natera | Prospera™ Transplant assessment

Natera's latest innovation:

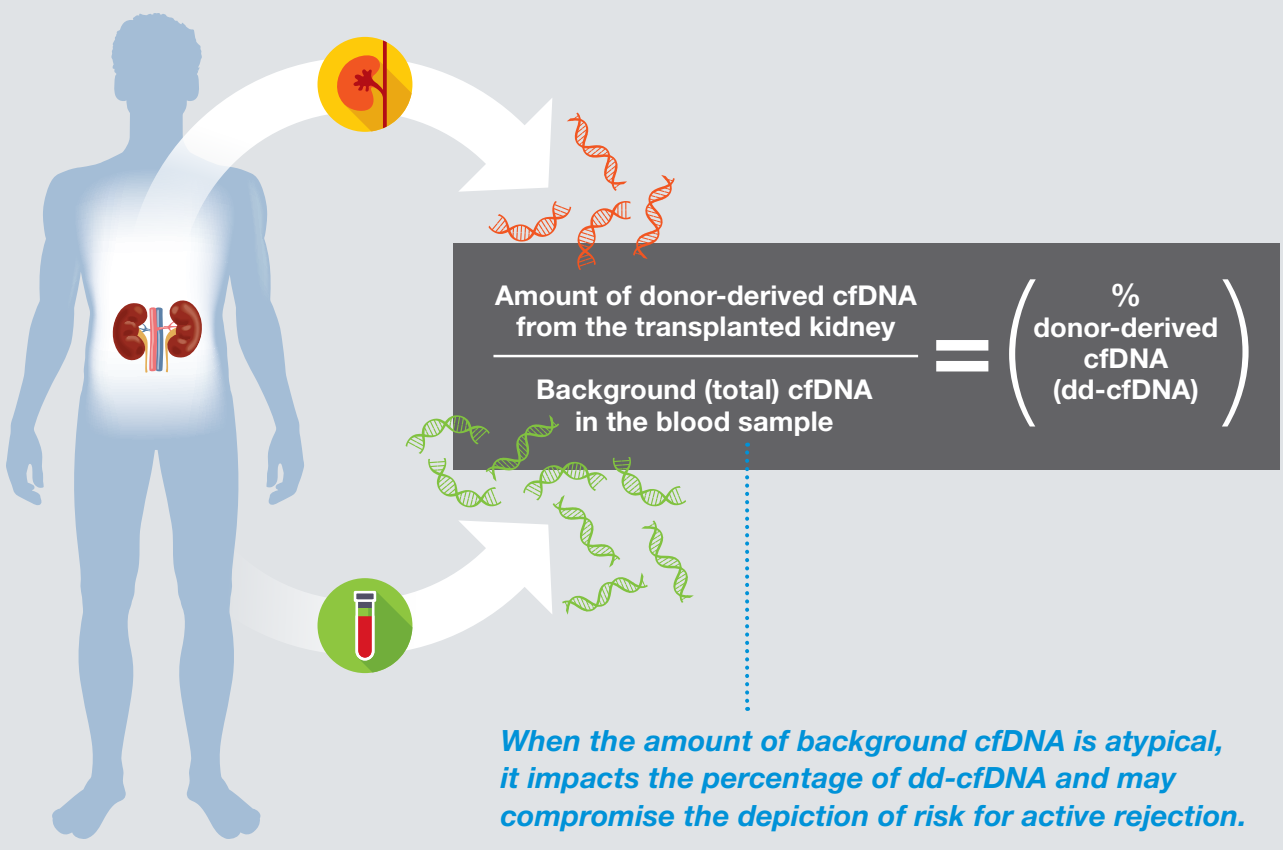
Quantification of background cell-free DNA to further refine transplant rejection risk

As the experts in cell-free DNA (cfDNA) testing, we have refined our workflow based on our findings from two million cfDNA tests to now include **a proprietary technique to quantify absolute background cfDNA** in a streamlined manner.

This enhancement provides additional information to the physician when assessing rejection and may assist in identifying patients at-risk of a false negative interpretation.

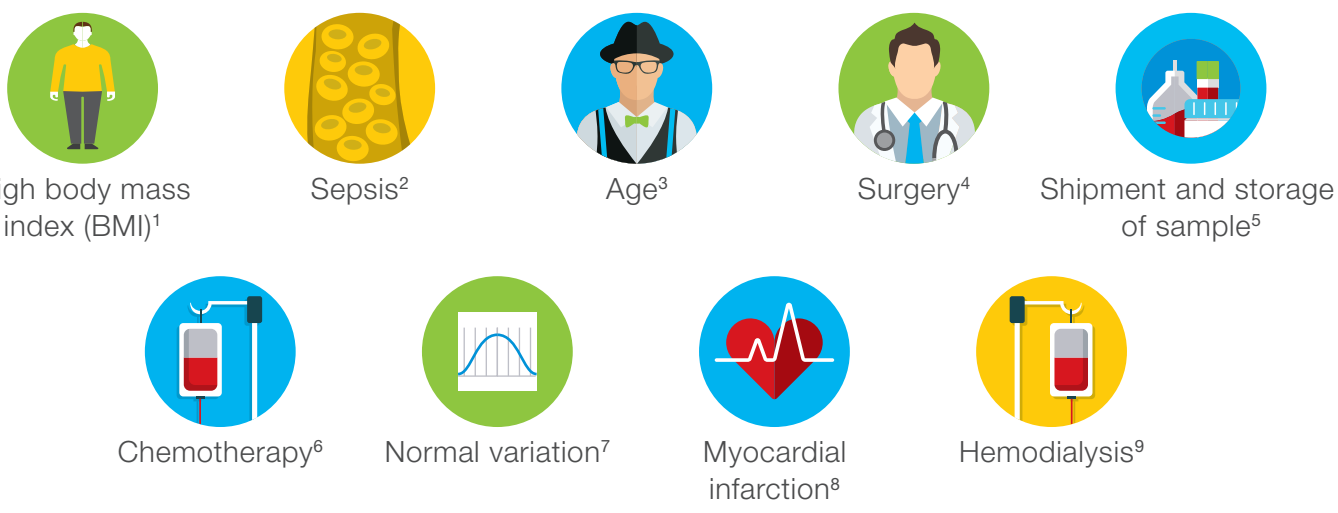
Defining background cell-free DNA and its influence on your result

Background cell-free DNA originates from the transplant recipient and is naturally occurring in variable amounts within the plasma.



When the amount of background cfDNA is atypical, it impacts the percentage of dd-cfDNA and may compromise the depiction of risk for active rejection.

Factors that may influence background DNA may include:



Providing greater precision for even more confidence in your Prospera result

Based on our leadership in cell-free DNA innovation, Natera has now introduced two novel techniques to provide greater precision.

1.

Proprietary Library Preparation

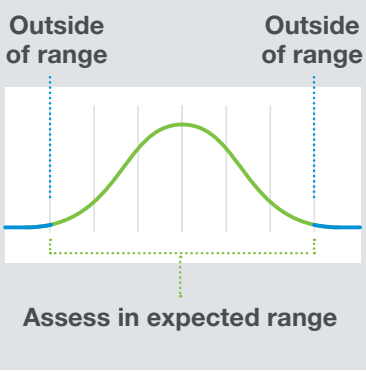
Proprietary library preparation step that adjusts for background cfDNA introduced into the sample during transport

+

2.

Quantifed Background CfDNA

Unique quantification method that can identify atypical background cfDNA that may influence the reported result for a particular patient



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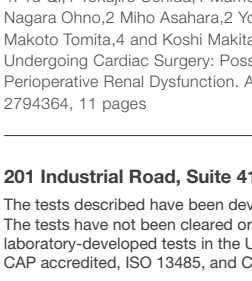
Potentially fewer false negative interpretations

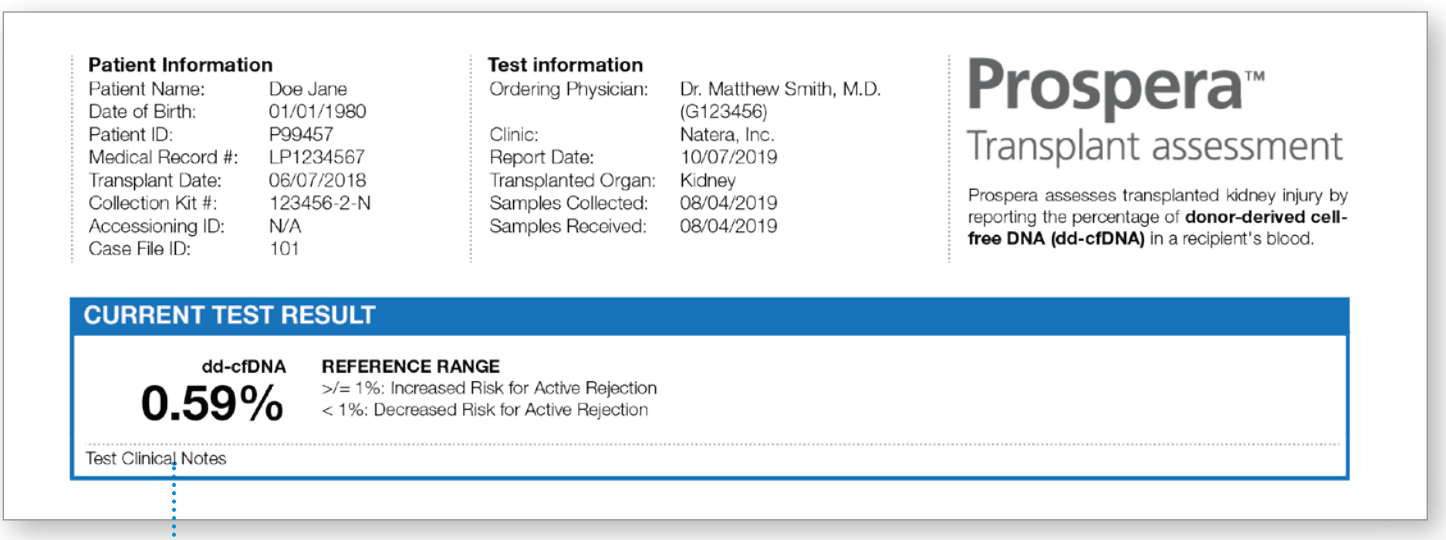
What you can expect with your Prospera results



As part of your Prospera report, Natera will notify you when a patient has atypically high background (total) DNA, indicating a risk for a potential false negative interpretation.

Our clinical team is here to discuss these findings further and how to apply these personalized results into your care decisions for each patient.

Patients may be eligible to participate in an ongoing research protocol, the Study for the Prediction of Active Rejection in Organs Using Donor-derived Cell-free DNA Detection **"SPARO,"** to improve the test performance and utility.



Patient Information

Patient Name:	Doe Jane
Date of Birth:	01/01/1980
Patient ID:	P99457
Medical Record #:	LP1234567
Transplant Date:	06/07/2018
Collection Kit #:	123456-2-N
Accessioning ID:	N/A
Case File ID:	101

Test information

Ordering Physician:	Dr. Matthew Smith, M.D. (G123456)
Clinic:	Natera, Inc.
Report Date:	10/07/2019
Transplanted Organ:	Kidney
Samples Collected:	08/04/2019
Samples Received:	08/04/2019

Prospera™ Transplant assessment

Prospera assesses transplanted kidney injury by reporting the percentage of **donor-derived cell-free DNA (dd-cfDNA)** in a recipient's blood.

CURRENT TEST RESULT

dd-cfDNA **0.59%**

REFERENCE RANGE
>/= 1%: Increased Risk for Active Rejection
< 1%: Decreased Risk for Active Rejection

Test Clinical Notes

Clinical notification if sample has atypical background cell-free DNA

Call us at 650.273.4468 to speak to our clinical team

References

1. Vora NL, Johnson KL, Basu S, Catalano PM, Hauguel-De Mouzon S, Bianchi DW. A multifactorial relationship exists between total circulating cell-free DNA levels and maternal BMI. Prenat Diagn. 2012;32(9):912–914. doi:10.1002/pd.3919
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Transplant assessment

Optimized surveillance for even more confident results

Precise cell-free DNA testing from the experts

Introducing Prospera™ Transplant Assessment for highly precise results you can trust

Prospera uses revolutionary tech to enhance the patient and physician's ability to assess otherwise undetected rejection events that might lead to kidney loss.

This could mean working closely with your care team for timely intervention, an informed treatment plan and appropriate maintenance of immunosuppression throughout the life of your transplanted kidney.

MORE INFORMED, BETTER PROTECTION

Transplant patients and physicians understand the importance of preserving the function of a newly transplanted kidney. Achieving this goal could mean avoiding dialysis or not going back on the transplant list. To do so—early, informed treatment of underlying disease and patient adherence to medications are essential.

Why is monitoring for active rejection important?

