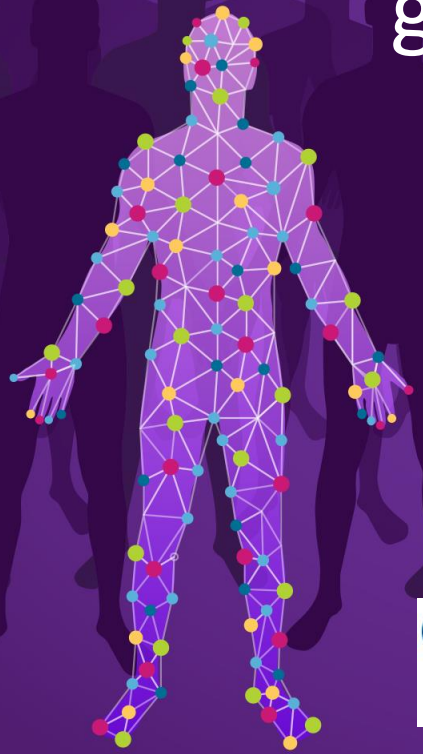
AST | AMERICAN SOCIETY OF TRANSPLANTATION

Biomarkers to assess risk and guide immunosuppression in kidney transplantation



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Icahn School of Medicine at Mount Sinai

CTOT Clinical Trials in Organ Transplantation

CEOT CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

NO SIZE FITS ALL: *Uncovering the Potential of Personalized Transplantation*

FEBRUARY 21–23, 2019 • ARIZONA BILTMORE • PHOENIX, AZ

Disclosure

Faculty: Peter Heeger

Relationships with commercial interests:

Grants/Research Support: Alexion Pharmaceuticals
Speakers Bureau/Honoraria: None
Consulting Fees: None
Other: None

Learning Objectives

To differentiate a biomarker from a surrogate endpoint

To explain the clinical utility of urinary CXCL9 testing among other biomarkers to diagnose kidney transplant rejection during CNI withdrawal

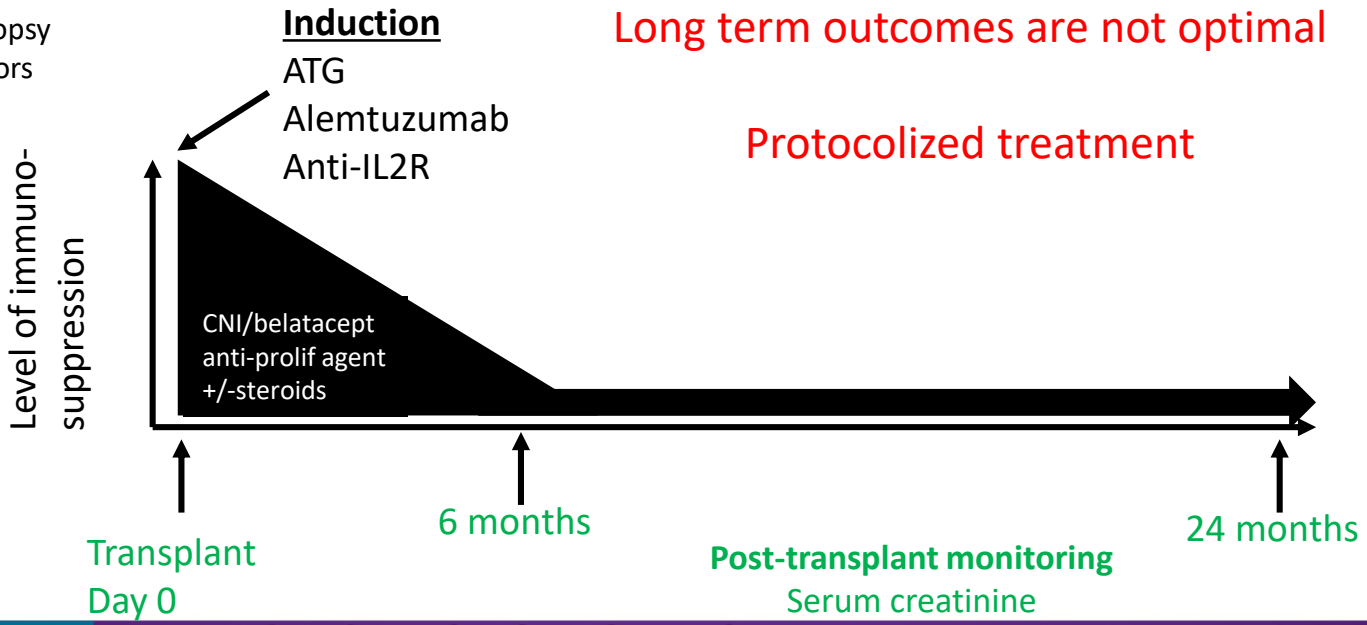
To explain the utility of pretransplant biomarkers as risk assessment tools for guiding CNI withdrawal post transplant

Definitions

- Biomarkers are anatomic, physiologic, biochemical, or molecular parameters that indicate, or are associated with an alteration in physiology and are of clinical significance (this doesn't necessarily mean they are clinically useful)
- Surrogate Markers can be defined as biomarkers that have established clinical utility
- Surrogate Endpoints are biomarkers used (in clinical trials) to evaluate the safety or effectiveness of a therapy and serve as alternatives to traditional endpoints.

