Non-invasive Monitoring of Transplant Recipients: Integrating Molecular Markers of Rejection into Clinical Practice – The New Frontier

Transplant Webinar
Online Clinical Session
Koç University Hospital
May 23rd, 2022

Michael Abecassis MD MBA
Dean, University of Arizona College of Medicine – Tucson
Professor, Departments of Surgery and Immunobiology

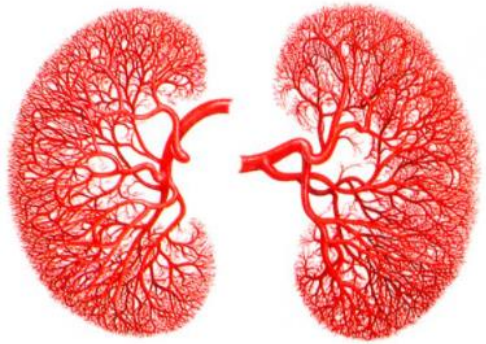
THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

DISCLOSURES

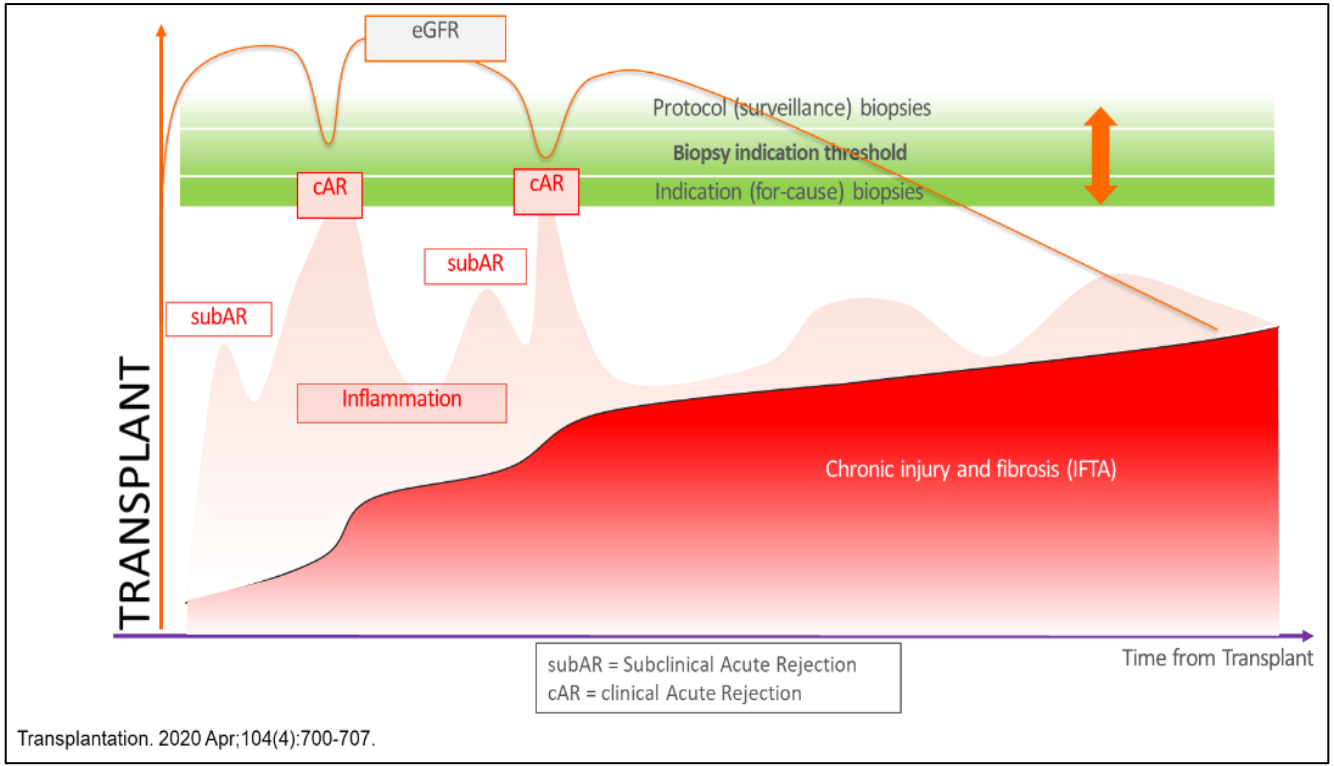
- Co-founder, Transplant Genomics Incorporated - TGI
- Clinical and Scientific Advisor, TGI/Eurofins Transplant Diagnostics
- Subcontract grant from Northwestern University Comprehensive Transplant Center – Eurofins Sponsored Research Agreement to University of Arizona College of Medicine – Tucson, Department of Surgery

KIDNEY TRANSPLANTATION



Non-invasive Monitoring of Transplant Recipients

The Ebb and Flow of Immune Status and Graft Injury following Kidney Transplantation



Transplantation. 2020 Apr;104(4):700-707.

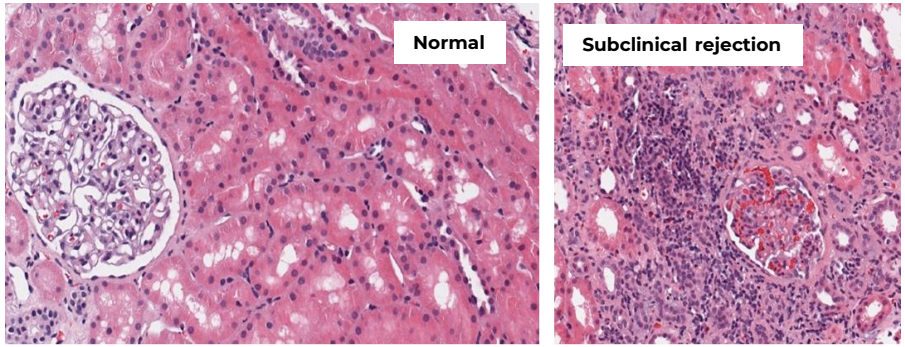
Sub-clinical Rejection (subAR)

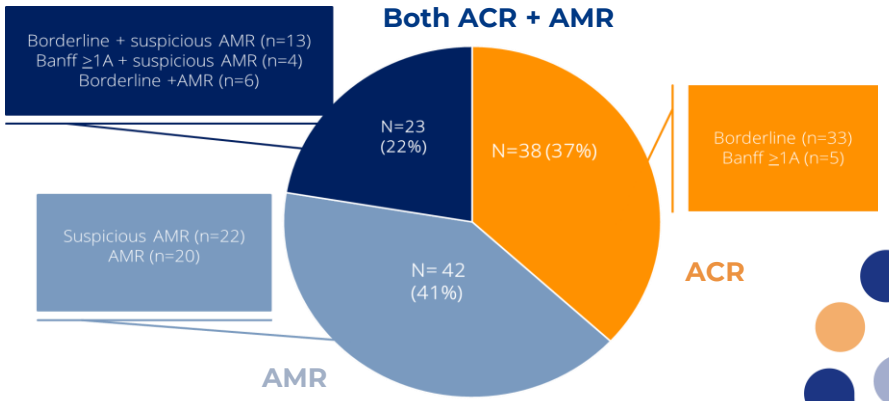
Definition: histological unsuspected (silent) rejection that can only detected on invasive surveillance biopsies in a patients with stable renal graft function