Transplant patients may develop complications after surgery—weeks, months or even years later.

“Active rejection” is one type of complication. It occurs when your immune system sees the transplanted kidney as foreign and attacks it. Certain changes in your blood tests may indicate possible rejection. In most cases, you may feel perfectly normal with no symptoms but still be experiencing active rejection of your kidney.

Knowing as soon as possible about rejection can help you and your care team develop a treatment plan to best protect your new kidney. That’s why accurate monitoring is so important.

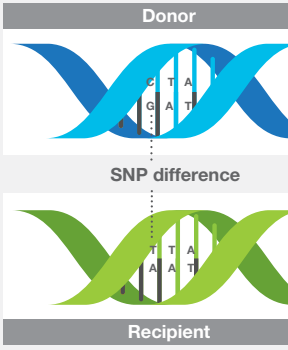
How does Prospera work?

From a single blood draw, Prospera measures the amount of donor DNA from your transplanted kidney in your blood. This helps your care team assess all types of rejection more precisely than available standard assessment tools.[1-4]

Recipient Blood Sample

Mix of donor and recipient cell-free DNA (cfDNA)

Precise Identification



>13,000 single-nucleotide polymo (SNPs) and advanced bioinforma to differentiate recipient and don

What do Prospera results show?

The Prospera result represents the percent of cell-free DNA in the patient's blood that originates from the donated kidney to determine whether or not you may be experiencing active rejection. It may also indicate other types of kidney injury you are experiencing. Like your other regular monitoring tests, Prospera is recommended for periodic use over time as directed by your doctor.

Your own personalized cell-free DNA baseline

Establishing a baseline tells you and your care team the "normal state" of your new kidney. You can measure new results against this baseline.

A way to track your cell-free DNA over time

Following your levels in the future reveals your new kidney's health.

If a Prospera result is above 1%[2]

This may mean that active rejection is occurring. Terms used to describe the various types of active rejection include:

- Antibody-mediated rejection
- T cell-mediated rejection
- Mixed rejection

To confirm a rejection or the type of rejection, you should consult your doctor.

If a Prospera result is in the normal range[2]

This may mean that your kidney is stable.

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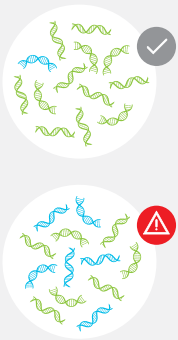
or cfDNA

Test Results

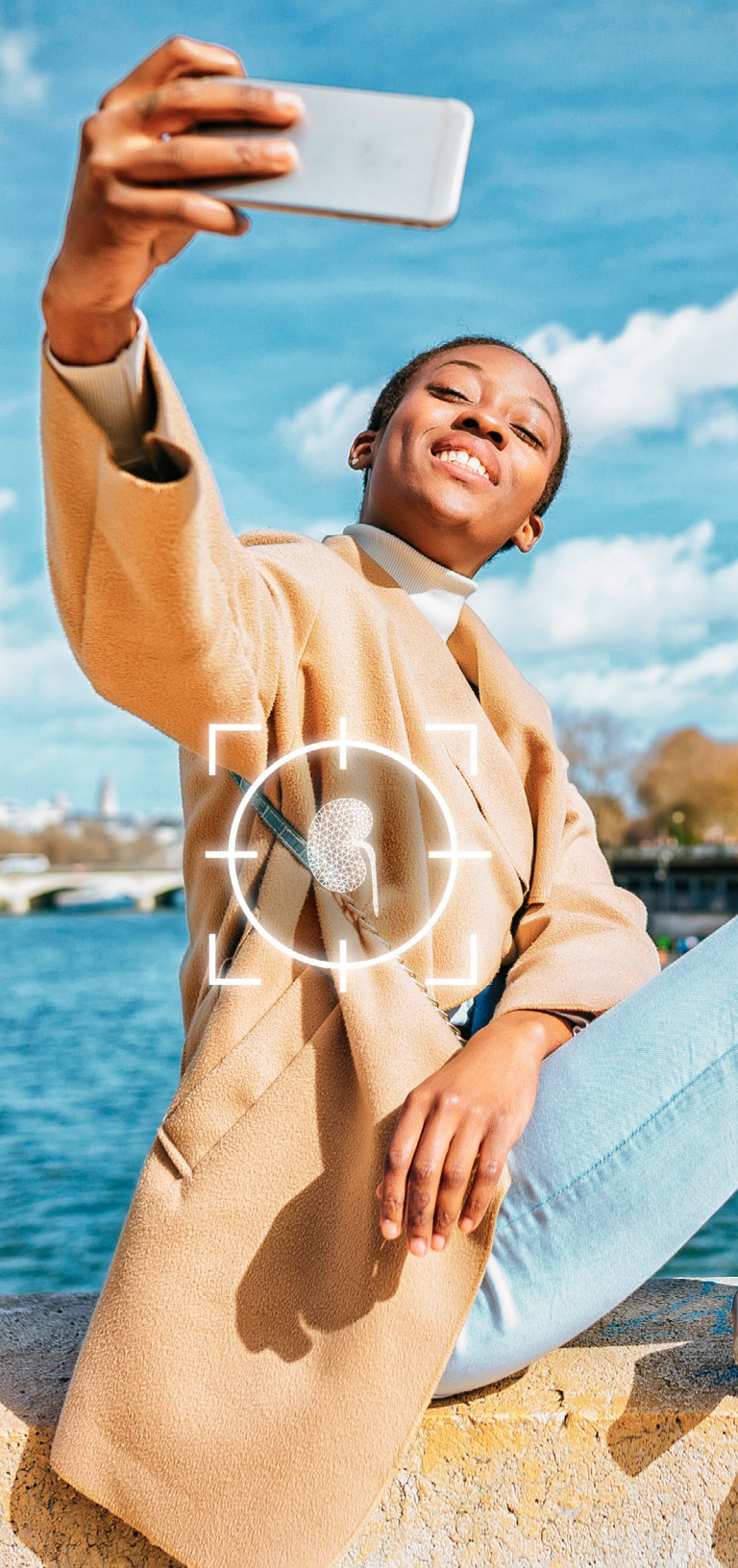


NO active rejection:
Minimal donor-derived cell-free DNA (dd-cfDNA) is released in a stable patient's blood.

OR

Active rejection:
Upon cell injury, more dd-cfDNA is released from the donor kidney.

Prospera reports the percentage of dd-cfDNA in a transplant recipient's blood



How do I get started?

Prospera is available through your doctor or care team at your transplant center. Not sure if your provider offers Prospera? Contact us at +1 650.273.4468.

Is Prospera covered by insurance?

Natera welcomes all insurances. Prospera is covered by Medicare for assessing potential kidney transplant rejection. The goal of Natera's billing department is to make the process transparent and easy for our patients. In the rare event you have financial responsibility for Prospera, Natera offers flexible financial assistance programs and will work closely with you to ensure there is no hardship on you or your family. In all cases, the Natera team is here to help you with any billing or reimbursement questions at +1 650.273.4468.

How does Natera support me?

We offer complete support through our ProsperaLink Program:

Always by your side: Natera's care team will guide you through the process of using Prospera and check-in with you at every milestone.

Flexible for your convenience: Our team coordinates blood draws around your schedule—at a certified laboratory near you or by a blood draw specialist who can come to you.

Transparent & accessible: Our proactive billing outreach and flexible payment plans, including assistance for financial hardship, help ensure no significant financial hardship in accessing Prospera.

Discover all the other ways Natera supports you by calling +1 650.273.4468.

Only from Natera

Our mission is to improve disease management for patients worldwide. We have been exploring and evolving our expertise in cell-free DNA (cfDNA) across reproductive health, cancer, and organ transplant care. We founded our company to use the most innovative technology for earlier detection of genetic conditions in pregnancies. In the years since, we have helped nearly two million families on their path to parenthood with solutions that tell them about the genetic health of their babies.

Built on Natera's pioneering technology, our first circulating tumor DNA (ctDNA) test is custom-built and personalized for each cancer patient. Now, we have refined our technology to assess tiny amounts of donor DNA in the blood of kidney transplant patients. And that's just the start.

With all the rapid advances taking place in cfDNA at Natera, there is one constant: our unwavering commitment to patients.

For more information about Prospera, call +1 650.273.4468 or visit natera.com/prospera.

REFERENCES

1 Altug Y, Liang N, Ram R, et al. Analytical validation of a single-nucleotide polymorphism-based donor-derived cell-free DNA assay for detecting rejection in kidney transplant patients. *Transplantation*. 2019

2 Sigdel TK, Archila FA, Constantin T, et al. Optimizing detection of kidney transplant injury by assessment of donor-derived cell-free DNA via massively multiplex PCR. *J Clin Med*. 2019;8(1):19.

3 Grskovic M, Hiller DJ, Eubank LA, et al. Validation of a clinical-grade assay to measure donor-derived cell-free DNA in solid organ transplant recipients. *J Mol Diagn*. 2016;18(6):890-902.

4 Bloom RD, Bromberg JS, Poggio ED, et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol*. 2017;28(7):2221-2232. doi: 10.1681/ASN.2016091034.

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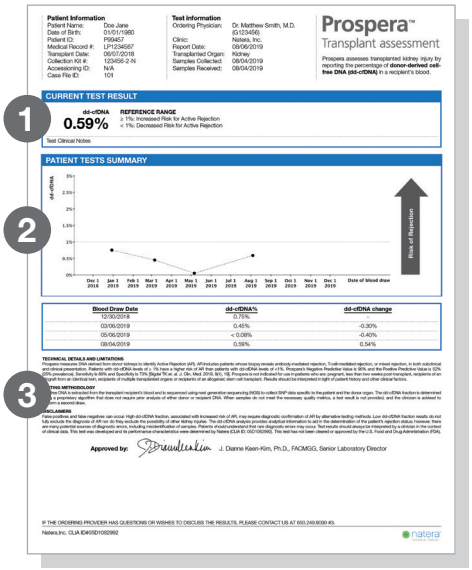


Clinician's guide to results

This guide is designed for clinicians and should be used as a supplement to the Prospera™ results reports. Prospera uses single-nucleotide polymorphism (SNP)-based technology to assess for active rejection (AR) by measuring the DNA derived from transplanted donor kidneys. AR includes antibody-mediated rejection, T-cell-mediated rejection, and mixed rejection in both subclinical and clinical presentations, as revealed in the biopsy. The Prospera result represents the percent of cell-free DNA in the recipient's blood that originates from the allograft. Prospera results and transplant rejection status should always be considered in the context of other significant clinical factors and physician judgment.

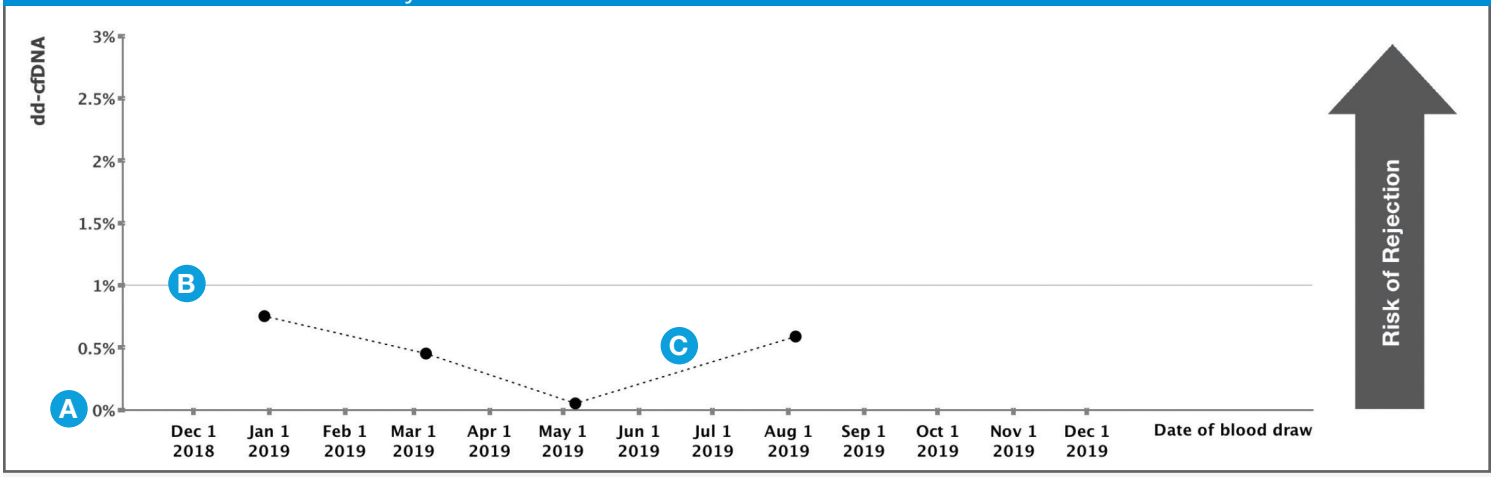
1 Current Test Result

dd-cfDNA
0.59%

REFERENCE RANGE
≥ 1%: Increased Risk for Active Rejection
< 1%: Decreased Risk for Active Rejection

The Current Test Result section reports the dd-cfDNA level obtained from the most recent Prospera draw. The reference range provides a general risk assessment derived from our published clinical validation study. Patients with dd-cfDNA levels ≥1% have a higher risk of AR than patients with dd-cfDNA levels of <1%.[1]

2 Patient Test Summary



A The X-axis highlights the date of blood draw; the Y-axis represents the % dd-cfDNA level at the date of the blood draw.

