriuretic peptide (BNP), its analogue N-terminal pro B-type natriuretic peptide (NT-proBNP), troponin T and I, renin, angiotensin, arginine vasopressin, C-reactive protein, and norepinephrine.[1,7,]

BNP and NT-proBNP are considered the reference standards for biomarkers in assessing heart failure patients. They have had a substantial impact on the standard of care for diagnosis of heart failure and are included in the recommendations of all major medical societies, including the American College of Cardiology Foundation and American Heart Association, [8,] European Society of Cardiology,[9,] and the Heart Failure Society of America.[10,] Although natriuretic peptide levels are not 100% specific for the clinical diagnosis of heart failure, elevated BNP or NT-proBNP levels in the presence of clinical signs and symptoms reliably identify the presence of structural heart disease due to remodeling and heightened risk for adverse events. Natriuretic peptides also can help in determining the prognosis of heart failure patients, with elevated blood levels portending a poorer prognosis.

In addition to diagnosing and assessing the prognosis of heart failure patients, blood levels of BNP or NT-proBNP have been proposed as an aid for managing patients diagnosed with chronic heart failure. [8,11,12,] Levels of either biomarker rise in response to myocardial damage and LV remodeling, whereas they tend to fall as drug therapy ameliorates symptoms of heart failure. Evidence from a large number of randomized controlled trials (RCTs) that have compared BNP- or NT-proBNP-guided therapy with clinically guided adjustment of pharmacologic treatment of patients who had chronic heart failure has been assessed in recent systematic reviews and meta-analyses. However, these analyses have not consistently reported a benefit for BNP-guided management. Savarese et al (2013) published the largest meta-analysis to date–a patient-level meta-analysis that evaluated 2686 patients from 12 RCTs.[11,] This meta-analysis showed that NT-proBNP-guided management was associated with significant reductions in all-cause mortality and heart failure-related hospitalization compared with clinically guided treatment. Although BNP-guided management in this meta-analysis was not associated with significant reductions in these parameters, differences in patient numbers and characteristics may explain the discrepancy. Troughton et al (2014) conducted a second patient-level meta-analysis that included 11 RCTs with 2000 patients randomized to natriuretic peptide-guided pharmacologic therapy or usual care.[12,] The results showed that, among patients 75 years of age or younger with chronic heart failure, most of whom had impaired left ventricular ejection fraction, natriuretic peptide-guided therapy was associated with significant reductions in all-cause mortality compared with clinically guided therapy. Natriuretic-guided therapy also was associated with significant reductions in hospitalization due to heart failure or cardiovascular disease.

Suppression of Tumorigenicity-2 Protein Biomarker

A protein biomarker, ST2, has elicited interest as a potential aid to predict prognosis and manage therapy of heart failure.[13,14,15,16,17,18,19,] This protein is a member of the interleukin-1 (IL-1) receptor family. It is found as a transmembrane isoform (ST2L) and a soluble isoform (sST2), both of which have circulating IL-33 as their primary ligand. ST2 is a unique biomarker that has pluripotent effects in vivo. Thus, binding between IL-33 and ST2L is believed to have an immunomodulatory function via T-helper type 2 lymphocytes and was initially described in the context of cell proliferation, inflammatory states, and autoimmune diseases.[20,] However, the IL-33/ST2L signaling cascade is also strongly induced through the mechanical strain of cardiac fibroblasts or cardiomyocytes. The net result is mitigation of adverse cardiac remodeling and myocardial fibrosis, which are key processes in the development of heart failure.[21,] The soluble isoform of ST2 is produced by lung epithelial cells and cardiomyocytes and is secreted into circulation in response to exogenous stimuli, mechanical stress, and cellular stretch. This form of ST2 binds to circulating IL-33, acting as a "decoy," thus inhibiting the IL-33-associated antiremodeling effects of the IL-33/ST2L signaling pathway. Thus, on a biologic level, IL-33/ST2L signaling plays a role in modulating the balance of inflammation and neurohormonal activation and is viewed as pivotal for protection from myocardial remodeling, whereas sST2 is viewed as attenuating this protection. In the clinic, blood concentrations of sST2 appear to correlate closely with adverse cardiac structure and functional changes consistent with remodeling in patients with heart failure, including abnormalities in filling pressures, chamber size, and systolic and diastolic function.[7,15,17,]

An enzyme-linked immunosorbent-based assay is commercially available for determining sST2 blood levels (Presage ST2 Assay).[18,] The manufacturer claims a limit of detection of 1.8 ng/mL for sST2, and a limit of quantification of 2.4 ng/mL, as determined according to Clinical and Laboratory Standards Institute guideline EP-17-A. Mueller and Dieplinger (2013) reported a limit of detection of 2.0 ng/mL for sST2 in their study.[18,] In the same study, the assay had a within-run coefficient of variation of 2.5% and a total coefficient of variation less than 4.0%, demonstrated linearity within the dynamic range of the assay calibration curve, and exhibited no relevant interference or cross-reactivity.

The ST2 biomarker is not intended to diagnosis heart failure because it is a relatively nonspecific marker that is increased in many other disparate conditions that may be associated with acute or chronic manifestations of heart failure.[17,18,] Although the natriuretic peptides (BNP, NT-proBNP) reflect different physiologic aspects of heart failure compared with sST2, they are considered the reference standard biomarkers when used with clinical findings to diagnose, prognosticate, and manage heart failure and as such are the comparator to sST2.

Heart Transplant Rejection
Most cardiac transplant recipients experience at least a single episode of rejection in the first year after transplantation. The International Society for Heart and Lung Transplantation (2005) modified its grading scheme for categorizing cardiac allograft rejection.[22,] The revised (R) categories are listed in Table 1.

Table 1. Revised Grading Schema for Cardiac Allograft Rejection

New Grade	Definition	Old Grade
0R	No rejection	
1R	Mild rejection	1A, 1B, and 2
2R	Moderate rejection	3A
3R	Severe rejection	3B and 4

Acute cellular rejection (ACR) is most likely to occur in the first 6 months after transplantation, with a significant decline in the incidence of rejection after this time. Although immunosuppressants are required on a life-long basis, dosing is adjusted based on graft function and the grade of ACR determined by histopathology. Endomyocardial biopsies are typically taken from the right ventricle via the jugular vein periodically during the first 6 to 12 months post transplant. The interval between biopsies varies among clinical centers. A typical schedule is weekly for the first month, once or twice monthly for the following 6 months, and several times (monthly to quarterly) between 6 months and 1-year post transplant. Surveillance biopsies may also be performed after the first postoperative year (eg, on a quarterly or semiannual basis). This practice, although common, has not been demonstrated to improve transplant outcomes. Some centers no longer routinely perform endomyocardial biopsies after 1 year in patients who are clinically stable.

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While the endomyocardial biopsy is the criterion standard for assessing heart transplant rejection, it is limited by a high degree of interobserver variability in the grading of results and potential morbidity that can occur with the biopsy procedure. Also, the severity of rejection may not always coincide with the grading of the rejection by biopsy. Finally, a biopsy cannot be used to identify patients at risk of rejection, limiting the ability to initiate therapy to interrupt the development of rejection. For these reasons, an endomyocardial biopsy is considered a flawed criterion standard by many. Therefore, noninvasive methods of detecting cellular rejection have been explored. It is hoped that noninvasive tests will assist in determining appropriate patient management and avoid overuse or underuse of treatment with steroids and other immunosuppressants that can occur with false-negative and false-positive biopsy reports. Two techniques are commercially available for the detection of heart transplant rejection.

NONINVASIVE HEART TRANSPLANT REJECTION TESTS

Presage ST2 Assay
In addition to its use as a potential aid to predict prognosis and manage therapy of heart failure, elevated serum ST2 levels have also been associated with an increased risk of antibody-mediated rejection (AMR) following a heart transplant. For this reason, ST2 has also been proposed as a prognostic marker post heart transplantation and as a test to predict acute cellular rejection (graft-versus-host disease). The Presage ST2 Assay, described above, is a commercially available sST2 test that has been investigated as a biomarker of heart transplant rejection.

Heartsbreath Test
The Heartsbreath test, a noninvasive test that measures breath markers of oxidative stress, has been developed to assist in the detection of heart transplant rejection. In heart transplant recipients, oxidative stress appears to accompany allograft rejection, which degrades membrane polyunsaturated fatty acids and evolving alkanes and methylalkanes that are, in turn, excreted as volatile organic compounds in breath. The Heartsbreath test analyzes the breath methylated alkane contour, which is derived from the abundance of C4 to C20 alkanes and monomethylalkanes and has been identified as a marker to detect grade 3 (clinically significant) heart transplant rejection.

HeartCare
Cell-free DNA (cfDNA), released by damaged cells, is normally present in healthy individuals.[23,] In patients who have received transplants, donor-derived cfDNA (dd-cfDNA) may be also present. It is proposed that allograft rejection, which is associated with damage to transplanted cells, may result in an increase in dd-cfDNA. HeartCare (CareDx) is a commercially-available test that combines AlloMap gene expression profiling with a next-generation sequencing assay that quantifies the fraction of dd-cfDNA in cardiac transplant recipients relative to total cfDNA. The AlloMap score, AlloMap score variability, and AlloSure % dd-cfDNA are reported.

Prospera

Prospera Heart (Natera) is a commercially available assay that uses massively multiplexed PCR (mmPCR) followed by next-generation sequencing (NGS) to quantify the fraction of dd-cfDNA in transplant recipients. Donor versus recipient cfDNA is differentiated via an advanced bioinformatics analysis of >13,000 single-nucleotide polymorphisms (SNPs) without the need for prior recipient or donor genotyping or computational adjustments for related donors.[24,] The Prospera Heart test reports the dd-cfDNA fraction in the patient's blood as a predictor of acute rejection, although the optimal dd-cfDNA cut-point is not described by the manufacturer.

myTAI_{HEART}

Using proprietary myTAI_{HEART} software (TAI Diagnostics), the myTAI_{HEART} test uses multiplexed, high-fidelity amplification followed by allele-specific qPCR of a panel of 94 highly informative bi-allelic single nucleotide polymorphisms (SNPs) and 2 controls to quantitatively genotype cfDNA in the patient's plasma after cardiac transplant, and accurately distinguish dd-cfDNA originating from the engrafted heart from cfDNA originating from the recipient's native cells.[25,] The ratio of dd-cfDNA to total cfDNA is reported as the donor fraction (%) and categorizes the patient as at low or increased risk of moderate (grade 2R) to severe (grade 3R) ACR : low donor fractions indicate less damage to the transplanted heart and a lower risk for rejection, while increased donor fractions indicate more damage to the transplanted heart and an increased risk for rejection. Testing with myTAI_{HEART} does not require a donor specimen. TAI Diagnostics suspended production of the myTAI_{HEART} test in 2020. As of September 2022, TAI Diagnostics appears to no longer be operational and it is unclear if myTAI_{HEART} will be available through another company in the future.

AlloMap

Another approach has focused on patterns of gene expression of immunomodulatory cells, as detected in the peripheral blood. For example, microarray technology permits the analysis of the expression of thousands of genes, including those with functions known or unknown. Patterns of gene expression can then be correlated with known clinical conditions, permitting a selection of a finite number of genes to compose a custom multigene test panel, which then can be evaluated using polymerase chain reaction techniques. AlloMap (CareDx) is a commercially available molecular expression test that has been developed to detect acute heart transplant rejection or the development of graft dysfunction. The test involves expression measurement of a panel of genes derived from peripheral blood cells and applies an algorithm to the results. The proprietary algorithm produces a single score that considers the contribution of each gene in the panel. The score ranges from 0 to 40. The AlloMap website states that a lower score indicates a lower risk of graft rejection; the website does not cite a specific cutoff for a positive test.[26,] All AlloMap testing is performed at the CareDx reference laboratory in California.

Other laboratory-tested biomarkers of heart transplant rejection have been evaluated. They include brain natriuretic peptide, troponin, and soluble inflammatory cytokines. Most have had low accuracy in diagnosing rejection. Preliminary studies have evaluated the association between heart transplant rejection and micro-RNAs or high-sensitivity cardiac troponin in cross-sectional analyses but the clinical use has not been evaluated.[27,28,]

Renal Transplant Rejection

Allograft dysfunction is typically asymptomatic and has a broad differential, including graft rejection. Diagnosis and rapid treatment are recommended to preserve graft function and prevent loss of the transplanted organ. For a primary kidney transplant from a deceased donor (accounting for about 70% of kidney donors), graft survival at 1 year is 93% ; at 5 years, graft survival is 74%.[29,30,]

Surveillance of transplant kidney function relies on routine monitoring of serum creatinine, urine protein levels, and urinalysis.[31,] Allograft dysfunction may also be demonstrated by a drop in urine output or, rarely, as pain over the transplant site. With clinical suspicion of allograft dysfunction, additional noninvasive workup including ultrasonography or radionuclide imaging may be used. A renal biopsy allows a definitive assessment of graft dysfunction and is typically a percutaneous procedure performed with ultrasonography or computed tomography guidance. Biopsy of a transplanted kidney is associated with fewer complications than biopsy of a native kidney because the allograft is typically transplanted more superficially than a native kidney. Renal biopsy is a low-risk invasive procedure that may result in bleeding complications; loss of a renal transplant, as a complication of renal biopsy, is rare.[32,]

Kidney biopsies allow for diagnosis of acute and chronic graft rejection, which may be graded using the Banff Classification.[33,34,] Pathologic assessment of biopsies demonstrating acute rejection allows clinicians to further distinguish between ACR and AMR, which are treated differently.

NONINVASIVE RENAL TRANSPLANT REJECTION TESTS

AlloSure
AlloSure Kidney (CareDx) is a commercially available, next-generation sequencing assay that quantifies the fraction of dd-cfDNA in renal transplant recipients relative to total cfDNA by measuring 266 single nucleotide variants. Separate genotyping of the donor or recipient is not required but patients who receive a kidney transplant from a monozygotic (identical) twin are not eligible for this test. The fraction of dd-cfDNA relative to total cfDNA present in the peripheral blood sample is cited in the report. For patients undergoing surveillance, a routine testing schedule is recommended for longitudinal monitoring.

Prospera
Prospera Kidney (Natera) is a commercially available assay that quantifies the fraction of dd-cfDNA in renal transplant recipients. The manufacturer recommends use of the Prospera test when there is clinical suspicion of active rejection and for regular surveillance of subclinical rejection in renal transplant recipients.[35,] In a surveillance scenario, regular testing is recommended at 1, 2, 3, 4, 6, 9, and 12 months after renal transplant or most recent rejection. [36,] Thereafter, the test should be repeated quarterly. The proportion of dd-cfDNA relative to total cfDNA is reported, with detection of ≥1% dd-cfDNA indicating increased risk for active rejection. The percent dd-cfDNA change between tests is also reported.

Lung Transplant Rejection

Despite advances in induction and maintenance immunosuppressive regimens, lung transplant recipients have a median overall survival of 6 years, with more than a third of patients receiving treatment for acute rejection in the first year after transplant.[37,38,] Acute cellular rejection, lymphocytic bronchiolitis, and AMR are all risk factors for subsequent development of chronic lung allograft dysfunction (CLAD). Pathologic grading of ACR is based on the histological assessment of perivascular and interstitial mononuclear cell infiltrates. Antibody-mediated rejection may be clinical (symptomatic or asymptomatic allograft dysfunction) or subclinical (normal allograft function). Key diagnostic criteria established via consensus by the International Society for Heart and Lung Transplantation include the presence of antibodies directed toward donor human leukocyte antigens and characteristic lung histology with or without evidence of complement 4d within the graft.[39,] The most common phenotype of CLAD is a persistent obstructive decline in lung function known as bronchiolitis obliterans syndrome (BOS), which is graded based on the degree of decrease in FEV_1. Approximately 50% of patients develop BOS within 5 years post-transplant. Median survival following a diagnosis of BOS is 3-5 years. Acute rejection may present with non-specific physical symptoms or be asymptomatic. However, the role of surveillance bronchoscopy for screening asymptomatic patients for acute rejection is controversial, and performance of surveillance bronchoscopies varies across transplant centers.

NONINVASIVE LUNG TRANSPLANT REJECTION TESTS

AlloSure

AlloSure Lung (CareDx) is a commercially available, NGS assay that quantifies the fraction of dd-cfDNA in lung transplant patients relative to total cfDNA by measuring single nucleotide polymorphisms. The test is intended to provide a direct, noninvasive measure of organ injury in lung transplant patients who are undergoing surveillance. Suggested thresholds for severe injury, injury, and quiescence are >0.9%, >0.5 to ≤0.9%, and <0.5%, respectively.[40,]

Prospera

Prospera Lung (Natera) is a commercially available assay that uses the same methodology as Propera Heart and Prospera Kidney to quantify the fraction of dd-cfDNA in transplant recipients. The Prospera Lung test reports the dd-cfDNA fraction in the patient's blood as a predictor of acute rejection, chronic rejection, or infection although the optimal dd-cfDNA cut-point for each outcome is not described by the manufacturer.[41,]

REGULATORY STATUS

The U.S. Food and Drug Administration (FDA) has cleared multiple biomarker tests for the detection of heart and renal allograft rejection. Table 2 provides a summary of the biomarker tests currently included in this policy that have FDA clearance.

Table 2. Select Biomarker Tests for Detection of Heart or Renal Allograft Rejection Cleared by the U.S. Food and Drug Administration

Test	Manufacturer	FDA Clearance Type, Product Number	FDA Clearance Date	Indicated Use
Heartsbreath™	Menssana Research	Humanitarian device exemption, H030004	2004	To aid in diagnosing grade 3 heart transplant rejection in patients who have received heart transplants within the preceding year. The device is intended as an adjunct to, and not as a substitute for, endomyocardial biopsy and is also limited to patients who have had endomyocardial biopsy within the previous month.
AlloMap® Molecular Expression Testing	CareDx, formerly XDx	510(k), k073482	2008	The test is to be used in conjunction with clinical assessment, for aiding in the identification of heart transplant recipients with stable allograft function and a low probability of moderate-to-severe transplant rejection. It is intended for patients at least 15 years old who are at least 2 months post transplant.
Presage® ST2 Assay kit	Critical Diagnostics	510(k), k093758	2011	For use with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure

FDA: Food and Drug Administration.

