

# Novel Rejection Biomarkers in Kidney Transplantation

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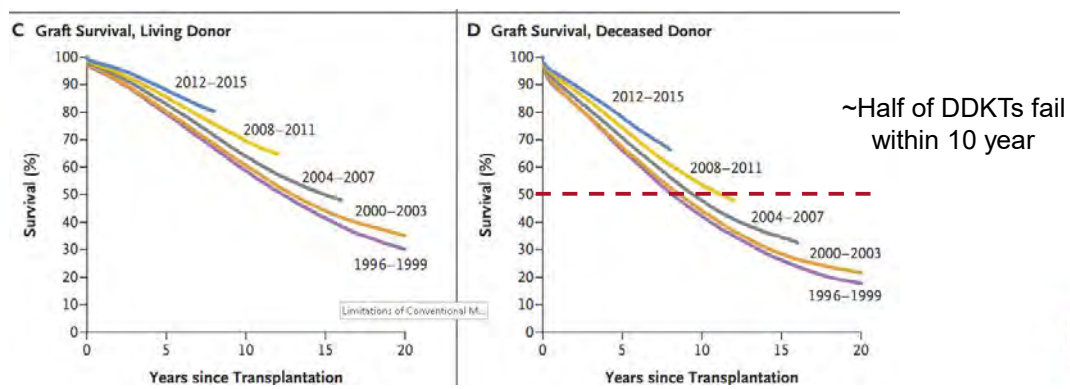


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## The Challenge: Unacceptable Rates of Long-Term Kidney Graft Failure

\*Kidney transplant is best treatment for ESKD

\*Advances in pre-, peri-, and post-transplant management have improved short-term (1-year outcomes)



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# Rejection is a Leading Cause of Late Graft Failure

## Conventional Approach to Allograft Monitoring

### BLOOD TESTS

\*Serial Measurements of serum creatinine (eGFR)

\*HLA Donor Specific Antibodies

\*Immunosuppression Drug Levels

### URINE TESTS

\*Urinalysis

\*Urine Protein:Creatinine Ratio

### BIOPSY

\*For-Cause Biopsies

\*Surveillance/Protocol Biopsies (e.g. 3 mo, 12mo)

\*\*Conventional strategies are not sufficient --development and implementation of novel biomarkers is an unmet need

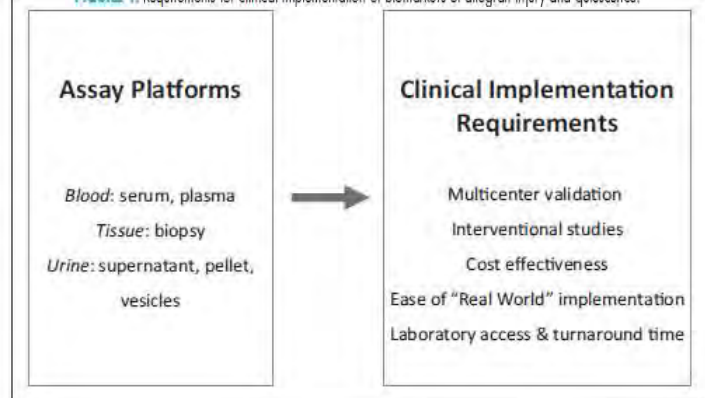


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## NOVEL BIOMARKERS: FROM DISCOVERY to CLINICAL IMPLEMENTATION



FIGURE 1. Requirements for clinical implementation of biomarkers of allograft injury and quiescence.



Westphal SG, Mannon RB. *Curr Opin Organ Transplant*, 2022.

