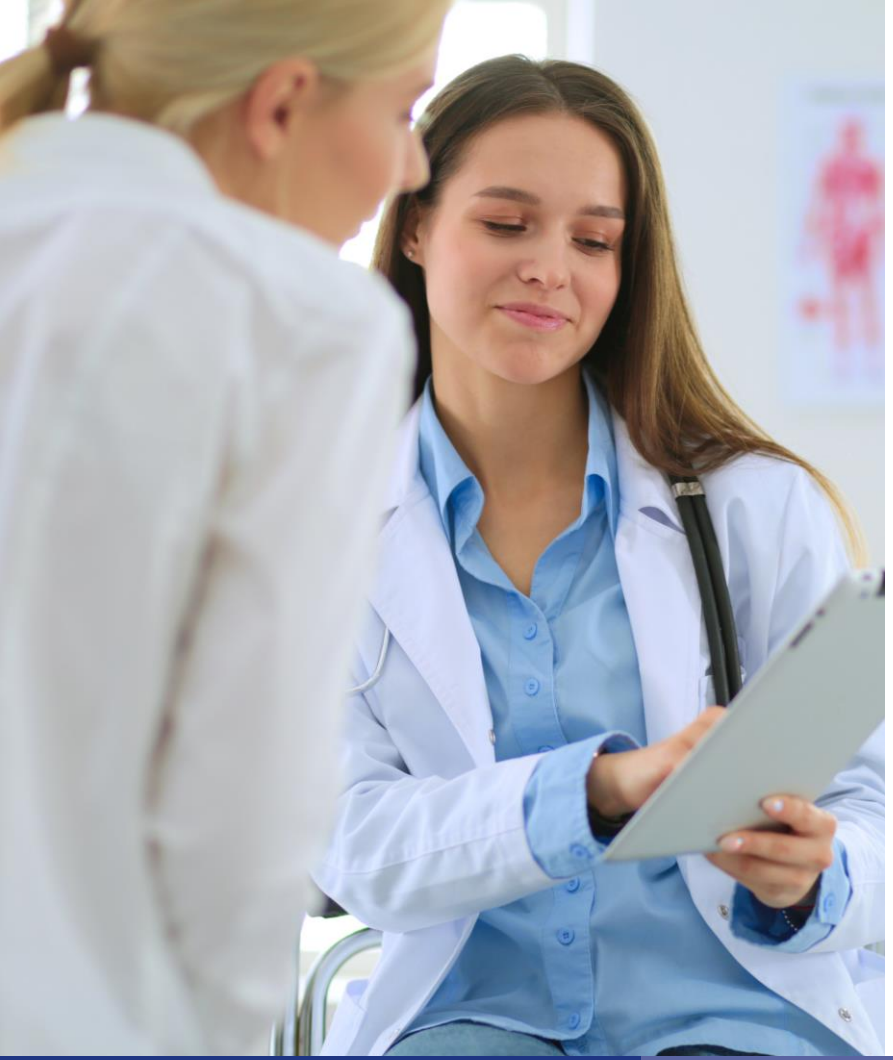

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Integrating Molecular Biomarkers into the Management of Kidney Transplant Recipients