Laboratory Developed Tests
There are also commercially available laboratory-developed biomarker tests for the detection of heart and renal allograft rejection. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. The AlloSure (CareDx), and Prospera (Natera) dd-cfDNA tests are regulated under the Clinical Laboratory Improvement Amendments standards.

myTAI$_{HEART}$ is also a laboratory developed test (LDT) developed for clinical diagnostic performance exclusively in the College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendment (CLIA) accredited TAI Diagnostics Clinical Reference Laboratory.[25,] This test was developed and its performance characteristics were determined by TAI Diagnostics.

These LDTs have not been cleared or approved by the FDA.

Other Tests

Other commercially available LDTs without FDA clearance or approval for use have been excluded from this evidence review when studies reporting on the clinical validity of the marketed version of the test could not be identified and/or where the test is marketed for research use only. Excluded tests and their descriptions are summarized for reference purposes in Table 3.

This policy does not address urine-based tests for allograft dysfunction (eg, One Lambda Laboratories' CXCL10).

Table 3. Biomarker Tests Excluded from Review

Test	Manufacturer	Technology	Indications for Use
AllosurePlus®	CareDx	dd-cfDNA	A post-transplant rejection risk prediction algorithm that incorporates AlloSure dd-cfDNA in an artificial intelligence supported model. The test is intended for use post-transplant to provide a personalized probability score and rejection status as a complement to conventional biopsy and standard clinical assessment.
KidneyCare®	CareDx	dd-cfDNA and GEP	Available as a research tool through the OKRA Registry.
AlloSeq® HCT	CareDx	NGS	To aid in the assessment of engraftment following HCT via NGS analysis of 202 biallelic SNPs. The fraction of recipient and donor genomic DNA is reported. The test is marketed for research use only.
AlloSeq® Tx17	CareDx	NGS	An NGS test utilizing Hybrid Capture Technology conducted pre-transplant to identify optimal transplant matches. The test sequences full HLA genes and other transplant-associated genes (KIR, MICA/B, C4, HPA, ABO). This test is marketed for research use only.
Viracor TRAC®	Eurofins	dd-cfDNA	To aid in the diagnosis of solid organ transplant rejection via NGS analysis. The fraction of dd-cfDNA is reported.[1]
TruGraf® Kidney	Eurofins	NGS	Identifies immune quiescence vs. risk for subclinical rejection in stable kidney transplant recipients to guide when a biopsy can be safely avoided.
OmniGraf® Liver	Eurofins	NGS	Identifies immune quiescence vs. risk for subclinical rejection in stable liver transplant recipients to guide when a biopsy can be safely avoided.
VitaGraf™ Kidney	Oncocyte Corporation	dd-cfDNA	Detection of kidney allograft injury and acute rejection based on dd-cfDNA fraction.

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Test	Manufacturer	Technology	Indications for Use
VitaGraf™ Liver	Oncocyte Corporation	dd-cfDNA	Detection of liver allograft injury and acute rejection based on dd-cfDNA fraction.
MMDx® Heart	Kashi Clinical Laboratories/Thermo Fisher	Tissue-based microarray	Tissue-based microarray mRNA gene expression test of 1283 genes post-transplant to provide a probability score of rejection as a complement to conventional biopsy processing. The test is not marketed to provide information for the diagnosis, prevention, or treatment of disease or to aid in the clinical decision-making process.
MMDx® Kidney	Kashi Clinical Laboratories/Thermo Fisher	Tissue-based microarray	Tissue-based microarray mRNA gene expression test of 1494 genes post-transplant to provide a probability score of rejection as a complement to conventional biopsy processing. The test is not marketed to provide information for the diagnosis, prevention, or treatment of disease or to aid in the clinical decision-making process.
MMDx® Lung	Kashi Clinical Laboratories/Thermo Fisher	Tissue-based microarray	Tissue-based microarray mRNA gene expression test post-transplant to provide a probability score of rejection as a complement to conventional biopsy processing. The test is not marketed to provide information for the diagnosis, prevention, or treatment of disease or to aid in the clinical decision-making process.

dd-cfDNA: donor-derived cell-free DNA; GEP: gene expression profiling; HCT: hematopoietic cell transplantation; HLA: human leukocyte antigen; MMDx: molecular microscope diagnostic system; NGS: next-generation sequencing; OKRA: Outcomes in KidneyCare in Renal Allografts; SNP: single-nucleotide polymorphism; TRAC: transplant rejection allograft check.

[1] Published studies reporting on the clinical validity of the marketed version of the test were not identified.

POLICY

A.   The use of the Presage ST2 Assay to evaluate the prognosis of individuals diagnosed with chronic heart failure is considered **experimental / investigational**.

B.   The use of the Presage ST2 Assay to guide management (e.g., pharmacologic, device-based, exercise) of individuals diagnosed with chronic heart failure is considered **experimental / investigational**.

C.   The use of the Presage ST2 Assay in the post cardiac transplantation period, including but not limited to predicting prognosis and predicting acute cellular rejection, is considered **experimental / investigational**.

D.   The measurement of volatile organic compounds to assist in the detection of moderate grade 2R (formerly grade 3) heart transplant rejection is considered **experimental / investigational**.

E.   The use of peripheral blood measurement of dd-cfDNA in the post cardiac transplantation period, including but not limited to predicting prognosis and predicting acute cellular rejection, is considered **experimental / investigational.**

F.   The use of peripheral blood gene expression profile tests or peripheral blood measurement of donor-derived cell-free DNA (dd-cfDNA), alone or in combination, in the management of individuals after heart transplantation, including but not limited to the detection of acute heart transplant rejection or heart transplant graft dysfunction, is considered **experimental / investigational**.

G.   The use of peripheral blood measurement of donor-derived cell-free DNA in the management of individuals after renal transplantation, including but not limited to the detection of acute renal transplant rejection or renal transplant graft dysfunction, is considered **experimental / investigational**.

H.   The use of peripheral blood measurement of dd-cfDNA in the management of individuals after lung transplantation, including but not limited to the detection of acute lung transplant rejection or lung transplant graft dysfunction, is considered **experimental / investigational**.

POLICY GUIDELINES
The U.S. Food and Drug Administration has indicated that the Heartsbreath (Menssana Research) test is only for use as an aid in the diagnosis of grade 3 (now known as grade 2R) heart transplant rejection in individuals who have received heart transplants within the preceding year and who have had endomyocardial biopsy within the previous month.

Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

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RATIONALE

This evidence review was created using searches of the PubMed database. The most recent literature update was performed through November 17, 2025.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

USE OF SOLUBLE SUPPRESSION OF TUMORIGENICITY-2 LEVELS IN CHRONIC HEART FAILURE PATIENTS

Clinical Context and Test Purpose

The purpose of the Soluble Suppression of Tumorigenicity-2 (sST2) assay is to determine prognosis and/or to guide management in individuals with chronic heart failure as an alternative to or an improvement on existing tests and clinical assessment.

The following PICO was used to select literature to inform this review.

Populations

The relevant population of interest is individuals with chronic heart failure.

Interventions

The test being considered is sST2 assay to determine prognosis and/or to guide management. Elevated sST2 levels are purported to predict a higher risk of poor outcomes.

Comparators

Comparators of interest include standard prognostic markers, including B-type natriuretic peptide levels and clinical assessment.

Outcomes

The general outcomes of interest are overall survival (OS), quality of life, and hospitalizations. Follow-up of 6-12 months would be appropriate to assess quality of life outcomes.

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Study Selection Criteria

For the evaluation of clinical validity of sST2 testing, methodologically credible studies were selected using the following principles:

- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Individual /sample clinical characteristics were described
- Individual selection criteria were described
- Included a validation cohort separate from the development cohort

REVIEW OF EVIDENCE

Subanalyses from Randomized Controlled Trials

A number of clinical studies in which sST2 blood levels were determined using the Assay have reported that there is an association between ST2 levels and adverse outcomes in patients diagnosed with chronic heart failure. A substantial body of biomarker evidence has been reported retrospectively from subsets of patients enrolled in randomized controlled trials (RCTs) of heart failure interventions. These RCTs include the Valsartan Heart Failure Trial (Val-HeFT)[42,]; Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training (HF-ACTION)[43,]; Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA)[44,]; and ProBNP Outpatient Tailored Chronic Heart Failure study (PROTECT).[45,] Although patients in these RCTs were well-characterized and generally well-matched between study arms, the trials were neither intended nor designed specifically to evaluate biomarkers as risk predictors. At present, no prospectively gathered evidence is available from an RCT in which sST2 levels were compared with levels of a B-type natriuretic peptide (BNP or N-terminal pro B-type natriuretic peptide [NT-proBNP]) to predict risk for adverse outcomes among well-defined cohorts of patients with diagnosed chronic heart failure. Key results of larger individual studies are summarized in Table 4.

Table 4. Summary of Selected Clinical Studies of sST2 to Predict Outcomes in Chronic Heart Failure Patients

Study	Population	Mean Age, y	Study Description and Biomarkers	Primary Endpoints	Mean FU	Synopsis of Findings
Ky et al (2011)[46,]	Ambulatory CHF (N = 1,141, 75% of Penn HF Study population)	56	Retrospective analysis of sST2 and NT-proBNP levels and their incremental usefulness over clinical SHFM	Mortality or cardiac transplant	2.8 y	• Elevated sST2 levels associated with increased risk (adjusted p=.002) • sST2 in plus NT-proBNP levels showed moderate improvement over SHFM in predicting outcomes (p=.017)

Study	Population	Mean Age, y	Study Description and Biomarkers	Primary Endpoints	Mean FU	Synopsis of Findings
Bayes-Genis et al (2012)[47,]	Ambulatory decompensated HF (N = 891)	70	Retrospective analysis of sST2 and NT-proBNP levels from consecutive series	Mortality	2.8 y	• Elevated sST2 and NT-proBNP levels provided independent and additive prognostic information for elevated risk of mortality (p<.001)
Broch et al (2012)[48,]	Ischemic CHF (N = 1,149, 30% of CORONA RCT)	72	Retrospective analysis of sST2, NT-proBNP, and CRP levels	CV mortality, nonfatal myocardial infarction or stroke	2.6 y	• Elevated sST2 levels were independently associated with increased risk for mortality, hospitalization due to HF, or any CV hospitalization (p<.001) • sST2 did not provide additive prognostic information vs. NT-proBNP
Felker et al (2013)[49,]	Ambulatory HF (N = 910, 39% of HF-ACTION RCT)	59	Retrospective analysis of sST2 and NT-proBNP levels	Mortality, hospitalization, functional capacity	2.5 y	• Elevated sST2 levels were independently associated with increased risk for mortality, hospitalization due to HF, or any CV hospitalization (p<.0001) • sST2 and NT-proBNP provided independent prognostic information • sST2 did not provide additive prognostic information vs. NT-proBNP
Gaggin et al (2013)[50,]	Recently decompensated CHF (n=151,	63	Retrospective analysis of sST2 and	Composite outcome (worsening HF,	0.8 y	• Elevated sST2 levels associated with increased risk for

Study	Population	Mean Age, y	Study Description and Biomarkers	Primary Endpoints	Mean FU	Synopsis of Findings
	100% of PROTECT RCT)		NT-proBNP levels	hospitalization for HF, clinically significant CV events)		adverse CV outcome (p<.001) • sST2 and NT-proBNP did not provide independent prognostic information
Anand et al (2014)[51,]	CHF (n=1,650, 33% of Val-HeFT RCT)	63	Retrospective analysis of sST2, NT-proBNP, and other biomarker levels	All-cause mortality and composite outcome (mortality, SCD with resuscitation, hospitalization for HF, or administration of IV inotropic or vasodilator drug for ≥4 h without hospitalization)		• Elevated sST2 levels were independently associated with increased risk of poor outcomes (p<.001) • Baseline sST2 levels did not provide substantial prognostic information when added to a clinical model that included NT-proBNP levels
Zhang et al (2015)[52,]	De novo HF or decompensated CHF (N = 1161)	58	Prospective analysis of sST2 in a hospitalized sample at 1 center in China	All-cause mortality	1 y	• Elevated sST2 levels were independently associated with increased risk of all-cause mortality (p<.001) after adjustment for clinical risk factors and NT-proBNP levels
Dupuy et al (2016)[53,]	HF for ≥6 mo (N = 178)	75	Prospective analysis of sST2, NT-proBNP, and other biomarker levels in a sample from 1 center in France	All-cause mortality and CV mortality	42 mo[a]	• Elevated sST2 levels were independently associated with increased risk for all-cause mortality and CV mortality (p<.001) • In multivariate analysis, sST2 and CRP significantly associated with all-cause mortality and CV mortality

CHF: chronic heart failure; CRP: C-reactive protein; CV: cardiovascular; FU: follow-up; HF: heart failure; IV: intravenous; NT-proBNP: N-terminal pro B-type natriuretic peptide; RCT: randomized controlled trial; SCD: sudden cardiac death; SHFM: Seattle Heart Failure Model; sST2: soluble suppression of tumorigenicity-2 .
[a] Median.

Meta-Analyses
Aimo et al (2017) pooled findings of studies on the prognostic value of sST2 for chronic heart failure in a meta-analysis.[54,] The meta-analysis selected 7 studies, including post hoc analyses of RCTs, and calculated the association between the Presage ST2 Assay and health outcomes. A pooled analysis of 7 studies found that sST2 was a statistically significant predictor of overall mortality (hazard ratio [HR] = 1.75; 95% confidence interval [CI], 1.37 to 2.22). Moreover, a pooled analysis of 5 studies found that sST2 was a significant predictor of cardiovascular mortality (HR = 1.79; 95% CI, 1.22 to 2.63).

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No evidence is available from randomized or nonrandomized controlled studies in which outcomes from groups of well-matched patients managed using serial changes in sST2 blood levels were compared with those managed using the reference standard of BNP or NT-proBNP levels.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

No inferences can be drawn about the clinical utility of sST2 levels for chronic heart failure.

Section Summary: Use of Soluble Suppression of Tumorigenicity-2 in Chronic Heart Failure Patients
Several analyses, mostly retrospective, have evaluated whether sST2 levels are associated with disease prognosis, especially mortality outcomes. Studies mainly found that elevated sST2 levels were statistically associated with an elevated risk of mortality. A pooled analysis of study results found that sST2 levels significantly predicted overall mortality and cardiovascular mortality. Several studies, however, found that sST2 test results did not provide additional prognostic information compared with BNP or NT-proBNP levels. In general, it appears that elevated sST2 levels predict higher risk of poor outcomes better than lower levels. The available evidence is limited by interstudy inconsistency and differences in patient characteristics, particularly the severity of heart failure, its etiology, duration, and treatment. Furthermore, most of the evidence was obtained from retrospective analyses of sST2 levels in subsets of larger patient cohorts

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within RCTs, potentially biasing the findings. The evidence primarily shows associations between elevated sST2 levels and poor outcomes, but does not go beyond that in demonstrating a clinical connection among biomarker status, treatment received, and clinical outcomes.

USE OF SOLUBLE ST2 SUPPRESSION OF TUMORIGENICITY-2 IN POST–HEART TRANSPLANTATION PATIENTS

Clinical Context and Test Purpose
The purpose of sST2 assay is to determine prognosis and/or to predict ACR in individuals with heart transplantation an alternative to or an improvement on existing tests.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with heart transplantation.

Interventions
The test being considered is sST2 assay to determine prognosis and/or to predict ACR.

Comparators
Comparators of interest include endomyocardial biopsy for predicting ACR.

Outcomes
The general outcomes of interest are OS, quality of life, and hospitalizations.

Table 5. Significant Outcomes for Post–Heart Transplantation Patients.

Outcomes	Details	Timing
Morbid events	Short-term and long-term events, such as acute cellular rejection, myocardial infarction, and stroke	30 days, 6 months, 1-5 years
Hospitalizations	Inpatient hospital admissions	30 days, 6 months, 1-5 years

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Study Selection Criteria
For the evaluation of clinical validity of sST2 testing, methodologically credible studies were selected using the following principles:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Individual /sample clinical characteristics were described
- Individual /sample selection criteria were described

- Included a validation cohort separate from the development cohort

Observational Studies

Serum ST2 levels have been proposed as a prognostic marker post heart transplantation and as a test to predict ACR (graft-versus-host disease). There is very little evidence available for these indications (summarized in Tables 6 and 7). Januzzi et al (2013) retrospectively assessed sST2 levels in 241 patients post–heart transplant.[55,] Over a follow-up out to 7 years, sST2 levels were predictive of total mortality (HR = 2.01; 95% CI, 1.15 to 3.51; p =.01). Soluble ST2 levels were also associated with risk of ACR , with a significant difference between the top and bottom quartiles of sST2 levels in the risk of rejection (p =.003).

Pascual-Figal et al (2011) reported on 26 patients post cardiac transplantation with and an acute rejection episode.[56,] Soluble ST2 levels were measured during the acute rejection episode and compared with levels measured when acute rejection was not present. Soluble ST2 levels were higher during the acute rejection episode (130 ng/mL) than during the nonrejection period (50 ng/mL; p=.002). Elevated sST2 levels greater than 68 ng/mL had a positive predictive value (PPV) of 53% and a negative predictive value (NPV) of 83% for the presence of ACR. The addition of sST2 levels to serum BNP resulted in incremental improvement in identifying rejection episodes.

Table 6. Summary of Key Nonrandomized Clinical Validity Study Characteristics

Study	Study Type	Country	Dates	Participants	Treatment	Follow-Up
Januzzi (2013)[55,]	Retrospective	United States	NR	Post–cardiac transplantation	sST2 levels assessment (n=241)	Median 7.1 years
Pascual-Figal (2011)[56,]	Retrospective	Spain	2002-2007	Post–cardiac transplantation with acute rejection	sST2 levels assessment (n=26)	Median 3 months

NR: not reported, sST2: soluble suppression of tumorigenicity-2.