B The 1% dd-cfDNA cutoff is marked as a reference.

C A dotted line connects dd-cfDNA values over time, but does not necessarily reflect a rate of change.

The Patient Test Summary depicts the patient's Prospera results over the previous 12-month period. For optimized clinical care, establishing a patient's baseline dd-cfDNA level may be beneficial. The chart below conveys the same information as in the graph above, but it adds specific draw dates and % dd-cfDNA change between draws.

Blood Draw Date	dd-cfDNA%	dd-cfDNA change
12/30/2018	0.75%	-
03/06/2019	0.45%	-0.30%
05/06/2019	< 0.08%	-0.40%
08/04/2019	0.59%	0.54%

3 Technical Details

	dd-cfDNA level ≥1.00(%)
Sensitivity – the ability of the test to correctly identify those patients **with** active rejection (true positives)	**88.7**
Specificity – the ability of the test to correctly identify those patients **without** active rejection (true negatives)	**72.6**
Positive predictive value (PPV)* – the chance that an individual is experiencing active rejection, given an increased risk result	**51.9**
Negative predictive value (NPV)* – the chance that the individual is truly stable, given a low-risk result	**95.1**

*PPV and NPV calculated based on a 25% prevalence of AR.

Limitations

Prospera is contraindicated:

- less than 24 hours after a biopsy or dialysis
- less than two weeks after transplant
- in pregnant women
- in recipients of multi-organ transplants or allogeneic stem cells
- in patients who have received an allograft from a genetically identical twin.

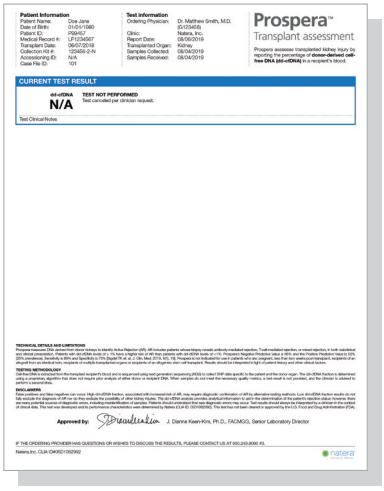
Results should be interpreted in the context of the entire clinical presentation because it is possible that other factors may influence dd-cfDNA results

Other Results

Test not performed (TNP)
The reason the test was not performed is indicated in the Current Test Result section and may include: sample receipt >8 days post draw; low blood volume (only one tube received instead of two); incorrect tube; or damaged sample. *If the test was not performed due to missing required information, please contact Natera to update. Otherwise, a new sample is required for testing.*

No results — submission of repeat specimen is required for testing
This result may be due to issues with laboratory processing or limitations of the testing algorithm. This result is likely sample-specific and is expected to resolve with a new sample.

No results — repeat sample is not indicated
These rare cases occur when an individual has a DNA pattern that cannot be interpreted clearly by this assay. This can be due to normal variation or to other clinical factors that may impact analysis. Please contact the Prospera clinical team with any additional patient information.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Transplant assessment
CURRENT TEST RESULT
dd-cfDNA
N/A
TEST NOT PERFORMED
Test cancelled per clinician request.

For additional assistance, you are encouraged to contact Prospera clinical support at **1.650.480.5007** or **transplantclinical@natera.com**.

References:
1. Sigdel TK, Acosta Archila F, Constantin T, et al. Optimizing detection of kidney transplant injury by assessment of donor-derived cell-free DNA via massively multiplex PCR. *J Clin Med*. 2019;8(1):19. doi: 10.3390/jcm8010019

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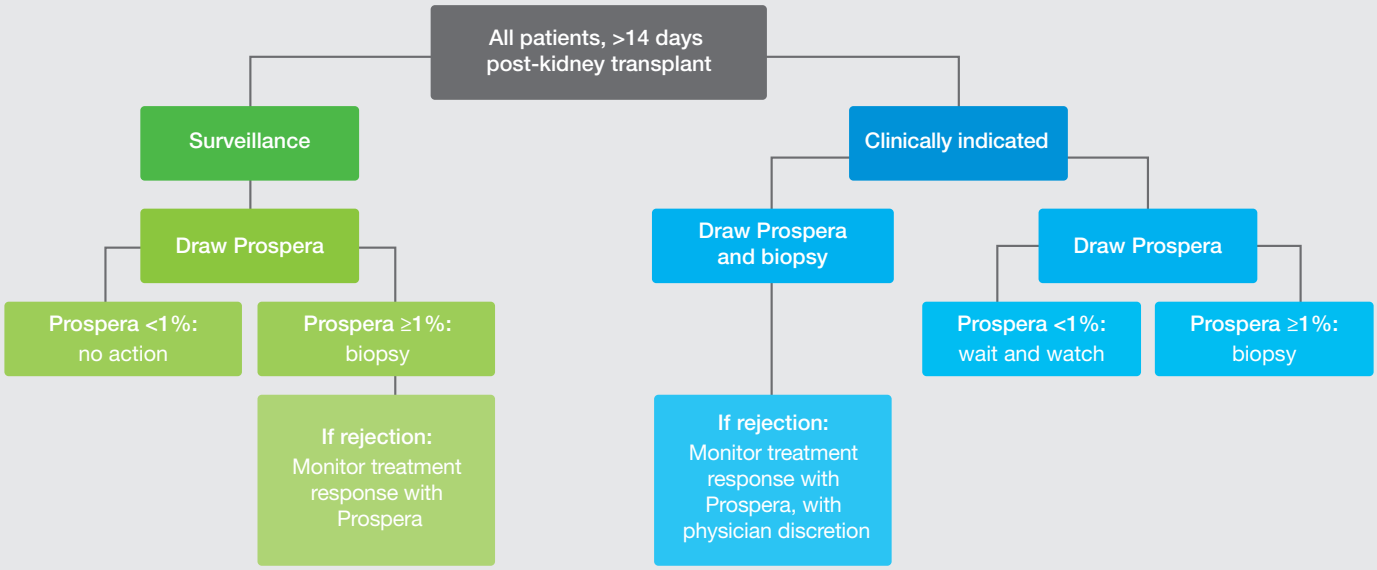
Indications for use

Prospera™ is a donor-derived cell-free DNA (dd-cfDNA) test for surveillance of rejection in post-renal-transplant patients. In a **surveillance** situation, Prospera testing is recommended at regular intervals: **1, 2, 3, 4, 6, 9,** and **12 months** after renal transplant or most recent rejection to establish an individual baseline for dd-cfDNA levels, and to detect subclinical rejection. It should then be repeated quarterly for the life of the transplant.

Proposed draw schedule (surveillance)

Prospera testing should also be considered in **clinically indicated** situations. The decision to order Prospera should be made in accordance with physician-assessed risk of active renal allograft rejection, including when a biopsy is considered or performed to evaluate suspected transplant rejection. Results should be interpreted alongside patient history and other clinical factors.

Example flow for ordering Prospera



If you would like to discuss your clinic's draw schedule and indications for use in more detail, please contact the Prospera clinical team at **transplantclinical@natera.com**.

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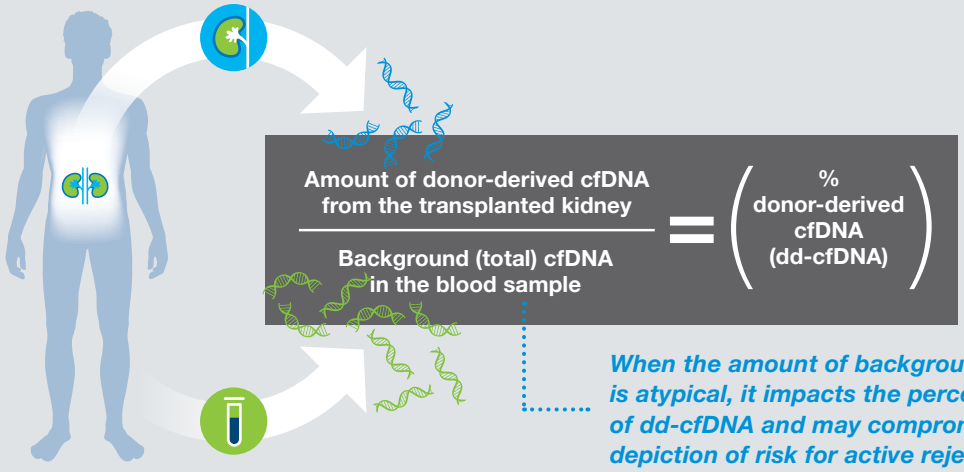

When the amount of background cfDNA is atypical, it impacts the percentage of dd-cfDNA and may compromise the depiction of risk for active rejection.

Factors that may influence background DNA may include:

- High body mass index (BMI)[1]
- Sepsis[2]
- Age[3]
- Surgery[4]
- Shipment and storage of sample[5]
- Chemotherapy[6]
- Normal variation[7]
- Myocardial infarction[8]
- Hemodialysis[9]
- Rejection

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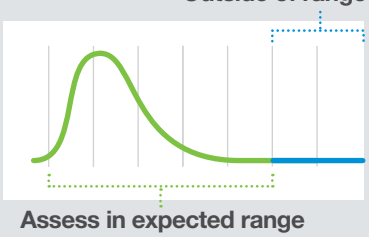

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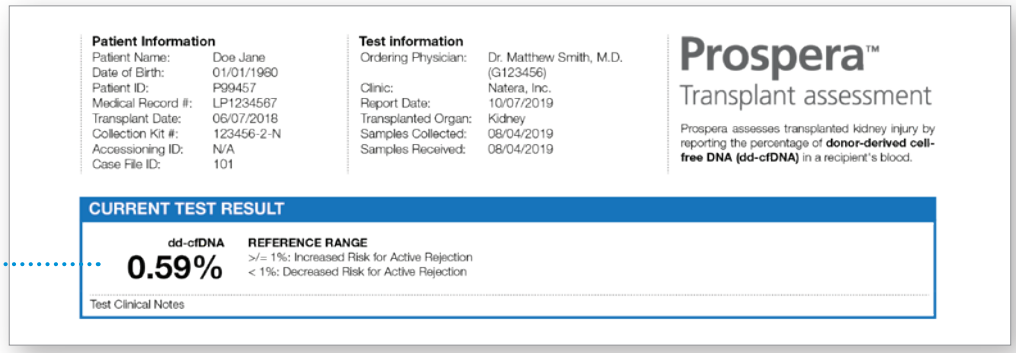

Patient Information

Patient Name:	Doe Jane
Date of Birth:	01/01/1980
Patient ID:	P99457
Medical Record #:	LP1234567
Transplant Date:	06/07/2018
Collection Kit #:	123456-2-N
Accessioning ID:	N/A
Case File ID:	101

Test information

Ordering Physician:	Dr. Matthew Smith, M.D. (G123456)
Clinic:	Natera, Inc.
Report Date:	10/07/2019
Transplanted Organ:	Kidney
Samples Collected:	08/04/2019
Samples Received:	08/04/2019

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Highlights from Prospera's clinical validation

Prospera™ is clinically validated to assess active rejection with great accuracy in the largest published donor-derived cell-free DNA (dd-cfDNA) validation study on renal transplant patients.