Clinical Significance: clear association with de-novo DSA and antibody-mediated rejection, chronic rejection and chronic graft loss

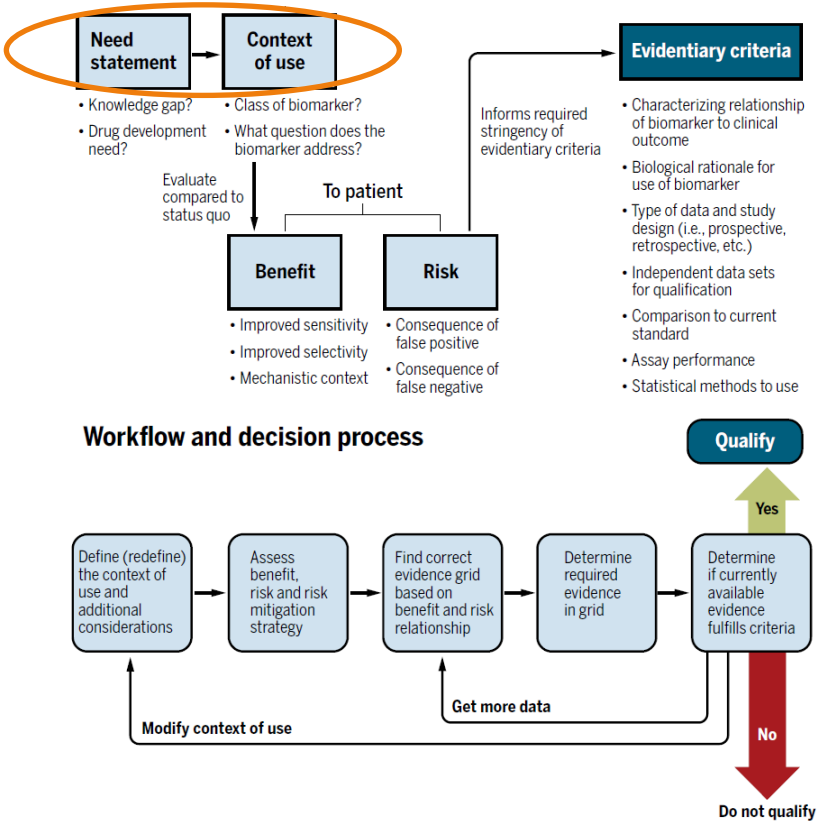
Outcome: worse graft function and graft and patient survival

Treatment: problematic because repeat invasive biopsies are impractical and serum creatinine is neither sensitive nor specific; 52% of subAR either persist or worsen with treatment in the context of stable renal function (Friedewald *AJT* 2018)





Biomarker Development



J. Liptak *Science and Translational Medicine* 2017

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Subclinical Rejection following Kidney Transplantation

Need statement: subAR (silent rejection) is bad for long-term graft outcomes, which have not improved in decades; the only way to currently diagnose subAR is through the use of empirically scheduled **invasive** surveillance biopsies, but **80+% of these invasive biopsies are negative** when used indiscriminately and therefore expose patients to unnecessary risk. Also, the only way to currently diagnose persistent subAR following treatment of subAR (>50%) is to repeat another invasive biopsy.

➢**Therefore, there is a clear need for non-invasive biomarkers to inform the use of surveillance biopsies in patients with stable renal function following kidney transplantation (KT).**

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

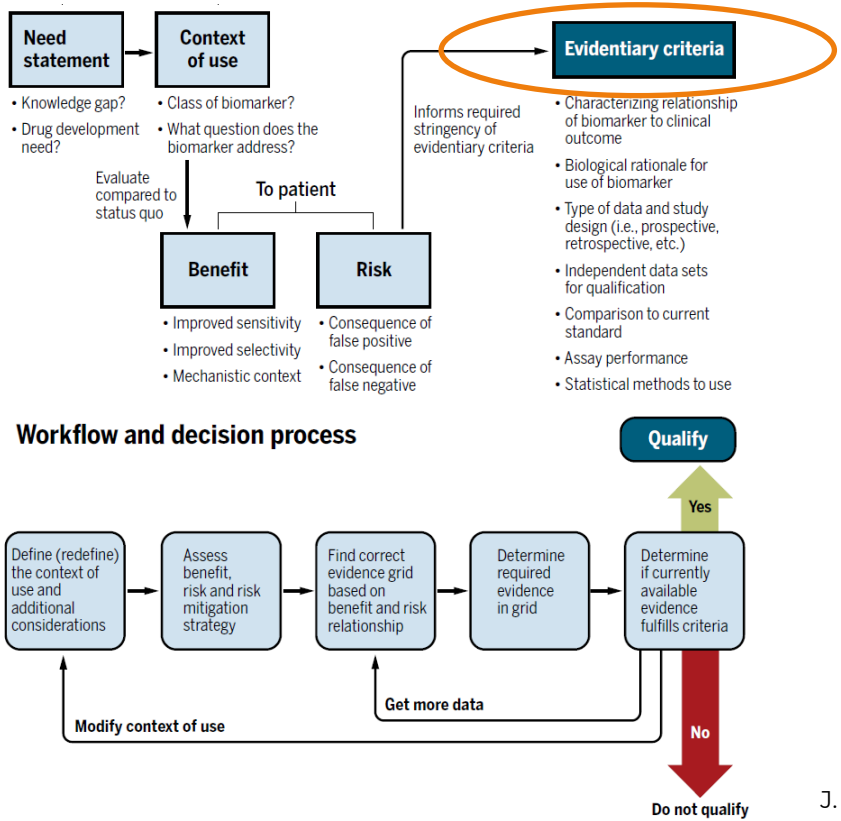
eurofins | Transplant Diagnostics

Subclinical Rejection following Kidney Transplantation

Context of use: to serially monitor patients following KT with blood-based biomarker tests that can be useful identifying patients with inadequate exposure to immunosuppression - IS) at a higher risk of harboring subAR (arc of disease is subAR → chronic rejection) in their grafts.

➢**Therefore, the objective would be to use biomarkers to guide the stratification of patients into a group that might more predictably or prognostically benefit from either a biomarker-guided biopsy or treatment for rejection in order to better individualize the management of IS.**