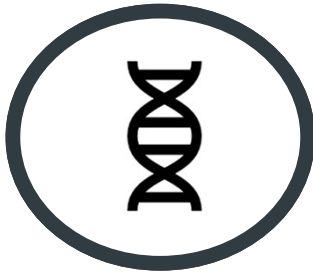
-Should be easily obtained, cost-effective, reliably indicate underlying biologic/pathologic process

-Diagnostic, predictive, prognostic, surrogate

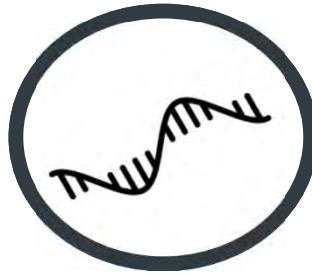


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## Novel Biomarkers in Kidney Transplantation



**DONOR-DERIVED  
CELL-FREE DNA  
(dd-cfDNA)**



**mRNA GENE  
EXPRESSION  
PROFILE (GEP)**



**URINE  
BIOMARKERS**

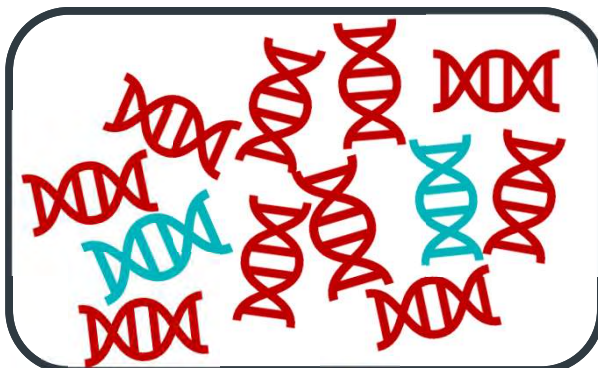


**TISSUE-BASED  
GENE EXPRESSION**



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## Donor-Derived Cell-Free DNA



 recipient derived     donor derived

Commercially  
Available Assays with  
CMS approval

1. Allosure® (CareDx)
2. Prospera™ (Natera)
3. TRAC® (Eurofins Viracor)
4. VitaGraft™ (Oncocyte)

Non-encapsulated cell-free DNA is released by cells undergoing injury/apoptosis

Modern high throughput technology (PCR, NGS) can compare differences in SNPs to quantify donor-derived vs. recipient-derived without donor genotyping



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## DIAGNOSTIC PERFORMANCE OF dd-cfDNA ASSAYS

| Study                           | Design   | Threshold        | Sensitivity | Specificity | NPV    | PPV    | AUC       |
|---------------------------------|--|------------------|-------------|-------------|--------|--------|-----------|
| <b>Allosure® (CareDx)</b>       |  |                  |             |             |        |        |           |
| DART <sup>1</sup>               | *Prospective, 107 for-cause biopsies<br>*Rejection prevalence = 25%  | 1.0%             | 59%         | 85%         | 84%    | 61%    | 0.74      |
| Admiral <sup>2</sup>            | *Prospective, Serial dd-cfDNA monitoring<br>*Surveillance and for-cause biopsies<br>*Rejection prevalence = 9.2% | 0.5%             | 78%         | 71%         | 90%    | 50%    | 0.80      |
| Gupta <sup>3</sup>              | *Prospective, n=208 biopsies (for cause and surveillance) (Rej Prevalence 38%)                                   | 0.82%            | 58-61%      | 83-90%      |        |        | 0.77-0.80 |
| <b>Prospera™ (Natera)</b>       |  |                  |             |             |        |        |           |
| Sigdel <sup>4</sup>             | *Biorepository (For Cause/Surveillance)<br>*Rejection Prevalence = 14%   | 1.0%             | 89%         | 73%         | 95%*   | 52%*-  | 0.87      |
| Trifecta <sup>5</sup>           | *Prospective, For-cause biopsies<br>*Rejection prevalence: 40%   | 1.0% or 78 cp/ml | 74-83%      | 81%         | 83-91% | 68-71% | 0.82-0.88 |
| <b>TRAC® (Eurofins Viracor)</b> |  |                  |             |             |        |        |           |
| Bixler <sup>6</sup>             | *Biorepository, For-Cause<br>*Rejection prevalence: 20%  | 0.69%            | 58%         | 84.5%       | 86%    | 55%    | 0.85      |
| <b>VitaGraft™ (Oncocyte)</b>    |  |                  |             |             |        |        |           |
| Oellerich <sup>7</sup>          | *Prospective, serial measurements<br>*Surveillance/For-cause biopsies<br>*Rejection prevalence: 8%               | 52 cp/ml         | 73%         | 73%         | 98%    | 13%    | 0.83      |