Published in *Journal of Clinical Medicine*, 2018

COHORT

Diverse patient population studied with a trusted partner

- Partnered with the University of California, San Francisco
- 217 biopsy-matched renal allograft samples
- Variety of ethnic and racial demographics
  - Hispanic/Latino (n=50)
  - Caucasian (n=74)
  - African American (n=31)
  - Asian (n=31)

DESIGN

Blinded, retrospective analysis using gold standard of kidney biopsy as truth

- Blinded pathology readings and analysis of blood samples
- Included same-day biopsy matched samples in primary analysis
- Reviewed by a single pathologist to ensure consistency and quality in the data
- Used prospectively selected, previously published cut-off of 1% dd-cfDNA to discriminate between active rejection and not active rejection

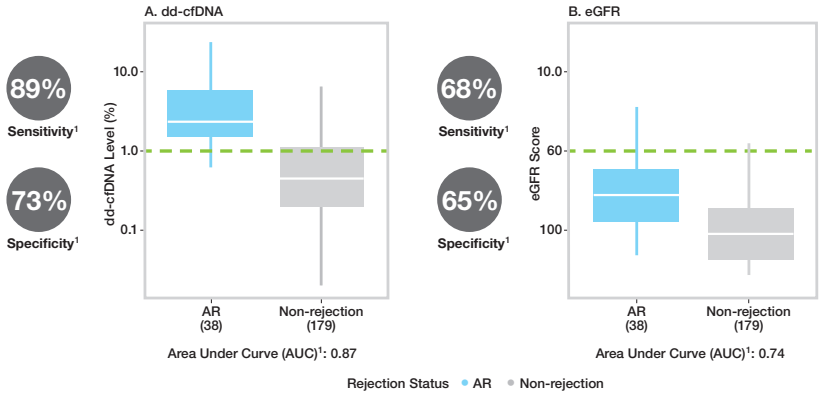
ANALYSIS

Classification based on recent guidelines

- Assessed active rejection in the following categories: antibody-mediated rejection (ABMR), T cell-mediated rejection (TCMR ≥ 1a) and mixed (ABMR/TCMR)
- Categorized biopsies with "borderline rejection" in the non-rejection group instead of active rejection, consistent with the latest BANFF 2017 criteria

Clinical Validation Results

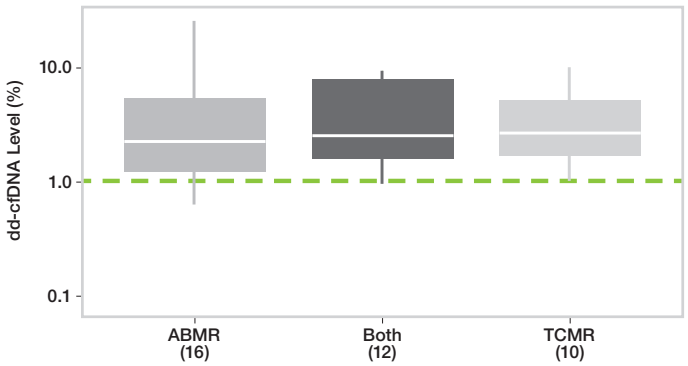
More sensitive and specific than standard screening tools[1]



Subanalysis:
Consistent performance regardless age

- Similar performance of sensitivity (89%), specificity (76%) and AUC (0.88) an **adults-only cohort** to the overall study population[4]
- Significantly higher predictive power (AUC of 0.84) compared to serum creatinine (AUC of 0.58) in assessing active rejection in an **adults-only, for-cause sub-cohort**. This serum creatinine performance of 0.58 AUC is in-line with other previously published serum creatinine performance metrics (0.542[2], 0.633[3])

Robust to all rejection types



- In contrast to other dd-cfDNA studies, Natera was able to differentiate T cell-mediated rejection from no rejection
- Median dd-cfDNA did not differ significantly between ABMR, TCMR and mixed rejection

Similar performance in stable and those with clinical signs of rejection

	Sensitivity	Negative predictive value*
Clinical AR	86%	93.8%
Subclinical AR	92%	98.3%**

* Assuming 25% AR Prevalence (higher risk population)
** Assuming 10% AR Prevalence in a low risk population

- Prospera is the first commercial cfDNA assay to publish performance in renal transplant surveillance situations, providing results that can enable physicians to manage patients with previously unsuspected rejection[1,2]

References:

1. Sigdel TK, Archila FA, Constantin T, et al. Optimizing detection of kidney transplant injury by assessment of donor-derived cell-free DNA via massively multiplex PCR. *Journal of Clinical Medicine*. 2019;8(1):19.
2. Bloom RD, et al. *J Am Soc Nephrol*. 2017 Jul;28(7):2221-2232.
3. Kaplan, et al. *Am Journal of Transplantation*. 2003; 3:1560-1565
4. Sigdel TK, Acosta Archila F, Navarro S, et al. Rapid Detection of Kidney Transplant Injury by Quantifying Donor-Derived Cell-Free DNA via Massively Multiplex PCR. Poster presented at: American Transplant Congress (ATC); 2019 June 01-05, Boston, MA.

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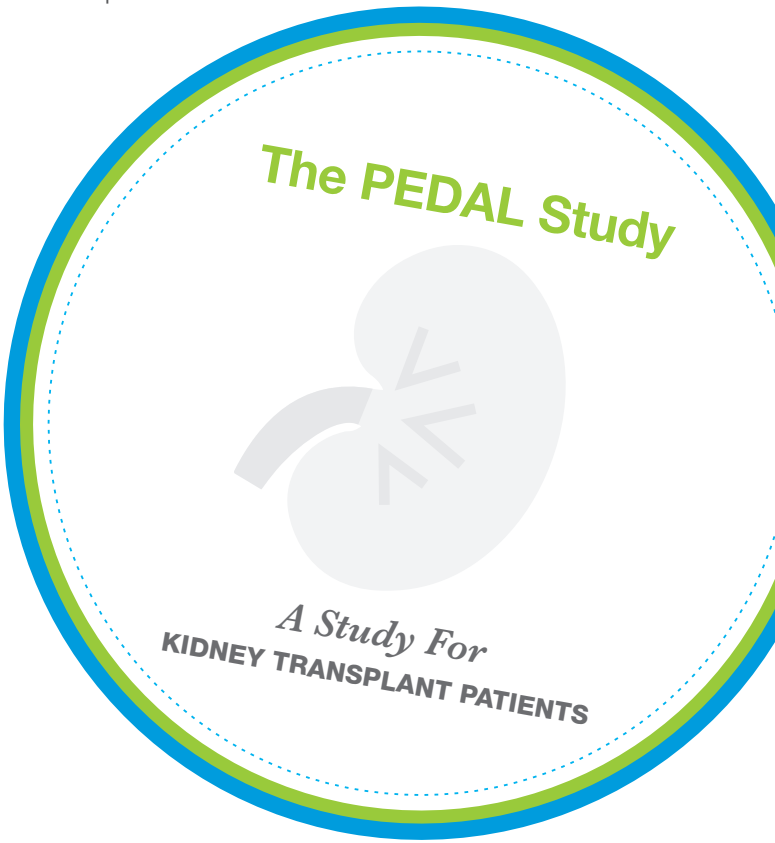
Explore a new technology for more-informed rejection assessment

Join us in understanding how the quantification of background cell-free DNA (cfDNA) may facilitate more precise rejection assessment and flag patients at high risk for false-negative interpretations.



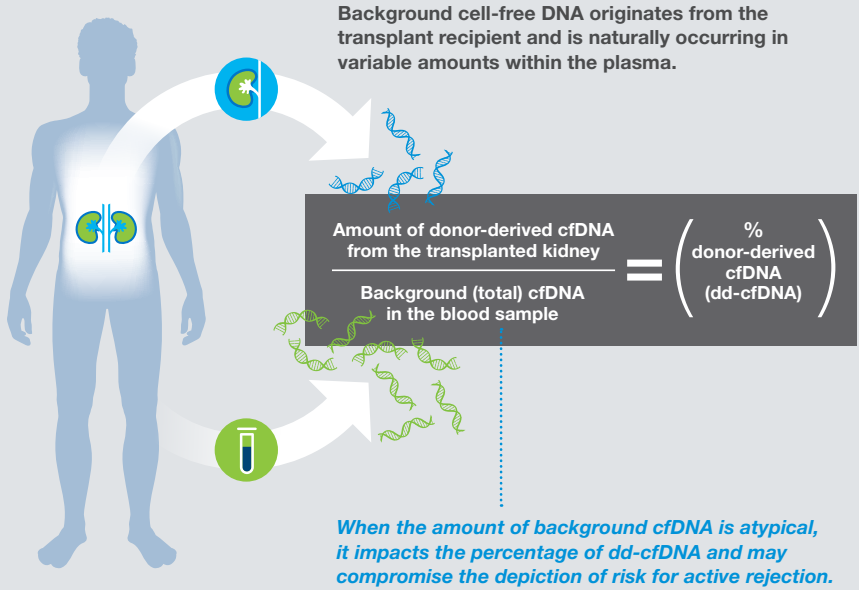
The PEDAL Study for Kidney Transplant Patients

Prospera **E**nhancement by **D**etecting Background Cell-free DNA **L**evels

PEDAL Study

Together with our study collaborators, we hope to gain a better understanding of how quantifying the absolute concentration of background cell-free DNA (cfDNA) may allow for a more precise and confident assessment of allograft rejection—especially in identifying patients at-risk of false-negative interpretations.

Defining background cell-free DNA and its influence on your result



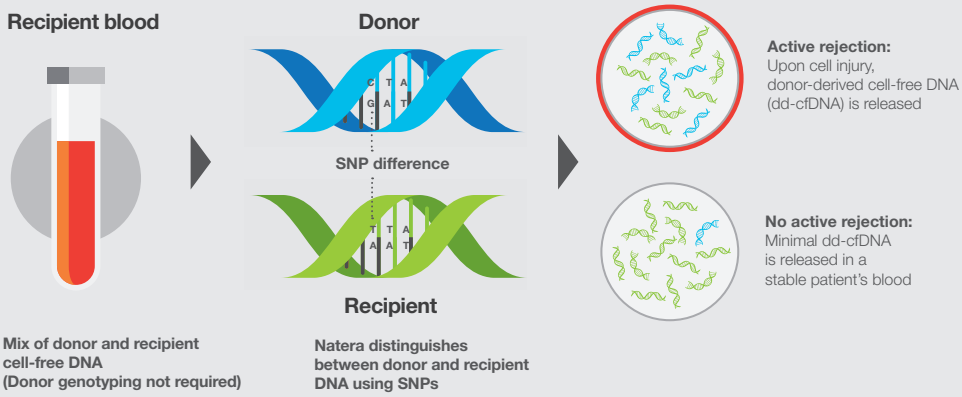
Let's understand how to more precisely assess for active rejection

Prospera™ and the PEDAL Study will delve deeper into how to better manage your transplant patients using cell-free DNA as a non-invasive biomarker for active rejection. The study will include 500 kidney transplant patients from 20 major U.S. centers to measure diagnostic capability of the update across three critical measures:

1. **Performance in both clinically indicated and surveillance biopsies**
2. **Ability to identify both antibody mediated rejection and T cell-mediated rejection**
3. **Correlation against clinical and/or histological resolution of rejection**

Prospera's core technology

Leveraging our findings from two million cfDNA tests, Prospera is designed to assess kidney transplant injury by evaluating the percentage of donor-derived cell-free DNA (dd-cfDNA) in a transplant recipient's blood. Too much dd-cfDNA in the recipient's blood is an early indication of potential organ rejection.



Based on our leadership in cfDNA innovation, Natera has now introduced two novel techniques for even greater precision in Prospera results:

Two novel techniques for greater precision

1.

Proprietary Library Preparation

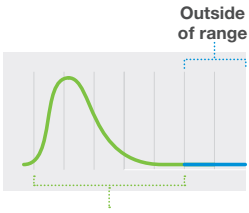
Proprietary library preparation step that adjusts for background cfDNA introduced into the sample during transport

+

2.

Quantifed Background CfDNA

Unique quantification method that can identify atypical background cfDNA that may influence the reported result for a particular patient



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Potentially fewer false negative interpretations

PEDAL STUDY ELIGIBILITY

Inclusion Criteria	Exclusion Criteria
Must be willing to provide informed consent	Cannot have other non-kidney transplanted organ(s)
Must be a kidney transplant recipient	Cannot be pregnant
	Cannot have genetically identical donor organs

We need your help to enable patients to thrive and prosper

With Prospera, we delivered a non-invasive way to identify rejection, giving you greater confidence in making treatment decisions for your organ transplantation patients. But we can do more—we're committed to continue refining this test to support you in bringing hope to these patients. Starting now.

Through PEDAL and other studies, we look to you as our partner in delivering innovations that offer a second chance to patients. Because together, we can make meaningful changes—in individual lives and the field of organ transplantation.

1 Organ Donation Statistics. U.S. Department of Health and Human Services. U.S. Government Information on Organ Donation and Transplantation. https://www.organdonor.gov/statistics-stories/statistics.html. Published March 31, 2016.
2 Kidney Disease Statistics for the United States. National Institute of Diabetes and Digestive and Kidney Diseases. https://www.niddk.nih.gov/health-information/health-statistics/kidney-disease. Published Dec. 1, 2016.
3 Stegall et al,Through a Glass Darkly: Seeking Clarity in Preventing Late Kidney Transplant Failure, *J Am Soc Nephrol*. 2015; 26 (1):20-9
4 Lamb et al, Long-term renal allograft survival in the United States: a critical reappraisal, *Am J of Transplantation*. 2011; Mar;11(3):450-62.5. Altug, et al.
5 Sigdel TK, et al. *J. Clin. Med*. 2019, 8, 19.