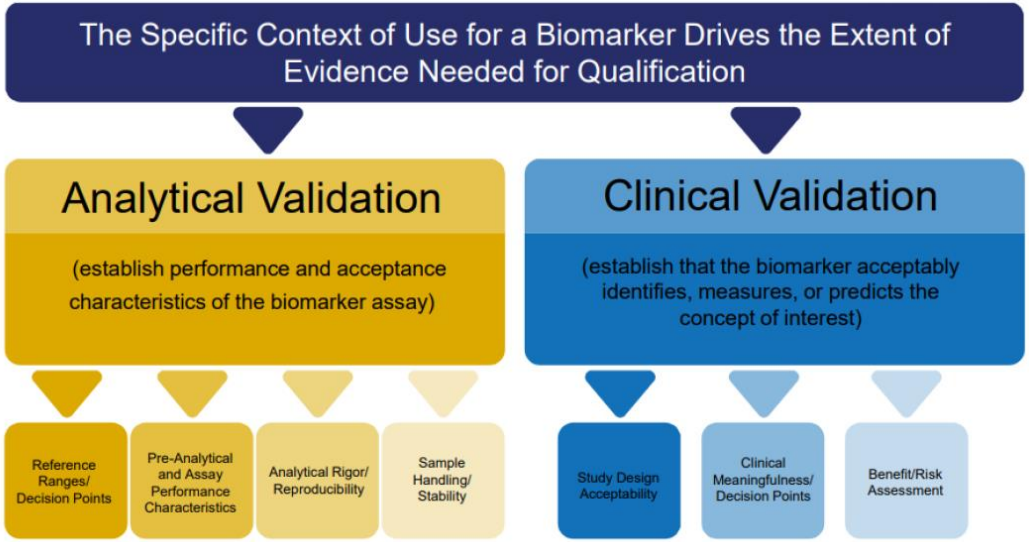
Biomarker Qualification



J. Liptak *Science and Translational Medicine* 2017

Evidentiary Criteria for Qualification

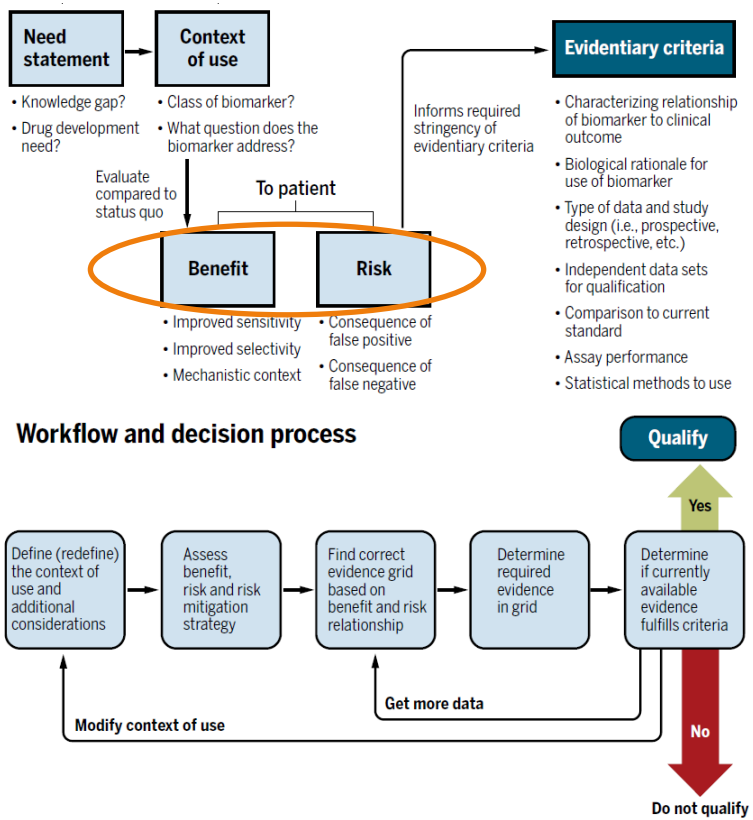
Level of Evidence Expected for a COU



Evidentiary Criteria for CTOT-08 Biomarker

- Relationship of the biomarker to clinical outcome
- Biological rationale for use of the biomarker
- Data and study design
- Reproducibility of data (independent dataset)
- Comparison to ‘gold standard’
- Assay performance
- Pre-specified statistical analysis

Biomarker Qualification



J. Liptak *Science and Translational Medicine* 2017

Benefit/Risk compared to standard

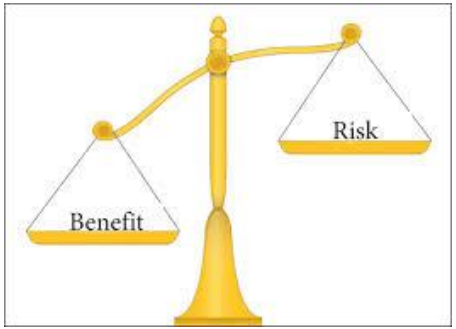
Potential Benefits – surveillance biopsies

- **Reduce the number of indiscriminate (empirically scheduled) surveillance biopsies** by **stratifying patients with stable renal function** following KT into groups at 'lower' vs. 'higher' risk of harboring subAR (silent rejection and graft injury) known to be associated with predictors of worse transplant outcomes
- **Reduce the number of negative (unnecessary) biopsies** and therefore reduce risk exposure of invasive biopsies
- **Stratify patients treated for subAR with stable function** who may not show histologic response to treatment potentially improving transplant outcomes

➢ **More personalized management of IS**

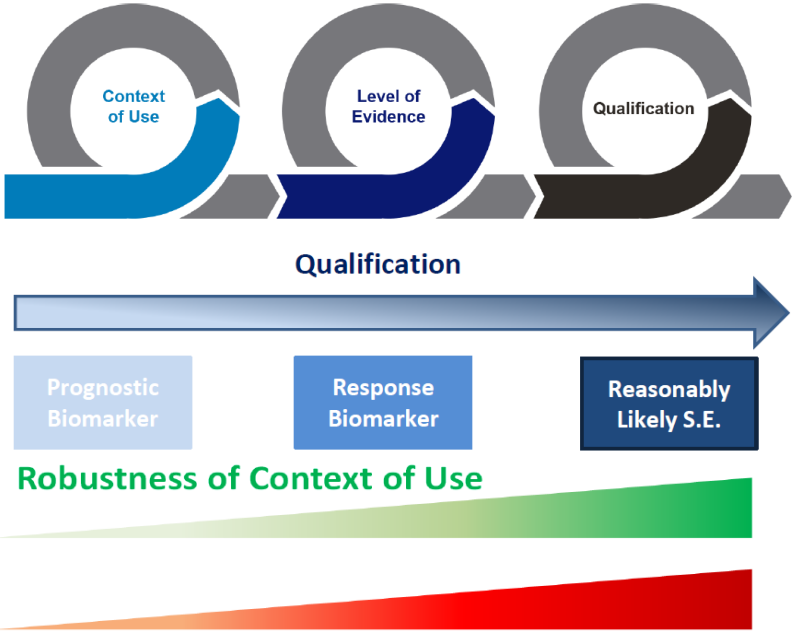
Potential Risks – no surveillance biopsies

- Increased number of biopsies in programs that do not currently use these or that use them selectively
- Increased risk of monitoring biopsies following treatment of subAR
- Over-immunosuppression secondary to unmasking of subAR leading to increased infection and malignancy



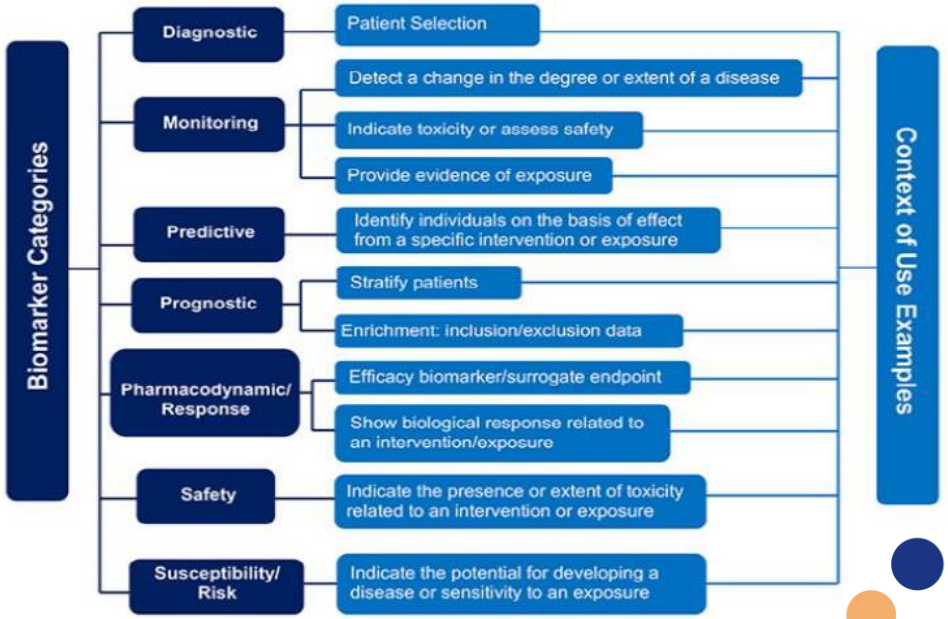
Evidentiary Criteria, Qualification and Categories