The need for biomarkers in transplantation

Pre-transplant
Risk assessment
HLA typing
Cross matching
Implantation biopsy
Clinical risk factors



Long term outcomes are not optimal

Protocolized treatment

Post-transplant monitoring
Serum creatinine

Biomarkers-potential uses

- Surrogate endpoints for clinical trials
- Risk assessment for post transplant outcomes
  - who is most likely to do badly (rejection/graft loss) and might require more/different immunosuppression
  - who is most likely to tolerate decreasing immunosuppression?
- Noninvasive diagnosis graft injury
  - Prevent morbidity of biopsy
  - Detect subclinical or incipient injury and or fibrosis
  - safety net for drug withdrawal studies
  - long term monitoring to detect changes in status
- Predict DGF
- Detect Immune tolerance

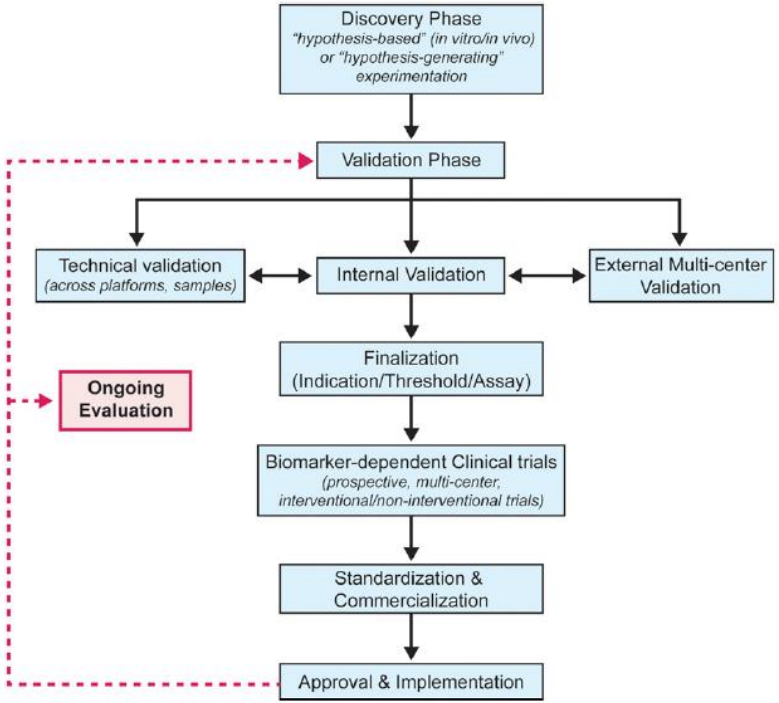
Biomarkers can support drug development & approval

- Trials cannot be done using hard endpoints of graft or patient survival because they would take too long, so we need surrogates of these hard endpoints
- AR is the only approved surrogate endpoint but
  - a) it occurs relatively infrequently and
  - b) graft failure occurs in the absence of AR
- Are there viable alternatives?

Candidate surrogate endpoints

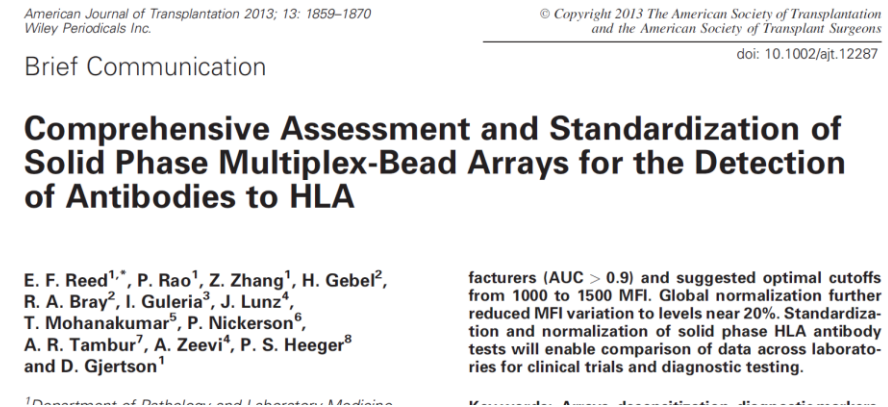
- De novo class II DSA
- Changes in eGFR during the first 2 years (kidney transplant)
- iBOX score (kidney transplant)
- IVUS measurements of cardiac vasculopathy (heart transplant)
- others

Beyond clinical trials
Moving Biomarkers Toward Clinical Implementation in Transplantation



Menon, Murphy, Heeger, JASN 2017

Multicenter validation and assay standardization are crucial



American Journal of Transplantation 2013; 13: 1859–1870
Wiley Periodicals Inc.

doi: 10.1002/ajt.12287

Brief Communication

Comprehensive Assessment and Standardization of Solid Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA

E. F. Reed[1,*], P. Rao[1], Z. Zhang[1], H. Gebel[2], R. A. Bray[2], I. Guleria[3], J. Lunz[4], T. Mohanakumar[5], P. Nickerson[6], A. R. Tambur[7], A. Zeevi[4], P. S. Heeger[8] and D. Gjertson[1]

facturers (AUC > 0.9) and suggested optimal cutoffs from 1000 to 1500 MFI. Global normalization further reduced MFI variation to levels near 20%. Standardization and normalization of solid phase HLA antibody tests will enable comparison of data across laboratories for clinical trials and diagnostic testing.