TruGraf, TRAC, OmniGraf

Application to real clinical cases

Spectrum of Immune Activation and Rejection Following Kidney Transplantation

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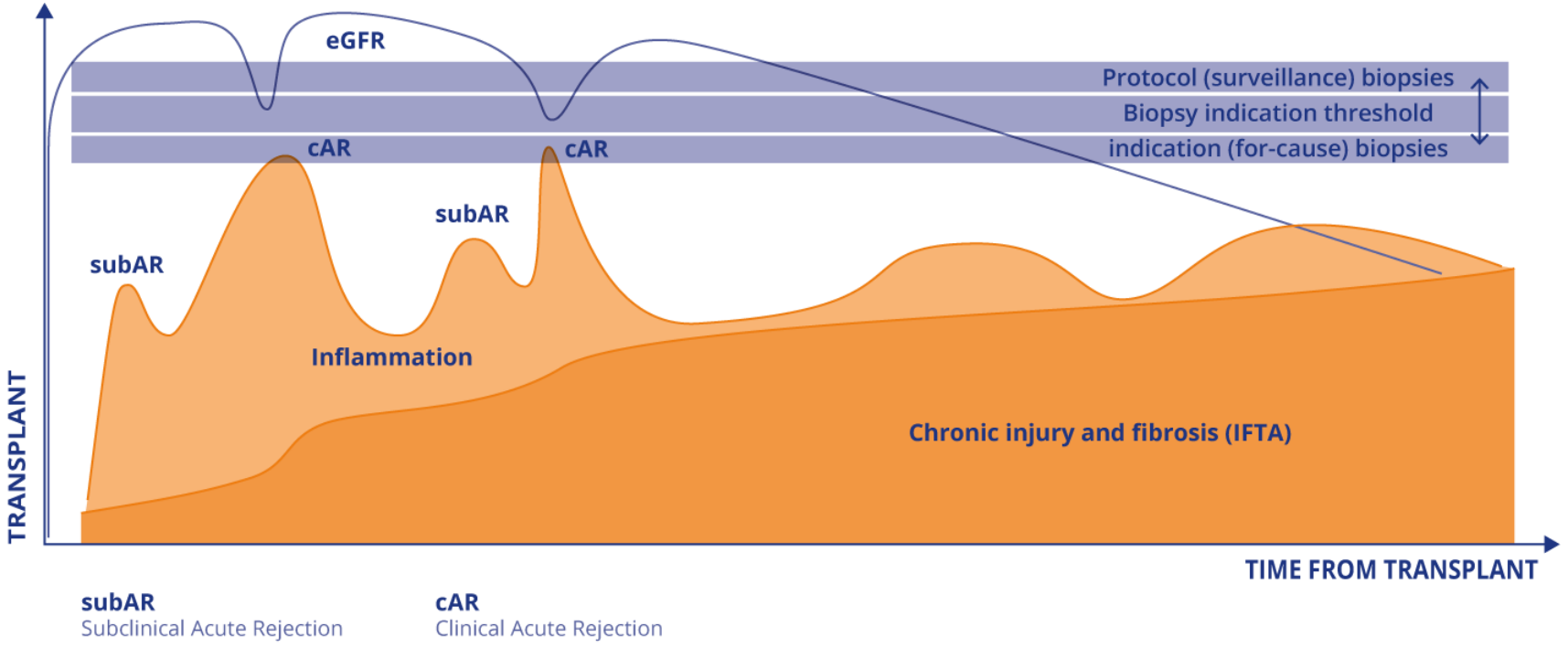

subAR
Subclinical Acute Rejection

cAR
Clinical Acute Rejection

25% of Stable Patients Will Experience Silent Subclinical Acute Rejection in the First Years Post-Transplant (CTOT '08)

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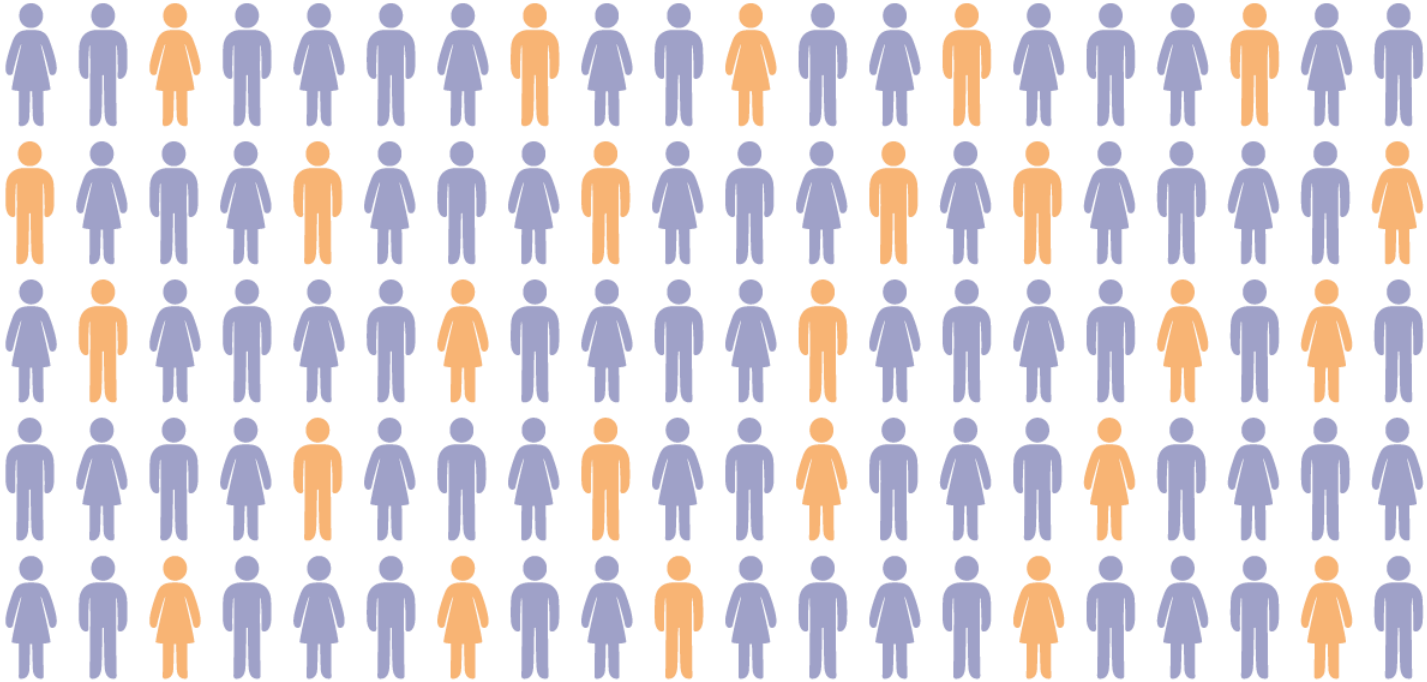