1. Bloom RD, et al. *JASN*, 2017      3. Gupta G, et al. *Transplantation*, 2022.      5. Halloran PF, et al. *Transplantation*, 2022.      7. Oellerich M, et al. *AJT*, 2019.  
 2. Bu L, et al. *KI*. 2022.      4. Sigdel TK, et al. *J Clin Med*, 2019.      6. Bixler E, et al. *Online Publication*, 2020.

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## DIAGNOSTIC PERFORMANCE OF dd-cfDNA ASSAYS

| Study                    | Diagnostic Ranges Across Assays               |  |
|--------------------------|---|--|
| Allosure® (CareDx)       | *Sensitivity: 58-89%      *Specificity 71-90% |  |
| DART <sup>1</sup>        |   |  |
| Admiral <sup>2</sup>     | *NPV: 68-98%      *PPV: 13-71%                |  |
| Gupta <sup>3</sup>       |   |  |
| Prospera™ (Natera)       | ROC-AUC: 0.74-0.88                            |  |
| Sigdel <sup>4</sup>      |   |  |
| Trifecta <sup>5</sup>    | *Sensitivity: 74-83%      *Specificity 81-91% |  |
| TRAC® (Eurofins Viracor) |   |  |
| Bixler <sup>6</sup>      | *NPV: 83-91%      *PPV: 52-68%                |  |
| VitaGraft™ (Oncocyte)    |   |  |
| Oellerich <sup>7</sup>   | *Sensitivity: 73%      *Specificity 73%       |  |
|                          |   |  |

dd-cfDNA AUC = 0.74

SCr AUC = 0.54

1. Bloom RD, et al. *JASN*, 2017      3. Gupta G, et al. *Transplantation*, 2022.      5. Halloran PF, et al. *Transplantation*, 2022.      7. Oellerich M, et al. *AJT*, 2019.  
 2. Bu L, et al. *KI*. 2022.      4. Sigdel TK, et al. *J Clin Med*, 2019.      6. Bixler E, et al. *Online Publication*, 2020.

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## Uses and Pitfalls of dd-cfDNA in kidney transplant

### DIAGNOSTIC

- Discrimination for ABMR >>TCMR (especially at higher thresholds and Banff 1a/Borderline)
- At low levels (e.g. <0.2%) good rule out test (strong NPV, especially in lower risk patient)
- Not specific for rejection (BKVN, pyelo, other injury can increase levels)

### PREDICTIVE/PROGNOSTIC

- Elevated dd-cfDNA levels associated with eGFR decline, de novo DSA development, and future rejection
- Potential to monitor response to treatment



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## Blood Gene Expression Profiles (GEP)

### Clinical Trials in Organ Transplantation 08 (CTOT-08)

\*Prospective, multicenter (discovery cohort and validation cohort)

\*Paired microarray based molecular GEP assay with surveillance biopsies (stable patients) (42% and 28% of subjects in the 2 cohorts had subclinical rejection w/in 24 mo)

\*Dichotomous result threshold: TX or Not-TX

| Data Set         | Paired Samples, n | TX: subAR (% subAR prevalence) | Probability Threshold | % Negative (spared biopsy) | NPV | True Negative | False Negative | % Positive (pickup subAR) | PPV |
|------------------|-------------------|--------------------------------|-----------------------|----------------------------|-----|---------------|----------------|---------------------------|-----|
| Discovery Set    | 530               | 400:130 (24.5)                 | 0.375                 | 74.7                       | 88  | 349           | 42             | 25.3                      | 61  |
| Validation set 1 | 138               | 96:42 (30.4)                   | 0.375                 | 71.7                       | 78  | 77            | 22             | 28.3                      | 51  |
| Validation set 2 | 129/138           | 93:36 (27.9)                   | 0.375                 | 72.1                       | 80  | 74            | 19             | 27.9                      | 47  |