- Representative example of CTOT biomarker standardization
- Rigorous analytical validation is critical
- Inter-laboratory reproducibility important

Pre-transplant biomarkers for risk stratification in transplantation

- Are there biomarkers that can be measured pre-transplant that can predict risk of post transplant rejection and/or graft function (beyond DSA)?
- If yes, one implication is that treatment strategies for high vs low risk patients could be individualized prior to transplant to optimize outcomes
- Yesterday we heard about pre transplant gene expression/non HLA mismatches
  - Sarwal, UCSF
  - Murphy, Mount Sinai

Donor-reactive Memory T cells and transplant outcome

- Memory cells are resistant to most immunosuppressant meds, are present at high frequency, have high functional avidity and respond rapidly to antigenic challenge
- Hypothesis: high frequencies of memory T cells reactive to donor HLA negatively impact transplant outcomes

Pre-transplant donor-reactive T cells and post-transplant outcome

IFNγ ELISPOT

	Pre-transplant donor reactive ELISPOT		
Variable	Negative (<25/300 K)	Positive (> 25/300 K)	*p* value
Acute cellular rejection	17%	50%	.036
GFR (MDRD) 12 months	55±20 ml/min/1.73 m^2	37±16 ml/min/1.73 m^2	.006
DGF	23%	31%	NS

Augustine et al, AJT 2005

Validation sets

- Other independent validation:
- Donor reactive IFNγ ELISPOT assays pre- and post-transplant correlate strongly with AR and 1 y eGFR
  - Berlin group (Volk, Reinke)
  - Barcelona (Grinyo, Bestard)
  - Results from multicenter CTOT-01 study

American Journal of Transplantation 2015; 15: 3166–3173
Wiley Periodicals Inc.

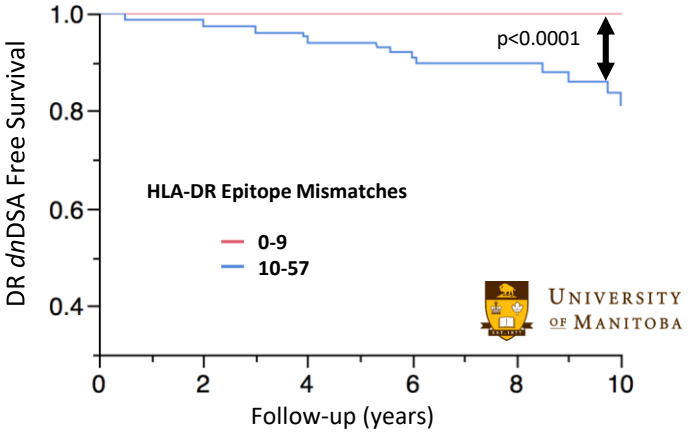
doi: 10.1111/ajt.13401

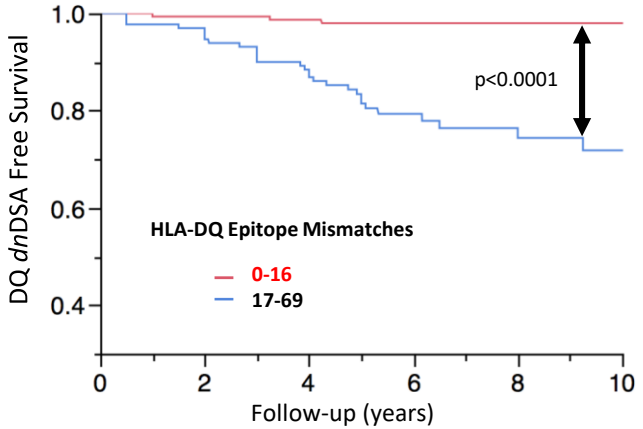
Interferon Gamma ELISPOT Testing as a Risk-Stratifying Biomarker for Kidney Transplant Injury: Results From the CTOT-01 Multicenter Study

Status: Pre-transplant donor-reactive IFNγ ELISPOT as a biomarker for post-transplant outcome

- Tested and validated by multiple groups
- Utility of using marker to guide therapy unknown
- Complex assay
- Requires customization (donor reactive)
- Some commercial interest

HLA EPITOPE Analysis (molecular mismatch) as a biomarker for developing DSA

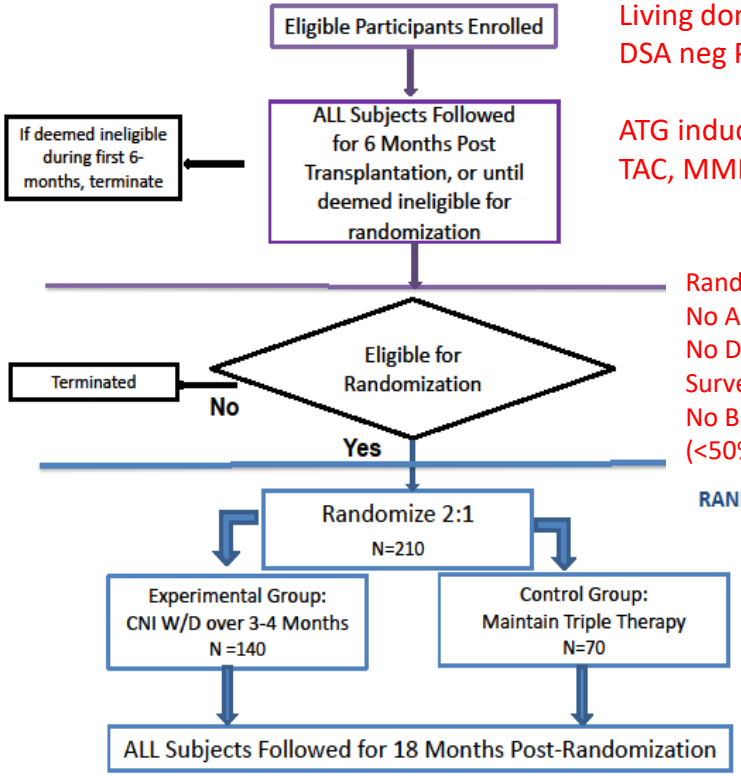




Epitope MM Load associated with *de novo* DR or DQ Donor specific antibody (DSA)

Can pre-transplant biomarkers predict those at highest risk for poor outcomes during changes in immunosuppression?