Table 7. Summary of Key Nonrandomized Clinical Validity Study Results

Study	Total Mortality	ST2 Levels	PPV	NPV
Januzzi (2013)[55,]			NR	NR
HR (95% CI)	2.02 (1.16-3.52)	≥ 30 ng/mL at 7-year follow-up	NA	NA
P-value	.01	NR	NA	NA
Pascual-Figal (2011)[56,]			53%	83%
Rejection Episode	NR	130 ng/mL (IQR 60-238 ng/mL)	NA	NA

Study	Total Mortality	ST2 Levels	PPV	NPV
Nonrejection Period	NR	50 ng/mL (IQR 28-80 ng/mL)	NA	NA
P-value	NR	.002	NA	NA

CI: confidence interval; HR: hazard ratio; IQR: interquartile range; NA: not applicable; NPV: negative predictive value; NR: not reported, PPV: positive predictive value.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs were identified using sST2 levels that directed patient management in heart transplantation patients and which assessed patient outcomes.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

No inferences can be drawn about the clinical utility of sST2 levels for patients with heart transplantation.

Section Summary: Use of Soluble Suppression of Tumorigenicity-2 sST2 in Post–Heart Transplantation Patients
Few studies are available, and they are observational and retrospective. No prospective studies were identified that provide high-quality evidence on the ability of sST2 levels to predict transplant outcomes. One retrospective study (N=241) found that sST2 levels were associated with ACR and mortality; another study (N=26) found that sST2 levels were higher during an acute rejection episode than before rejection.

MEASUREMENT OF VOLATILE ORGANIC COMPOUNDS FOR HEART TRANSPLANT

Clinical Context and Test Purpose
The purpose of measuring volatile organic compounds in individuals with a heart transplant is to assess for heart allograft rejection in a noninvasive manner.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with a heart transplant.

Interventions
The test being considered measures volatile organic compounds to assess for allograft rejection.

Comparators
The following test is currently being used to diagnose heart allograft rejection: routine endomyocardial biopsy.

Outcomes
The general outcomes of interest are OS, test validity, morbid events, and hospitalizations. Follow-up over months to years is necessary to monitor for signs of allograft rejection.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Study Selection Criteria
For the evaluation of the clinical validity of measuring volatile organic compounds, studies that met the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard (describe the reference standard)
- Individual /sample clinical characteristics were described
- Individual /sample selection criteria were described.

Observational Studies
The U.S. Food and Drug Administration approval of the Heartsbreath test was based on the results of the Heart Allograft Rejection: Detection with Breath Alkanes in Low Levels (HARDBALL) study sponsored by the National Heart, Lung, and Blood Institute.[57,] The HARDBALL study was a 3-year, multicenter study of 1061 breath samples in 539 heart transplant patients. Before the scheduled endomyocardial biopsy, patient breath was analyzed by gas chromatography and mass spectroscopy for volatile organic compounds. The amount of C4 to C20 alkanes and monomethylalkanes was used to derive the marker for rejection, known as the breath methylated alkane contour. The breath methylated alkane contour results were compared with subsequent biopsy results, as interpreted by 2 readers using the International Society for Heart and Lung Transplantation biopsy grading system as the criterion standard for rejection.[22,]

The authors of the HARDBALL study reported that the abundance of breath markers that measured oxidative stress was significantly greater in grade 0, 1, or 2 rejection than in healthy normal persons. In contrast, in grade 3 rejection, the abundance of breath markers that measure oxidative stress was found to be reduced, most likely due to accelerated catabolism of alkanes and methylalkanes that make up the breath methylated alkane contour. The authors also reported that in identifying grade 3 rejection, the NPV of the breath test (97.2%) was similar to

endomyocardial biopsy (96.7%) and that the breath test could potentially reduce the total number of biopsies performed to assess for rejection in patients at low-risk for grade 3 rejection. The sensitivity of the breath test was 78.6% vs 42.4% with biopsy. However, the breath test had a lower specificity (62.4%) and a lower PPV (5.6%) in assessing grade 3 rejection than a biopsy (specificity, 97%; PPV = 45.2%). In addition, the breath test was not evaluated in grade 4 rejection.

Findings from the HARDBALL study were published by Phillips et al (2004). No subsequent studies evaluating the use of the Heartsbreath test to assess for graft rejection were identified in literature updates.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs assessing the measurement of volatile organic compounds to diagnose cardiac allograft rejection were identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of measuring volatile organic compounds to assess for cardiac allograft rejection has not been established, a chain of evidence to support clinical utility cannot be constructed.

Section Summary: Measurement of Volatile Organic Compounds for Heart Transplant
A published study found that for identifying grade 3 (now grade 2R) rejection, the NPV of the breath test the study evaluated (97.2%) was similar to endomyocardial biopsy (96.7%), and the sensitivity of the breath test (78.6%) was better than that for biopsy (42.4%). However, the breath test had a lower specificity (62.4%) and a lower PPV (5.6%) in assessing grade 3 rejection than a biopsy (specificity, 97%; PPV = 45.2%). The breath test was also not evaluated for grade 4 rejection. At present, no studies evaluating the clinical utility for the measurement of volatile organic compound testing for heart transplant have been identified.

DONOR-DERIVED CELL-FREE DNA TESTING FOR HEART TRANSPLANT

Clinical Context and Test Purpose
The purpose of donor-derived cell-free DNA (dd-cfDNA) testing in individuals with a heart transplant is to assess for allograft rejection.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with heart transplants.

Interventions
The test being considered is dd-cfDNA testing to assess for allograft rejection (i.e., AlloSure, Prospera, myTAI$_{HEART)}$.

The AlloSure and Prospera tests report the fraction of dd-cfDNA, with both tests using a proposed high-risk of active transplant rejection cutoff of ≥0.15%. Clinical interpretation of alternate thresholds for quiescence (<0.12%), injury (0.20%) and severe injury (0.35%) have also been proposed.

The myTAI$_{HEART}$ test uses proprietary software to quantitatively genotype cfDNA in the patient's plasma after cardiac transplant, and distinguish dd-cfDNA originating from the engrafted heart from cfDNA originating from the recipient's native cells. Production of the myTAI$_{HEART}$ test was halted in 2020.

Comparators
The following test is currently being used to diagnose cardiac allograft rejection: routine endomyocardial biopsy.

Outcomes
The general outcomes of interest are OS, test validity, morbid events, and hospitalizations. Follow-up over months to years is needed to monitor for signs of allograft rejection.

Beneficial outcomes resulting from a true-negative test result are avoiding unnecessary subsequent biopsy. Harmful outcomes resulting from a false-positive result may include unnecessary biopsy or unnecessary treatment. Harmful outcomes from a false-negative result are increased risk of adverse transplant outcomes.

In a triage scenario, the test would need to identify precisely a group of individuals that could safely forgo biopsy; therefore, the sensitivity, NPV, and negative likelihood ratio are key test performance characteristics.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Study Selection Criteria
For the evaluation of the clinical validity of dd-cfDNA testing, studies that met the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)

- Included a suitable reference standard (describe the reference standard)
- Individual /sample clinical characteristics were described
- Individual /sample selection criteria were described.

AlloSure

Khush et al (2019) published performance characteristics for the AlloSure Heart dd-cfDNA test as assessed in the D-OAR prospective, multicenter registry study.[58,] Patients already undergoing AlloMap testing for surveillance were eligible for inclusion; however following a protocol amendment, dd-cfDNA specimens were only obtained in patients with clinical suspicion of rejection and a planned for-cause biopsy after 2016 through 2018. The majority of dd-cfDNA samples (81%) were drawn in the first-year post-transplant. The D-OAR cohort included 841 biopsy-paired dd-cfDNA results, of which 587 were performed for routine surveillance of rejection. Overall, cell-mediated rejection and AMR were biopsy-confirmed in 17 and 18 cases, respectively. The area under the curve (AUC) for detecting acute rejection was 0.64 (95% CI, 0.52 to 0.75). At a 0.2% cutoff for dd-cfDNA, the sensitivity, specificity, PPV, and NPV for detection of acute rejection was 80%, 44%, 8.9%, and 97.1% respectively. For the subgroup of patients undergoing surveillance, the sensitivity, specificity, PPV, and NPV were 38.1%, 84.0%, 8.1%, and 97.3%, with a corresponding AUC of 0.61 (95% CI, 0.46 to 0.74). Among for-cause samples, the sensitivity, specificity, PPV, and NPV were 53.8%, 76.1%, 11.6%, and 96.6%, respectively. The study is limited by the protocol changes designed to increase the number of observed rejection events overall and low availability of concurrent dd-cfDNA results with respect to biopsy specimens (58%).

Richmond et al (2023) published data on pediatric (n=60) and adult (n=61) heart transplant recipients (median age, 24.3) prospectively enrolled at 8 participating centers from August 2016 to October 2017 and followed for up to 12 months.[59,] All patients had samples from 1 or more endomyocardial biopsies post-transplantation with Allosure dd-cfDNA testing within 24 hours prior to biopsy. dd-cfDNA level was blinded to participants and investigators over the study period. Median dd-cfDNA was found to be significantly higher in the patients who had biopsy-defined allograft rejection (ACR or AMR) compared with healthy allograft participants (0.21% versus 09%, p<.0001). An AUC analysis yielded an AUC of 0.78 using a pre-defined dd-cfDNA threshold of 14% and resulted in a test sensitivity of 67% and a specificity of 79% (NPV = 94% and PPV = 34%), a sub-group analysis satisfying patients into adult of pediatric patients found similar results (AUC of the adult cohort = 0.81; AUC of the pediatric cohort =0.79).

Prospera Heart

Kim et al (2022)[60,] assessed the clinical validity of the Prospera Heart dd-cfDNA test versus endocardial biopsy for prediction of acute heart transplant rejection. The study included 811 samples (703 prospectively collected and 108 retrospectively collected) from 223 heart transplant patients with a planned biopsy from 2 U.S. centers. The median patient age was 54 years and 27% were female. Race/ethnicity of the study population was: 54% White, 21% Hispanic, 12% Black, 6% Asian and 5% other race/ethnicity. The majority (91% [737/811]) of reference standard biopsies were conducted for surveillance, and median dd-cfDNA was lower in the surveillance samples (0.04%) than the for-cause samples (0.22%). The time from transplant to biopsy was 10 weeks, and the total prevalence of acute rejection was 9.0%. Median dd-cfDNA % was 0.58% in patients with acute rejection, although fractions varied according to rejection

type/grade and were higher in those with antibody mediated rejection (median range 0.44% to 3.43%) than those with ACR (median range 0.045% to 0.13%). In patients without acute rejection, dd-cfDNA % was 0.04. Diagnostic accuracy for 3 dd-cfDNA fractions were explored: 0.12%, 0.15% and 0.20%. At a cut-off off of 0.12%, sensitivity was 86.6%, specificity was 72.0%, PPV was 23.4%, and NPV 98.2%. Corresponding values at a dd-cfDNA cut-of of 0.15% were 78.6%, 76.9%, 25.1% and 97.3%, and 78.6%, 82.1%, 30.3% and 97.5% at a dd-cfDNA cut-off of 0.20%. This resulted in an AUC for detection of acute rejection of 0.86 (95% CI 0.77 to 0.96). The optimal dd-cfDNA fraction for detection of heart transplant rejection has yet to be established. Limitations of the study include potential selection bias, as only patients with a scheduled biopsy were included in the study, and study authors noted that the prevalence of acute rejection in the study cohort was higher than in other cohorts.

A retrospective cohort study conducted by Rodgers et al (2023) compared dd-cfDNA testing with Allosure, which examines 405 single nucleotide polymorphisms (SNPs), to Prospera, which evaluates 13,292 SNPs, in 112 heart transplant patients.[61,] Participants were enrolled from October 2020 to January 2022 and had a median age of 60 years (IQR, 47 to 65 years). Both tests used a dd-cfDNA threshold value of 15%. Testing with Allosure resulted in a low sensitivity (39%) and high specificity (82%) for identification of acute rejection; the Prospera test had similar characteristics with sensitivity at an identical 39% and a negligible difference in specificity (84%). Between-group comparisons showed no difference between the 2 tests in this small cohort. PPV with the Allosure test was 6.2% compared to 7% in Prospera testing (p=.7) and NPV was 98% for both tests (p=.76).

myTAI_{HEART}

myTAI$_{HEART}$

In a study funded by TAI Diagnostics, Inc., North et al (2020) performed a blinded clinical validation study on 158 matched pairs of endomyocardial biopsy-plasma samples collected from 76 volunteer adult and pediatric heart transplant recipients (ages 2 months or older, and 8 days or more post-transplant) between June of 2010 and Aug 2016 from 2 Milwaukee transplant centers.[25,] Based on ACR grade as defined by the 2004 International Society for Heart and Lung Transplantation (ISHLT) classification, ROC analysis was performed to evaluate diagnostic accuracy across all possible dd-cfDNA % cutoffs. To maximize diagnostic accuracy, Youden's Index was used to select the optimal cutoff, found to correspond to a donor fraction value of 0.32%. Using this cutoff, clinical performance characteristics of the assay included a NPV of 100.00% for grade 2R or higher ACR , with 100.00% sensitivity and 75.48% specificity; AUC for this analysis was 0.842, indicative of robust ability of the donor fraction assay to rule out 2R or greater ACR for donor fraction values less than 0.32%. There was no statistically significant correlation of donor fraction with age. Donor fraction elevation can also be caused by other forms of injury to the donor heart such as ACR 1R, AMR , and presence of coronary artery vasculopathy (CAV), thereby requiring correlation of myTAI$_{HEART}$ results with other clinical indicators.

In study funded by a grant from the National Institutes of Health and TAI Diagnostics, Inc., Richmond et al (2019) assessed 174 post-cardiac transplant patients from 7 centers (ages 2.4 months-73.4 years) with myTAI$_{HEART}$ testing (before transplant; 1, 4, and 7 days following transplant; and at discharge from transplant hospitalization) using blinded analysis of biopsy-paired samples.[62,] All the patients were followed for at least 1 year. dd-cfDNA was higher in ACR 1R/2R (n = 15) than ACR 0R (healthy) (n = 42) (p =.02); an optimal donor fraction threshold (0.3%) was determined by the use of ROC analysis, revealing an AUC of 0.81 with a sensitivity of 0.65, specificity of 0.93, and an NPV of 81.8% for the absence of any allograft rejection.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs assessing the measurement of dd-cfDNA to diagnose cardiac allograft rejection were identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of measuring dd-cfDNA to assess for cardiac allograft rejection has not been established, a chain of evidence to support clinical utility cannot be constructed.

Section Summary: Measurement of Donor-Derived Cell-Free DNA for Heart Transplant
Studies measuring dd-cfDNA suggest that the dd-cfDNA fraction is elevated in acute rejection, but optimal fraction cut-offs for detection of acute rejection have not been established. Using dd-cfDNA thresholds ranging from 0.12% to 0.32% resulted in NPVs ranging from 82% to 98% and AUCs ranging from 0.61 to 0.86 in 5 studies. At present, no studies evaluating the clinical utility for the measurement of dd-cfDNA for heart transplant rejection have been identified.

GENE EXPRESSION PROFILING AND DONOR-DERIVED CELL-FREE DNA TESTING FOR HEART TRANSPLANT

Clinical Context and Test Purpose
The purpose of gene expression profiling (GEP) and donor-derived cell-free DNA (dd-cfDNA) testing in individuals with a heart transplant is to assess for allograft rejection.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest are individuals with heart transplants.

Interventions
The test being considered is GEP to assess for allograft rejection (ie, AlloMap), used alone or in combination with AlloSure Heart dd-cfDNA testing. The combination of these tests is commercially marketed as HeartCare (CareDx).

AlloMap test results are reported on a scale from 0 to 40, with a proposed high-risk cutoff of ≥ 30 for patients < 6 months post-transplant and ≥34 for patients ≥6 months post-transplant. The HeartCare report provides the AlloMap score, AlloMap score variability, and AlloSure percent dd-cfDNA. Direct guidance for the combined interpretation of results is not provided in the HeartCare report, but potential clinical implications of concordant and discordant test scenarios have been proposed.[63,]

Comparators
The following test is currently being used to diagnose cardiac allograft rejection: routine endomyocardial biopsy.

Outcomes
The general outcomes of interest are OS, test validity, morbid events, and hospitalizations. Follow-up over months to years is needed to monitor for signs of allograft rejection.

Beneficial outcomes resulting from a true-negative test result are avoiding unnecessary subsequent biopsy. Harmful outcomes resulting from a false-positive result may include unnecessary biopsy or unnecessary treatment. Harmful outcomes from a false-negative result are increased risk of adverse transplant outcomes.

In a triage scenario, the test would need to identify precisely a group of patients that could safely forgo biopsy; therefore, the sensitivity, NPV, and negative likelihood ratio are key test performance characteristics.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Study Selection Criteria
For the evaluation of the clinical validity of GEP testing, studies that met the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard (describe the reference standard)
- Individual /sample clinical characteristics were described
- Individual /sample selection criteria were described.

ALLOMAP

Systematic Reviews
A TEC Assessment (2011) reviewed the evidence on the use of GEP using the AlloMap test.[64,] The Assessment concluded that the evidence was insufficient to permit conclusions about the effect of the AlloMap test on health outcomes. Key evidence in the TEC Assessment is described below.

Nonrandomized Studies

Patterns of gene expression for the development of the AlloMap test were studied in the Cardiac Allograft Rejection Gene Expression Observation (CARGO) study, which included 8 U.S. cardiac transplant centers enrolling 629 cardiac transplant recipients.[65,] The study included the discovery and validation phases. In the discovery phase, patient blood samples were obtained during the endomyocardial biopsy, and the expression levels of more than 7000 genes involved in immune responses were assayed and compared with the biopsy results. A subset of 252 candidate genes was identified, from which a panel of 11 genes was selected for evaluation. A proprietary algorithm was applied to the results, producing a single score that considers the contribution of each gene in the panel.