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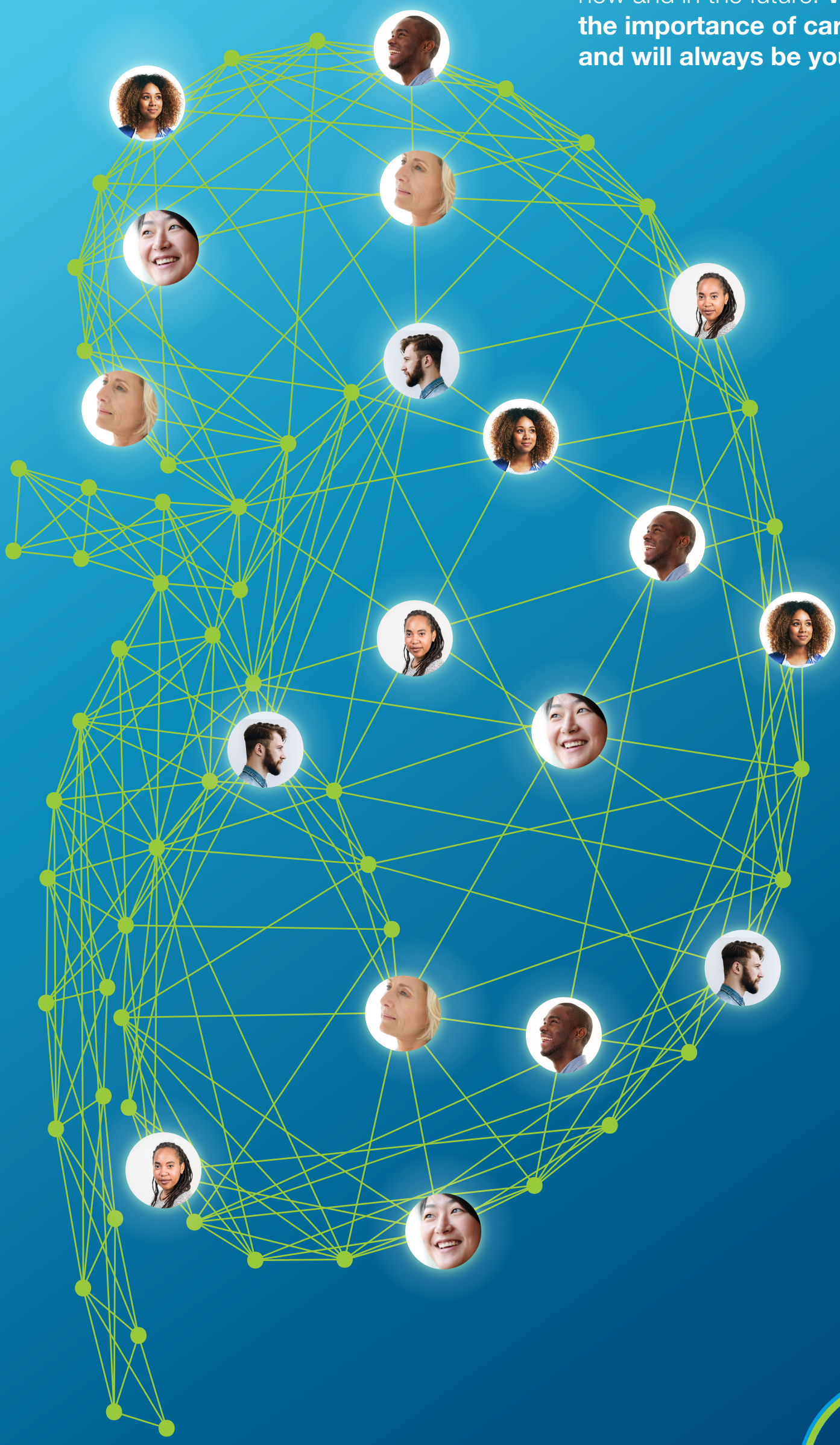
The PEDAL Study for Kidney Transplant Patients

Prospera **E**nhancement by **D**etecting Background cfDNA **L**evels

A kidney transplant is the greatest gift that can be given or received. Such a precious treasure must be cared for with the utmost diligence and attention.

A precise, non-invasive biomarker for rejection gives you the confidence you need to know that all is well. Natera will continue to refine and improve Prospera now and in the future. **We understand the importance of caring for patients and will always be your best partner.**



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Transplant assessment

The PEDAL Study
A Study For
KIDNEY TRANSPLANT PATIENTS

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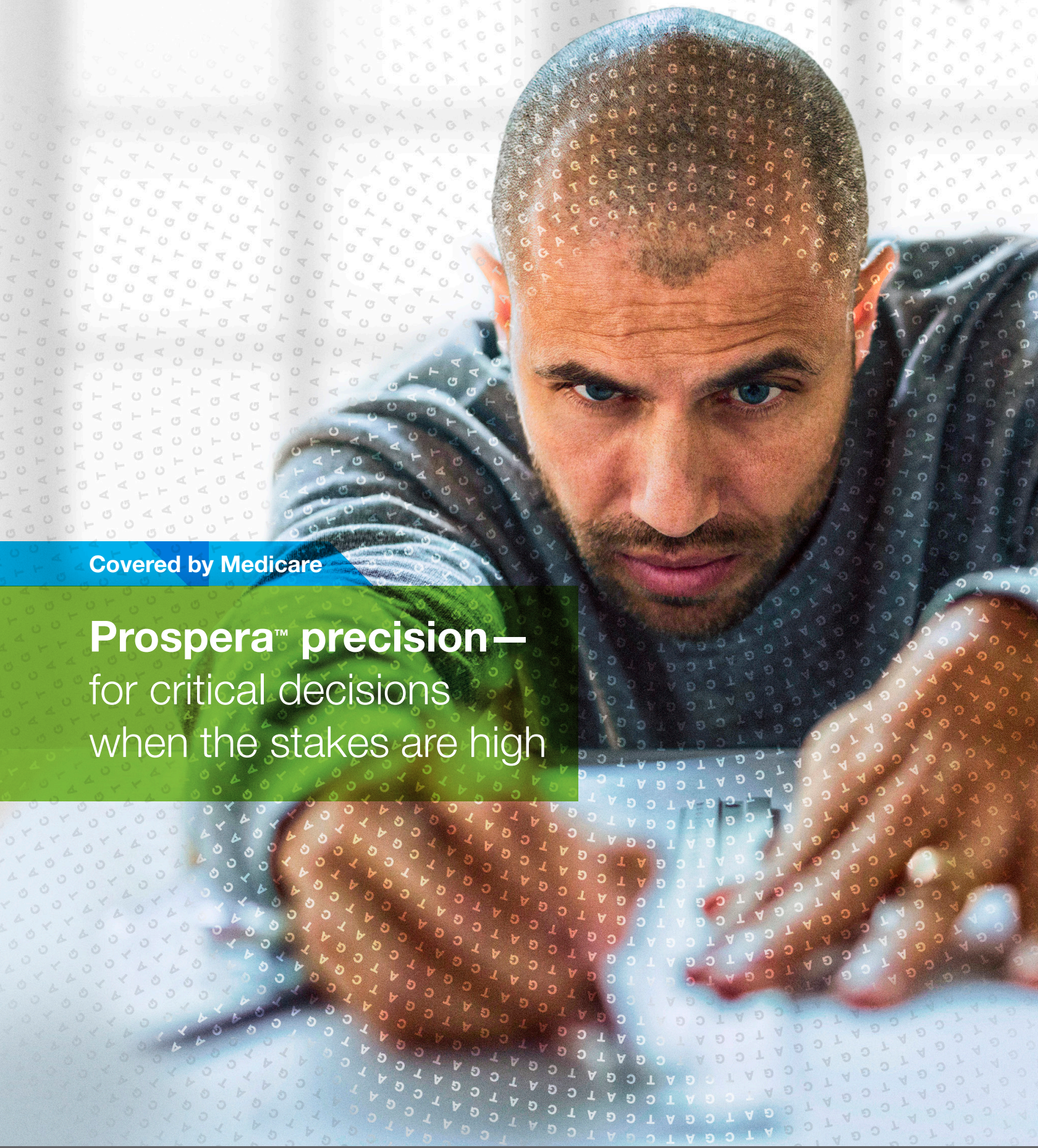
Transplant assessment

NEW
Available now



Covered by Medicare

Prospera™ precision—
for critical decisions
when the stakes are high

Introducing Prospera

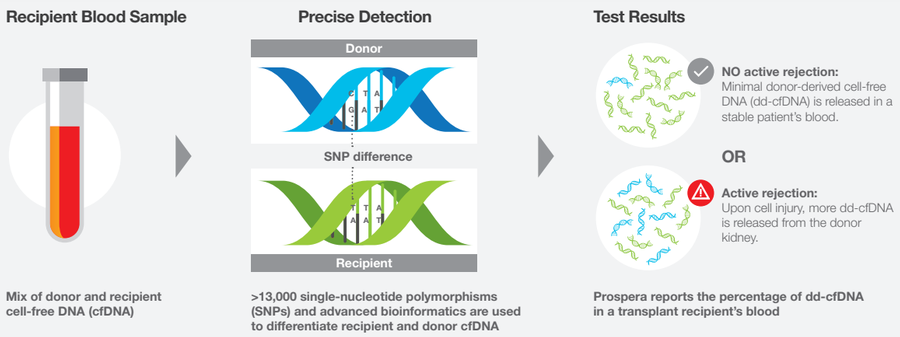
Prospera is powered by highly optimized, proprietary cell-free DNA (cfDNA) technology. As part of your tool kit, Prospera assesses all types of kidney transplant rejection[2] with great precision.[1,3]

- Simpler and less invasive than biopsy
- More sensitive and specific than current assessment tools across all types of rejection[2,4,5]
- Up to 5x less variability than first-generation donor-derived cell-free DNA technology[1,3]
- Covered by Medicare for all kidney transplant recipients

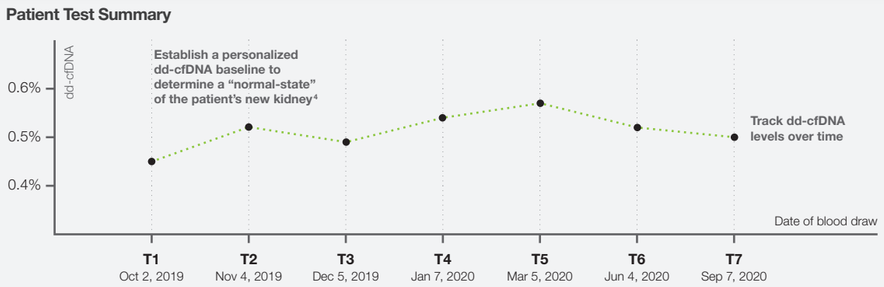
Powering clear and confident decisions

Developed by Natera with our trusted legacy in cfDNA, Prospera is optimized to be the most precise cfDNA tool for early, clinically meaningful rejection assessment.[1,3]

HOW PROSPERA WORKS



PERSONALIZED ASSESSMENT

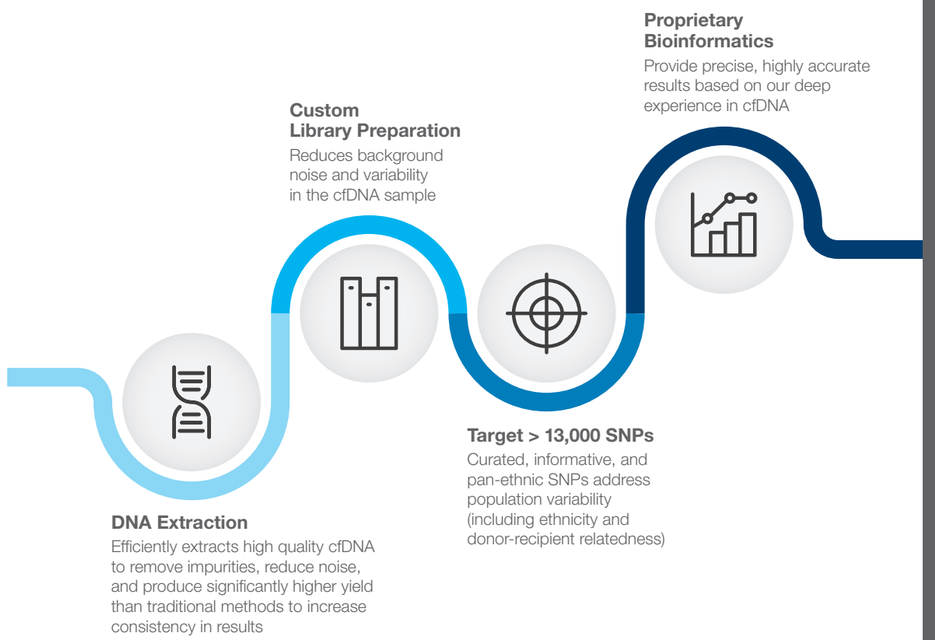


Experts in cell-free DNA. Optimized for transplantation.