CTOT09
TAC withdrawal in low risk, stable recipients of first living donor kidneys



Living donor transplants
DSA neg PRA<30%

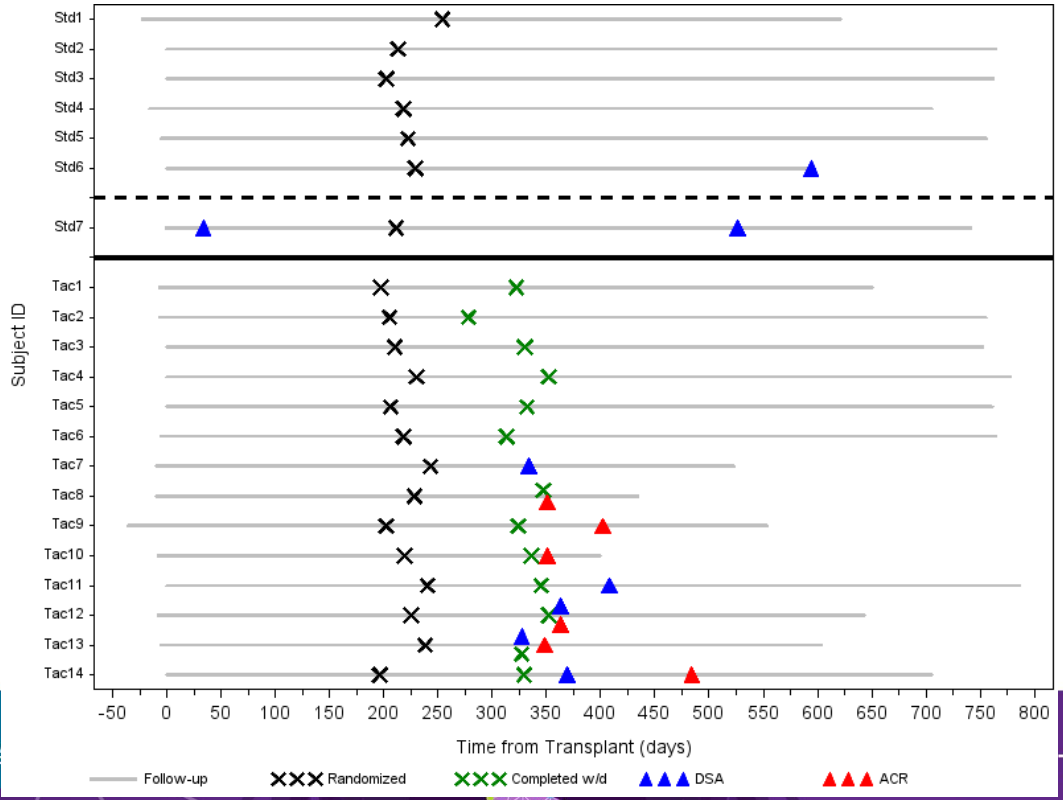
ATG induction
TAC, MMF, Pred

Randomize at 6 mo if:
No ACR
No DSA
Surveillance biopsy normal
No BKV, on 1500/day MMF
(<50% of enrollees reached randomization)

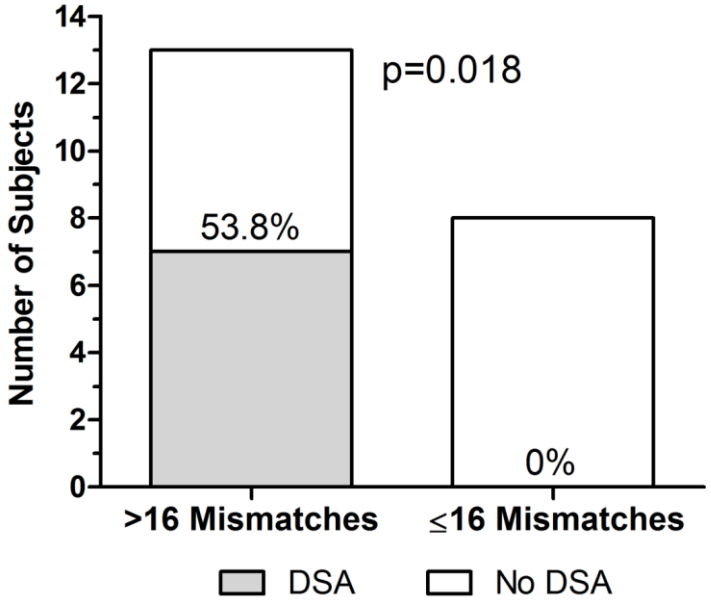
RANDOMIZATION

Study terminated due to absence of equipoise

Confirms standard clinical risk assessment is inadequate!