Friedewald JJ, Kurian SM, Heilman RL, et al. . Am J Transplant. 2019;19:98–109.

5 Consequences of Undetected and Untreated Sub Clinical Rejection

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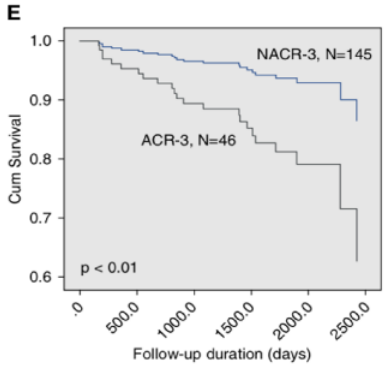
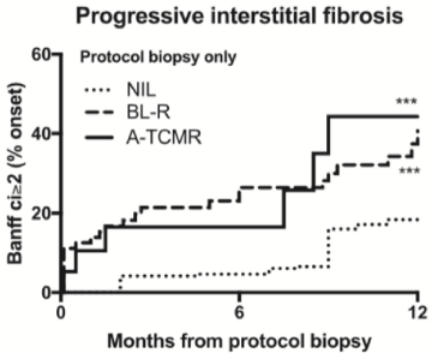
- More likely to form de novo DSA
- Higher risk of going on to develop clinical Acute Rejection
- More likely to develop graft fibrosis (IFTA 2+)
- More rapid loss of GFR
- More likely to lose the graft at 5 years



Graft Survival in GOCAR



Differentiated Genomic Testing Technologies for Different Clinical Applications

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TRUGRAF®

Pre-Injury (Earliest Immune Activation) Proactive Gene Expression

Gene expression profiling characterizes different gene expression states from circulating blood cells.

The gene expression profiles of immune system quiescence and early silent sub-clinical rejection can be differentiated by TruGraf.

Stable Renal Function

VIRACOR TRAC®

Significantly Elevated Levels of dd-cfDNA - Kidney Injury

Donor derived cell-free DNA is released into the blood that originates from organs experiencing injury and death.

When the transplanted graft is experiencing injury donor derived cell free DNA increases in the blood. A significant increase is required to overcome daily normal variations.