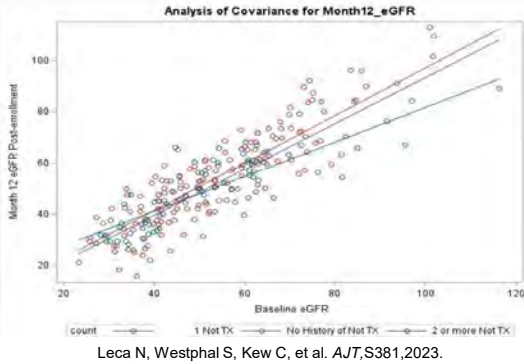
Friedewald JJ, et al. *AJT*, 2019.



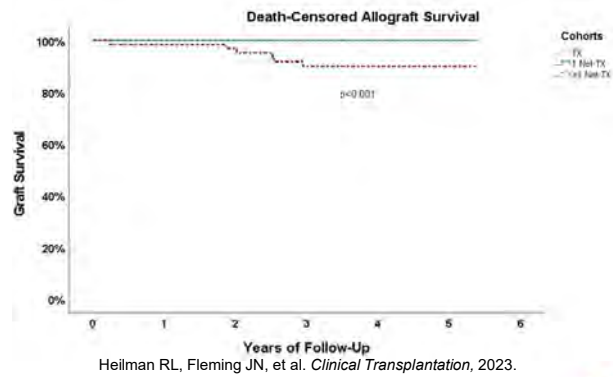
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# TruGraf (Transplant Genomics) GEP Assay Correlates with Outcomes

>1 Not-TX results -> greater loss of eGFR and dnDSA formation



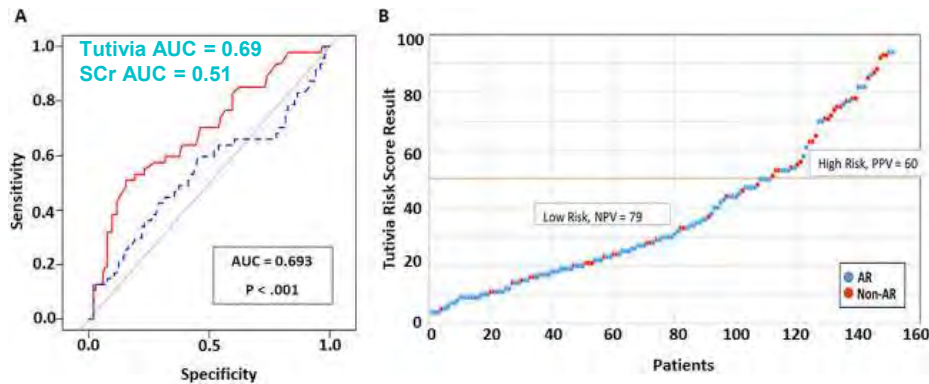
>1 Not-TX results -> increased rates of rejection and lower death-censored graft survival



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## Blood Gene Expression Profile: Tutivia™ (Verici Dx)

\*17-gene mRNA signature developed from GoCAR study validated in recent prospective study with paired surveillance/for-cause biopsy samples (rejection rate 31%)



Bestard O, Augustin J, Wee A, Poggio E, Mannon R, et al. *AJT*, 2024.

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## Tissue Based Gene Expression: Results from the Molecular Microscope

Case: 39 yo 9 mo post DDKT. SCr = 0.8 mg/dl (stable). dd-cfDNA 3.6%. DR52 DSA (MFI 1365).  
Histology – peritubular capillaritis (very rare/scant C4d + staining)

### Pure Molecular Interpretation (Results Summary)

Abnormal kidney transplant biopsy 274 days post-transplant. Severe fully developed ABMR. No TCMR. Mild inflammation with minimal AKI and atrophy-fibrosis.  
Note: the Molecular Microscope® Diagnostic System cannot exclude primary glomerular diseases.