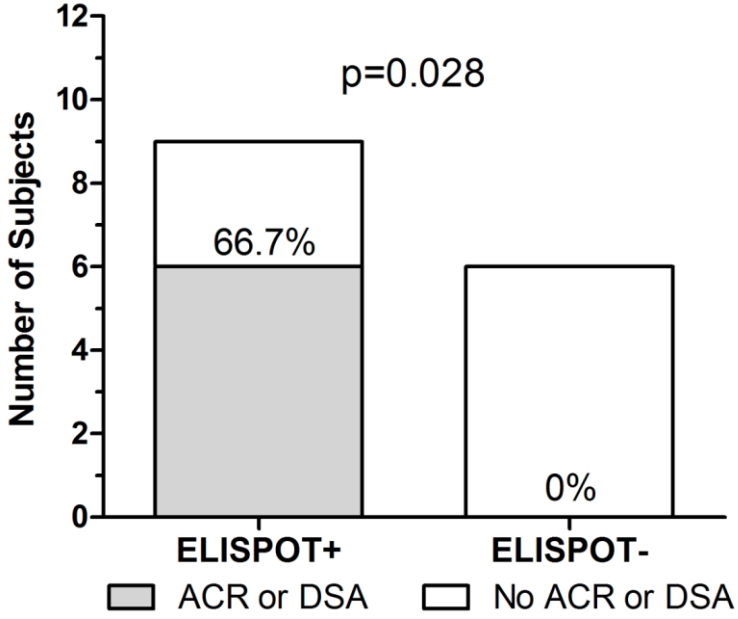


Pre-transplant risk assessment
High epitope load associates with development of de novo DQ DSA in the CTOT09 cohort



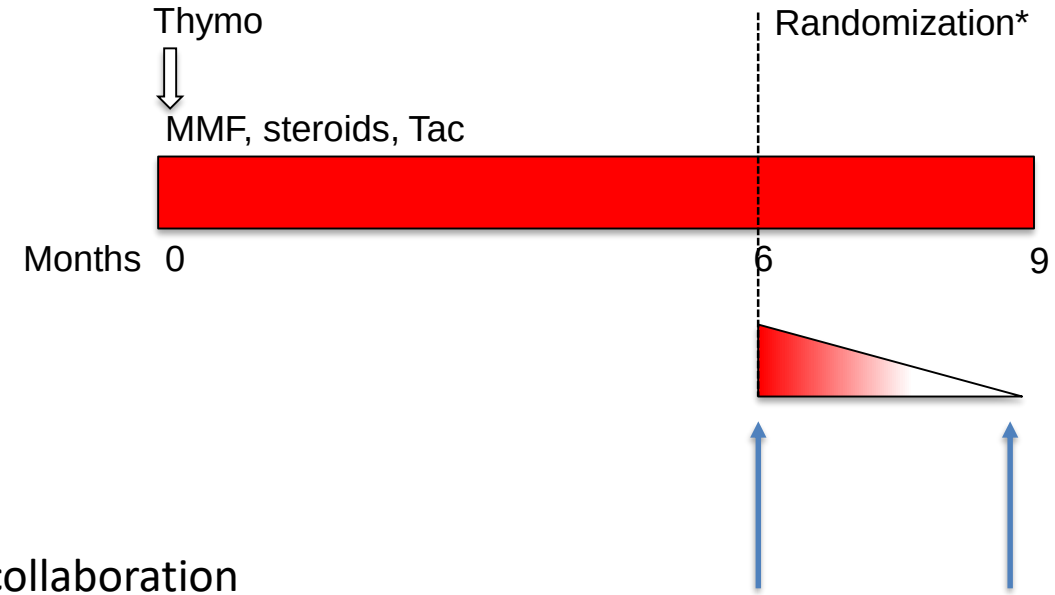
Hricik et al J Am Soc Nephrology 2015

Pre transplant risk assessment
ACR/DSA upon withdrawal associated with high pre-transplant anti-donor IFNγ ELISPOTs in the CTOT-09 cohort



Hricik et al J Am Soc Nephrology 2015

Do peripheral blood gene expression profiles obtained before and after withdrawal provide insight?