Qualification



Expectations:
Data, Evidentiary, and Regulatory

BEST: Biomarker EndpointS and Other Tools



THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Graft Injury vs Immune Status

Donor-derived Cell-free DNA (dd-cfDNA)

- Cell-free DNA released **after cellular injury and death**
- **Not specific** to a particular disease or etiology
- With increased use and study, seems **most sensitive to detecting microvascular injury** (as opposed to tubular or interstitial inflammation) in both clinical and sub-clinical rejection

1. Antibody-mediated rejection
2. Vascular cellular rejection (Banff 1B+)
3. Thrombotic microangiopathy
4. Vasculitis

Gene Expression Profile (GEP)

- Differential gene expression differences specific rejection vs non-rejection phenotypes (paired biopsies) that **reflect peripheral blood signals of immune activation vs quiescence**
- With increased use and study, seems **most sensitive and specific for immune activation in stable kidney transplant recipients** (subAR)

1. T-cell as well as antibody-mediated and mixed rejection
2. Validated in stable patients

Performance Metrics of Individual and Combined Molecular Markers

Sub-clinical (silent) Rejection in Stable Kidney Transplant Recipients

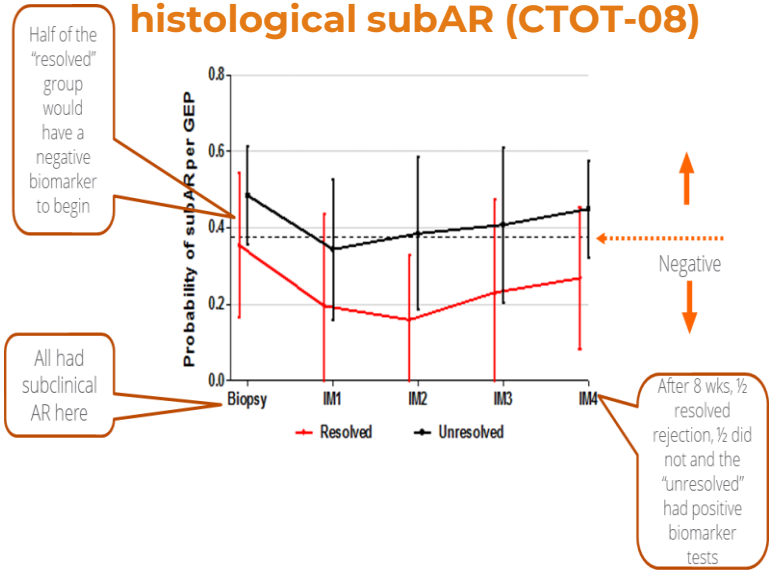
Diagnostic performance	TruGraf Alone (95% CI)	Viracor TRAC (95% CI)	+ TruGraf OR TRAC (95% CI)	OmniGraf + TruGraf AND TRAC (95% CI)
Sensitivity	0.72 (0.68-0.83)	0.47 (0.34-0.59)	0.69 (0.58-0.79)	0.77 (0.71-0.80)
Specificity	0.85 (0.80-0.89)	0.88 (0.84-0.92)	0.74 (0.69-0.80)	0.94 (0.92-1)
PPV	0.65 (0.61- 0.70)	0.56 (0.44-0.67)	0.46 (0.37-0.55)	0.89 (0.84-0.95)
NPV	0.91 (0.86-0.94)	0.84 (0.80-0.88)	0.94 (0.92-1)	0.81 (0.63-0.95)
Accuracy	0.75	0.78	0.73	0.85
False Positive Rate	8%	12%	6%	4.5%

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Serial TruGraf monitoring

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

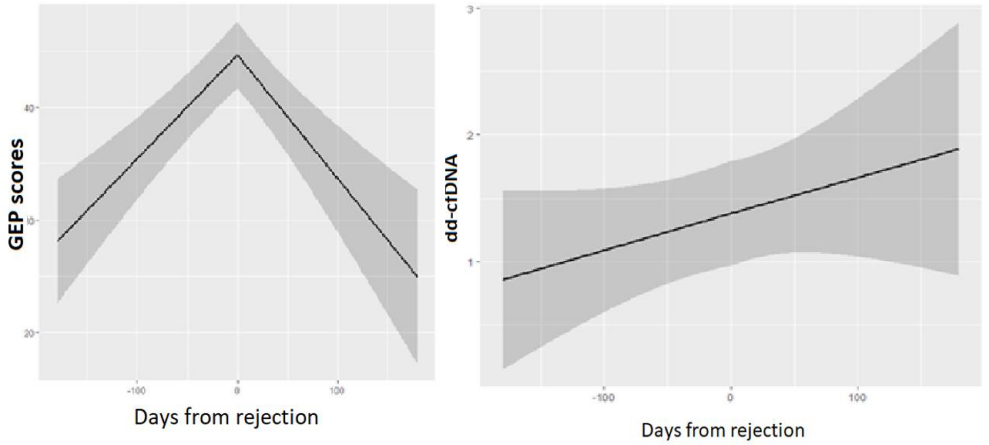
Friedewald et al, Am J Transplant. 2018 Jul 9. doi: 10.1111/ajt.15011.

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

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).

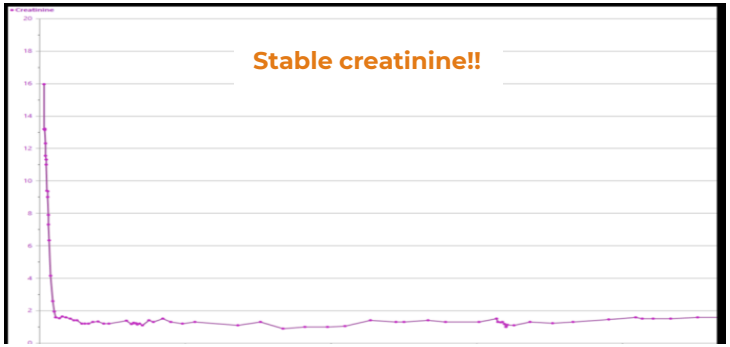


THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Case #1: Early Subclinical ACR Recognition and Treatment

- **40-year-old 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:

Month 6 – TX
Month 8 – not TX
Month 9 – not TX

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

Month 12 – not TX
Month 14 – TX
Month 17 – TX

- Added low dose sirolimus to regimen

Month 20 – Not TX

Tacrolimus levels 5-7.