To improve the management of organ transplantation, cancer, and reproductive health, Natera is harnessing the power of cfDNA from a single blood sample and a methodology that uses single-nucleotide polymorphisms (SNPs) for non-invasive testing.

Refined workflow. Only from Natera.

Natera's core technology and finely tuned workflows cut through the noise to deliver superior clinical and analytical performance.[1,2]



~2M
cfDNA tests performed

>100
clinicians, PhD's, and scientists

90
countries worldwide

CAP
accredited

CLIA
certified

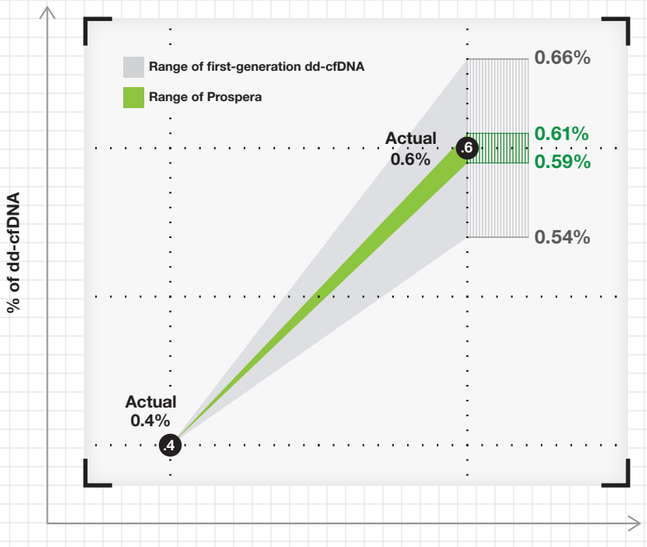
With all of the rapid advances taking place in cfDNA at Natera, our unwavering commitment to improving the health and care of your patients remains constant.

Unparalleled precision. Optimized by Prospera.

1 Highly optimized to significantly reduce variability

Based on analytical validation data, Prospera exhibited **up to 5x less variability** in results.[1,3]

Patient Test Summary Example*



*Depicted ranges are ±1 standard deviation from actual dd-cfDNA level based on coefficient of variations[1,3]

2 Now—catch ALL rejection types with a single blood draw

Prospera's unique ability to identify T cell-mediated rejection gives a more comprehensive view of your patient's rejection status.[2,5]

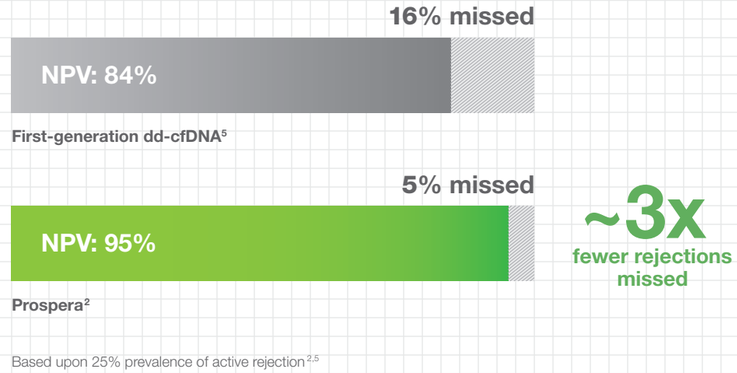
Rejection Types	Prospera[2]	First-generation dd-cfDNA[6]
Antibody-mediated rejection (ABMR)	✓ Yes	✓ Yes
T cell-mediated rejection (TCMR) ≥ IA	✓ Yes	✗ No

Prospera is the first cfDNA assay to publish performance in surveillance situations, providing results that can enable physicians to manage patients with previously unsuspected rejection.[2]

3 Lower risk of missing active rejection

In the event of a result with dd-cfDNA level <1%, Prospera's likelihood of a patient not experiencing active rejection† outperforms existing options.[2,5]

Comparison of Negative Predictive Values (NPV) from published validation studies

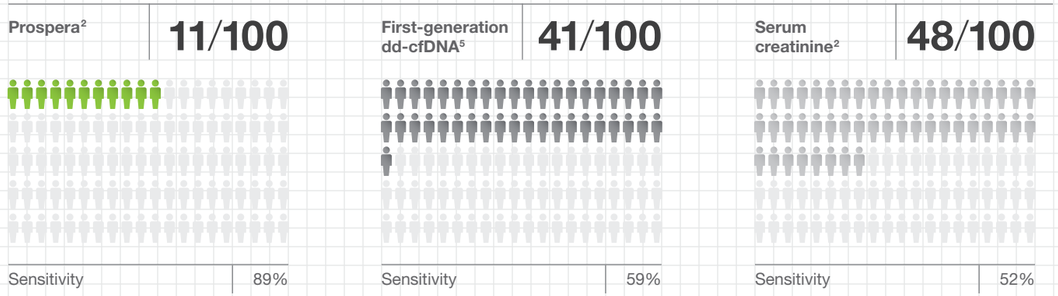


Based upon 25% prevalence of active rejection[2,5]

4 Ultra-sensitive for more accurate classification

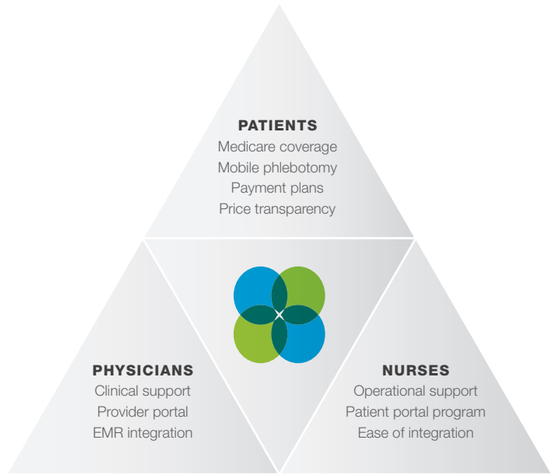
When comparing published clinical validation studies, Prospera demonstrated better performance in correctly classifying patients with active rejection—including cell-mediated rejection.[2,5] Other tests may incorrectly classify patients experiencing active rejection as normal (up to 1 out of 2 cases).[5]

Of 100 active rejection cases, the number of patients who would be missed, with dd-cfDNA <1%‡



† ‡Using a 1% dd-cfDNA threshold

Patients first. Partners always.



Pledging ongoing support and resources

Natera offers outstanding support for your patients

- Medicare coverage for Prospera to renal transplant patients
- Proactive billing outreach and price transparency
- Convenient complimentary phlebotomy services—either on-site, via mobile phlebotomy or at any of the >1,000 patient services blood draw centers

We also back you and other physicians with resources

- Direct support from clinical staff to discuss your patients' results
- Prospera Provider Portal plus EMR integration options so you can easily order, track and receive patients' reports

Our initiatives are tailored for your transplant nurses and coordinators

- ProsperaLink Program of dedicated Natera nurses and patient care coordinators
- Dedicated operations team to ensure ease of integration into your current patient care workflow

Natera welcomes all insurances. Prospera is covered by Medicare for assessing potential kidney transplant rejection. The goal of Natera's billing department is to make the process transparent and easy for our patients. In the rare event your patient has financial responsibility for Prospera, Natera offers flexible financial assistance programs and will work closely with your patient to ensure there is no hardship on them or their family.

In all cases, the Natera team is here to help you, your staff, and your patients with any billing or reimbursement questions and needs at **+1 650.273.4468**.

Prospera precision. Setting a new standard through research.

Best-in-class transplant care depends on best-in-class assessment. Prospera is the most advanced cfDNA solution for assessing transplant rejection—reinforced by ongoing research efforts:

Sigdel et al
Clinical Validation
Published 2019

- Conducted with the University of California, San Francisco
- Largest biopsy-matched study conducted in renal transplantation assessing the use of cfDNA
- First to publish performance of cfDNA testing in subclinical, surveillance setting

ProActive Registry Study
Now enrolling

- Largest clinical utility study evaluating cfDNA; includes more than 3,000 kidney transplant patients studied over three years
- Long-term assessment of high-risk recipients for up to five years post-transplantation
- Personalized transplant management protocols using cfDNA data

Research with MMDx
(Molecular Microscope Diagnostic System)
Now enrolling

- Global, prospective multicenter study under the leadership of Dr. Philip Halloran
- 300 patients to be comprehensively evaluated with clinical information, cfDNA measures, biopsies, molecular microscope, evaluations, and donor-specific antibodies (DSA)
- Integrated data analysis to better inform non-invasive and interventional management in kidney transplantation

REFERENCES

1 Altug Y, Liang N, Ram R, et al. Analytical validation of a single-nucleotide polymorphism-based donor-derived cell-free DNA assay for detecting rejection in kidney transplant patients [published online February 19, 2019]. *Transplantation*. 2019. doi: 10.1097/TP.0000000000002665
2 Sigdel TK, Archila FA, Constantin T, et al. Optimizing detection of kidney transplant injury by assessment of donor-derived cell-free DNA via massively multiplex PCR. *J Clin Med*. 2018;8(1):pii E19.
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4 Bromberg JS, Brennan DC, Poggio E, et al. Biological variation of donor-derived cell-free DNA in renal transplant recipients: clinical implications. *J Appl Lab Med*. 2017;2(3):309-321.
5 Bloom RD, Bromberg JS, Poggio ED, et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol*. 2017;28(7):2221-2232. doi: 10.1681/ASN.2016091034.
6 Huang E, Sethi S, Peng A, et al. Early clinical experience using donor-derived cell-free DNA to detect rejection in kidney transplant recipients. *Am J Transplant*. 2019; 19:1663-1670.

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CASE STUDIES

How quantifying background cell-free DNA enables even greater precision with Prospera for rejection assessment

As the experts in cell-free DNA (cfDNA) testing, Natera has refined our workflow based on findings from two million cfDNA tests to now include **a first-in-class technique capable of quantifying background cfDNA** in a streamlined manner.

When assessing rejection via the Prospera™ transplant assessment test, this pioneering enhancement provides additional information, which may enable an even more precise and confident assessment—particularly in flagging patients at-risk of false-negative interpretations.

PROSPERA CASE STUDIES*

Even more precise, holistic assessment with background cfDNA

PATIENT 1

Meet Kirk

- Male
- Late 70s
- End-stage renal disease (ESRD)
- Transplant in mid-2018

THE JOURNEY

Transplant surgery	Post-transplant		
	6 months	7 months	14 months
Mid 2018	Elevated creatinine levels, indicating acute T cell-mediated rejection (TCMR)	Tested positive for BK viremia, which was immediately treated and resolved	Admitted for herpetic and cytomegalovirus (CMV) esophagitis and was treated with intravenous ganciclovir

CLINICAL ASSESSMENT WITH PROSPERA

The Prospera result revealed a low **donor-derived cell-free DNA (dd-cfDNA) fraction at 0.38%**, indicating a decreased risk for active rejection.

Further Prospera analysis quantified background cfDNA, **revealing a level 21x the median**—and thereby flagging an increased risk of a false-negative interpretation.

Based on Prospera's enhanced reporting, percutaneous kidney transplant biopsy was performed; the result **confirmed chronic cellular rejection** (via Banff criteria).

THE TAKEAWAY

Viral infections can cause an atypical increase in recipient background cfDNA. This inflation may lead to an artificially deflated percentage of dd-cfDNA.

Prospera's novel ability to quantify background cfDNA highlighted an increased risk for a false-negative interpretation.

PATIENT 2

Meet Janice

- Female
- Early 60s
- End-stage renal disease (ESRD)
- Transplant in early 2017

THE JOURNEY

Transplant surgery	Post-transplant
	3 years
Early 2017 from a deceased donor	Assessed with Prospera during a routine visit

CLINICAL ASSESSMENT WITH PROSPERA

Prospera result showed a **dd-cfDNA of 0.28%**, potentially a decreased risk for active rejection.

The report also flagged atypical background cfDNA levels that were elevated at **~7x the median.**

The resulting percutaneous kidney transplant biopsy **revealed BK virus-associated nephropathy and T cell-mediated rejection.**

THE TAKEAWAY

BK virus-associated active injury may contribute to atypical background cfDNA levels.

Prospera's latest enhancement allows for physicians to more effectively identify active rejection that would have otherwise been missed.