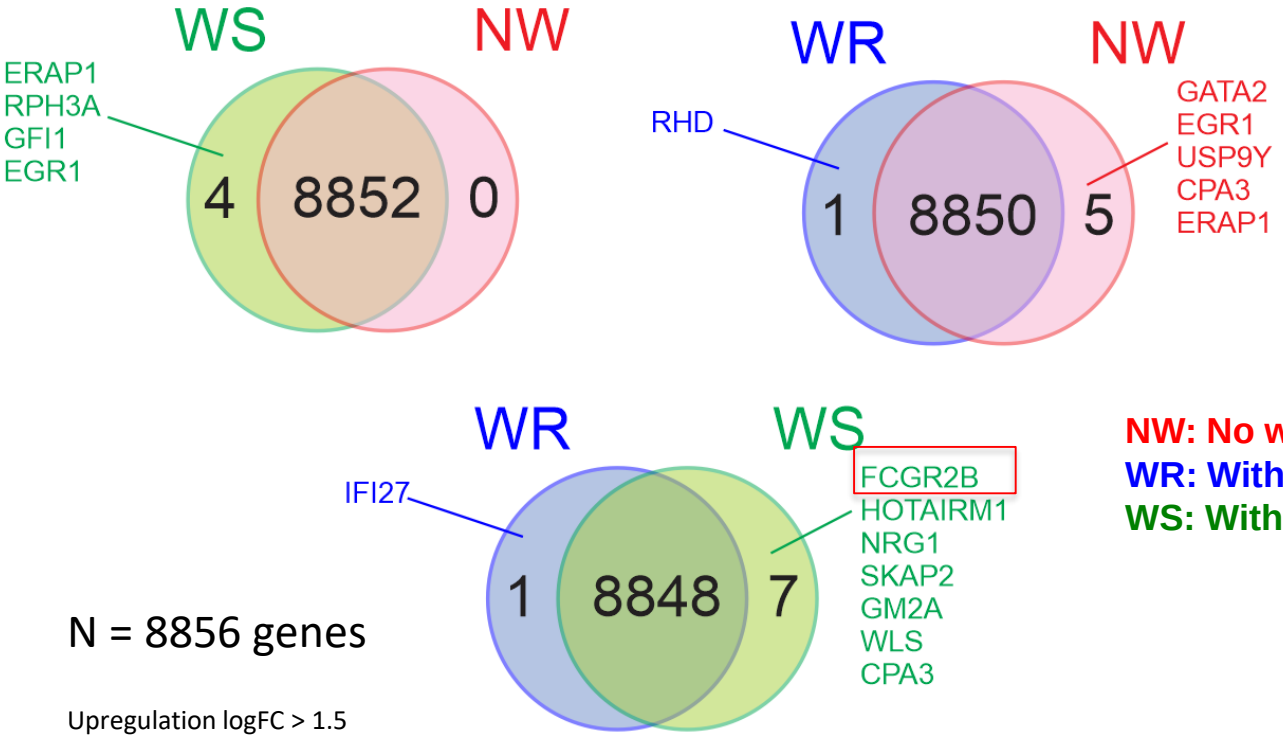


Work done in collaboration with Dan Salomon, Scripps

PBMC RNA Affimetryx Array analysis

Few Differences in Transcriptional Programs at Baseline (before randomization)

Differential Gene Expression

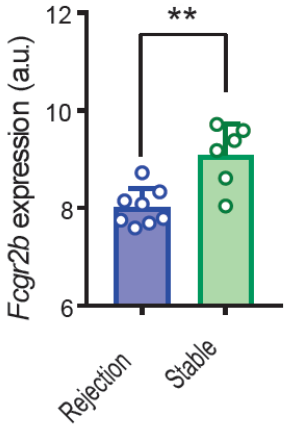


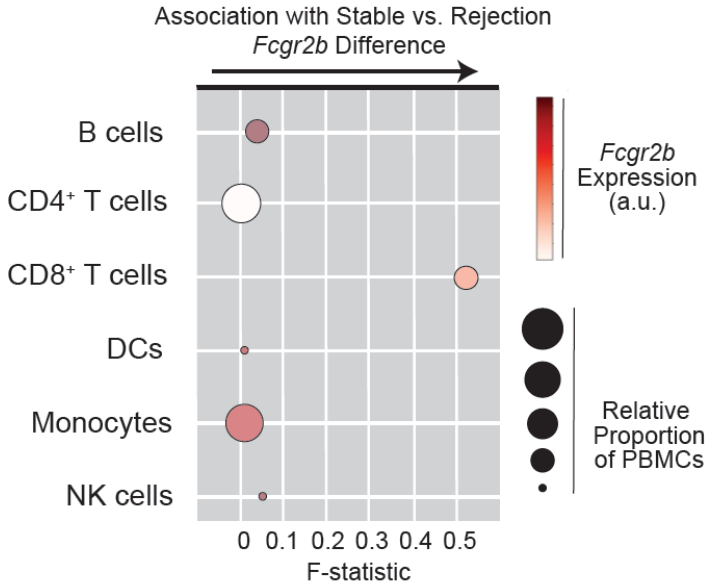
NW: No withdrawal (n=4)
WR: Withdrawal AR (n=8)
WS: Withdrawal stable (n=6)

N = 8856 genes

Upregulation logFC > 1.5
Downregulation flogFC < -1
p<0.001 (no adjustment)