Renal Dysfunction

Opportunities for Intervention

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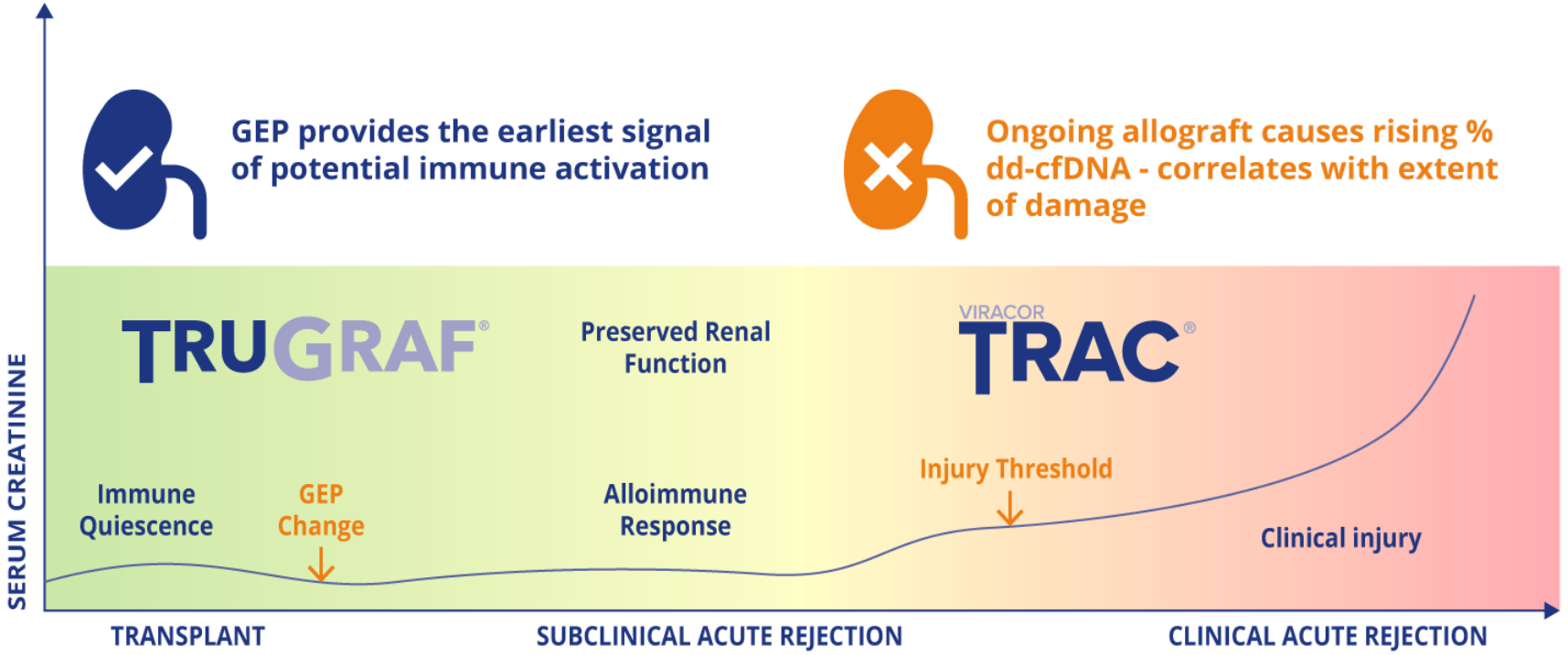

1 in 4 Stable Kidney Transplant patients can have "Silent" Subclinical Rejection

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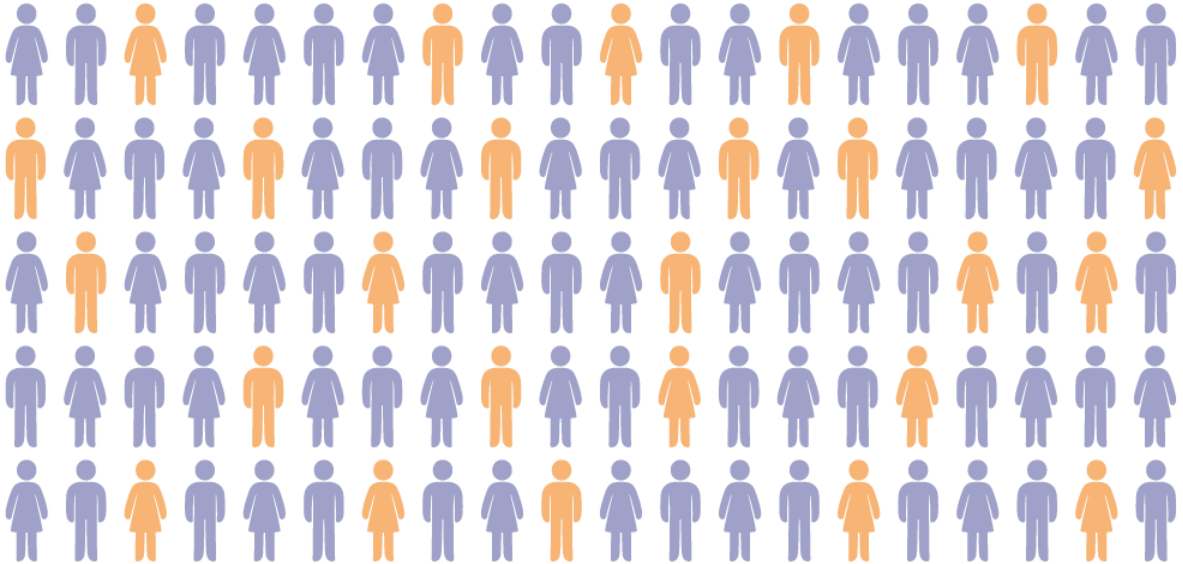
Which patients are silently rejecting?