- Dose boosted, levels 7-8 range

Month 24 – TX

Creatinine stable, no DSA

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

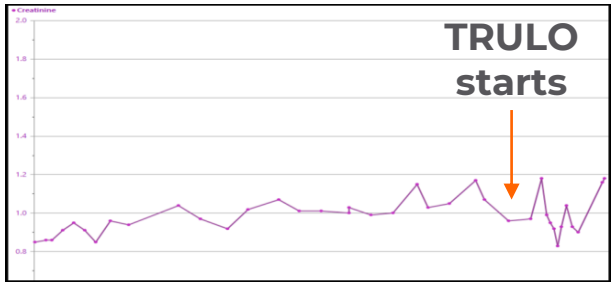
eurofins | Transplant Diagnostics

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

- **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**

TruGraf Monitoring Initiated:

- Month 3 – TX
- Month 12 – TX
- Month 18 – TX
- Month 24 – TX; no DSA



Patient enrolls in TRULO Registry Trial using OmniGraf (TruGraf & dd-cfDNA):

Month 30

- TruGraf – TX
- TRAC – 4.45% (>0.7%)

Month 31 (Repeat)

- TruGraf – TX
- TRAC – 4.47%

➢ Biopsy shows 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

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Case #3 – Value of One – Combined Testing

- **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:**
- TruGraf not-TX
- TRAC 4.05%

➢Biopsy: Chronic active AMR (ptc 2, g1, cg3, c4d+) with i0, t0, ci1, ct1; DSA positive (strong DR and DQ)

Treatment and Response:

➢ 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
- Monitored OmniGraf monthly during treatment
- TRAC results – 4.05% → 4.79% → 7.94% (TruGraf remained not-TX)

➢ Added Belatacept

- 3 months later, TruGraf – TX
- TRAC 7.94% → 3.6%
- Creatinine stable at 1.2, UPC now 1.0

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

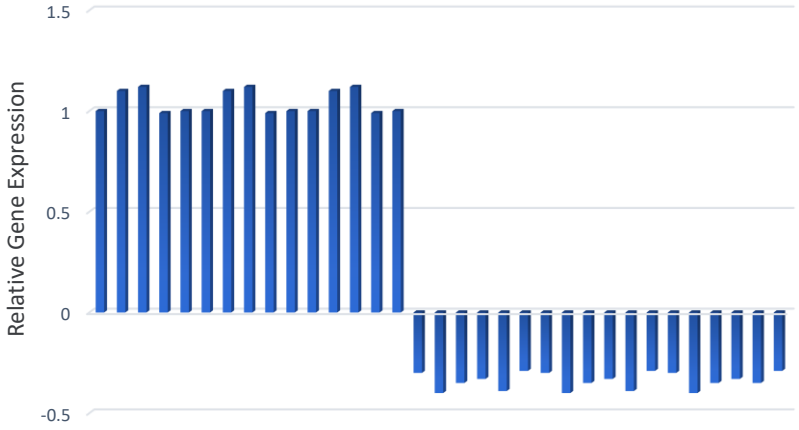
eurofins | Transplant Diagnostics

Technology Evolution from Microarray to PCR

TRUGRAF® Blood Gene Expression Test

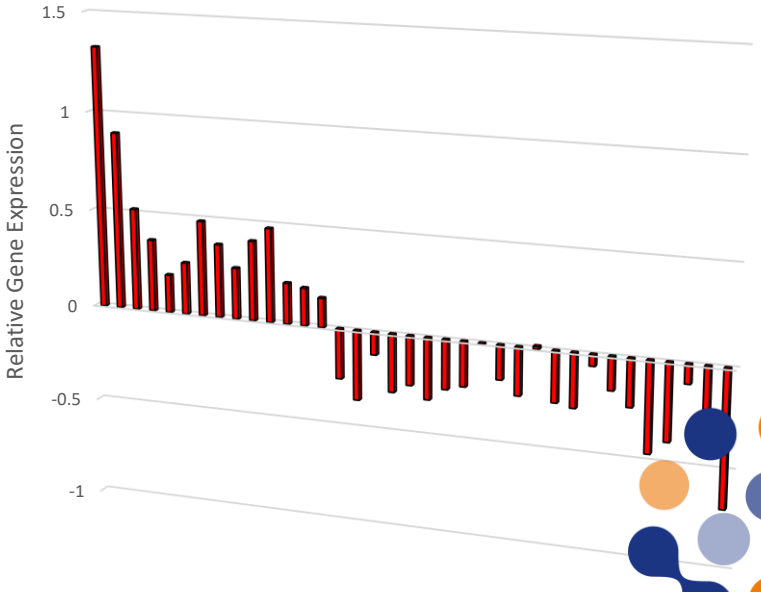
Microarray

Log Fold Change



Microfluidics PCR

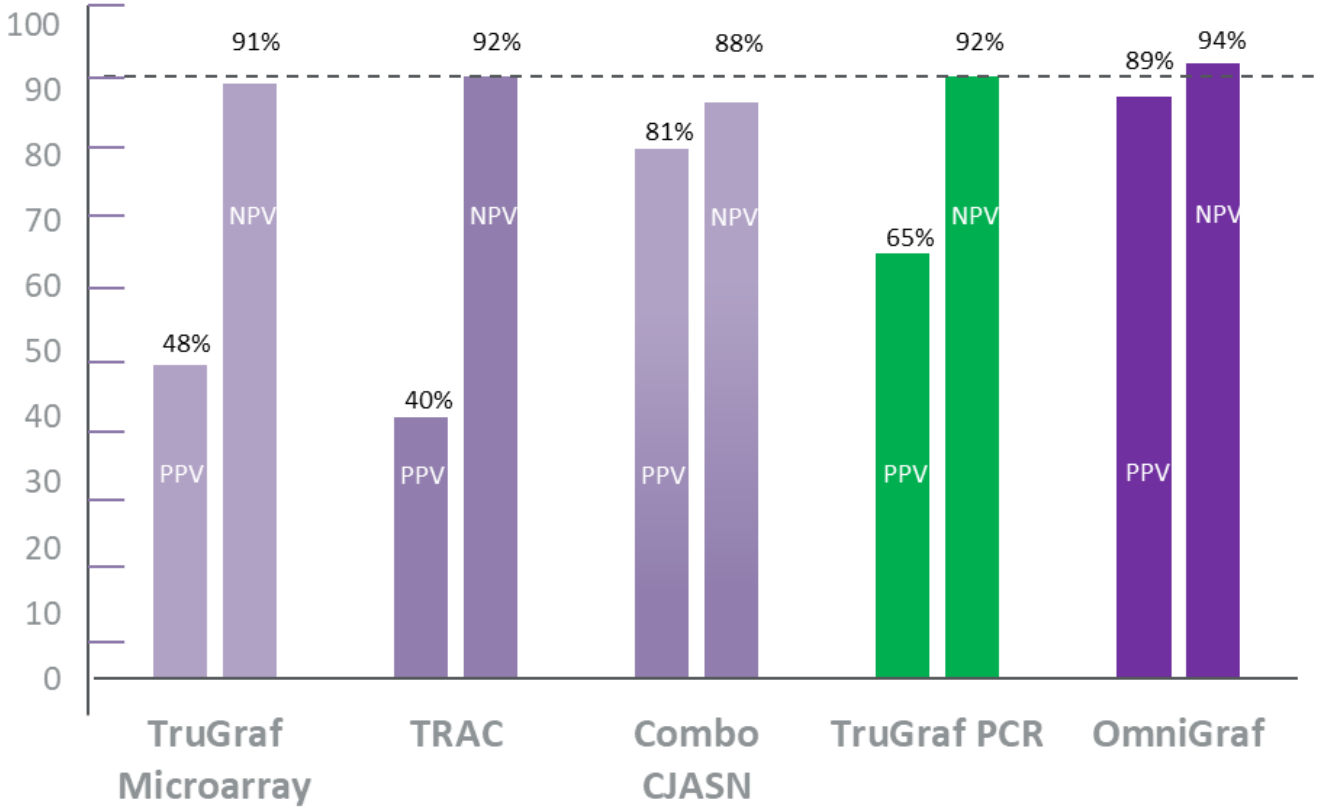
Log Fold Change



THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Increasing Biomarker Diagnostic Performance to a New Standard



THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Combining Tests – The power of ONE

OMNIGRAF™ = TRUGRAF® + VIRACOR TRAC®

89% PPV

Positive Predictive Value

94% NPV

Negative Predictive Value

One 6ml Routine Blood Draw

One Easy-to-Interpret Longitudinal Report

eurofins | Transplant Genomics

https://transplantgenomics.com/omnigraf-kidney/

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Summary - Integrating Molecular Markers into Clinical Practice

Abrogating Routine Surveillance Biopsies in Stable Kidney Transplant Recipients

A world divided: two standards of care – surveillance biopsies for any stable patient

- Logistical difficulties with routine 'protocol' biopsies
- No definitive studies have demonstrated effectiveness of treating sub-clinical rejection mainly due to lack of ability to diagnose untreated rejection in the setting of normal creatinine and reluctance to re-biopsy a stable patient
- No prior validated marker of 'high risk patient' to guide 'surveillance biopsy for cause'

A world united: meeting in the middle for stable patients recognizing the impact of undetected and therefore untreated sub-clinical (silent) rejection on long-term outcomes

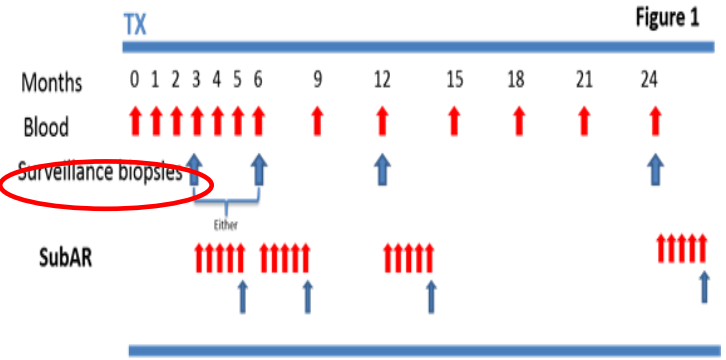
- Individual rule-out tests (high NPV) for T-cell (TruGraf) vs. antibody mediated (TRAC) silent rejection – biomarker-guided biopsy only for stratified "high risk" stable patients – 25-30% with positive test(s)
- Combined test (**OmniGraf**) as both rule-out and rule-in test (high NPV and PPV) - increases accuracy of positive test, further decreasing or essentially transforming all 'surveillance' biopsies into 'for-cause' biopsies for stable patients similar to patients with graft dysfunction

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

eurofins | Transplant Diagnostics

Non-invasive Monitoring of Transplant Recipients

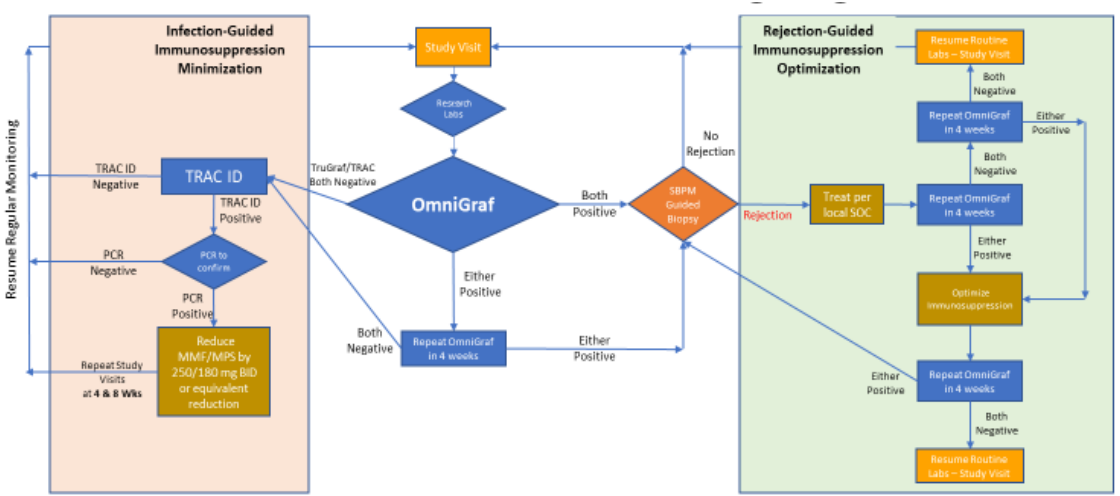
CTOT-08: genomic signatures of subAR in serial samples from multi-center prospective study of 300 LTR



Paired samples used for both TruGraf and TRAC validation; histology and biomarker independently correlated with clinical and histological outcomes; external validation of both biomarkers

Clinical Trial Testing Algorithm – Study Arm
SURVEIL (Serial Biomarker Profile Monitoring – SBPM)

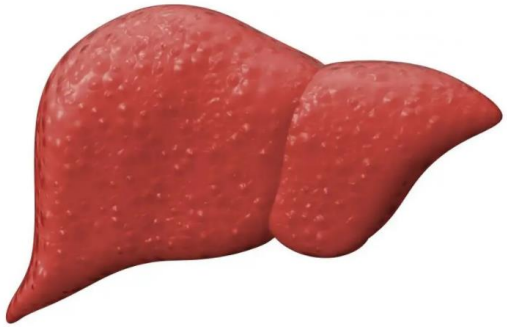
Patients randomized to SOC vs SURVEIL; OmniGraf and TRAC-ID



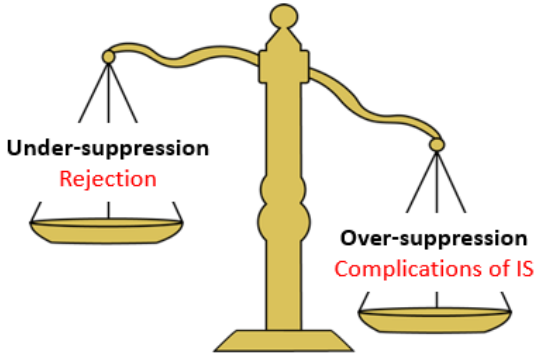
Overimmunosuppression

Underimmunosuppresion

LIVER TRANSPLANTATION



The Clinical Conundrum of Immunosuppression (IS) in Liver Transplant Recipients



- Following life-saving liver transplantation (LT), routine baseline immunosuppression (IS) is universally accepted as routine, safe, efficacious and effective, leading to excellent clinical outcomes.
- In some patients, IS can lead to life-threatening renal, infectious, metabolic, and oncologic complications leading to significant morbidity and mortality.
- Most clinicians reduce IS either routinely over time following LT, or in response to IS complications

➢ There is no currently generally accepted or validated approach to managing IS reduction other than to monitor patients for graft dysfunction (acute rejection), which is associated with worse outcomes