The validation phase of the CARGO study, published by Deng et al (2006), was prospective, blinded, and enrolled 270 patients.[65,] Primary validation was conducted using samples from 63 patients independent from discovery phases of the study and enriched for biopsy-proven evidence of rejection. A prospectively defined test cutoff value of 20 resulted in a sensitivity of 84% of patients with moderate/severe rejection but a specificity of 38%. Of note, in the "training set" used in the study, these rates were 80% and 59%, respectively. The authors evaluated the 11-gene expression profile on 281 samples collected at 1 year or more from 166 patients who were representative of the expected distribution of rejection in the target population (and not involved in discovery or validation phases of the study). When a test cutoff of 30 was used, the NPV (no moderate/severe rejection) was 99.6%; however, only 3.2% of specimens had grade 3 or higher rejection. In this population, grade 1B scores were found to be significantly higher than grade 0, 1A, and 2 scores but were similar to grade 3 scores.

A second prospective multicenter study evaluating the clinical validity of GEP with the AlloMap test (CARGO II) was published by Crespo-Leiro et al (2016).[66,] The study enrolled 499 heart transplant recipients undergoing surveillance for allograft rejection. The reference standard for rejection status was histologic grade from an endomyocardial biopsy performed on the same day as blood samples were collected. Blood samples need to be collected 55 days or more post transplant, more than 30 days after blood transfusion, more than 21 days after administration of prednisone 20 mg/day or more, and more than 60 days after treatment for a prior rejection. Patients had a total of 1579 eligible blood samples for which paired GEP scores and endomyocardial biopsy rejection grades were available.

As in the original CARGO study, the proportion of cases of rejection was small. The prevalence of moderate-to-severe rejection (grade 2R/>3A) reported by local pathologists was 3.2%, which was reduced to 2.0% when confirmation from 1 or more other independent pathologists was required. At a GEP cutoff of 34, for patients who were at least 2 to 6 months post transplant, the sensitivity of GEP for detecting grade 2R/>3A was 25.0%, and the specificity was 88.7%. The PPV and NPV were 4.0% and 98.4%, respectively. Using the same cutoff of 34, for patients more than 6 months post transplant, the sensitivity of GEP was 25.0%, the specificity was 88.8%, the PPV was 4.3%, and the NPV was 98.3%. The number of true-positives used in the above calculations was 5 (9.1%) of 55 for patients at least 2 to 6 months post transplant and 6 (10.2%) of 59 for patients more than 6 months post transplant.

Kanwar et al (2021) published data from the Outcomes AlloMap Registry (OAR) indicating that asymptomatic or active cytomegalovirus infection is associated with significantly higher AlloMap scores among heart transplant recipients compared to those without infection, even in the absence of acute rejection, potentially resulting in unnecessary biopsies among surveillance patients.[67,] Donor-derived cell-free DNA levels measured by the AlloSure Heart test available for a small subset of samples (5.3%) were not significantly different between groups. The authors conclude that further assessment of the combined use of AlloMap and AlloSure scores is required to determine if this will improve differentiating infection-related from rejection-related immune activation. The combined use of these tests, commercially available as HeartCare (CareDx), is addressed in the following section.

HeartCare
The commercially available HeartCare (CareDx) test combines AlloMap GEP testing with AlloSure Heart measurement of percent dd-cfDNA. The combined use of GEP and dd-cfDNA testing for surveillance of acute rejection was assessed in a single-center, retrospective study conducted by Gondi et al (2021) between February 2019 and March 2020.[68,] Patients (N=153) were required to be ≥ 55 days post transplant, hemodynamically stable, ≥ 15 years of age, and single-organ recipients. The majority of patients were male (74.5%) and white (78.4%) with an average age of 54.5 years. Patients were assessed once monthly between 2 and 12 months, every 3 months between 12 and 24 months, and every 6 months between 24 and 36 months post transplant. Pre-specified thresholds for GEP scores were ≥ 30 for patients < 6 months post transplant and ≥ 34 for patients ≥ 6 months post-transplant. The pre-specified threshold for percent dd-cfDNA was ≥ 0.20% based on a prior study of the AlloSure test by Khush et al (2019),[58,] described in the following section. In patients < 6 months post-transplant, endomyocardial biopsy was performed regardless of test results. For patients ≥ 6 months post-transplant who received both GEP and dd-cfDNA testing, endomyocardial biopsy was canceled in patients with dd-cfDNA < 0.20% regardless of AlloMap score. In patients with positive AlloMap scores but negative dd-cfDNA, endomyocardial biopsy could be performed or deferred in favor of repeat dd-cfDNA testing. Among 495 samples, overall test result distributions were 59.6% for patients negative on both tests, 12.3% for patients positive by dd-cfDNA only, 22.6% for patients positive by GEP only, and 5.5% positive by both GEP and dd-cfDNA. The combined testing approach resulted in a 12.7% reduction (48 biopsies) in endomyocardial biopsy volume compared to GEP testing alone. Among the 172 biopsies performed, 2 patients with cell-mediated rejection were identified, with corresponding dual-positive tests. Two patients with AMR were identified, with corresponding tests that were only positive by dd-cfDNA. The study is limited by its retrospective design, incomplete evaluation of performance characteristics, and lack of reporting on health outcomes.

The Surveillance HeartCare Outcomes Registry (SHORE) evaluated dual testing with GEP and dd-cfDNA for acute cellular rejection (ACR) surveillance in heart transplant patients from 2018 to 2021.[69,] This multi-center, prospective study, involving 2,077 participants, assessed the utility of combined GEP/dd-cfDNA testing compared to single-test approaches. The incidence of biopsy-proven ACR varied by test result, with dual-negative results yielding the lowest rate (1.5%; 95% CI,1.1% to 2.0%), GEP-positive/dd-cfDNA-negative results at 1.9% (95% CI, 1.4% to 2.6%), dd-cfDNA-positive/GEP-negative results at 4.3% (95% CI, 2.8% to 6.6%), and dual-positive results showing the highest rate of 9.2% (95% CI, 7.1% to 11.9%). Sensitivity for detecting ACR was 59.0% (95% CI, 51.3% to 66.3%) for GEP alone, 44.7% (95% CI, 37.3% to 52.4%) for dd-

cfDNA alone, and 32.3% (95% CI, 25.6% to 39.9%) for dual-positive testing. Specificity for dual-positive testing was 91.7% (95% CI, 91.0% to 92.4%), yielding a positive likelihood ratio of 3.90 (95% CI, 3.08 to 4.96), which exceeded that of GEP (1.37) and dd-cfDNA (2.91) individually. Follow-up biopsy testing after dual noninvasive surveillance showed that dual-negative results were followed by biopsies in only 8.8% of cases (95% CI, 8.2% to 9.4%), whereas dual-positive results prompted biopsies in 35.4% (95% CI, 32.0% to 38.9%). In this registry cohort, the frequency of biopsies declined over time, with second-year biopsies decreasing from 1.5 biopsies per patient in 2017-2018 to 0.9 in 2021. Despite the reduction in biopsy volume, there was a 94.9% survival at two years with only 2.7% experiencing graft dysfunction. Limitations include site-based variability in biopsy interpretation, biopsies not performed at a consistent time post-transplantation, exclusion of antibody-mediated rejection (AMR) data from sensitivity analyses, and non-experimental study design.

Kim et al (2025) reported an updated SHORE registry analysis focused on AMR among 2,240 adult heart transplant recipients from 2017 to 2022, with verified biopsy, dd-cfDNA, echocardiographic graft function, and DSA data.[70,] Across 24,768 biopsies, AMR was identified in 2.6%, but incidence varied substantially by clinical context (eg, 1.1% in biopsies with normal graft function and no known DSA versus 20.4% in biopsies with graft dysfunction and known DSA). When analyses were restricted to paired dd-cfDNA/biopsy samples ≥55 days post-transplant, dd-cfDNA performance for detecting any biopsy-proven AMR depended on the positivity threshold: at 0.20%, sensitivity was 62.8% (95% CI, 54.2% to 70.6%) and specificity was 84.1% (95% CI, 83.2% to 84.9%), while at 0.50% sensitivity was 52.7% (95% CI, 44.1% to 61.1%) with improved specificity of 92.8% (95% CI, 92.2% to 93.3%). Teuteberg et al (2025) reported additional SHORE registry outcomes in adult heart-only recipients transplanted between January 1, 2017, and December 31, 2022 (n=1,934). [71,] In the 12 months following the most abnormal combined GEP and dd-cfDNA testing result during months 2 to 6 post-transplant, graft dysfunction occurred in 166 patients and cardiovascular death in 19 patients. Dual-positive (GEP+/dd-cfDNA+) results were associated with the highest incidence of graft dysfunction or cardiovascular death (15.5%) with a HR of 1.59 (95% CI, 1.08 to 2.32; p=.017) compared to those who were not GEP+/dd-cfDNA+.

Moeller et al (2025) reported a single-center retrospective review of adult single-organ heart transplant recipients monitored with paired dd-cfDNA and peripheral GEP from January 2019 through July 2023, evaluating both contemporaneous endomyocardial biopsy (EMB) findings and longer-term outcomes.[72,] A total of 2,388 HeartCare samples from 257 patients were categorized as dual-negative (dd-cfDNA-/GEP-; n=1,437), dual-positive (dd-cfDNA+/GEP+; n=157), or discordant (dd-cfDNA-/GEP+; n=419 and dd-cfDNA+/GEP-; n=375), using dd-cfDNA positivity defined as ≥0.12% and GEP positivity defined as >30 in the first 5 months post-transplant and >34 thereafter. EMB was paired with 693 samples (29%) and was performed more frequently in dual-positive and dd-cfDNA+/GEP- samples. Biopsy-proven rejection rates among biopsied samples were highest in dual-positive (15.1%) and dd-cfDNA+/pGEP- (10.9%) groups compared with dual-negative (3.8%) and dd-cfDNA-/GEP+ (3.7%) groups (p<.001). For detection of biopsy-confirmed rejection, dual-positive testing showed a sensitivity of 26.5% and specificity of 93.8% with a positive likelihood ratio of 4.31, while dd-cfDNA alone demonstrated higher sensitivity (67%) than GEP alone (36.7%). Over a median follow-up of 29 months (IQR, 16 to 42), mortality was 3.5% and graft dysfunction 2.3%; in mixed-effects Cox models, dual-positive

results were associated with substantially higher mortality risk versus dual-negative results (HR, 6.1; 95% CI, 2.5 to 14.8; p<.001), and dd-cfDNA+/pGEP− discordance was also associated with increased mortality (HR, 3.3; 95% CI, 1.3 to 8.3; p=.013), whereas GEP positivity without dd-cfDNA elevation was not statistically associated with mortality (HR, 2.1; p=.13). dd-cfDNA+/pGEP- discordance was additionally associated with higher graft dysfunction risk (HR, 2.2; 95% CI 1.01 to 4.6; p=.047 at 0.12%), while other groups showed no consistent increase. Limitations included a retrospective, single-center design; biopsy pairing not defined by protocol; low overall event rates; potential confounding from practice variation and variation in the timing of testing.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

ALLOMAP

Randomized Controlled Trials
Kobashigawa et al (2015) published the results of a pilot RCT evaluating the use of the AlloMap test in patients who were 55 days to 6 months post transplant.[73,] The trial design was similar to that of the Invasive Monitoring Attenuation through Gene Expression (IMAGE) RCT, discussed next. Sixty subjects were randomized to rejection monitoring with AlloMap or with endomyocardial biopsy at prespecified intervals of 55 days and 3, 4, 5, 6, 8, 10, and 12 months post transplant. The threshold for a positive AlloMap test was set at 30 for patients 2 to 6 months post transplant and 34 for patients after 6 months post transplant, based on data from the CARGO study. Endomyocardial biopsy outside of the scheduled visits was obtained in either group if there was clinical or echocardiographic evidence of graft dysfunction and for the AlloMap group if the score was above the specified threshold. The incidence of the primary outcome at 18 months post transplant (a composite outcome of the first occurrence of any of the following: death or retransplant, rejection with hemodynamic compromise, or allograft dysfunction due to other causes) did not differ significantly between the AlloMap and biopsy groups (10% vs 17%; p =.44). The number of biopsy-proven rejection episodes (International Society for Heart and Lung Transplantation grading system ≥ 2R) within the first 18 months did not differ significantly between groups (3 in the AlloMap group vs 1 in the biopsy group; p =.31). Of the rejections in the AlloMap group, 1 was detected after an elevated routine AlloMap test, while 2 were detected after patients presenting with hemodynamic compromise. As in the IMAGE study, a high proportion of rejection episodes were detected by clinical signs or symptoms (however, this study had only 3 rejection episodes in the AlloMap group).

In 2010, the results of the IMAGE study were published.[74,75,] This was an industry-sponsored, nonblinded, noninferiority RCT that compared outcomes in 602 patients managed with the

AlloMap test (n=297) or with routine endomyocardial biopsies (n=305). The trial included adults from 13 centers who underwent cardiac transplantation between 1 and 5 years prior to participating, were clinically stable and had a left ventricular ejection fraction of at least 45%. To increase enrollment, the trial protocol was later amended to include patients who had undergone transplantation between 6 months and 1 year prior to participating; this subgroup ultimately comprised only 15% of the final sample (n=87). Each transplant center used its own protocol for determining the intervals for routine testing. At all sites, patients in both groups underwent clinical and echocardiographic assessments in addition to the assigned surveillance strategy. According to the study protocol, patients underwent biopsy if they had signs or symptoms of rejection or allograft dysfunction at clinic visits (or between visits) or if the echocardiogram showed a left ventricular ejection fraction decrease of at least 25% compared with the initial visit. Additionally, patients in the AlloMap group underwent biopsy if their test score was above a specified threshold; however, if they had 2 elevated scores with no evidence of rejection found on 2 previous biopsies, no additional biopsies were required. The AlloMap test score varied from 0 to 40, with higher scores indicating a higher risk of transplant rejection. The investigators initially used 30 as the cutoff for a positive score; the protocol was amended to use a cutoff of 34 to minimize the number of biopsies needed. Fifteen patients in the AlloMap group and 26 in the biopsy group did not complete the trial.

The primary outcome was a composite variable: (1) the first occurrence of rejection with hemodynamic compromise; (2) graft dysfunction due to other causes; (3) death; or (4) retransplantation. Use of the AlloMap test was considered noninferior to the biopsy strategy if the 1-sided upper boundary of the 95% CI for the hazard ratio comparing the 2 strategies was less than the prespecified margin of 2.054. The margin was derived using the estimate of a 5% event rate per year in the biopsy group, taken from published observational studies, and allowing for an event rate of up to 10% per year in the AlloMap group.

According to Kaplan-Meier analysis, the 2-year event rate was 14.5% in the AlloMap group and 15.3% in the biopsy group. The corresponding hazard ratio was 1.04 (95% CI, 0.67 to 1.68). The upper boundary of the CI of the hazard ratio (1.68) fell within the prespecified noninferiority margin (2.054); thus, GEP was considered noninferior to endomyocardial biopsy. Death from all causes, a secondary outcome, did not differ significantly between groups. There were 13 (6.3%) deaths in the AlloMap group and 12 (5.5%) in the biopsy group (p =.82). During follow-up, there were 34 treated episodes of graft rejection in the AlloMap group. Only 6 (18%) of the 34 patients with graft rejection presented solely with elevated AlloMap scores. Twenty (59%) patients presented with clinical signs/symptoms and/or graft dysfunction on echocardiogram and 7 patients had an elevated AlloMap score plus clinical signs/symptoms with or without graft dysfunction on echocardiogram. In the biopsy group, 22 patients were detected solely due to an abnormal biopsy.

A total of 409 biopsies were performed in the AlloMap group and 1249 in the biopsy group. Most biopsies in the AlloMap group (67%) were performed because of elevated gene profiling scores. Another 17% were performed due to clinical or echocardiographic manifestations of graft dysfunction, and 13% were performed as part of routine follow-up after treatment for rejection. There was 1 (0.3%) adverse event associated with biopsy in the AlloMap group and 4 (1.4%) in the biopsy group. In terms of quality of life, the physical health and mental health summary

scores of the 12-Item Short-Form Health Survey were similar in the 2 groups at baseline and did not differ significantly between groups at 2 years.

A limitation of the trial was that the threshold for a positive AlloMap test was changed partway through the study; thus, the optimal test cutoff remains unclear. Moreover, the trial was not blinded, which could have affected treatment decisions based on clinical findings, such as whether to recommend a biopsy. In addition, the study did not include a group that only received clinical and echocardiographic assessment, so the value of AlloMap testing beyond that of clinical management alone cannot be determined. The uncertain incremental benefit of the AlloMap test is highlighted by the finding that only 6 of the 34 treated episodes of graft rejection detected during follow-up in the AlloMap group were initially identified solely due to an elevated GEP score. Since 22 episodes of asymptomatic rejection were detected in the biopsy group, the AlloMap test does not appear to be a sensitive test, possibly missing more than half of the episodes of asymptomatic rejection. Because clinical outcomes were similar in the 2 groups, there are at least 2 possible explanations: the clinical outcome of the study may not be sensitive to missed episodes of rejection, or it is not necessary to treat asymptomatic rejection. In addition, the trial was only statistically powered to rule out more than a doubling of the rate of the clinical outcome, which some may believe is an insufficient margin of noninferiority. Finally, only 15% of the final study sample had undergone transplantation less than 1 year before study participation; therefore, findings might not be generalizable to the population of patients 6 to 12 months post transplant.

HeartCare
Direct evidence of clinical utility was not identified for the HeartCare test.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of GEP testing, alone or in combination with dd-cfDNA testing, to assess for cardiac allograft rejection has not been established, a chain of evidence to support clinical utility cannot be constructed.

SUBSECTION SUMMARY: GENE EXPRESSION PROFILING FOR HEART TRANSPLANT

AlloMap
The 2 studies (CARGO, CARGO II) examining the diagnostic performance of GEP using the AlloMap test for detecting moderate or severe rejection were flawed by lack of a consistent threshold (ie, 20, 30, or 34) for determining positivity and by a small number of positive cases. In the available studies, although the NPVs were relatively high (ie, at least 88%), the performance characteristics were calculated based on the detection of 10 or fewer cases of rejection each. Moreover, the PPV in the CARGO II study was only 4.0% for patients who were at least 2 to 6 months post transplant and 4.3% for patients more than 6 months post transplant. The ability of the AlloMap test to differentiate between infection-related and rejection-related graft injury has also been called into question.