Percent cases<sup>1</sup>  
78%

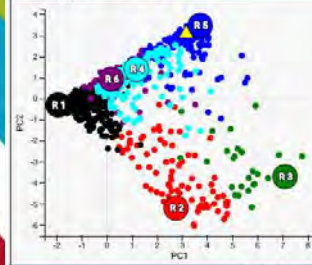
### Result Details

#### Biopsy Rejection and Injury Scores

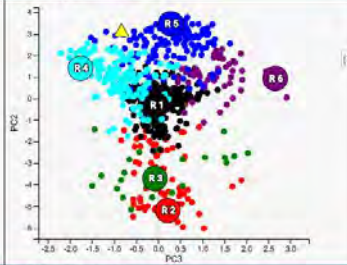
|                  | Classifier / Gene Sets                   | Biopsy Score | Range of Values <sup>2</sup> | Upper Limit of Normal <sup>2</sup> | Interpretation |
|------------------|--|--------------|------------------------------|------------------------------------|----------------|
| Injury Scores    | Global Disturbance Score                 | -0.40        | -3.8 – 5.8                   | 0.02                               | Mild           |
|                  | Acute Kidney Injury (AKI) Score          | -0.28        | -0.6 – 1.6                   | 0.47                               | Minimal        |
|                  | Atrophy-Fibrosis Score                   | 0.15         | 0 – 1                        | 0.47                               | Minimal        |
| Rejection Scores | Rejection Score                          | 0.92         | 0 – 1                        | 0.30                               | Severe         |
|                  | T Cell Mediated Rejection (TCMR) Score   | 0.01         | 0 – 1                        | 0.10                               | Normal         |
|                  | Antibody-Mediated Rejection (ABMR) Score | 0.92         | 0 – 1                        | 0.20                               | Severe         |

### Archetypal Analysis (please see Archetypal Analysis Description on Page 2 for details)

#### Current Biopsy vs Reference Set: PC2 vs PC1



#### Current Biopsy vs Reference Set: PC2 vs PC3



| Rejection phenotype <sup>3</sup> (six scores, R1-R6, adding up to 1.0) | Score |
|--|-------|
| R1 Non-rejecting   | 0.00  |
| R2 TCMR  | 0.00  |
| R3 Mixed Rejection   | 0.00  |
| R4 Early-Stage ABMR (EABMR)  | 0.30  |
| R5 Fully-Developed ABMR (FABMR)  | 0.70  |
| R6 Late-Stage ABMR (LABMR)   | 0.00  |
| All ABMR (Sum of R4, R5, and R6)                                       | 1.00  |

### Additional Biopsy Scores

| Classifier / Gene Sets                  | Biopsy Score                                     | Range of Values <sup>2</sup> | Upper Limit of Normal <sup>2</sup> | Interpretation |         |
|---|--|------------------------------|------------------------------------|----------------|---------|
| TCMR-1                                  | 0.01   | 0 – 1                        | 0.10                               | Normal         |         |
| TCMR-2                                  | 0.01   | 0 – 1                        | 0.10                               | Normal         |         |
| Mean of 2 TCMR Classifiers              | 0.01   | 0 – 1                        | 0.10                               | Normal         |         |
| Rejection Related                       | 0.92   | 0 – 1                        | 0.30                               | Severe         |         |
| Injury-Scoring Related                  | AKI Score  | -0.28                        | -0.6 – 1.6                         | 0.47           | Minimal |
|   | Atrophy-Fibrosis Score                           | 0.15                         | 0 – 1                              | 0.47           | Minimal |
| ABMR Related                            | ABMR-1   | 0.92                         | 0 – 1                              | 0.20           | Severe  |
|   | ABMR-2   | 0.96                         | 0 – 1                              | 0.20           | Severe  |
|   | ABMR-3   | 0.99                         | 0 – 1                              | 0.20           | Severe  |
|   | Mean of 3 ABMR Classifiers                       | 0.96                         | 0 – 1                              | 0.20           | Severe  |
| Classifiers based on Histologic Lesions | Glomerulitis (g) = 0 probability                 | 0.93                         | 0 – 1                              | 0.28           | Severe  |
|   | Transcubular Glomerulopathy (tg) = 0 probability | 0.53                         | 0 – 1                              | 0.18           | Severe  |
|   | Peritubular capillaritis (pts) = 0               | 0.88                         | 0 – 1                              | 0.36           | Severe  |
|   | ISG+ positive probability                        | 0.88                         | 0 – 1                              | 0.49           | Severe  |
|   | Interstitial inflammation (i) = 1 probability    | 0.04                         | 0 – 1                              | 0.07           | Normal  |
|   | Tubulitis (t) = 1 probability                    | 0.06                         | 0 – 1                              | 0.19           | Normal  |
|   | Tubular atrophy (a) = 1 probability              | 0.08                         | 0 – 1                              | 0.52           | Normal  |
|   | Arteriosclerosis (ar) = 0 probability            | 0.33                         | 0 – 1                              | 0.70           | Normal  |