PATIENT 3

Meet Scotty

- Male
- Late 70s
- End-stage renal disease (ESRD)
- Transplant in mid-2018

THE JOURNEY

Transplant surgery	Post-transplant	
	1 month	6 months
Late 2019 from an unrelated, living donor	Diagnosed with dengue fever, followed by acute allograft dysfunction	A biopsy was performed revealing active antibody-mediated rejection. He was then treated with plasmapheresis and intravenous immunoglobulin with clinical resolution

CLINICAL ASSESSMENT WITH PROSPERA

At 7 months post-transplant, he received a Prospera result of **0.16% dd-cfDNA level**, indicative of a decreased risk for active rejection.

The Prospera result also revealed a heightened level of background cfDNA at **~13X the median**.

A biopsy thereafter **showed resolution of ABMR and borderline acute cellular rejection.**

THE TAKEAWAY

For the first time, further evaluation of background cfDNA levels enabled the physician to identify signs of borderline acute cellular rejection.

This additional information by Prospera can provide a more complete clinical assessment of your transplant patient.

PATIENT 4

Meet Sharon

- Female
- Late 50s
- End-stage renal disease (ESRD)
- secondary to PKD

THE JOURNEY

Transplant surgery	Post-transplant		
	11 months	11 months, 1 week	11 months, 1.5 weeks
Late 2018 from a deceased donor	Presented with four days of worsening diffuse muscle pain	Progressed to a temperature of 101°F (asymptomatic previously). Visited her local emergency room	Tested positive for COVID-19 and intubated at her transplant center. Renal function deteriorated, immunosuppression was closely managed

CLINICAL ASSESSMENT WITH PROSPERA

Prospera was used to assess rejection status on the 20th day of her hospital stay.

The Prospera result showed **0.07% dd-cfDNA** with a heightened level of background cfDNA at **~57X the median.**

A second Prospera test was drawn on the 25th day of her hospital stay with a result of **0.25% dd-cfDNA** and a decreased level of background cfDNA at **~15x the median.**

THE TAKEAWAY

COVID-19 may cause very elevated background cfDNA. Therefore, patients are at-risk for a false negative interpretation, especially when immunosuppression is reduced in response to the infection.

By reporting high background level, Natera proactively alerts the physician if the result may yield a false negative in a high-risk patient.

* Deidentified patient names and details

About Prospera, Transplant Assessment Test

Powered by highly optimized, proprietary cfDNA technology, Prospera enables you to:

Catch all rejection types in a single blood draw: Prospera's unique ability to identify T cell-mediated rejection (TCMR) gives a more holistic view of your patient's rejection status.[1]

Minimize risk of missing active rejection: Prospera is three times less likely to miss an active rejection** than the first-generation donor-derived cell-free DNA test (Negative Predictive Value of 95% vs 84%).[1, 2]

More accurately classify active rejection: Prospera demonstrated better performance than the first-generation dd-cfDNA test (sensitivity of 89% vs 59%) to identify patients with active rejection.[1, 2]

References

** 25% prevalence of active rejection

1 Sigdel TK, Archila FA, Constantin T, et al. Optimizing detection of kidney transplant injury by assessment of donor-derived cell-free DNA via massively multiplex PCR. J Clin Med. 2019;8(1):19.

2 Bloom RD, Bromberg JS, Poggio ED, et al. Cell-free DNA and active rejection in kidney allografts. J Am Soc Nephrol. 2017;28(7):2221-2232. doi: 10.1681/ASN.2016091034.

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Prospera™
Now quantify the risk
A cfDNA background check everytime

May 2020

How quantifying background cell-free DNA (cfDNA) is raising the bar for precision in rejection assessment

Introduction

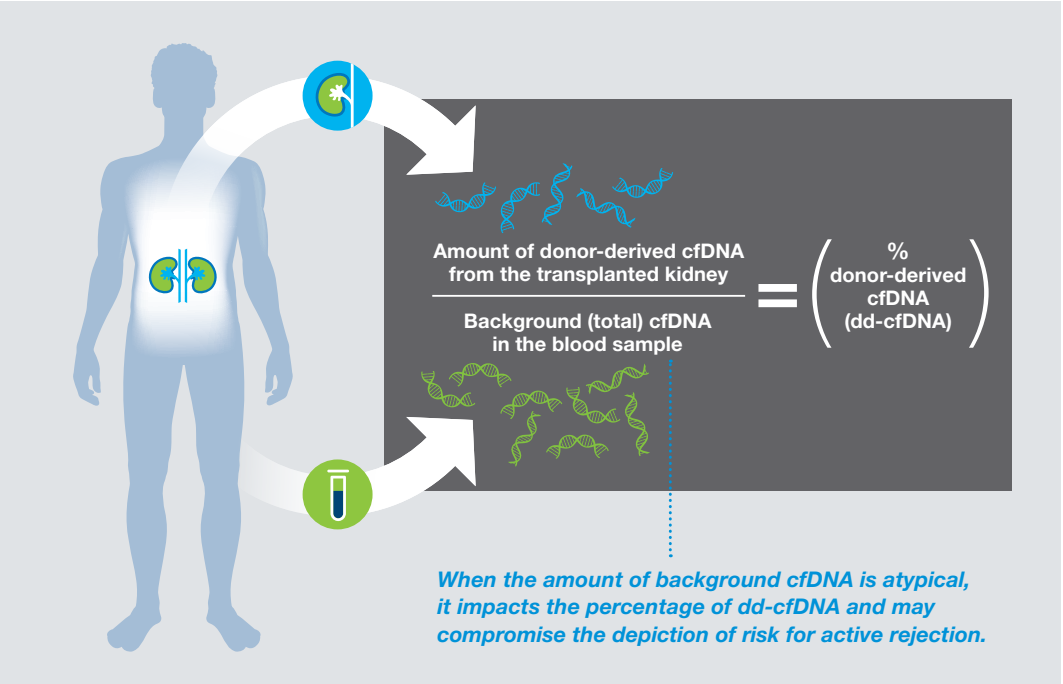
Non-invasive monitoring using cell-free DNA (cfDNA) technology is an established method for distinguishing between patient DNA and non-patient DNA that is also in the bloodstream, such as cfDNA from a fetus (prenatal), tumor (oncology), or donor (transplantation).[1–16] Donor-derived cfDNA (dd-cfDNA) is a proven biomarker in kidney and heart transplantation for identifying active rejection.[1–6,13–16] Existing commercial assays report dd-cfDNA results as a percentage of total cfDNA. However, results reported in this manner may not provide a full picture of rejection risk due to background cfDNA levels that can be affected by many factors. Atypically high levels of recipient cfDNA may lead to a decreased dd-cfDNA proportion, and a potential false negative interpretation; less frequently, atypically low cfDNA levels can lead to false positive results.

Natera's Prospera™ transplant assessment test is powered by highly optimized, proprietary cfDNA technology. It has now been enhanced with an exclusive technique, making Prospera the first test of its kind to quantify absolute background cfDNA.

This enhancement benefits physicians by identifying patients with atypical background cfDNA levels – thereby flagging them as at-risk for false-negative reporting and potentially missed rejections.

More? Less? Why the amount of background cfDNA matters

Background cfDNA originates from the transplant recipient and is naturally occurring in variable amounts within the plasma.



About the Prospera transplant assessment test

Prospera assesses all types of kidney transplant rejection[1] with great precision.[2,3] From a single blood draw, Prospera measures the amount of donor cfDNA from the transplanted kidney in the patient's blood. Using more than 13,000 single-nucleotide polymorphisms (SNPs) and advanced bioinformatics, the assay can differentiate donor and recipient cfDNA to provide a result as a percentage of dd-cfDNA in a transplant recipient's blood. A greater percentage of dd-cfDNA in a recipient's blood may signify that the transplanted organ is shedding more DNA than ideal, thereby indicating an increased risk of rejection.

Prospera's performance was evaluated in a blinded, large scale study of 217 biopsy-matched renal allograft plasma samples using a prospectively determined cut-off of 1% dd-cfDNA or greater.[1] Sigdel et al[1] demonstrated Prospera's superior accuracy in identifying active rejection over current standard-of-care biomarkers (estimated glomerular filtration rate and serum creatinine). Comparative statistics for Prospera include sensitivity of 89% vs 52%; specificity of 73% vs 68% and area under the curve (AUC) of 0.87 vs 0.68, respectively.[1] Test performance in the validation study was independent of donor type (related/unrelated, living/deceased), rejection type (antibody mediated rejection/T cell-mediated rejection/combination) and clinical presentation (clinical/subclinical).[1]

The first to optimize on precision and accuracy

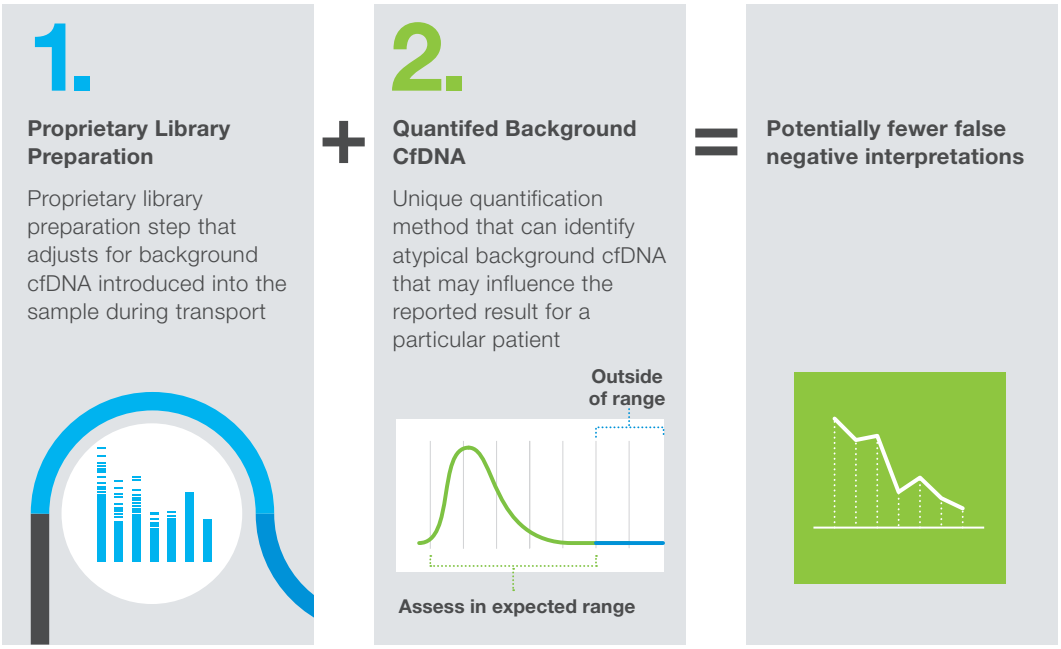
Natera has processed more than two million cfDNA tests and is the first laboratory to introduce two novel techniques that together deliver increased precision and further expand confidence in Prospera results.

Technique 1: Proprietary library preparation. This technique results in higher yield, higher quality DNA than standard cfDNA tests. It accounts for additional cfDNA that may be released into the sample during collection and transport.

Technique 2: Quantification of background cfDNA. This technique identifies atypical levels of background cfDNA that may influence the reported result for a particular patient.

Applying both techniques may yield potentially fewer false negative interpretations.