The most direct evidence on the clinical utility of GEP using the AlloMap test comes from a large RCT comparing a GEP-directed strategy with an endomyocardial biopsy-directed strategy for detecting rejection; it found that the GEP-directed strategy was noninferior. However, given the high proportion of rejection episodes in the GEP-directed strategy group detected by clinical signs/symptoms, the evidence is insufficient to determine that health outcomes are improved because of the uncertain incremental benefit of GEP. In addition, a minority of subjects assessed were in the first year post transplant. Results from a pilot RCT would suggest that GEP may have a role in evaluating for heart transplant rejection beginning at 55 days post transplant, but the trial was insufficiently powered to permit firm conclusions about the noninferiority of early GEP use.

SUBSECTION SUMMARY: GENE EXPRESSION PROFILING WITH DONOR-DERIVED CELL-FREE DNA TESTING FOR HEART TRANSPLANT

HeartCare
One retrospective study assessing the combined use of GEP testing with AlloMap and dd-cfDNA testing with AlloSure Heart reported a 12.7% reduction in endomyocardial biopsy volume when combined testing was used compared to AlloMap alone. However, this observation is limited by a lack of reporting on long-term health outcomes and incomplete diagnostic performance assessment for combined testing. One registry study evaluated paired GEP (AlloMap) and dd-cfDNA (AlloSure Heart) surveillance in 2,077 heart transplant recipients (2018 to 2021) and found dual testing stratified ACR risk (lowest with dual-negative, highest with dual-positive) while biopsy use declined over time with high 2-year survival (94.9%) and low graft dysfunction (2.7%). An updated analysis in 2,240 adults (2017 to 2022) showed dd-cfDNA performance for AMR varied by threshold (0.20% more sensitive, 0.50% more specific) and a prognostic analysis (n=1,934) linked dual-positive results to the highest 12-month risk of graft dysfunction or cardiovascular death (15.5%; HR 1.59). A single-center retrospective study of HeartCare testing reported biopsy-proven rejection was most frequent in dual-positive and dd-cfDNA+/GEP- groups, with dual-positive results showing low sensitivity but high specificity for biopsy-confirmed rejection, and dual-positive and dd-cfDNA+/GEP- discordant results were associated with higher subsequent mortality (and graft dysfunction for dd-cfDNA+/GEP-).

DONOR-DERIVED CELL-FREE DNA TESTING FOR RENAL TRANSPLANT

Clinical Context and Test Purpose
The purpose of dd-cfDNA testing in individuals with renal transplant who are undergoing surveillance or have clinical suspicion of allograft rejection is to detect allograft rejection.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest are individuals with renal transplants who are undergoing surveillance or who have a clinical suspicion of allograft rejection.

Clinical suspicion of allograft rejection may be indicated by clinical symptoms (eg, pain) or dynamic changes in laboratory parameters.

Allograft dysfunction is typically asymptomatic and has a broad differential, including graft rejection. Diagnosis and rapid treatment are recommended to preserve graft function and prevent loss of the transplanted organ.

Interventions
The test being considered is dd-cfDNA testing to assess for renal allograft rejection (ie, AlloSure or Prospera).

Various clinical pathways have been proposed for these tests. Use of the Prospera test is recommended when there is clinical suspicion of active rejection and for regular surveillance of subclinical rejection.[35,] In a surveillance scenario, regular testing is recommended at 1, 2, 3, 4, 6, 9, and 12 months after renal transplant or most recent rejection. Thereafter, the test should be repeated quarterly. The proportion of dd-cfDNA relative to total cfDNA is reported, with detection of ≥ 1% dd-cfDNA indicating increased risk for active rejection. The percent dd-cfDNA change between tests is also reported.[24,] In the surveillance scenario, individuals with a negative result may avoid biopsy and it is recommended that a positive test result is incorporated with clinical findings to determine whether a biopsy is indicated. When there is clinical suspicion of rejection, testing is recommended as an adjunct to biopsy for treatment response monitoring, or as a rule-out test for biopsy.

For the AlloSure test, various dd-cfDNA thresholds are suggested depending on the clinical scenario and include the detection of AMR in patients with donor-specific antibodies (DSA), the detection of "likely" active rejection, the prediction of adverse outcomes as an adjunct to biopsy-confirmed T cell-mediated (TCMR) 1A/borderline rejections, and for the exclusion of active rejection.[76,] A routine testing schedule is also recommended, and details regarding its clinical rationale have been published.[77,]

Comparators
The following test is currently being used to confirm a clinical suspicion of allograft rejection: renal biopsy. The adoption of protocol (ie, surveillance) biopsies varies across transplant centers and its use is not standardized.

Clinical suspicion of allograft rejection may be indicated by physical symptoms and/or dynamic changes in laboratory parameters (eg, serum creatinine, estimated glomerular filtration rate [eGFR], DSA).

Outcomes
The general outcomes of interest are OS, test validity, morbid events, and hospitalizations. Follow-up over months to years is needed to monitor for signs of allograft rejection.

For a primary kidney transplant, graft survival at 1 year is 94.7%; at 5 years, graft survival is 78.6%.[29,]

Beneficial outcomes resulting from a true-negative test result are avoiding unnecessary subsequent biopsy. Harmful outcomes resulting from a false-positive result may include an

unnecessary biopsy or unnecessary treatment. Harmful outcomes from a false-negative result are increased risk of adverse transplant outcomes.

In a triage scenario, the test would need to identify precisely a group of individuals that could safely forgo biopsy; therefore, the sensitivity, NPV, and negative likelihood ratio are key test performance characteristics.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse)

Study Selection Criteria
For the evaluation of the clinical validity of dd-cfDNA testing, studies that met the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard (describe the reference standard)
- Individual /sample clinical characteristics were described
- Individual /sample selection criteria were described.

Meta-Analyses
Two meta-analyses were identified which assessed the clinical validity of dd-cfDNA testing.[78,79,] Both studies quantitatively synthesized the findings from 9 observational studies to determine the diagnostic accuracy of dd-cfDNA as a potential marker of graft rejection following kidney transplantation. Xiao et al (2021) calculated a pooled sensitivity of 0.70 (95% CI, 0.57-0.81; I^2, 65) and specificity of 0.78 (0.70-0.84; I^2, 75) from 6 studies evaluating the diagnostic accuracy of dd-cfDNA for any rejection episode.[79,] The area under the receiver operating characteristics curve (AUC) was 0.81 (95% CI, 0.77 to 0.84; I^2, 65) with an overall diagnostic odds ratio (DOR) of 8.18 (95% CI, 5.11 to 13.09). Similar pooled estimates were calculated for 5 studies discriminating AMR. The authors reported a pooled sensitivity of 0.84 (95% CI, 0.75 to 0.90; I^2, 0) and a specificity of 0.80 (95% CI, 0.75 to 0.84; I^2, 4) with an AUC of 0.89 (95% CI, 0.86 to 0.91) and overall DOR of 20.48 (95% CI, 10.76 to 38.99). Overall, the authors found greater value in dd-cfDNA as a biomarker for AMR in patients with suspected renal dysfunction than in discriminating a main rejection episode and cite the need for more large-scale, prospective research on the topic.

Wijvliet et al (2020) performed stratified analyses of dd-cfDNA fraction and calculated pooled median estimates in the following patient groups: patients, patients without rejection at indication biopsy, patients with pure T cell-mediated rejection, and patients with a component AMR.[78,] In stable patients (n=1149; 5 studies), the median dd-cfDNA fraction was 0.29% (95% CI, 0.21% to 0.45%) and in the AMR group (n=89; 6 studies) the average was nearly 10 times greater (2.5%; 95% CI, 1.4% to 2.9%). In T cell-mediated rejection patients (n=35; 4 studies), the weighted median was found to be 0.27% (95% CI, 0.26% to 2.69%) and in patients without rejection (n=225; 4 studies) the weighted median was 0.57% (95% CI, 0.30 to 0.67). The authors also calculated the weighted median differences in medians (WMDMs) between groups and found that median dd-cfDNA fractions were significantly higher in patients with AMBR than in

patients without rejection (1.89%; 95% CI, 1.02 to 2.6), stable patients (2.3%; 95% CI, 1.8 to 2.69). However, no significant difference was observed for WMDMs between AMR patients and T cell-mediated rejection patients or for comparing T cell-mediated rejection to stable patients. This review had moderate heterogeneity for most between-group comparisons. Overall, higher dd-cfDNA fractions were found in patients with AMR than in individuals without rejection or stable patients, but a less clear relationship was established for T cell-mediated rejection and other investigated subgroups.

Xing et al. (2024) conducted a meta-analysis of 22 studies to assess the diagnostic accuracy of dd-cfDNA in detecting kidney allograft rejection, with results analyzed separately for antibody-mediated rejection (AMR) and any rejection.[80,] For any rejection, the pooled sensitivity was 59% (95% CI, 48% to 69%), specificity was 83% (95% CI, 76% to 88%), and the AUC was 0.80 (95% CI, 0.76 to 0.83); for AMR, sensitivity was 81% (95% CI, 72% to 87%), specificity was 80% (95% CI, 73% to 85%), and the AUC was 0.87 (95% CI, 0.84 to 0.90). Significant heterogeneity across studies was attributed to differences in study design, dd-cfDNA thresholds, and sample sizes. Subgroup analyses highlighted trade-offs in diagnostic performance based on dd-cfDNA thresholds, with a 1% threshold offering higher specificity (85%) but lower sensitivity (57%) compared to a 0.5% threshold, which provided higher sensitivity (78%) but lower specificity (66%). Despite the high study heterogeneity, the authors concluded that dd-cfDNA shows promise as a biomarker for kidney allograft rejection and emphasized the need for further prospective studies to establish an optimal threshold.

Observational Studies
Major study results are summarized in Table 8.

AlloSure
Development of the AlloSure test was conducted in the multicenter prospective Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients (DART) study by Bloom et al (2017), which both recruited patients who were less than 3 months after renal transplant (n=245) and recruited renal transplant patients requiring a biopsy for suspicion of graft rejection (n=139).[81,] For the primary analysis, an active rejection was defined as the combined categories of T cell-mediated rejection, acute/active AMR, and chronic/active AMR as defined by the Banff working groups. Only patients undergoing biopsy were considered; further exclusion of biopsies that were not for cause or had an inadequate or incomplete collection of biopsies or corresponding blood samples or had prior allograft in situ. These exclusions resulted in the main study cohort of 102 patients (107 biopsies). Within this population, acute rejection was noted in 27 patients (27 biopsies). After statistical analysis accounting for multiple biopsies from the same patient, the threshold dd-cfDNA fraction corresponding to acute rejection was set to 1.0% or higher. In the main study group, this resulted in a sensitivity of 59% (95% CI, 44% to 74%) and specificity of 85% (95% CI, 79% to 81%) for detecting active rejection versus no rejection. Using the original data set including all biopsies performed for clinical suspicion of rejection, 58 cases of acute rejection were diagnosed in 204 biopsies (170 patients). This PPV was 61% and the NPV 84%. Biopsies performed for surveillance (n=34 biopsies) were excluded from analysis in this study, as only 1 biopsy for surveillance demonstrated acute rejection. Study limitations included the absence of a validation data set.

Huang et al (2019) conducted a smaller single center that recruited 63 renal transplant patients with suspicion of rejection that had AlloSure assessment of dd-cfDNA within 30 days of an allograft biopsy.[82,] Median years from transplant to dd-cfDNA measurement was 2.0 (interquartile range, 0.3 to 6.5). Within this population, biopsy found acute rejection in 34 (54%) of patients; 10 (15.9%) were cell-mediated only, 22 (25.4%) were antibody-mediated only, and 2 (3.2%) were mixed cell-mediated and antibody-mediated. In contrast to the study by Bloom et al (2017), the optimal threshold for a positive dd-cfDNA result was identified as ≥ 0.74%. For the outcome of any rejection (ie, cell-mediated, antibody-mediated, or mixed), use of this threshold was associated with an overall sensitivity of 79.4%, specificity of 72.4%, PPV of 77.1%, and NPV of 75.0%. Discrimination of rejection differed by biopsy findings, however. For the subgroup of patients with AMR , the sensitivity was 100%, specificity was 71.8%, PPV was 68.6%, and NPV was 100%. The dd-cfDNA test did not discriminate rejection in patients with cell-mediated rejection, as evidenced by an AUC of 0.43 (95% CI, 0.17 to 0.66). The major limitations of this study are its small sample size and single-center setting.

Stites et al (2020) assessed clinical outcomes in 79 patients diagnosed with TCMR 1A/borderline rejection with simultaneous AlloSure assessment of dd-cfDNA across 11 centers between June 2017 and May 2019.[83,] Timing of testing with respect to the date of transplantation was not reported. Elevated levels of dd-cfDNA (≥ 0.5%) were detected in 42 (53.2%) patients. No statistically significant differences between dd-cfDNA distributions when stratified by protocol versus for-cause biopsies was detected (p =.7307). Elevated levels of dd-cfDNA were associated with adverse clinical outcomes compared to patients with low levels (< 0.5%), including decline in eGFR (8.5% versus 0%; p =.004), de novo DSA formation (40% versus 2.7%; p <.0001), and future or persistent rejection (21.4% versus 0%; p =.003). The authors hypothesize that the use of dd-cfDNA may complement histological evaluation and risk stratify patients with TCMR 1A or borderline rejection identified on biopsy and propose the use of reference ranges as opposed to absolute dd-cfDNA cutoff thresholds.

Additional analyses of the DART study have reported on associations between first-year AlloSure dd-cfDNA fraction or serial variability and subsequent eGFR decline (Sawinski et al [2021]),[84,] and combined use of dd-cfDNA and DSA testing to diagnose active AMR (Jordan et al [2018], Mayer et al [2021]).[85,86,]

Puliyanda et al (2021) conducted a prospective study of 67 pediatric renal transplant recipients enrolled across 2 medical centers between 2017 and 2019.[87,] Patients had a median age of 11 years (interquartile range [IQR], 4 to 13) and median time post-transplant to first AlloSure dd-cfDNA measurement was 55.6 months. Nineteen patients (28.4%) received dd-cfDNA testing in the absence of clinical suspicion of rejection. Median dd-cfDNA scores in the surveillance versus for-cause cohorts were 0.37% (IQR, 0.19% to 1.10%) and 0.47% (IQR, 0.24% to 2.15%), respectively. Among patients undergoing surveillance, 26.3% (5/19 patients) had a dd-cfDNA score >1% with biopsies indicating 4 cases of AMR and 1 case of mixed rejection. Among patients with clinical suspicion of rejection, 43.8% (21/48 patients) had dd-cfDNA scores >1%. All for-cause biopsies showed evidence of rejection, including 11 cases of AMR, 2 cases of T cell-mediated rejection, and 8 cases of mixed rejection. An additional 7 patients with clinical suspicion of rejection underwent biopsy despite dd-cfDNA scores < 1%, revealing 4 cases without

rejection, 1 case with AMR, 1 case with cell-mediated rejection, and 1 case of mixed rejection. Among all patients with biopsy-matched results (33/67), dd-cfDNA >1% was associated with a sensitivity of 86% and specificity of 100%, with a corresponding AUC of 0.996 (p =.002). No significant difference in serum creatinine change from baseline to testing was identified for those with rejection compared to those without. The study is limited by the small sample size and lack of biopsy-matched data for a complete assessment of false negatives. The authors also note that the 1% dd-cfDNA cutoff threshold was used based on prior studies in adults and it is unclear if this is appropriate for the pediatric population. Additionally, the authors suggest that relative increases in dd-cfDNA, as opposed to absolute values, may be more valuable in the pediatric population, given that appropriate cutoff thresholds may depend on child age and size.

Dandamudi et al (2022) conducted a prospective study of 57 pediatric renal transplant recipients enrolled in a single center from 2013 to 2019.[88,] Patients had a median age of 14 years (IQR, 7.5 to 16) and time post-transplantation to first Allosure dd-cfDNA measurement was within 30 days and through 12 months post-transplantation. The authors attempted to correlate dd-cfDNA scores to biopsy-proved T cell-mediated rejection (including sub-clinical rejection). Twenty-two of the patients had biopsy-proven rejection, and cfDNA median levels were higher in these patients (0.91%, IQR, 0.54% to 1.2%) than in the patients without biopsy-proven rejection (median, 0.22%; IQR, 0.14% to 0.45%; p<.001). An area under the receiver operating characteristic curve (AUC) value of.82 (95% CI, 0.71 to 0.93) was found between dd-cfDNA level and biopsy-proven rejection. Using a cut-off of 1%, cfDNA had a high specificity (96%; 95% CI, 90% to 99%) and low sensitivity (33%; 95% CI, 19% to 52%). A lower cut-off of 0.5% dd-cfDNA had a lower specificity (79%; 95% CI, 69% to 87%) but had a higher sensitivity (78%; 95% CI, 59% to 89%).

Bu et al (2022) evaluated data from 1092 kidney transplant recipients at 7 centers from June 2016 to January 2020 as part of the ADMIRAL study (NCT0456605).[89,] All patients were monitored with Allosure dd-cfDNA as part of their standard care. A total of 1092 adult kidney transplant recipients (mean age 49.5 years) were followed for a period of up to 3 years to determine the association of dd-cfDNA with evidence of allograft rejection identified histologically. Using a cfDNA threshold of 0.5%, the authors found an increase in the risk of the development of donor-specific antibodies (hazard ratio [HR], 2.7). Having a dd-cfDNA result of more than 0.5% on more than 1 test predicted a reduction in eGFR over 3 years (HR, 1.97). The presence of allograft rejection was established using results from 203 patients who had a biopsy to pain with cfDNA results. Amongst patients with no rejection on biopsy, a median dd-cfDNA level of 0.23% (IQR, 0.19% to 0.64%) was lower than that observed in individuals with biopsy-defined cellular or humoral rejection (1.6%; IQR, 0.68% to 2.6%; p<.0001). Median dd-cfDNA levels had an area under the receiver operating characteristic curve (AUROC) of 0.8 for graft rejection (95% CI, 0.72 to 0.87) and was found to be more predictive than the AUROC of median creatinine levels in this sample of patients. Performance characteristics of the Allosure test at a dd-cfDNA threshold of 0.5% resulted in a sensitivity of 78% and specificity of 71%; using a dd-cfDNA cut-off of 1.0 reduced the test sensitivity to 58% but improved the specificity to 82%.