FcgR2b has coinhibitory functions on CD8+ T cells



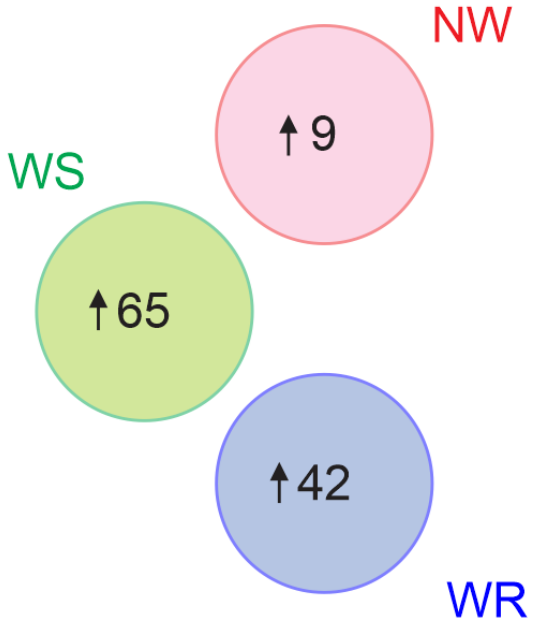


Work done in collaboration with Mandy Ford, Emory

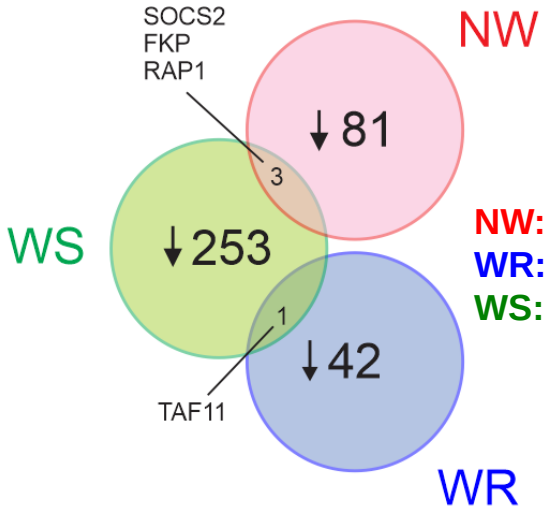
Evolution of the transcriptional program between 0 and 3 months post-randomization

- The three groups have distinct upregulated transcriptional programs.
- The Withdrawal Stable group is characterized by a large downregulatory transcriptional program.

Upregulated genes



Downregulated genes



NW: No withdrawal (n=4)
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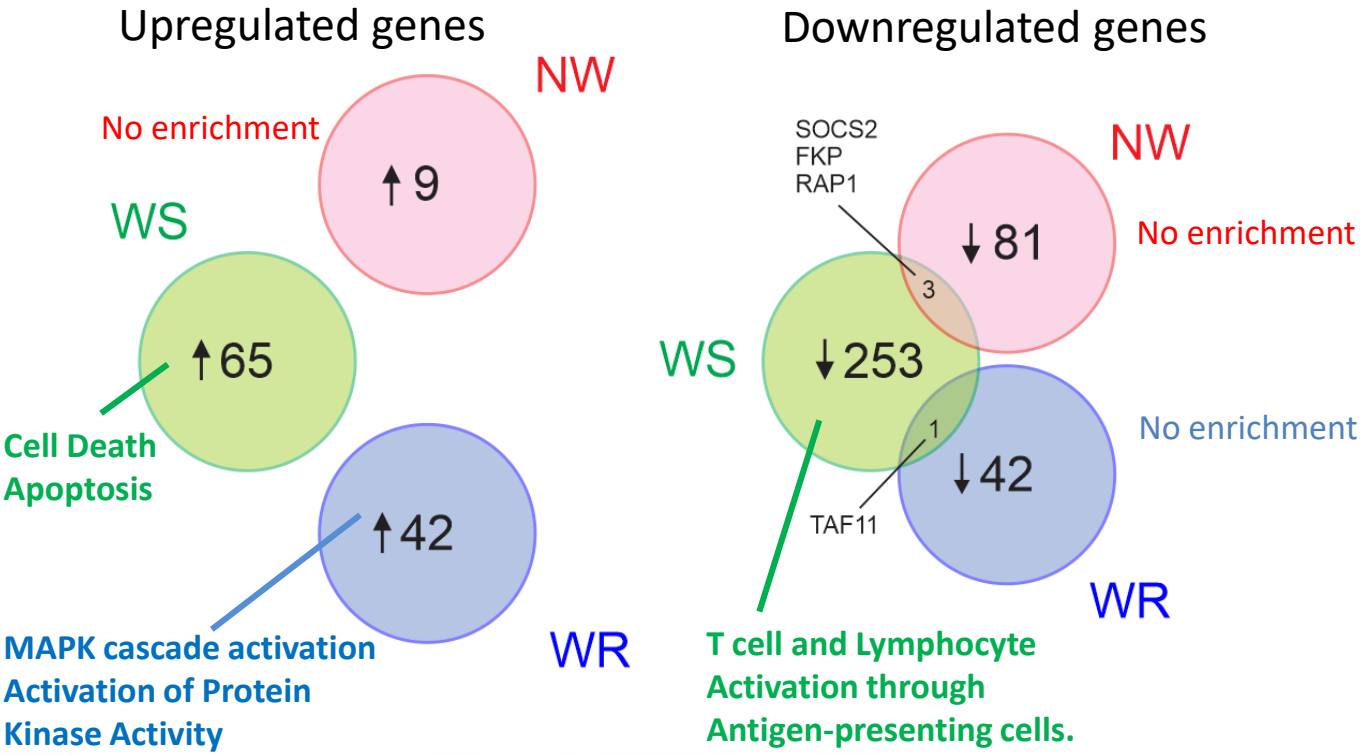
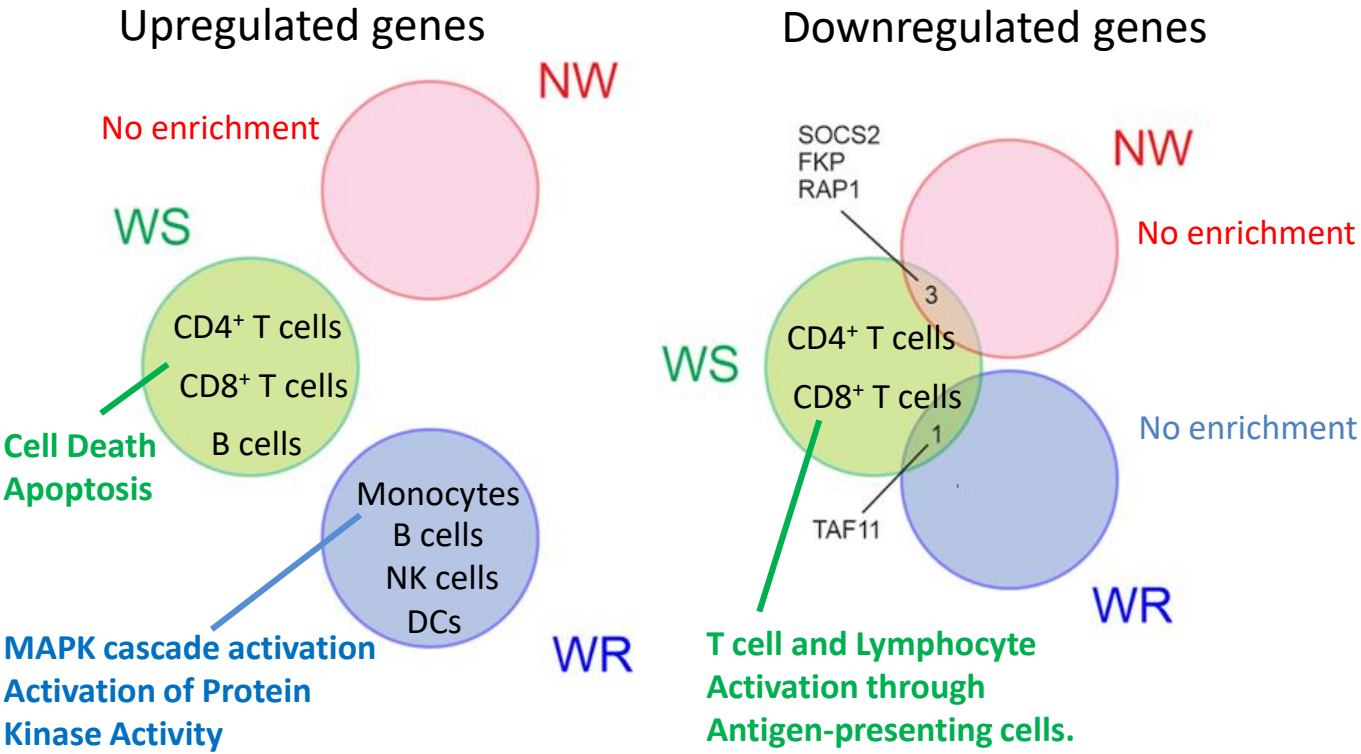
Evolution of the transcriptional program between 0 and 3 months post-randomization