For Classifiers:  
TCMR-1 TCMR vs everything else.  
ABMR-1 ABMR vs everything else.  
ABMR-2 ABMR vs everything else, with Mixed/TG/ABMR suspicious withfield

TCMR-2 TCMR vs everything else, with ISG/Borderline/Mixed withfield.  
ABMR-2 ABMR vs everything else, with TG/ABMR suspicious withfield.

### Nearest Neighbor Analysis

| Rank | order of the most common histologic diagnosis in the 50 nearest molecular neighbors. | Percentage |
|------|--|------------|
| 1    | ABMR   | 65%        |
| 2    | NOADA  | 12%        |
| 3    | TG   | 6%         |
| 4    | Mixed  | 4%         |
| 5    | GN   | 4%         |

| Mean molecular scores in the 50 nearest molecular neighbors. | Score |
|--|-------|
| R4   | 0.54  |
| ABMR   | 0.92  |
| Atrophy-Fibrosis   | 0.2   |
| Acute Kidney Injury  | 0.13  |
| TCMR   | 0.02  |



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## Urine Biomarkers

mRNA GENE EXPRESSION

CHEMOKINES (CXCL9 & CXCL10)



Exosomes and Vesicle RNA

Others: miRNA, dd-cfDNA, peptides

\*Non-invasive and easy to collect  
\*May best approximate allograft microenvironment



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LCD Reference Article    Billing and Coding Article

## Billing and Coding: MoIDX: Molecular Testing for Solid Organ Allograft Rejection

<https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleid=58061&ver=18&=>

### Intended Use Requirements for Coverage:

#### For-Cause:

1. "Concern for rejection and **would otherwise obtain for-cause biopsy**"
2. "Assess adequacy of immunosuppression and **would otherwise obtain biopsy** (or considering doing so"
3. "Assess the probability of rejection with concerning clinical information (clinical pre-tests that **inform whether a subsequent biopsy would likely be avoided**"
4. "Results of an inconclusive biopsy (wherein the **test may subsequently preclude another biopsy**"

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LCD Reference Article    Billing and Coding Article

## Billing and Coding: MoIDX: Molecular Testing for Solid Organ Allograft Rejection

<https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleid=58061&ver=18&=>

### Intended Use Requirements for Coverage:

#### Surveillance:

"If a patient population exists such that they are at particularly high risk for rejection, and the **pre-test clinical information would otherwise indicate a biopsy** to evaluate the allograft health based on the risk of that population, the use of these services may fit within the intent of the policy coverage language"...