Huang and colleagues (2023) reported a retrospective cohort study of all kidney transplant patients at a single center who received testing with Allosure.[82,] A total of 317 individuals who underwent kidney transplantation were included in this study (median age, 55 years) and were

defined as either low (<0.5%, n=239), moderate (0.5% to <1%, n=43), or high (≥1%, n=35) based on dd-cfDNA threshold levels. The rejection rate was established by comparing the 62 participants who underwent a biopsy; patients in the low dd-cfDNA group had a rejection rate of only 5% which was statistically less than that observed in the high dd-cfDNA group (17%; p=.01) but did not vary significantly in the moderate dd-cfDNA group (12%; p=.13). Although each group did not experience a significant change in eGFR from baseline levels, a linear mixed-effects model of eGFR over time found that dd-cfDNA category had a significant interaction when comparing both the moderate to low (p=.005) and low to high (p=.048) after adjustments for age, donor type, and history of donor-specific antibodies.

Moein et al. (2024) conducted a retrospective cohort study evaluating the diagnostic performance of transcriptomic kidney profile testing and dd-cfDNA testing in detecting kidney allograft rejection at a single center.[90,] The study included 136 kidney transplant recipients, of which 127 also underwent concurrent dd-cfDNA testing. Biopsy results identified 33 cases of rejection, comprising AMR (42.4%), TCMR (36.4%), and mixed rejection (21.2%). Transcriptomic kidney profile testing demonstrated an 82.35% sensitivity and 85.49% specificity, while dd-cfDNA testing showed a sensitivity of 56.66% and specificity of 85.56%. The combined use of positive transcriptomic and dd-cfDNA tests detected 51.51% of rejections, whereas combined negative tests were observed in 70.21% of biopsies without rejection. The PPV and NPV for dd-cfDNA testing were 54.83% and 86.45%, respectively, compared to 52.83% and 92.77% for transcriptomic testing. The study found moderate agreement between transcriptomic kidney profile testing and biopsy results (kappa, 0.50; p<.01) and fair agreement between dd-cfDNA and biopsy results (kappa, 0.37; p<.01). Transcriptomic testing identified 90.47% of AMR cases but only 57.89% of TCMR cases, whereas dd-cfDNA testing identified 66% of ABMR and 36.84% of TCMR cases. Limitations included potentially limited generalizability due to the single-center nature of the study and the retrospective study design.

Aubert et al. (2024) evaluated the association of dd-cfDNA with kidney AMR in a multinational observational study involving 2,882 recipients across 14 centers.[91,] Among the derivation cohort (n=1134), dd-cfDNA levels were significantly elevated in patients with active AMR (mean, 1.15% ± 0.15%) and TCMR (mean, 2.03% ± 1.13%) compared to stable recipients (mean, 0.36% ± 0.02%; p<.0001). The odds ratio (OR) for rejection associated with dd-cfDNA was 2.28 (95% CI, 1.90 to 2.74; p<.001) in multivariable analysis. The inclusion of dd-cfDNA improved the AUC for rejection prediction from 0.78 (95% CI, 0.74 to 0.81) to 0.82 (95% CI, 0.78 to 0.85; p=.0011) over standard of care parameters. Validation in the external cohort (n=1748) confirmed these findings, with an AUC increase from 0.743 to 0.842 (p<.001). For subclinical rejection, dd-cfDNA levels demonstrated an OR of 2.20 (95% CI, 1.66 to 2.95; p<.001) and reclassified 66.3% of subclinical cases over standard monitoring (p<.001). Sensitivity analyses showed dd-cfDNA associated with AMR at different post-transplantation intervals, including early (<3 months OR, 2.37; 95% CI, 1.51 to 3.73; p<.001) and late (>1 year OR, 2.52; 95% CI, 1.94 to 3.32; p<.001) time points. The study's limitations include a lack of longitudinal monitoring data for a subset of patients, lack of information on drug adherence, and non-experimental study design.

Aravamudhan et al (2025) conducted a retrospective single-center study of 271 kidney/pancreas transplant recipients who underwent dd-cfDNA testing at the University of Minnesota Medical Center between January 2019 and February 2024, including 256 tested with AlloSure and 15

tested with Prospera.[92,] In the subgroup with paired DSA and biopsy results available within 1 year of dd-cfDNA testing (N=107; AlloSure n=96, Prospera n=11, both assays n=2), diagnostic performance was assessed against biopsy using a 1% dd-cfDNA positivity threshold with calculation of sensitivity, specificity, PPV/NPV, and ROC/AUC. At the 1% cutoff, AlloSure had low sensitivity (19%) but higher specificity (80%), with a PPV of 21% and an NPV of 77%. Discrimination by ROC was similar across platforms (AUC, 0.86; 95% CI, 0.83 to 0.94 for AlloSure and 0.80; 95% CI, 0.61 to 0.99 for Prospera). Limitations included limited generalizability due to the single-center design, potential confounding from the inclusion of a minority of individuals with combined kidney and other organ transplants, and the retrospective study design.

Bromberg et al (2025) analyzed data from the Kidney Allograft Outcomes AlloSure Registry, a multicenter cohort of 1,743 kidney transplant recipients across 56 sites, and evaluated whether dd-cfDNA testing improved the diagnostic yield of kidney allograft biopsies in both surveillance and for-cause settings.[93,] Using dd-cfDNA thresholds of ≥1.0%, ≥0.5%, or ≥0.5% with a ≥61% relative increase from a prior value, dd-cfDNA demonstrated moderate discrimination for biopsy-proven acute rejection versus no rejection (AUC, 0.789; 95% CI, 0.743 to 0.836). In surveillance biopsies, rejection occurred in 39% with elevated dd-cfDNA versus 7% with nonelevated dd-cfDNA, and in for-cause biopsies, rejection occurred in 47% with elevated dd-cfDNA versus 12% with nonelevated dd-cfDNA. Among biopsies performed without dd-cfDNA elevation, most showed no rejection, with borderline changes or mild T cell-mediated rejection (TCMR 1A) predominating, whereas more severe rejection phenotypes (eg, ABMR, mixed rejection, higher-grade TCMR) were more often associated with dd-cfDNA elevation. dd-cfDNA elevations were observed up to 4 months prior to biopsy-confirmed ABMR (and up to 1 month prior to TCMR). Limitations included the observational registry design, lack of mandated testing schedules and protocol biopsies, and limited short-term outcome/response-to-treatment analyses.

Hogan et al (2025) evaluated the diagnostic performance of dd-cfDNA (AlloSure or AlloSeq cfDNA) in an unselected, two-center cohort of 196 pediatric kidney transplant recipients (mean age at transplantation 12 years) who underwent 367 kidney allograft biopsies with concomitant dd-cfDNA testing.[94,] Half of biopsies were performed for surveillance (58.4%), and biopsy diagnoses included 60 acute rejection episodes (27 TCMR, 27 AMR, 6 mixed) and 56 borderline rejections. dd-cfDNA levels increased with Banff inflammatory lesion severity and were independently associated with biopsy-proven rejection after adjustment for standard clinical markers (adjusted OR, 1.89). For the composite outcome of biopsy-proven acute rejection (including borderline), dd-cfDNA demonstrated fair discrimination (AUC, 0.76; 95% CI, 0.69 to 0.81), with similar AUCs when stratified by rejection phenotype (TCMR AUC, 0.73; 95% CI, 0.63 to 0.84; AMR AUC, 0.79; 95% CI, 0.70 to 0.89) and for borderline lesions (AUC, 0.75; 95% CI, 0.66 to 0.83). Using a cutoff of 0.5% for dd-cfDNA yielded higher sensitivity but lower specificity (Sensitivity, 78%; Specificity, 61%; PPV, 49%; NPV, 85%), whereas a 1% threshold shifted toward higher specificity with lower sensitivity (Sensitivity, 59%; Specificity, 84%; PPV, 64%; NPV, 81%). Performance differed by biopsy indication. In clinically indicated, for-cause, samples, sensitivity and specificity were 87% and 41% at 0.5%, and 65% and 72% at a 1% cut-off. Whereas in routine surveillance samples, sensitivity and specificity were 56% and 68% at 0.5% and 47% and 89% at 1% (with NPVs 89% and 90%, respectively). In a modeled analysis, adding dd-cfDNA to a standard-of-care prediction model improved discrimination for rejection,

increasing AUC from 0.80 to 0.84 (p=.01). Key limitations include the retrospective study design, modest event rate, and lack of longitudinal dd-cfDNA trajectories to assess temporal changes.

Prospera Kidney

Sigdel et al (2019) evaluated the diagnostic accuracy of the Prospera Kidney dd-cfDNA test in a retrospective analysis of 300 biorepository plasma samples from kidney transplant recipients at a single academic medical center.[95,] Of the 300 samples (193 patients), 217 were biopsy-matched with 38 cases of active rejection, 72 cases of borderline rejection, 82 with stable allografts, and 15 cases of other kidney injuries. The sample cohort was demographically diverse, including women (42.5%), Hispanic and Latino patients (34.6%), Black or African American patients (14%), and pediatric patients (20%). Indication for renal transplantation was unknown in 45.6% of samples. The majority of samples (72.3%) were drawn on the day of surveillance (n = 114 [52.5%] patients) or clinically indicated biopsy (n=103 [47.5%] patients). Timing of tests with respect to the date of transplantation was not reported. Biopsies were evaluated by a single pathologist according to 2017 Banff criteria and classified as active rejection or non-rejection (ie, borderline rejection, other injury, or stable allograft status). Median dd-cfDNA levels were significantly higher in biopsy-proven active rejection (2.32%) versus non-rejection subgroups (0.47%; p <.0001). All subtypes of active rejection could be detected, and median dd-cfDNA did not differ significantly between antibody-mediated (2.2%), T cell-mediated (2.7%), and combined subtypes (2.6%).

Sigdel et al (2019) also assessed the performance characteristics of eGFR, which was calculated as a function of serum creatinine with adjustments for age, sex, and race based on the Modification of Diet in Renal Disease (MDRD) Study equation.[95,] At a cutoff threshold of < 60, the sensitivity and specificity for eGFR were lower compared to dd-cfDNA, at 67.8% (95% CI, 51.3% to 84.2%) and 65.3% (95% CI, 57.6% and 73.0%), respectively, with a corresponding AUC of 0.74 (95% CI, 0.66 to 0.83). However, the relevance of absolute eGFR measurements is limited as dynamic changes in laboratory parameters (eg, serum creatinine elevation, eGFR decline) are used to flag impaired kidney function in clinical practice in the transplant population. Separate eGFR estimates in the for-cause subgroup were not reported. Major limitations of this study include its retrospective design and single-center setting. While the dd-cfDNA cutoff was prespecified, it was based on prior studies of the AlloSure test and may not be optimized for Prospera.

Bunnapradist et al (2021) noted that while % dd-cfDNA is a promising noninvasive biomarker for detecting renal allograft rejection, levels can be artificially depressed by high levels of circulating cfDNA, which may be observed in patients who are obese, have recently undergone surgery, have medical complications, or receive certain medications, potentially leading to false-negative results.[96,] The authors suggested that a combination of dd-cfDNA fraction and absolute quantity thresholds may improve the sensitivity of allograft rejection while maintaining high specificity.

Preliminary results from the ongoing Trifecta study (NCT04239703) published by Halloran et al (2022) provide assessment of combined dd-cfDNA fraction and absolute values for prediction of active kidney allograft rejection.[97,] The study reported data from 218 individuals included in a test set (median age 51 years) enrolled from December 2019 to July 2021. Thirty-eight patients were female and 17% were Black or African American; other race or ethnicity data were not reported.

The mean post-transplant time was 1,439 days (3.9 years). The study used a training set (n=149) to identify optimal % dd-cfDNA (≥1%) and absolute values cut-offs (≥78 cp/mL). Accuracy of dd-cfDNA testing was compared with the Molecular Microscope Diagnostic System (MMDx) and histological analysis using Banff criteria as reference standards. The use of 2 reference standards in this study is based on previous Trifecta analysis that suggested a strong correlation between dd-cfDNA fraction and molecular changes due to rejection assessed using MMDx.[98,]

Bromberg et al (2024) assessed the utility of dd-cfDNA as an early indicator of kidney transplant rejection in the ProActive registry study (NCT04091984), which included 424 patients with 1,013 dd-cfDNA tests conducted within 6 months before biopsy.[99,] Among the cohort, 6.1% of biopsies revealed AMR, 14.6% showed TCMR, and 79.3% indicated non-rejection. The study found dd-cfDNA levels were significantly elevated up to 5 months before AMR biopsies (median, 1.04%; IQR 0.45% to 1.18%) and 2 months before TCMR biopsies, compared with non-rejection biopsies (median, 0.24%; IQR 0.12% to 0.69%; p<.001). Sensitivity and specificity for dd-cfDNA in predicting AMR were 77% and 84%, respectively, with an AUC of 0.88 for all rejection types. Limitations included the retrospective design, variability in biopsy timing, and the absence of standardized protocols for patient management.

Table 8. Diagnostic Performance Characteristics of dd-cfDNA Tests for Detection of Active Kidney Allograft Rejection

Study; dd-cfDNA threshold	Biopsy-Matched Samples	Prevalence, n (%)[a]	Sensitivity, % (95% CI)	Specificity, % (95% CI)	AUC (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Allosure							
Bloom et al (2017) (≥1%)[81,]							
For-cause, dd-cfDNA	107	27 (25.2)	59 (44 to 74)	85 (79 to 81)	0.74 (0.61 to 0.86)	61 (NR)	84 (NR)
For-cause, SCr	204	58 (28.4)	NR	NR	0.54 (0.43 to 0.66)	NR	NR
Huang et al (2019) (≥0.74%)[82,]							
For-cause, any rejection	63 (patients)	34 (54)	79.4 (NR)	72.4 (NR)	0.71 (0.58 to 0.85)	77.1 (NR)	75 (NR)
For-cause, CMR	63 (patients)	10 (16)	NR	NR	0.42 (0.17	NR	NR

Study; dd-cfDNA threshold	Biopsy-Matched Samples	Prevalence, n (%)[a]	Sensitivity, % (95% CI)	Specificity, % (95% CI)	AUC (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
					to 0.66)		
Moein et al (2024) (≥1%)[90,]							
AMR/TCMR or Mixed Rejection	127	34 (24.3)	56.6 (NR)	85.6 (NR)	NR	52.8 (NR)	86.4 (NR)
Aubert et al (2024)[91,]							
AMR/TCMR or Mixed Rejection	1415	227 (16)	NR	NR	0.82 (0.78 to 0.85)	NR	NR
Aravamudhan et al (2025) (≥1%) [92,]							
AMR/TCMR or Mixed Rejection	96	21 (21.9)[b]	19 (NR)	80 (NR)	0.86 (0.83 to 0.94)	21 (NR)	77 (NR)
Bromberg et al (2025) (≥1% **or** ≥0.5% with ≥61% RCV) [93,]							
AMR/TCMR or Mixed Rejection	1070[b]	208 (19.4)[b]	60 (49 to 70)	85 (81 to 89)	0.789 (0.743 to 0.836)	60 (49 to 70)	85 (81 to 89)
Hogan et al (2025) (≥1%)							
AMR/TCMR or Mixed Rejection	367	60 (16.3)[b]	59 (NR)	84 (NR)	0.76 (0.69 to 0.81)	64 (NR)	81 (NR)
Prospera							
Sigdel et al (2019) (≥1%)[95,]							

Contains Public Information

Study; dd-cfDNA threshold	Biopsy-Matched Samples	Prevalence, n (%)[a]	Sensitivity, % (95% CI)	Specificity, % (95% CI)	AUC (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Overall, dd-cfDNA	217	33 (17.5)	88.7 (77.7 to 99.8)	72.6 (65.4 to 79.8)	0.87 (0.80 to 0.95)	52.0 (44.7 to 59.2)[c]	95.1 (90.5 to 99.7)[c]
Overall, eGFR	217	33 (17.5)	67.8 (51.3 to 84.2)	65.3 (57.6 to 73.0)	0.74 (0.66 to 0.83)	39.4 (31.6 to 47.3)[c]	85.9 (75.9 to 92.2)[c]
Surveillance, dd-cfDNA	114	12 (11.4)	92.3 (64.0 to 99.8)	75.2 (65.7 to 83.3)	0.89 (0.79 to 0.99)	55.4 (46.2 to 64.7)[c]32.4 (24.8 to 41.1)[d]	96.7 (90.6 to 99.9)[c]98.7 (92.0 to 99.8)[d]
For-cause, dd-cfDNA	103	25 (24.3)	84.0 (63.9 to 95.5)[b]	68.0 (56.4 to 78.1)[b]	NR	45.7 (36.8 to 54.8)[d]	93.0 (84.2 to 97.1)[d]
Halloran et al (2022)[97,] ≥1%; ≥78 cp/mL							
dd-cfDNA % + absolute quantity; MMDx criteria	218	71 (32.6)	83.1% (95% CI NR)	81.0% (95% CI NR)	0.88 (95% CI NR)	67.8% (95% CI NR)	90.8% (95% CI NR)
dd-cfDNA % + absolute quantity; Banff criteria	213	83 (39.0)	73.5% (95% CI NR)	80.8% (95% CI NR)	0.82 (95% CI NR)	70.9% (95% CI NR)	82.7% (95% CI NR)
Bromberg et al (2024)[99,]							
Any rejection	249	58 (23.2)	79.3% (66.6 to 88.8)	85.3% (79.5 to 90)	0.88	32.6% (20.0 to 47.3)	97.9% (94.8 to 99.4)
AMR	249	19 (7.6)	94.7% (74 to 99.9)	NR	NR	36.6% (22.7 to 52.3)	99.4% (96.8 to 100)
TCMR	249	39 (15.6)	71.8% (55.1 to 85)	NR	NR	30.4% (17.6 to 46)	97.1% (93.6 to 99)

Study; dd-cfDNA threshold	Biopsy-Matched Samples	Prevalence, n (%)[a]	Sensitivity, % (95% CI)	Specificity, % (95% CI)	AUC (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Aravamudhan et al (2025) (≥1%)[92,]							
AMR/TCMR or Mixed Rejection	11	5 (45.5)[b]	75 (NR)	60 (NR)	0.80 (0.61 to 0.99)	60 (NR)	75 (NR)

AUC: area under the receiver-operating curve; AMR: antibody mediated rejection; CI: confidence interval; CMR: cell-mediated rejection; dd-cfDNA: donor-derived cell-free DNA; eGFR: estimated glomerular filtration rate; MMDx: molecular microscope diagnostic system; NPV: negative predictive value; NR: not reported; PPV: positive predictive value; SCr: serum creatinine; TCMR: T-cell mediated rejection.
[a] Study disease prevalence.
[b] Calculated based on reported case numbers.
[c] Projected value as reported based on assumed disease prevalence of 25% in an at-risk population.
[d] Calculated value based on study disease prevalence.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs assessing the clinical utility of dd-cfDNA (ie, AlloSure, Prospera) testing to diagnose renal allograft rejection were identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of dd-cfDNA (ie, AlloSure, Prospera) testing to assess for renal allograft rejection has not been established, a chain of evidence to support clinical utility cannot be constructed.