TRUGRAF®

TruGraf outperformed TRAC on Patients with TCMR

VIRACOR TRAC®

TRAC is more sensitive to Patients with AMR



Increasing Biomarker Diagnostic Performance to a New Standard

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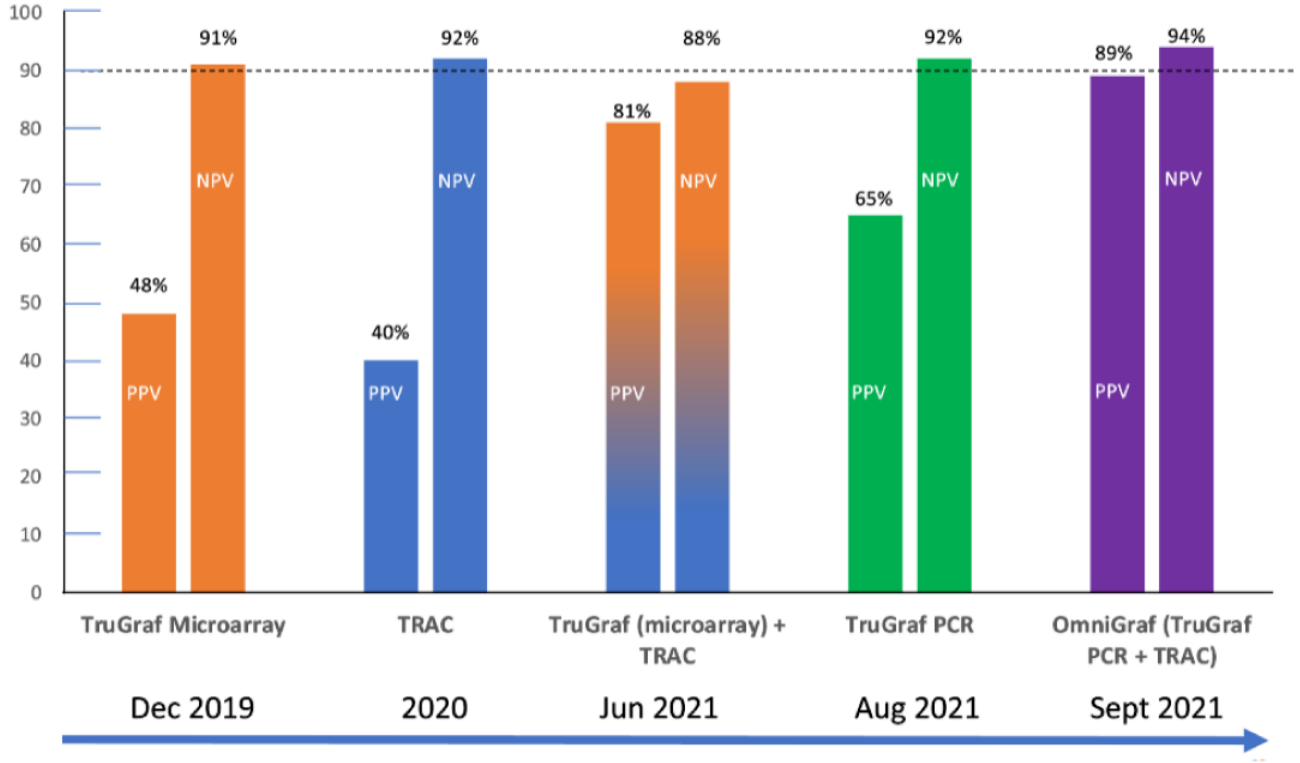