"As such, the use of a **molecular test for surveillance (protocol) testing can only be reasonably seen as compliant with the policy if the patient would otherwise receive a surveillance (protocol) biopsy**. Providers **must demonstrate that such a practice (for protocol biopsies) would otherwise be performed** to meet policy requirements. Other uses of surveillance testing are not compliant with the policy language"

#### Other:

- \*Test/biopsy cannot be simultaneous or within "short window"
- \*Only one molecular test can be used for a given encounter



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**MolDx Reimbursement Decision or Molecular Testing in Organ Transplant Causes Confusion, Concern**

Jun 27, 2023 | Premium

NEW YORK — A recent billing article concerning Medicare reimbursement for solid organ transplant patients has caused confusion and concern among transplant surgeons and patients alike.

**Medicare's Organ Transplant Funding**

Patients and doctors explain the damage from denying tests in the name of saving money.



**ASTS**  
American Society of Transplant Surgeons

Saving and improving lives with transplant

STAT+  
**Transplant patients say new Medicare guidance puts their donated organs at risk**



March 21, 2023

Gabriel A. Bien-Willner, MD, PhD  
Medical Director, MolDX  
Chief Medical Officer, Palmetto GBA

RE: Article - Billing and Coding: MolDX: Molecular Testing for Solid Organ Allograft Rejection (A58019)

Dear Dr. Biel-Willner,

As President of the American Society of Transplant Surgeons (ASTS), I am writing to express ASTS' deep concerns about the document entitled, *Article - Billing and Coding: MolDX: Molecular Testing for Solid Organ Allograft Rejection (A58019)* (the "Billing Article") (Appendix A). ASTS is a medical specialty society representing approximately 2,000 professionals dedicated to excellence in transplant surgery and to the patients that we serve. Our mission is the advancement of the art and science of transplant surgery through patient care, research, education, and advocacy.

We appreciate that data for the rapidly emerging field of molecular diagnostic testing is still maturing and that the global costs associated with such testing are significant. However, we feel strongly that molecular diagnostic testing may provide massive clinical and economic benefits in the early detection and management of solid organ allograft rejection. Utilizing molecular testing for detection of allograft injury is an emerging standard of care that can directly aid in clinical decision making and may improve patient and allograft survival. We support the access of transplant patients to these diagnostic technologies and believe that continued Medicare coverage of these tools is critical to further refine their utility and cost effectiveness. We have recently issued an ASTS White Paper on molecular diagnostic testing (**Appendix B**), which provides additional background that was not made public in time to be taken into consideration by MolDX when it formulated the Billing Article.

We have scientific, ethical, and process-related concerns about the Billing Article, which are enumerated below. We are puzzled that the coverage limitations set forth in the Billing Article have been put forward at a time when CMS has clearly acknowledged that transplantation is the best, and most cost-effective, treatment option for those with ESRD, and without consideration of the potential chilling impact on innovation in the field or continuity of care for patients. **We request that MolDX delay the implementation of the Billing Article** until the Billing Article's modifications of limitations on coverage for

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## 2024 Nebraska Medicine Surveillance/Biomarker Protocol

\*\*applies to stable patients (e.g. stable renal function w/out new proteinuria, DSA, etc.)

Conventional Surveillance

**Group 1:  
Conventional  
Monitoring Only**

Low Immunologic Risk

\*Age >65 + unsensitized

\*Age < 65 + unsensitized + zero DR/DQ match

\*Zero antigen mismatch

\*Physician discretion

Biomarker/Biopsy Surveillance

**Group 2:  
Biomarker  
Surveillance**

Most Patients

\*Gene Expression Profile at month 3, 6, 12, 18, 24

\*Response to abnl GEP

A. Biopsy  
B. Repeat GEP 1-3 in mo.  
C. dd-cfDNA  
D. Other eval (DSA, BK, etc)

**Group 3:  
Biopsy  
Surveillance**

Default

\*Surveillance biopsy at:

3-6 months  
6-12 months  
12-24 month

No biopsy if risk prohibitive

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## 2024 Nebraska Medicine: Utilization of For-Cause Novel Biomarkers

### For-Cause

If there is clinical, laboratory or physiologic suspicion of rejection, a biopsy will be performed

OR

dd-cfDNA (any of the assays) obtained to decide if a biopsy should be performed (results used in conjunction with clinical picture to decide need for biopsy)

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