➢ There is a clear need for molecular markers to help inform IS reduction in LT recipients

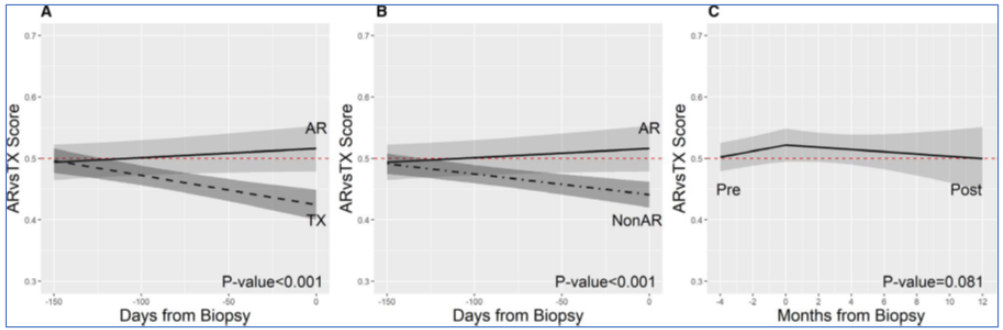
Performance Metrics of Gene Expression and cfDNA Molecular Markers

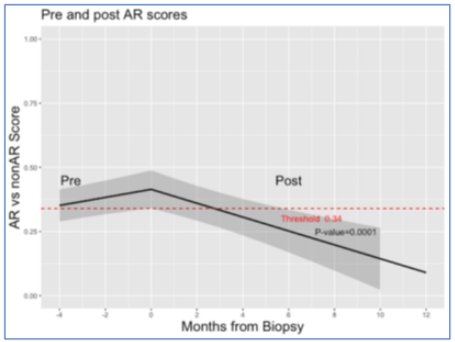
AR - Acute Rejection**; TX –** normal**; non-AR –** all other causes of graft dysfunction

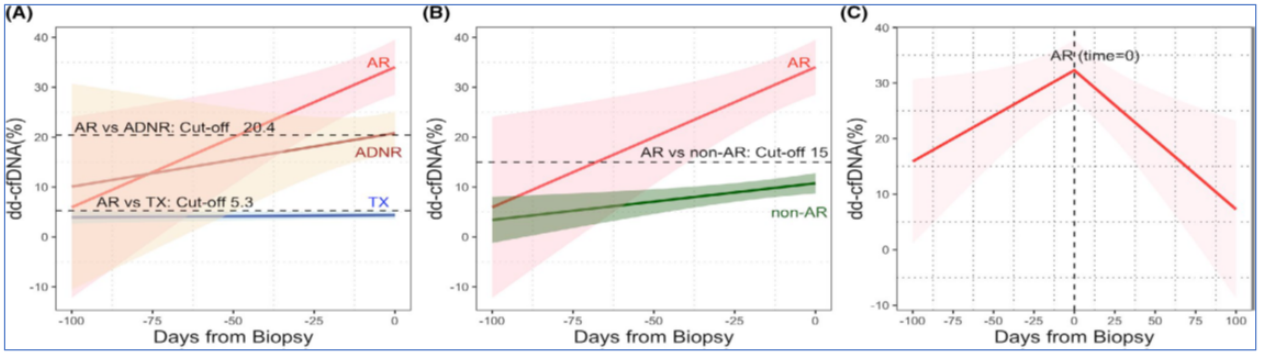
Diagnostic performance	TruGraf Liver Alone** (AR vs TX) (95% CI)	Viracor Liver TRAC** (AR vs TX) (95% CI)	TruGraf Liver Alone** (AR vs non-AR) (95% CI)	Viracor Liver TRAC** (AR vs non-AR) (95% CI)
Sensitivity	0.57 (0.43-0.69)	1.00 (0.70-1.00)	0.67 (0.53-0.79)	0.88 (0.62-0.98)
Specificity	0.82 (0.73-0.89)	0.80 (0.62-0.92)	0.73 (0.63-0.79)	0.80 (0.66-0.90)
PPV	0.47 (0.38- 0.59)	0.63 (0.45-0.77)	0.48 (0.38- 0.60)	0.53 (0.45-0.72)
NPV	0.87 (0.84-0.94)	1.00	0.86 (0.83-0.93)	0.95 (0.84-0.99)
Accuracy	0.77	0.85	0.72	0.82
False Positive Rate	18%	20%	17%	20%

**Analysis for the combination of TruGraf Liver and TRAC Liver is ongoing

Trends of TruGraf and TRAC over time by Clinical Phenotype







Levitsky J… Abecassis M; *AJT* 2020; Levitsky J… Abecassis M; *Transplantation* 2021; Levitsky… Abecassis M; *AJT* 2021.

New Frontier - Integrating Molecular Markers into Clinical Practice

Standardized IS Reduction in LT recipients using Biomarker-informed Approach

Current State: no standardized approach to the clinician's conundrum regarding IS reduction following Liver Transplantation (LT) other than to monitor for and treat acute rejection with graft dysfunction when it occurs resulting in worse clinical outcomes.

- No role for invasive surveillance biopsies
- Wide variation in practice with no standardized (best practices) approach to IS reduction for either routine IS management, or management or IS complication
- Despite excellent outcomes of life-saving LT, unmitigated IS complications result in both graft loss and patient death

Future State: standardized approach to IS reduction following LT using novel molecular biomarkers, recently developed and validated that signal transition from immune quiescence to immune activation and graft injury prior to graft dysfunction (i.e., prior to elevation of liver function tests)

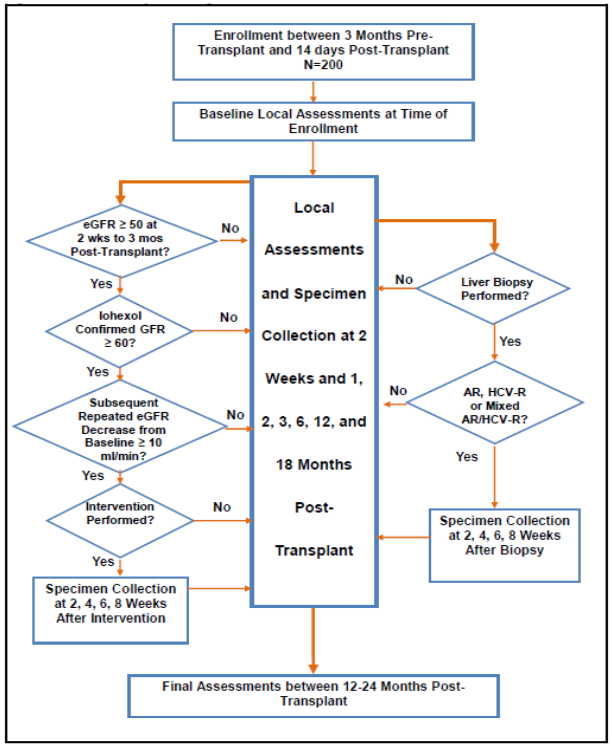
- Serial monitoring of LT recipients with non-invasive molecular tests with excellent performance metrics can now be performed routinely in stable LT recipients as part of center-specific IS reduction protocols
- Early IS reduction following LT can now be safely performed in patients undergoing LT with compromised renal function but who do not warrant simultaneous liver-kidney transplants
- The National Institutes of Health have recently funded a multi-center US multi-million-dollar study that will attempt to standardize IS reduction approaches using these biomarkers following LT