Increasing Biomarker Diagnostic Performance to a New Standard

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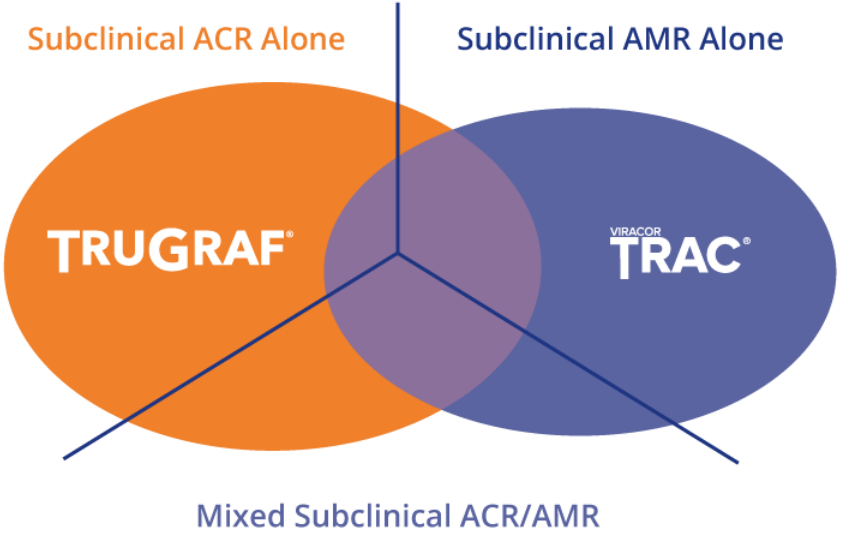
OMNIGRAF™ →

TRUGRAF®
Microfluidic gene expression classification of the 120 specific genes that express during subclinical acute rejection.

+

VIRACOR TRAC®
Next-generation sequencing of donor-derived cell-free DNA, analyzing the whole genome (100,000+ SNPs) for evaluating clinical acute rejection.



OmniGraf: The Power of One

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One All-inclusive
Sample Collection Kit

One 6ml
Routine Blood Draw

One Overnight
Shipment

One Easy-to-Interpret
Longitudinal Report

OMNIGRAF™

Improved Diagnostic Confidence for the Nephrologist

- Single panel-based Test Request Form
- Blood GEP preferentially detects cellular/mixed rejection
- cfDNA preferentially detects antibody/mixed rejection
- The combination of tests is complementary, detecting more rejection episodes
- When both tests are positive or negative the PPV/NPV increases compared to either test alone
- Single report with longitudinal resulting

Clinical Case #1
Early Subclinical ACR Recognition and Treatment

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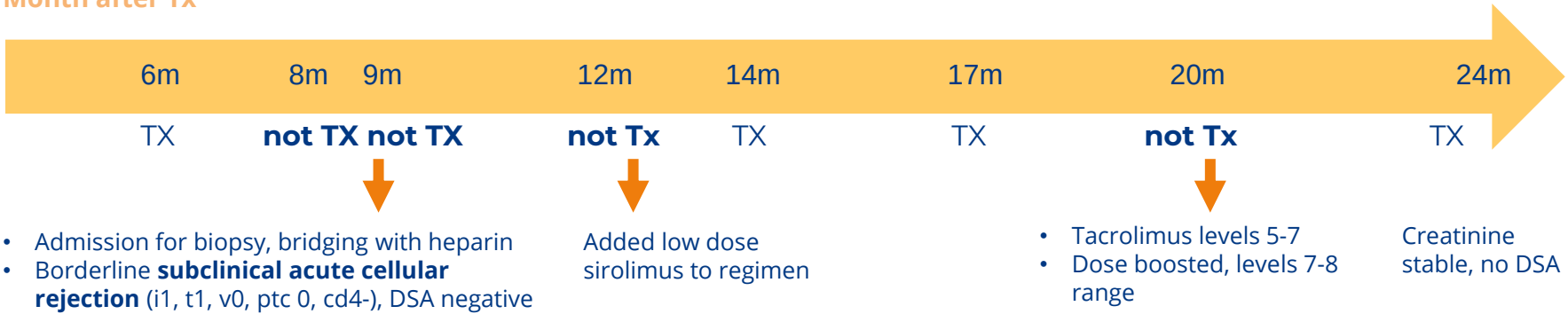
- 40-yo-female; ESRD from GN, prior transplant complicated by early thrombosis and removal (APLS)
- Sensitized, second transplant from living donor, no pretransplant DSA
- On chronic anticoagulation with warfarin for APLS
- Creatinine stable at 1.2-1.5 mg/dl

TruGraf Monitoring Initiated Given Risk of Surveillance Biopsies:



- Admission for biopsy, bridging with heparin
- Borderline **subclinical acute cellular rejection** (i1, t1, v0, ptc 0, cd4-), DSA negative
- Pulse steroids, increased baseline IS