Section Summary: Donor-Derived Cell-Free DNA Testing for Renal Transplant
Three meta-analyses were identified which pooled observational data from up to 22 studies. One reported an pooled sensitivity to detect graft rejection after kidney transplant of 70% with a specificity of 78%, and another found sensitivities of 59% with a specificity of 83%. The third meta-analysis reported that AMR was significantly associated with higher weighted median differences in dd-cfDNA fraction than in patients without rejection or in stable patients. Twelve studies of the Allosure test, using dd-cfDNA threshold values from ≥0.5% to ≥1%, established a

range of sensitivities from 19% to 86% with specificities of 72% to 100%. This corresponded to PPVs ranging from 21% to 77% and NPVs from 75% to 86%. Seven studies provided information on the area under the curve of Allosure to detect graft rejection which ranged from.76 to.996. A retrospective study of the Prospera test reported a PPV and NPV or 52% and 95% respectively using a ≥1% dd-cfDNA threshold. A second, retrospective study using the same dd-cfDNA threshold found a sensitivity and specificity of 77% and 84%, respectively, with an AUC of 0.88 for all rejection types. A third, prospective Prospera study reported PPVs of 68% and 71% and NPVs 91% and 83% using combined dd-cfDNA fraction and absolute quantity compared with 2 different reference standards. Larger prospective studies validating dd-cfDNA thresholds for active rejection are needed to develop conclusions for each test. At present, no studies evaluating the clinical utility for AlloSure or Prospera dd-cfDNA testing were identified.

DONOR-DERIVED CELL-FREE DNA TESTING FOR LUNG TRANSPLANT

Clinical Context and Test Purpose
The purpose of dd-cfDNA testing in individuals with lung transplant who are undergoing surveillance is to detect allograft rejection.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest are individuals with lung transplants who are undergoing surveillance for allograft rejection.

Interventions
The test being considered is dd-cfDNA testing to assess for lung allograft rejection.

A regular testing schedule is recommended for patients undergoing surveillance, with monthly testing in the first-year post-transplant and quarterly in the years 2-3. The proportion of dd-cfDNA relative to total cfDNA is reported. The report also notes that a threshold of >0.85% dd-cfDNA is associated with a higher probability of ACR , chronic lung allograft dysfunction (CLAD), and AMR and that the NPV is maximized at a % dd-cfDNA cutoff of 0.20%.

Comparators
The following test is currently being used to confirm a clinical suspicion of allograft rejection: bronchoscopy with transbronchial biopsy.

Outcomes
The general outcomes of interest are OS, test validity, morbid events, and hospitalizations. Follow-up over months to years is needed to monitor for signs of allograft rejection.

Beneficial outcomes resulting from a true-negative test result are avoiding unnecessary subsequent biopsy. Harmful outcomes resulting from a false-positive result may include an unnecessary biopsy or unnecessary treatment. Harmful outcomes from a false-negative result are increased risk of adverse transplant outcomes.

In a triage scenario, the test would need to identify precisely a group of patients that could safely forgo biopsy; therefore, the sensitivity, NPV, and negative likelihood ratio are key test performance characteristics.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse)

Study Selection Criteria
For the evaluation of the clinical validity of dd-cfDNA testing, studies that met the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard (describe the reference standard)
- Individual /sample clinical characteristics were described
- Individual /sample selection criteria were described.

OBSERVATIONAL STUDIES

AlloSure
Sayah et al (2020) conducted a pilot study investigating the ability of AlloSure dd-cfDNA testing to detect ACR.[100,] Biopsy-matched biorepository samples from 69 lung transplant recipients who had previously enrolled in the multicenter Lung Allograft Gene Expression Observational (LARGO) Study were evaluated. Diagnostic cohorts included patients with respiratory allograft infection (n=26), normal histopathology without infection or rejection (n=30), and ACR without concurrent infection (n=13). Samples were obtained between > 14 days and < 1-year post-transplant, and samples associated with potential concurrent infection with rejection were excluded. Median dd-cfDNA levels were 0.485% (IQR, 0.220 to 0.790) in the normal cohort, 1.52% (IQR, 0.520 to 2.550) in the ACR cohort, and 0.595% (IQR, 0.270 to 1.170) in the infection cohort. While dd-cfDNA levels were significantly higher in the ACR cohort compared to the normal cohort (p =.026), samples associated with infection were not significantly different from the normal (p =.282) or ACR (p=.1) cohorts. The AUC for detection of ACR was 0.717 (95% CI, 0.547 to 0.887; p=.025). At a threshold of 0.87% dd-cfDNA and an estimated prevalence rate of 25%, sensitivity for ACR was 73.1% (95% CI, 52.2% to 88.4%), specificity was 52.9% (95% CI, 27.8% to 77.0%), positive likelihood ratio was 1.55, negative likelihood ratio was 0.51, PPV was 34.1%, and NPV was 85.5%. The study is limited by the small sample size and use of archived samples, and raises concerns regarding the ability of AlloSure dd-cfDNA testing to detect AMR and to discriminate between infection and rejection.

Khush et al (2021) evaluated 107 biorepository plasma samples from 38 lung transplant recipients enrolled in the Genome Transplant Dynamics Study via AlloSure dd-cfDNA testing.[101,] The study cohort included 14 patients (22 samples) with ACR confirmed by histopathology, 6 patients (7 samples) treated for ACR without a confirmed histopathological diagnosis, 6 patients (8 samples) with obstructive CLAD, 7 patients (9 samples) with AMR , 22 patients (33 samples) with infection without rejection, and 18 patients (28 samples) with stable allografts. The median dd-cfDNA levels in the ACR (0.91%; IQR, 0.39% to 2.07%) and CLAD

(2.06%; IQR, 0.97% to 3.34%) cohorts were significantly higher compared to the stable cohort (p =.02, respectively). However, the AMR cohort was not statistically different when compared with the stable cohort (p =.07). The median dd-cfDNA level in an aggregated rejection cohort, composed of ACR , AMR, and CLAD samples, was approximately 3-fold higher when compared to the stable cohort (1.06% versus 0.38%). At a dd-cfDNA threshold of 0.85%, the sensitivity for this spectrum of rejection was 55.6%, specificity was 75.8%, PPV was 43.3%, and NPV was 83.6%. The study is limited by the small sample size and use of archived samples. The authors suggest that AlloSure dd-cfDNA testing may have clinical utility as a plasma marker of "tissue injury" and that the 0.85% dd-cfDNA threshold requires further prospective clinical validation.

A retrospective study conducted by Keller et al (2022) included 157 patients enrolled in a post-transplant home surveillance program that included the AlloSure test for detection of acute allograft rejection.[102,] The study analyzed data from patients at 4 U.S. centers. Data were collected from March to September 2020, during the COVID-19 pandemic at a time when in-office visits were limited and routine, surveillance bronchoscopy was deferred. Home monitoring was intended to identify those patients most at risk for acute rejection for triage to bronchoscopy. Study inclusion was limited to adults >18 years between 30 days and 3 years post-transplant. Of the total cohort, the mean age was 59 years and the majority were male (54%) and White race (64%). Eighteen percent were Black, 3% Asian, and 15 % other race/ethnicity. The mean time since transplantation as 13 months, and 82% underwent bilateral transplantation. Diagnosis of ACR, AMR, infection, or a composite of these outcomes (Acute Lung Allograft Dysfunction [ALAD]), was made based on biopsy and/or clinical diagnosis. Mean dd-cfDNA % was 1.6% for acute rejection (ACR+AMR) and 1.7% for ALAD. In comparison, the mean dd-cfDNA in stable patients was 0.37%. Using a dd-cfDNA cut-off of 1.0% for detection of ALAD, the sensitivity was 73.9%, specificity 87.7%, PPV 43.4% and NPV 96.5%. Of the 157 patients with dd-cfDNA measurement for surveillance, 52 also had a contemporaneous reference standard surveillance bronchoscopy independent of dd-cfDNA level (i.e. patients who were not triaged to bronchoscopy). When analysis was limited to this subgroup, diagnostic performance declined slightly: 76.2% sensitivity, 70.0% specificity, 66.7% PPV and 79.2% NPV. The study was limited by the small sample size, particularly the limited number of unselected patients who underwent both dd-cfDNA testing and bronchoscopy.

Prospera Lung
Rosenheck et al (2022) assessed the predictive ability of dd-cfDNA testing using the Prospera test for lung transplant rejection.[103,] The study included 195 samples from 103 patients, who were predominantly White (93%) and male (60%); mean age was 62 years. Black and Hispanic patients comprised 6% and 1% of the study population, respectively. The median time since lung transplant was 198 days, and most patients (85%) underwent lung biopsy for routine transplant surveillance. Consistent with other dd-cfDNA studies, median dd-cfDNA % was higher in patients with acute rejection (AR), which included ACR (1.43%) or AMR (2.50%), than those who were stable (0.46%). Prevalence of acute rejection was 28% (29/103), and prevalence of CLAD or neutrophilic-responsive allograft dysfunction (NRAD) was 21% (22/103); patients could be included in both diagnostic groups. Using a dd-cfDNA threshold of ≥1% for prediction of acute rejection, sensitivity was 89.1% and specificity was 82.9%, resulting in an AUC of 0.91 (95% CI 0.83 to 0.98). PPV was 51.9% and NPV was 97.3%. For a combined measure that included AR, CLAD/NRAD, and infection, sensitivity was 59.9%, specificity 83.9%, AUC 0.76, PPV 43.6%, and

NPV 91.0%. As with other dd-cfDNA studies in lung transplantation, this study was limited by the small sample size though unlike other studies samples were collected prospectively.

Arunachalam et al (2024) evaluated the utility of dd-cfDNA as a noninvasive biomarker for monitoring acute rejection after single lung transplantation in a multicenter analysis involving 257 test results from 103 patients across 6 centers.[104,] The study applied a dd-cfDNA threshold of ≥1.0%, corrected for single-lung transplant, at a median of 233 days post-transplant. The authors observed elevated dd-cfDNA fractions in acute rejection (median 1.80%; IQR, 1.04% to 3.56%), infections (1.10%; IQR, 0.52% to 1.74%), and chronic lung allograft dysfunction (CLAD, 0.96%; IQR 0.75% to 1.06%) compared to stable patients (0.46%; IQR, 0.20% to 0.72%; p <.0001). Sensitivity for dd-cfDNA in detecting acute rejection was 77.8% (95% CI, 58.3% to 94.1%), with a specificity of 84.6% (95% CI, 76.0% to 92%), a PPV of 38.3% (95% CI, 26.9% to 55.0%), a NPV of 96.8% (95% CI, 94.2% to 99.2%), and AUC of 0.85 (95% CI, 0.72 to 0.95). Limitations include the retrospective design, the relatively small number of samples which prohibited some sub-group analyses, and reliance on histopathologic confirmation, which may miss subclinical events.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from RCTs.

No RCTs assessing the clinical utility of dd-cfDNA (ie, AlloSure or Prospera) testing to diagnose lung allograft rejection were identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because the clinical validity of dd-cfDNA testing to assess for lung allograft rejection has not been established, a chain of evidence to support clinical utility cannot be constructed.

Section Summary: Donor-Derived Cell-Free DNA Testing for Lung Transplant
Five small diagnostic accuracy studies of dd-cfDNA testing with AlloSure or Prospera utilizing biorepository (3 studies) or prospectively collected samples (2 studies) were identified. At a threshold of 0.87% dd-cfDNA, the PPV and NPV for detecting ACR in the first study were 34.1% and 85.5%, respectively. A second study reported a PPV of 43.3% and NPV of 83.6% at a dd-cfDNA cutoff of 0.85% for an aggregate rejection cohort composed of patients with ACR, AMR, and CLAD. In the third study, using a dd-cfDNA cut-off of 1.0%, PPV was 51.9% and NPV was 97.3% for acute rejection, and 43.6%, and 91.0% for acute rejection, CLAD/NRAD or infection. One study that used dd-cfDNA testing as part of a home surveillance program found a PPV

43.4% and NPV 96.5% for detection of ACR, AMR or infection, but when limited to patients with a contemporaneous reference standard surveillance bronchoscopy independent of dd-cfDNA level PPV 66.7% and NPV was 79.2%. One study assessed dd-cfDNA for detecting acute rejection following single lung transplantation, using a cut-off adjusted for single-organ transplantation; the study reported a sensitivity of 77.8% and specificity of 84.6%, with a PPV of 38.3%, NPV of 96.8%, and an AUC of 0.85. Larger and additional prospective studies validating the dd-cfDNA threshold for active rejection are needed to develop conclusions. At present, no studies evaluating the clinical utility for AlloSure or Prospera dd-cfDNA testing were identified.

SUPPLEMENTAL INFORMATION
The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Clinical Input From Physician Specialty Societies and Academic Medical Centers
While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

2012 Input
In response to requests, input was received from 7 academic medical centers and 1 specialty society while this policy was under review in 2012. Input was mixed on whether AlloMap should be investigational. Four reviewers agreed with the investigational status, 1 disagreed, and 3 indicated it was a split decision/other. Reviewers generally agreed that the sensitivity and specificity have not yet been adequately defined for AlloMap and that the negative predictive value was not sufficiently high to preclude the need for biopsy. There was mixed input about the need for surveillance cardiac biopsies to be performed in the absence of clinical signs and/or symptoms of rejection.

2008 Input
In response to requests, input was received from 2 academic medical centers and 2 physician specialty societies while this policy was under review in 2008. Three reviewers agreed that these approaches for monitoring heart transplant rejection are considered investigational. The American College of Cardiology disagreed with the policy, stating that the College considers the available laboratory tests to have good potential to diagnose heart transplant rejection and reduce the frequency of invasive biopsies performed on heart transplant patients, although questions remained as to their role in clinical practice.

Practice Guidelines and Position Statements
Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American College of Cardiology et al

In 2022, the American College of Cardiology, American Heart Association, and Heart Failure Society issued an updated guideline for the management of heart failure.[8,] The 2022 guideline replaced a 2013 guideline[1,] and a 2017 focused guideline update.[105,] The guideline states measurement of natriuretic peptide levels may be useful for diagnosis, risk stratification, and prognosis of heart failure. The use of soluble suppression of tumorigenicity-2 is not discussed specifically, though the guideline notes that "a widening array of biomarkers including markers of myocardial injury, inflammation, oxidative stress, vascular dysfunction, and matrix remodeling have been shown to provide incremental prognostic information over natriuretic peptides but remain without evidence of an incremental management benefit."

American Society of Transplant Surgeons

In 2024, the American Society of Transplant Surgeons (ASTS) issued a position statement on the role of dd-cfDNA in kidney transplant surveillance.[106,] The authors summarized, "The accumulated evidence supporting the use of dd-cfDNA argues that its use should no longer be considered investigational. The use of dd-cfDNA is evidence-based and validated, and a prime example of much needed innovation in transplant organ surveillance. It has demonstrated utility in the early detection of allograft injury and assistance with clinical decision-making regarding allograft biopsy and treatment initiation." Regarding the timing of dd-cfDNA testing in kidney transplant, the ASTS states, "This protocol recommended testing at monthly intervals from month 1-4, and then quarterly thereafter. There are no data to support cessation of surveillance testing at any timepoint after transplant, and it is intuitively obvious that, as the risk of allograft injury or acute rejection does not disappear, testing should continue indefinitely at some frequency."

The following recommendations regarding the clinical utility and decision analysis were issued:
- "The most data have been accumulated in adult transplant recipients, and these recommendations are therefore most applicable to adult patient populations.
- We suggest that clinicians consider measuring serial dd-cfDNA levels in kidney transplant recipients with stable renal allograft function to exclude the presence of subclinical antibody-mediated rejection.
- We recommend that clinicians measure dd-cfDNA levels in kidney transplant recipients with acute allograft dysfunction to exclude the presence of rejection, particularly antibody-mediated rejection (ABMR).
- We do not recommend the use of blood gene expression profiling (GEP) in kidney transplant recipients for the purpose of diagnosing or excluding sub-clinical rejection, as adequate evidence supporting such use is still lacking.
- We do not recommend the use of blood GEP to diagnose or exclude the presence of acute graft rejection in kidney transplant recipients with acute allograft dysfunction given the paucity of data to support this practice.
- We recommend that dd-cfDNA may be utilized to rule out subclinical rejection in heart transplant recipients.
- We recommend that clinicians utilize peripheral blood GEP as a non-invasive diagnostic tool to rule out acute cellular rejection in stable, low-risk, adult heart transplant recipients who are over 55 days status post heart transplantation."