Figure 1: Two new techniques for enhanced Prospera results

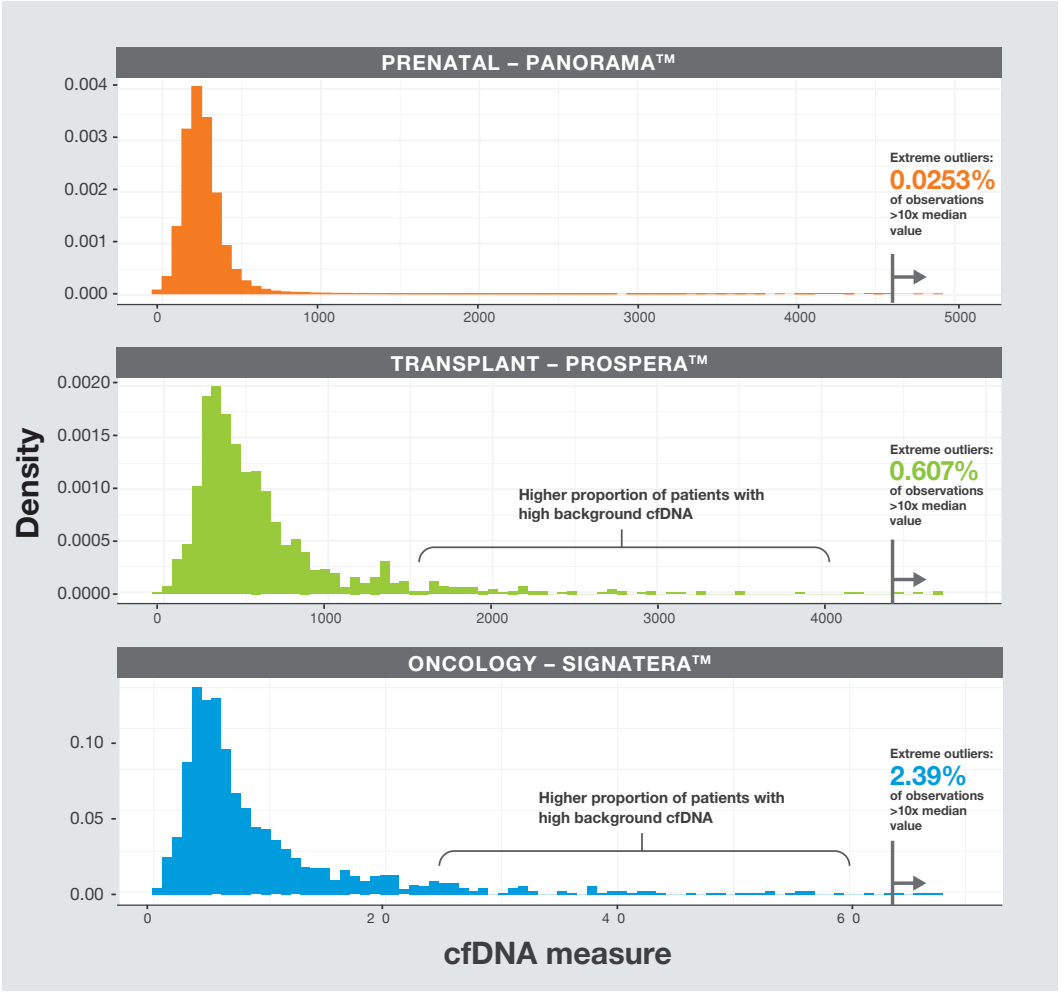


Enabling the quantification of background cfDNA

Natera scientists reviewed more than 20,000 cfDNA cases in prenatal, ~ 1,000 cfDNA cases in oncology, and ~1,000 cfDNA cases in transplantation to identify an "expected range" of background DNA in a patient.

The graphs shown in Figure 2 compare results from three sets of tests.

Figure 2: Defining "expected range" of background cfDNA

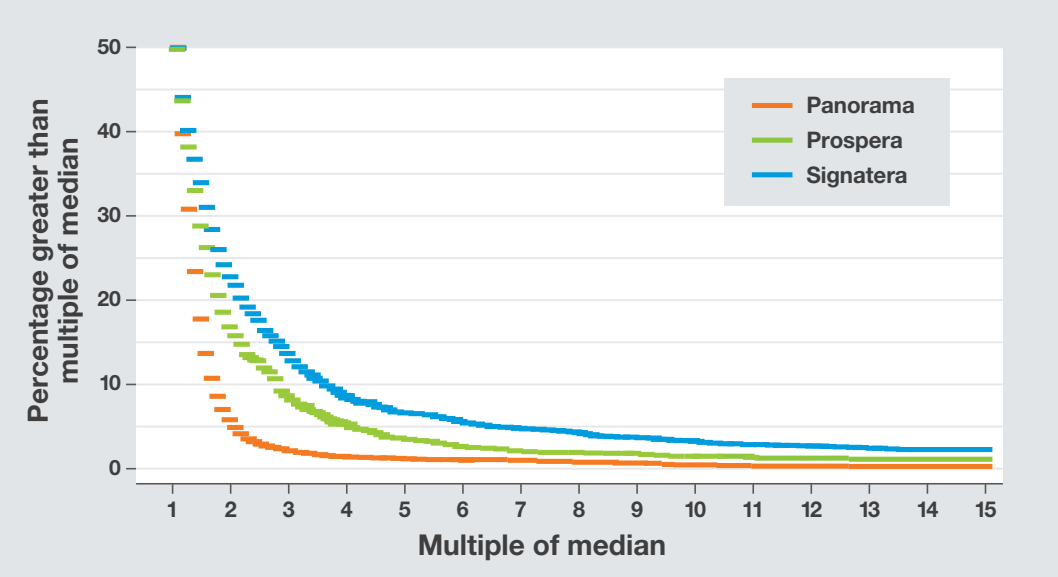


Panorama™ results, a non-invasive prenatal screening test to identify chromosomal variants, represent an overall healthy population of pregnant women. This data was compared to results from cancer patients using the Signatera™ minimal residual disease test and an indirect measurement of total background cfDNA results from Prospera on transplant patients.

Further analysis of the three data sets showed that the transplant and oncology patients were more likely to have very high cfDNA measurements relative to the median. For example, the fraction of transplant patients with cfDNA measurement more than 8 times the median was about 33 times greater compared to the fraction of Panorama patients. Figure 3 shows the percentage of patients above a certain multiple of the median, for the three patient groups. The actual medians are approximately: 487.98 per mL for Prospera ratio, 266.43 per mL for Panorama ratio, and 6.825 ng/mL for Signatera total cfDNA.

Figure 3:
Direct and indirect measurements of background cfDNA

How heavy are the tails for various tail lengths

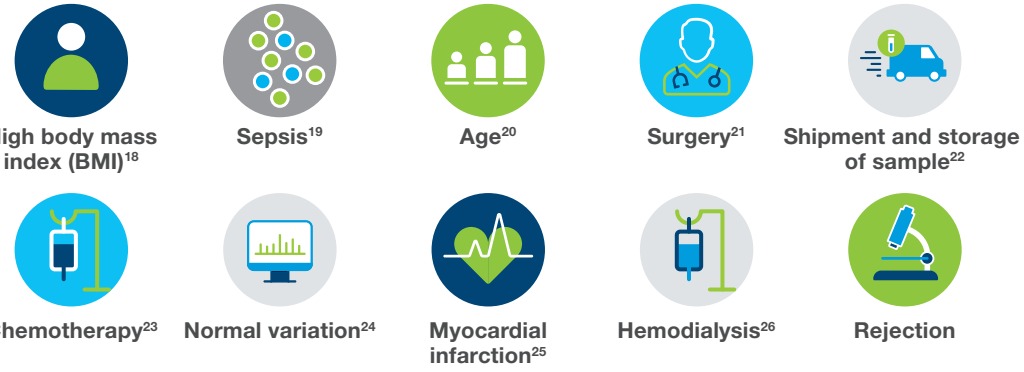


This data suggests that patients with certain medical problems such as cancer and kidney disease may have unusually high background cfDNA levels. Conversely, a healthy population of pregnant women was used to define "normal ranges." **This finding painted a picture we could not ignore: A fraction-based rejection assay may not be sufficiently precise for all transplant patients.**

Potential factors influencing background DNA

A recent literature review highlighted several factors that may cause atypical levels of background cfDNA in a patient's sample. In a review of 80 patients with severe sepsis, total cfDNA levels had a better prognostic utility than MODS or APACHE II scores, with an AUC for ICU mortality of 0.97.[27] This suggests that infection can elevate cfDNA levels. Further, a study in mice showed that fat cells can degenerate and elevate cfDNA levels, which in turn can directly cause inflammation.[28] Also, cfDNa levels seem to increase after dialysis and may predict mortality in these patients.[28,29]

In this regard, preliminary data suggests that any inflammatory process in the body including infection can cause a surge in total cfDNA.



Case studies support findings

Natera's review of internal data revealed similar drivers contributing to the variability of cfDNA levels, including viral infection.

Case Study 1:

Meet Kirk*

Kirk

The patient: A male in his late 70s with end-stage renal disease (ESRD)

The journey:

- In mid-2018, the patient underwent a kidney transplant.
- At six months post-transplant surgery, his creatinine levels were elevated, indicating acute T cell-mediated rejection (TCMR).
- At seven months post-transplant surgery, he tested positive for BK viremia, which was immediately treated and resolved.
- At 14 months post transplant surgery, he was admitted for herpetic and cytomegalovirus (CMV) esophagitis and was treated with intravenous ganciclovir.

Clinical assessment with Prospera:

- The Prospera result revealed a low donor-derived cell-free DNA (dd-cfDNA) fraction at 0.38%, indicating a decreased risk for active rejection.
- Further Prospera analysis quantified background cfDNA, revealing a level 21x the median – and thereby flagging an increased risk of a false-negative interpretation.
- Based on Prospera's enhanced reporting, percutaneous kidney transplant biopsy was performed; the result confirmed chronic cellular rejection (via Banff criteria).

The takeaway:

Viral infections can cause an atypical increase in recipient background cfDNA. This inflation may lead to an artificially deflated percentage of donor-derived cfDNA.

Prospera's novel ability to quantify background cfDNA highlighted an increased risk for a false-negative interpretation. This prompted a crucial biopsy confirming active rejection that may have otherwise been missed.

Case Study 2:

Meet Janice*

Janice

The patient: A female in her early 60s with end-stage renal disease

The journey:

- In early 2017, she received a kidney transplant from a deceased donor.
- Three years post-transplant surgery, she was assessed with Prospera during a routine visit.

Clinical assessment with Prospera:

- Prospera result showed a donor fraction of 0.28%, potentially a decreased risk for active rejection.
- The report also flagged atypical background cfDNA levels that were elevated at ~ 7x the median.
- The resulting percutaneous kidney transplant biopsy revealed BK virus-associated nephropathy and T cell-mediated rejection.

The takeaway:

BK virus-associated active injury may contribute to atypical background cfDNA levels.

Prospera's latest enhancement allows for physicians to more effectively identify active rejection that would have otherwise been missed.

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Case Study 3: Meet Scotty*

Scotty

The patient: A male in his early 50s with end-stage renal disease

The journey:

- In late 2019, he obtained a kidney transplant from an unrelated, living donor.
- One month post-transplant, he was diagnosed with dengue fever, followed by acute allograft dysfunction.
- At 6 months post-transplant, a biopsy was performed revealing active antibody-mediated rejection. He was then treated with plasmapheresis and intravenous immunoglobulin with clinical resolution.

Clinical assessment with Prospera:

- At 7 months post-transplant, he received a Prospera result of 0.16% dd-cfDNA level, indicative of a decreased risk for active rejection.
- The Prospera result also revealed a heightened level of background cfDNA at ~13X the median.
- A biopsy thereafter showed resolution of ABMR and borderline acute cellular rejection.

The takeaway:

For the first time, further evaluation of background cfDNA levels enabled the physician to identify signs of borderline acute cellular rejection.

This additional information by Prospera can provide a more complete clinical assessment of your transplant patient.

Case Study 4: Meet Leia*

Leia

The patient: A female in her late 50s with end-stage renal disease secondary to polycystic kidney disease (PKD)

The journey:

- In late 2018, she received a kidney transplant from a deceased donor.
- At 11 months post-transplant, she presented with four days of worsening diffuse muscle pain.
- With normal labs in the prior week, her symptoms progressed with a temperature of 101 F so she visited her local physician and was sent to the local emergency room.
- After being tested as positive by COVID-19 nasopharyngeal swab, she was soon transferred to her transplant center where her respiratory status worsened and she was intubated.
- Progressing to septic shock requiring vasopressor therapy, her renal function deteriorated and immunosuppression dosages were closely managed.

Clinical assessment with Prospera:

- Prospera was used to assess rejection status on the 20th day of her hospital stay
- The Prospera result showed 0.07% dd-cfDNA with a heightened level of background cfDNA at ~57x the median.
- A second Prospera test was drawn on the 25th day of her hospital stay with a result of 0.25% dd-cfDNA and a decreased level of background cfDNA at ~ 15x the median.

The takeaway:

COVID-19 may cause very elevated background cfDNA. Therefore, these patients are at-risk for a false negative interpretation, especially when immunosuppression is reduced in response to the infection.

By reporting high background levels, Natera proactively alerts the physician if the result may yield a false negative in a high-risk patient.

*** Deidentified patient names and details**

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Conclusion

Given the variability of cfDNA in several pathogenic states and its role as an inflammatory mediator, a dd-cfDNA-based assay that expresses results as a percentage of the total "background" cfDNA must necessarily be inaccurate in some patients. This is a metric that is variable and may be influenced by clinical or treatment-related factors. Based on published studies and data derived from the large number of tests performed by Natera, transplant patients appear to have more variability in background cfDNA levels than a non-transplant population. Variability in this metric may be influenced by clinical or treatment-related factors.

More specifically, recent data from Natera suggests a correlation between background cfDNA levels and multiple factors, including patient weight, medications, recent surgery and medical complications. For example, patients with viral infections may have atypically high background cfDNA levels.

If using a dd-cfDNA-based assay that expresses results as a percentage of total background cfDNA, it is important to flag patients with atypical levels of background cfDNA, as this will affect the final dd-cfDNA result. Most commonly, an unusually high level can result in an artificially low dd-cfDNA result, increasing the risk for false-negative interpretations and missed opportunities to preemptively spot rejections.

As such, responsible laboratories leveraging dd-cfDNA technology should consider both the proportion of dd-cfDNA and the background cfDNA levels when reporting results.

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