Added low dose sirolimus to regimen

- Tacrolimus levels 5-7
- Dose boosted, levels 7-8 range

Creatinine stable, no DSA

Clinical Case #1
Early Subclinical ACR Recognition and Treatment

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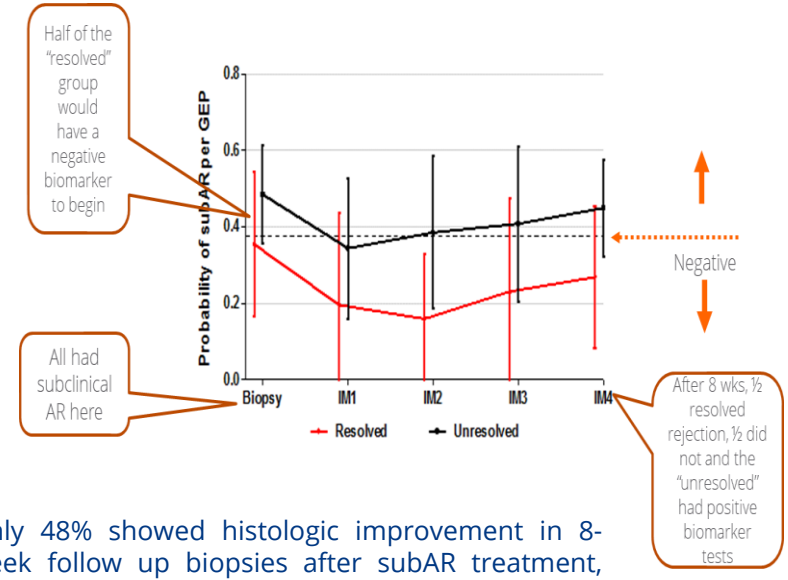
Serial TruGraf Monitoring following histological subAR (CTOT-08)



Only 48% showed histologic improvement in 8-week follow up biopsies after subAR treatment, and only 25% of patients with both a positive biopsy and TruGraf test showed improvement

The Value and Significance of Serial TruGraf Monitoring

Table 3. Odds Ratio of Progression to BPAR Based on Serial TruGraf Testing (n=38) (TX = negative result)

Monthly Testing →→	Repeat #1	Repeat #2	Odds ratio of subsequent BPAR
Not-TX	TX	TX	Reference (n=15)
Not-TX	TX	Not-TX	9.333, 95% CI [0.624, 139.581], p=0.106 (n=5)
Not-TX	Not-TX	TX	2.333, 95% CI [0.124, 43.794], p=0.571 (n=7)
Not-TX	Not-TX	Not-TX	28.0, 95% CI [1.208, 648.844], p=0.038 (n=3)

- A not-TX followed by repeat not-TX 4 or 8 weeks later was associated with a higher odds ratio of having an episode of clinical acute rejection
- Recognizing early subclinical ACR is half the battle – effective treatment is key and highlights the importance of follow up testing

Friedewald et al, Am J Transplant. 2018 Jul 9. doi: 10.1111/ajt.15011.

Clinical Case #2
Clinical Utility of dd-cfDNA (TRAC) for Pure AMR Memory Response

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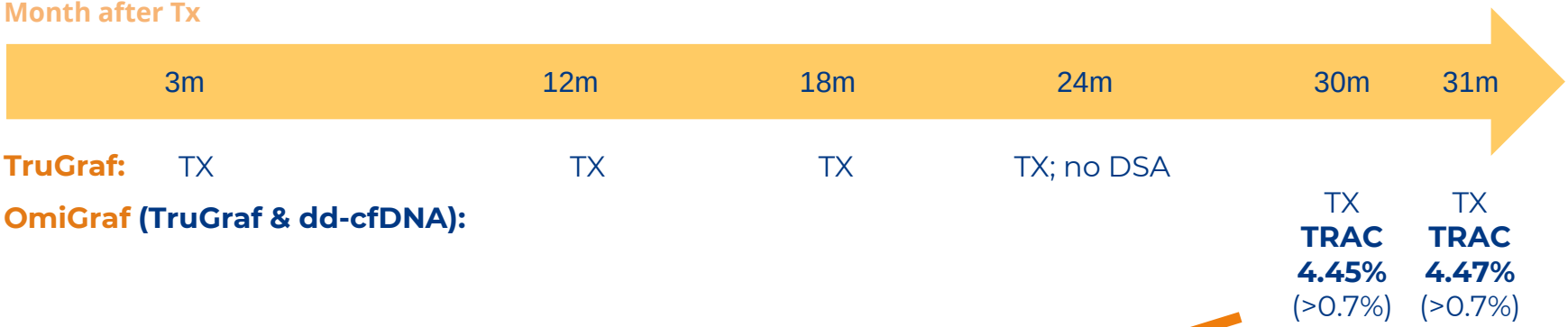
- 60-yo-female, living donor
- No pretransplant DSA identified (was not listed for long so not a long “history” of DSA testing)
- Uneventful transplant, excellent graft function
- Immunosuppression changed from tacrolimus/MMF to everolimus/MMF due to alopecia in 1st year post transplant