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

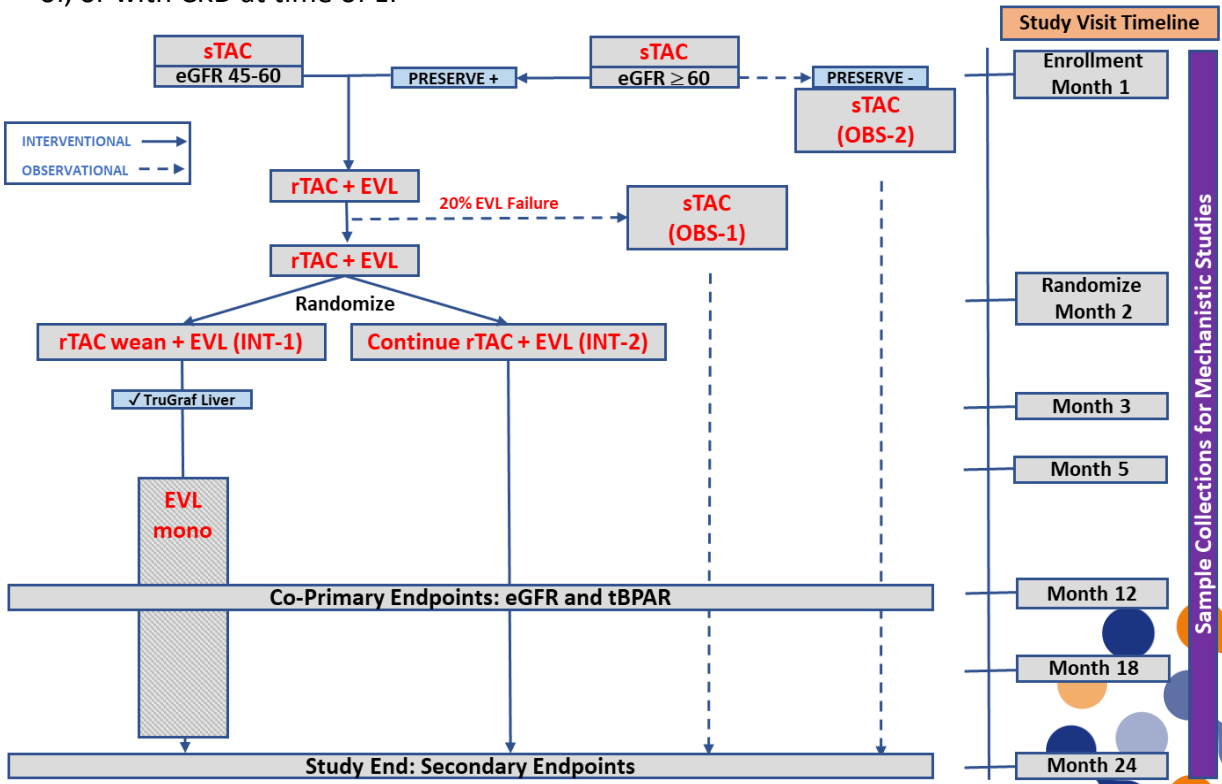
eurofins | Transplant Diagnostics

Non-invasive Monitoring of Transplant Recipients

CTOT-14: genomic signatures of AR and CKD in serial samples from multi-center prospective study of 202 LTR



CTOT-43: interventional biomarker-guided multi-center CNI minimization in 450 LTR at risk of, or with CKD at time of LT



Conclusions

We have reached *A New Frontier* of *integrating molecular biomarkers into clinical practice for kidney and liver transplant recipients*, delivering on the promise of precision medicine: "*when molecular diagnostics detect actionable differences operating in individual patients, that can inform management and improve clinical outcomes.*" (*Daniel R. Salomon* - 1953-2016).

Biomarkers: responding to a need statement with a specific context of use (COU)

Kidney transplant recipients:
Need: non-invasive monitoring to detect silent rejection in stable patients
COU: safe, routine, and non-invasive serial monitoring of stable patients to detect sub-clinical (silent) rejection, known to result in chronic graft injury and loss

Liver transplant recipients:
Need: non-invasive approach to inform IS reduction either routinely or for-cause
COU: safe, routine, and non-invasive biomarker-informed approach for standardized IS reduction protocols where clinician can assess immune and graft status prior to, and during IS reduction decreasing risk of rejection and graft dysfunction

References

- Friedewald JJ, Kurian SM, Heilman RL, Whisenant TC, Poggio ED, Marsh C, Baliga P, Odim J, Brown MM, Ikle DN, Armstrong BD, Charette JI, Brietigam SS, Sustento-Reodica N, Zhao L, Kandpal M, Salomon DR, Abecassis MM, Clinical Trials in Organ T: **Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant**. Am J Transplant. 2019
- Park S, Guo K, Heilman RL, Poggio ED, Taber DJ, Marsh CL, Kurian SM, Kleiboeker S, Weems J, Holman J, Zhao L, Sinha R, Brietigam S, Rebello C, Abecassis MM, Friedewald JJ: **Combining Blood Gene Expression and Cell-free DNA to Diagnose Subclinical Rejection in Kidney Transplant Recipients**. Clin J Am Soc Nephrol. 2021
- Khilnani C, Heeger PS (Editorial): **Two Can Be Better Than One: Improving Noninvasive Diagnostics in Kidney Transplantation**. Clin J Am Soc Nephrol. 2021
- Lee DM, Abecassis MM, Friedewald JJ, Rose S, First MR: **Kidney Graft Surveillance Biopsy Utilization and Trends: Results From a Survey of High-Volume Transplant Centers**. Transplant Proc, 2020
- Mannon RB, Morris RE, Abecassis M, Axelrod D, Bala S, Friedman GS, Heeger PS, Lentine KL, Loupy A, Murphy B, Nickerson P, Sarwal M, O'Doherty I, Spear N, Karpen SR. **Use of biomarkers to improve immunosuppressive drug development and outcomes in renal organ transplantation**: A meeting report. Am J Transplant. 2020

References

- Levitsky J, Asrani SK, Schiano T, Moss A, Chavin K, Miller C, Guo K, Zhao L, Kandpal M, Bridges N, Brown M, Armstrong B, Kurian S, Demetris AJ, Abecassis M; Clinical Trials in Organ Transplantation - 14 Consortium. **Discovery and validation of a novel blood-based molecular biomarker of rejection following liver transplantation**. Am J Transplant. 2020
- Levitsky J, Kandpal M, Guo K, Zhao L, Kurian S, Whisenant T, Abecassis M. **Prediction of Liver Transplant Rejection with a Biologically Relevant Gene Expression Signature**. Transplantation. 2021
- Levitsky, J, Kandpal M, Guo K, Kleiboeker S, Sinha R, Abecassis M; **Donor-derived cell-free DNA levels predict graft injury in liver transplant recipients**. Am J Transplant. 2021
- Levitsky J, Asrani SK, Klintmalm G, Schiano T, Moss A, Chavin K, Miller C, Guo K, Zhao L, Jennings LW, Brown M, Armstrong B, Abecassis M; **Discovery and Validation of a Biomarker Model (PRESERVE) Predictive of Renal Outcomes After Liver Transplantation**. Hepatology. 2020

Thanks!

info@transplantgenomics.com