"Caveats and recommendations for future studies:
- None of these recommendations should be construed as recommending one biomarker over another in the same diagnostic niche.
- We strongly recommend ongoing clinical studies to clarify the scenarios in which molecular diagnostic studies should be utilized.
- We specifically recommend that studies be carried out to evaluate the potential role of dd-cfDNA surveillance in kidney transplant recipients to improve long-term allograft survival.
- We recommend studies be carried out to evaluate the potential global cost effectiveness of clinical use of dd-cfDNA testing in surveillance and for-cause settings."

European Association of Urology

The European Association of Urology (2024) provided guidance on renal transplantation including post-transplantation follow-up.

Table 9. Guidelines for Renal Transplant Recipient Follow-up

Recommendation	SOR
Provide lifelong regular post-transplant follow-up by an experienced and trained transplant specialist at least every six to twelve months.	Strong
Advise patients on appropriate lifestyle changes, potential complications, and the importance of adherence to their immunosuppressive regimen.	Strong
Regularly monitor (approximately every four to eight weeks) serum creatinine, estimated glomerular filtration rate, blood pressure, urinary protein excretion, immunosuppression and complications after renal transplantation. Changes in these parameters over time should trigger further diagnostic work-up including renal biopsy, a search for infectious causes and anti-HLA antibodies.	Strong
Perform an ultrasound of the graft, in case of graft dysfunction, to rule out obstruction and renal artery stenosis.	Strong
In patients with interstitial fibrosis and tubular atrophy undergoing calcineurin inhibitor (CNI) therapy and/or with histological signs suggestive for CNI toxicity (e.g. arteriolar hyalinosis, striped fibrosis) consider CNI reduction or withdrawal.	Strong
Initiate appropriate medical treatment, e.g. tight control of hypertension, diabetes, proteinuria, cardiac risk factors, infections, and other complications according to current guidelines	Strong

SOR: strength of recommendation.

European Society for Organ Transplantation

In 2024, the European Society for Organ Transplantation issued updated guidelines for the care of heart transplant recipients.[107,] The guidelines included the following recommendations including a strong recommendation for GEP based on a moderate level of evidence (see Table 10).
- 'Peripheral blood GEP assay (marketed in United States as Allomap®) is a reliable non-invasive diagnostic tool to rule out acute cellular rejection in stable, low-risk heart transplant recipients >15 years of age who are >55 days post HT.'

Table 10. Guidelines for Renal Transplant Recipient Follow-up

PICO question	Recommendation	Level of evidence	
In heart transplant patients with stable graft function, are GEP and dd-cfDNA reliable surveillance tools for subclinical acute rejection monitoring, compared to endomyocardial biopsy?	GEP	Strong for	Moderate
	Cf-ddDNA	Weak for	Low
In heart transplant patients, are dd-cfDNA and GEP reliable methods to monitor for cardiac allograft vasculopathy as compared with standard diagnostic methods?		Weak against	Very Low
In heart transplant patients with stable graft function, is dd-cfDNA or GEP a reliable marker to stratify prognosis as compared to standard clinical classifiers?		Weak against	Very Low
Is dd-cfDNA a reliable marker to diagnose/monitor a) clinical and subclinical acute rejection or b) infection of the graft in lung transplant patients, compared with standard diagnostic methods (surveillance bronchoscopy with TBB for histopathology and bronchoalveolar lavage for microbiology testing)?	5A	Weak for	Low
	5B	Weak for	Very Low
Is dd-cfDNA a reliable therapeutic marker to monitor treatment response for acute rejection or infection of the graft in lung transplant patients, compared with standard diagnostic methods (i.e., follow-up surveillance TBBx)?		Weak against	Very Low
Is dd-cfDNA a reliable marker to stratify prognosis of lung transplant recipients for chronic lung allograft dysfunction (CLAD), as compared to standard clinical classifiers?		Weak for	Very Low

International Society of Heart and Lung Transplantation
In 2022, the International Society of Heart and Lung Transplantation issued updated guidelines for the care of heart transplant recipients. Key recommendations relevant to rejection surveillance and noninvasive testing are summarized in Table 11.[108, 11]. A subsequent 2025 update discusses the potential utility of dd-cfDNA and Molecular Microscope (MMDx) findings in the evaluation and management of post-transplant donor-specific antibodies (DSA), but does not assign a specific class of recommendation or level of evidence.[109,]

'Several variables should be considered to treat patients with post-transplant DSA, including cardiac dysfunction by imaging, abnormal hemodynamics, an increasing MFI trend in DSA, the presence of early DSA, antibody attributes (C1q+ binding, detection of DSA in dilution assay), biopsy-proven pAMR ≥2, and abnormal molecular findings (dd-cfDNA and/or the MMDx) as available.'

Table 11. Guidelines for Postoperative Care of Heart Transplant Recipients

Recommendation	COR	LOE
"It is reasonable to perform periodic EMB during the first 3 to 12 postoperative months for surveillance of HT rejection."	IIa	C
"After the first post-operative year, it is reasonable to continue EMB surveillance in patients who are at higher risk for late acute rejection..."	IIa	C
"Gene Expression Profiling (GEP) (i.e., AlloMap) of peripheral blood can be used in low-risk patients between 2 months and 5 years after HT to identify adult recipients who have low risk of current ACR to reduce the frequency of EMB. Data in children does not allow a general recommendation of GEP as a routine tool at present."	IIa	B

ACR: acute cellular rejection; COR: class of recommendation; EMB: endomyocardial biopsy; HT: heart transplant; LOE: level of evidence.

Kidney Disease Improving Global Outcomes

The Kidney Disease Improving Global Outcomes (2009) issued guidelines for the care of kidney transplant recipients.[110,] The guidelines included the following recommendations (see Table 12).

Table 12. Guidelines for Biopsy in Renal Transplant Recipients

Recommendation	SOR	LOE
"We recommend kidney allograft biopsy when there is a persistent, unexplained increase in serum creatinine."	Level 1	C
"We suggest kidney allograft biopsy when serum creatinine has not returned to baseline after treatment of acute rejection."	Level 2	D
"We suggest kidney allograft biopsy every 7-10 days during delayed function."	Level 2	C
"We suggest kidney allograft biopsy if expected kidney function is not achieved within the first 1-2 months after transplantation."	Level 2	D
"We suggest kidney allograft biopsy when there is new onset of proteinuria."	Level 2	C
"We suggest kidney allograft biopsy when there is unexplained proteinuria ≥3.0 g/g creatinine or ≥3.0 g per 24 hours."	Level 2	C

LOE: level of evidence; SOR: strength of recommendation.

U.S. Preventive Services Task Force Recommendations
Not applicable.

Ongoing and Unpublished Clinical Trials
Some currently ongoing and unpublished trials that might influence this review are listed in Table 1 3.

Table 1 3. Summary of Key Active Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
AlloMap			
NCT04855422[a]	Assessing Benchmarks For Allosure And AlloMap Testing in Simultaneous Kidney & Pancreas Transplant Recipients.	50	Dec 2026 (recruiting)
HeartCare			
NCT05459181[a]	Molecular Outcome Surveillance Using AlloSure and AlloMap Guided Immunomodulation in Cardiac Transplant (MOSAIC)	930	Sep 2026
NCT03695601[a]	Surveillance HeartCare Outcomes Registry (SHORE)	2743	Nov 2026 (active, not recruiting)
AlloSure (Kidney)			
NCT04855422[a]	Assessing Benchmarks For Allosure And AlloMap Testing in Simultaneous Kidney & Pancreas Transplant Recipients.	50	Dec 2026 (recruiting)
NCT03326076[a]	Evaluation of Patient Outcomes From the Kidney Allograft Outcomes AlloSure Registry (KOAR)	4000	Dec 2025 (active, not recruiting)
AlloSure (Lung)			
NCT05050955[a]	Allosure Lung Assessment and Metagenomics Outcomes Study (ALAMO)	1500	Dec 2028 (active, not recruiting)
Prospera (Kidney)			
NCT04239703[a]	Trifecta-Kidney cfDNA-MMDx Study: Comparing the DD-cfDNA Test to MMDx Microarray Test, Central HLA Antibody Test, and Histology	300	Dec 2026 (recruiting)
NCT04091984[a]	The PROspera Kidney Transplant ACTIVE Rejection Assessment Registry (ProActive)	5000	Oct 2027 (recruiting)
Prospera (Heart)			
NCT04707872[a]	Trifecta-Heart cfDNA-MMDx Study: Comparing the DD-cfDNA test to MMDx Microarray Test and Central HLA Antibody Test	300	Jul 2027 (recruiting)
NCT05205551	Prospera Test Evaluation in Cardiac Transplant (ProTECT)	411	Dec 2027 (active, not recruiting)
Prospera (Lung)			

Contains Public Information

NCT No.	Trial Name	Planned Enrollment	Completion Date
NCT05837663[a]	Trifecta-Lung cfDNA-MMDx Study: Comparing the Dd-cfDNA Test to MMDx Microarray Test and Central HLA Antibody Test	600	Dec 2027 (recruiting)
NCT05170425[a]	Observational Registry Study With Sub-analysis (Patients Previously Randomized to LAMBDA 001) to Assess ProsperaTM Performance for Detection of CLAD After Lung Transplant (LAMBDA 002)	154	May 2027 (active, not recruiting)
Unpublished			
NCT01833195[a]	Outcomes AlloMap Registry: the Long-term Management and Outcomes of Heart Transplant Recipients With AlloMap Testing (OAR)	2444	Feb 2020 (unknown)
NCT02178943[a]	Utility of Donor-Derived Cell-free DNA in Association With Gene-Expression Profiling (AlloMap®) in Heart Transplant Recipients (D-OAR)	100	Feb 2020 (unknown)
NCT04566055[a]	Assessing AlloSure dd-cfDNA Monitoring Insights of Renal Allografts With Longitudinal Surveillance (ADMIRAL)	1000	Oct 2020 (unknown)
NCT04318587[a]	Assessment of Donor Derived Cell Free DNA and Utility in Lung Transplantation	40	Dec 2023 (terminated)
NCT04057742[a]	AlloSure for the Monitoring of Antibody Mediated Processes After Kidney Transplantation (All-MAP)	69	Dec 2024 (completed)
NCT04601155[a]	Transition of Renal Patients Using AlloSure Into Community Kidney Care (TRACK)	3500	Oct 2023 (completed)
NCT03984747[a]	Study for the Prediction of Active Rejection in Organs Using Donor-derived Cell-free DNA Detection (SPARO)	500	Aug 2024 (completed)

NCT: national clinical trial.

[a] Denotes industry-sponsored or cosponsored trial.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. This may not be a comprehensive list of procedure codes applicable to this policy.

Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

The code(s) listed below are medically necessary ONLY if the procedure is performed according to the "Policy" section of this document.

CPT/HCPCS	
81595	Cardiology (heart transplant), mRNA, gene expression profiling by real-time quantitative PCR of 20 genes (11 content and 9 housekeeping), utilizing subfraction of peripheral blood, algorithm reported as a rejection risk score.
83006	Growth stimulation expressed gene 2 (ST , Interleukin 1 receptor like-1
0018M	Transplantation medicine (allograft rejection, renal), measurement of donor and third-party-induced CD154+T-cytotoxic memory cells, utilizing whole peripheral blood, algorithm reported as a rejection risk score
0055U	Cardiology (heart transplant), cell-free DNA, PCR assay of 96 DNA target sequences (94 single nucleotide polymorphism targets and two control targets), plasma [Test name-myTAIHEART, Manufacturer-Tai Diagnostics Inc.]
0087U	Cardiology (heart transplant), mRNA gene expression profiling by microarray of 1283 genes, transplant biopsy tissue, allograft rejection and injury algorithm reported as a probability score [Test name- Molecular Microscope® MMDx—Heart; Manufacturer— Kashi Clinical Laboratories]
0088U	Transplantation medicine (kidney allograft rejection), microarray gene expression profiling of 1494 genes, utilizing transplant biopsy tissue, algorithm reported as a probability score for rejection [Test name- Molecular Microscope® MMDx—Kidney; Manufacturer— Kashi Clinical Laboratories]
0118U	Transplantation medicine, quantification of donor-derived cell-free DNA using whole genome next generation sequencing, plasma, reported as percentage of donor-derived cell-free DNA in the total cell-free DNA
0221U	Red cell antigen (ABO blood group) genotyping (ABO), gene analysis, next generation sequencing, ABO (ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase) gene
0493U	Transplantation medicine, quantification of donor-derived cell-free DNA (cfDNA) using nextgeneration sequencing, plasma, reported as percentage of donor derived cell-free DNA
0540U	Transplantation medicine, quantification of donor-derived cell-free DNA using next-generation sequencing analysis of plasma, reported as percentage of donor-derived

	cell-free DNA to determine probability of rejection (AlloSure® from CareDx® Laboratory)

REVISIONS	
03-12-2013	Policy added to the bcbsks.com web site.
10-16-2013	Description section reviewed
	In Policy section: ▪ Added to item B, "including but not limited to" to read, "B. The evaluation of genetic expression in the peripheral blood including, but not limited to, the detection of acute heart transplant rejection or graft dysfunction is considered experimental / investigational."
	Rationale section updated
	References updated
01-01-2016	Published 12-14-2015. Effective 01-01-2016.
	Description section updated
	In Policy section: ▪ In Item B removed "The evaluation of genetic expression in the peripheral blood" and added The use of peripheral blood genetic profiling tests in the management of patients after heart transplantation" and "heart transplant" to read "The use of peripheral blood genetic profiling tests in the management of patients after heart transplantation including, but not limited to, the detection of acute heart transplant rejection or heart transplant graft dysfunction is considered experimental / investigational." These wording updates clarify the policy statement, but do not change the original intent.
	Rationale section updated
	In Coding section: ▪ Added CPT Code: 81595 (Effective January 1, 2016) ▪ Removed CPT Code: 86849 (Effective January 1, 2016) · Updated Coding notations
	References updated
07-01-2018	In Coding section: ▪ Added PLA Code: 0051U
09-12-2018	Description section updated
	In Policy section: ▪ In Item A removed "with the Heartsbreath test" and added "2R (formerly grade" to read "The measurement of volatile organic compounds to assist in the detection of grade 2R (formerly grade 3) heart transplant rejection is considered experimental / investigational." ▪ In Item B replaced "genetic" with "gene expression" · Updated Policy Guidelines
	Rationale section updated

REVISIONS	
	In Coding section: ▪ Removed CPT Code: 93505 (the code does not pertain to the policy) ▪ Updated Coding nomenclature: 0051U · Updated Coding notations.
	References updated
12-20-2018	Policy published 11-20-2018. Policy effective 12-20-2018.
	Title changed "Laboratory Tests for Heart and Kidney Transplant Rejection" from "Laboratory Tests for Heart Transplant Rejection"
	Description section updated
	In Policy section: ▪ Added "C. The use of peripheral blood measurement of donor-derived cell-free DNA in the management of patients after renal transplantation, including but not limited to the detection of acute renal transplant rejection or renal transplant graft dysfunction, is considered experimental / investigational."
	Rationale section updated
	In Coding section: ▪ Added CPT Code: 81599 ▪ Coding notations removed
	References updated
07-01-2019	In Coding section: ▪ Added PLA Codes: 0087U, 0088U
07-01-2019	In Coding section: ▪ Added PLA Code: 0118U
05-18-2020	Description section updated
	Rationale section updated
	References updated
02-04-2022	Changed Title To: "Laboratory Tests Post Transplant and for Heart Failure"
	Updated Description Section
	In Policy Section Added: ▪ Section A: The use of the Presage ST2 Assay to evaluate the prognosis of patients diagnosed with chronic heart failure is considered experimental / investigational. ▪ Section B: The use of the Presage ST2 Assay to guide management (e.g., pharmacologic, device-based, exercise) of patients diagnosed with chronic heart failure is considered experimental / investigational. ▪ Section C: The use of the Presage ST2 Assay in the post cardiac transplantation period, including but not limited to predicting prognosis and predicting acute cellular rejection, is considered experimental / investigational. ▪ Section D:The use of the myTAIHEART assay in the post cardiac transplantation period, including but not limited to predicting prognosis and predicting acute cellular rejection, is considered experimental / investigational. ▪ Section E: Added word "moderate"

Contains Public Information

REVISIONS	
	- Section F: Added "or peripheral blood measurement of donor-derived cell-free DNA (dd-cfDNA), alone or in combination" - Section H: The use of peripheral blood measurement of dd-cfDNA in the management of patients after lung transplantation, including but not limited to the detection of acute lung transplant rejection or lung transplant graft dysfunction, is considered experimental / investigational.
	Updated Rationale Section
	Updated Coding Section: - Added: 83006, 0018M, 0221U - Removed 81599, 0085T
	Updated References Section
04-01-2022	In Coding Section Added: 0319U, 0320U (new codes 04-01-2022)
12-13-2022	Updated Description Section
	Updated Policy Section - Added statement "The use of peripheral blood measurement of dd-cfDNA in the post cardiac transplantation period, including but not limited to predicting prognosis and predicting acute cellular rejection, is considered experimental / investigational." - Removed statement: "The use of the myTAIHEART assay in the post cardiac transplantation period, including but not limited to predicting prognosis and predicting acute cellular rejection, is considered experimental / investigational."
	Updated Rationale Section
	Updated References Section
11-17-2023	Updated Description Section
	Updated Rationale Section
	Updated Coding Section - Removed ICD-10 Diagnoses Box - Removed 0319U and 0320U
	Updated References Section
10-01-2024	Updated Coding Section Added 0493U (eff. 10-01-2024)
01-05-2026	Updated Description Section
	Updated Rationale Section
	Updated Reference Section
02-24-2026	Updated Description Section
	Updated Rationale Section
	Updated Coding Section Added 0540U
	Updated Reference Section

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