Biopsy showed acute subclinical antibody mediated rejection
(g2, ptc 2, i1, t1, v0, cg0), c4d ++, no TG; no proteinuria;
New HLA-C (>1:1024) and DQ (1:1) donor specific antibodies
Treated with steroids, TPE/IVIg, anti-CD20; Converted EVL to CsA + belatacept
TRAC 4.45%→3.26%→2.89%

HLA – C antibody remains at high titer, DQ currently undetectable

Clinical Case #3
Value of One – Combined Testing

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- 30-yo-male, 10 years post DDKT
- History of ACR in the first 2 years post-transplant, treated “successfully”
- Stable kidney function for 7 years (1.0-1.1 mg/dl). UPC 0.25 (>0.5)
- Tacrolimus, MMF maintenance, Tacro levels 4-6 ng/ml

OmniGraf sent at annual visit at month m:

	m	m+1	m+2	m+5
TruGraf:	**not-TX**	**not-TX**	**not-TX**	TX
TRAC:	**4.05%**	**4.79%**	**7.94%**	**3.60%**

Added Belatacept

Creatinine stable at 1.2, UPC now 1.0

- Biopsy: Chronic active AMR (ptc 2, g1, cg3, c4d+) with i0, t0, ci1, ct1; DSA positive (strong DR and DQ)
- Converted to tacrolimus and sirolimus based regimen, added prednisone
- Treated with IVIg 1 gm/kg weekly x 4 and anti-CD20
- Developed severe headaches prompting ER visit – LP (aseptic meningitis)
- Held further IVIg

Clinical Case #3
Value of One – Combined Testing

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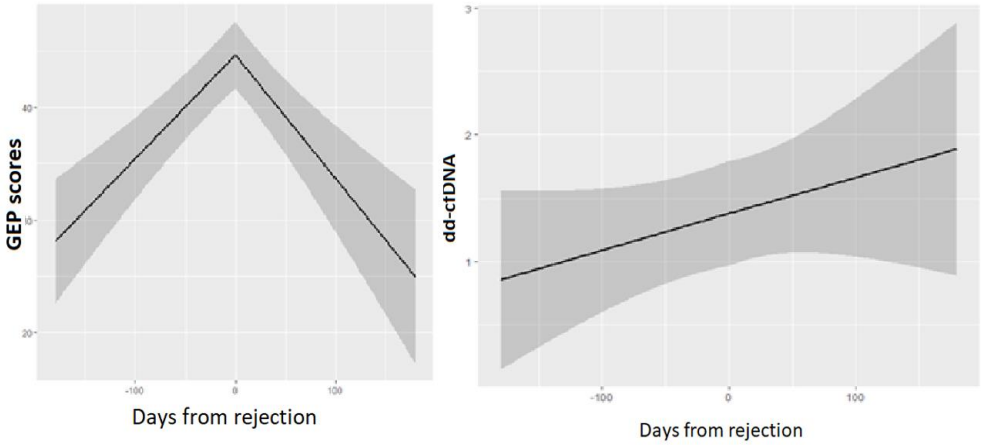
Trends in GEP Probability Scores and dd-cfDNA Scores preceding and following treatment of subAR

- A total of 1,314 blood samples were assessed.
- The longitudinal changes of GEP scores at a sample level are shown in the Figure.
- The slope of GEP scores was significantly different after subAR (slope difference = -0.201, p-value <0.001)
- dd-cfDNA continued to rise even after subAR
- There were no significant changes to the slope of dd-cfDNA between pre-subAR and post subAR (0, p-value = 0.98).



Trajectory of Gene Expression Profile and Donor-Derived Cell-Free DNA Before and After Subclinical Acute Rejection; Sook Park MD[1], Zachary Dietch MD[1], Kexin Guo[1] , Lihui Zhao PhD[1], John Friedewald MD - ASN 2021 Presentation

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Thanks!