Gene Ontology (GO) Term Enrichment



Evolution of the transcriptional program between 0 and 3 months post-randomization

CellCODE analysis (SPV estimation + interaction model + GO term enrichment)



Gene expression profiling prior to and during Tac withdrawal has potential to guide decision-making

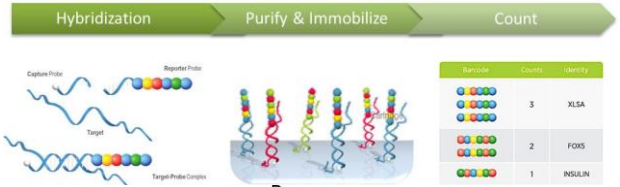
Post transplant biomarkers

- Urine
  - Gene expression (PCR/nanostring)
  - cfDNA
  - Protein (chemokines)
- Blood
  - Gene expression patterns
  - cfDNA
  - others

The NEW ENGLAND JOURNAL of MEDICINE

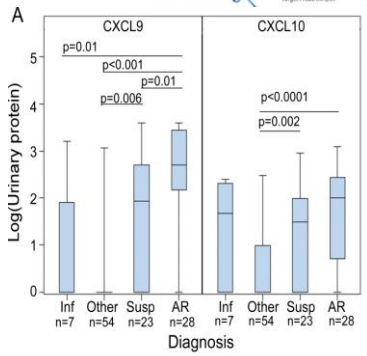
ORIGINAL ARTICLE

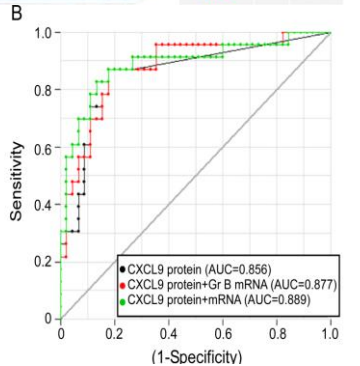
Urinary-Cell mRNA Profile and Acute Cellular Rejection in Kidney Allografts



Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant

John J. Friedewald[1] | Sunil M. Kurian[2] | Raymond L. Heilman[3] | Thomas C. Whisenant[4] | Emilio D. Poggio[5] | Christopher Marsh[2] | Prabhakar Baliga[6] | Jonah Odim[7] | Merideth M. Brown[7] | David N. Ikle[8] | Brian D. Armstrong[8] | jane I. charette[1] | Susan S. Brietigam[1] | Nedjema Sustento-Reodica[1] | Lihui Zhao[1] | Manoj Kandpal[1] | Daniel R. Salomon[2†] | Michael M. Abecassis[1] | for the Clinical Trials in Organ Transplantation 08 (CTOT-08)





Identification of Common Blood Gene Signatures for the Diagnosis of Renal and Cardiac Acute Allograft Rejection

Li Li[1*], Kiran Khush[2*], Szu-Chuan Hsieh[1,3], Lihua Ying[1], Helen Luikart[2], Tara Sigdel[1,3], Silke Roedder[1,3], Andrew Yang[2], Hannah Valantine[2*], Minnie M. Sarwal[1,3*]

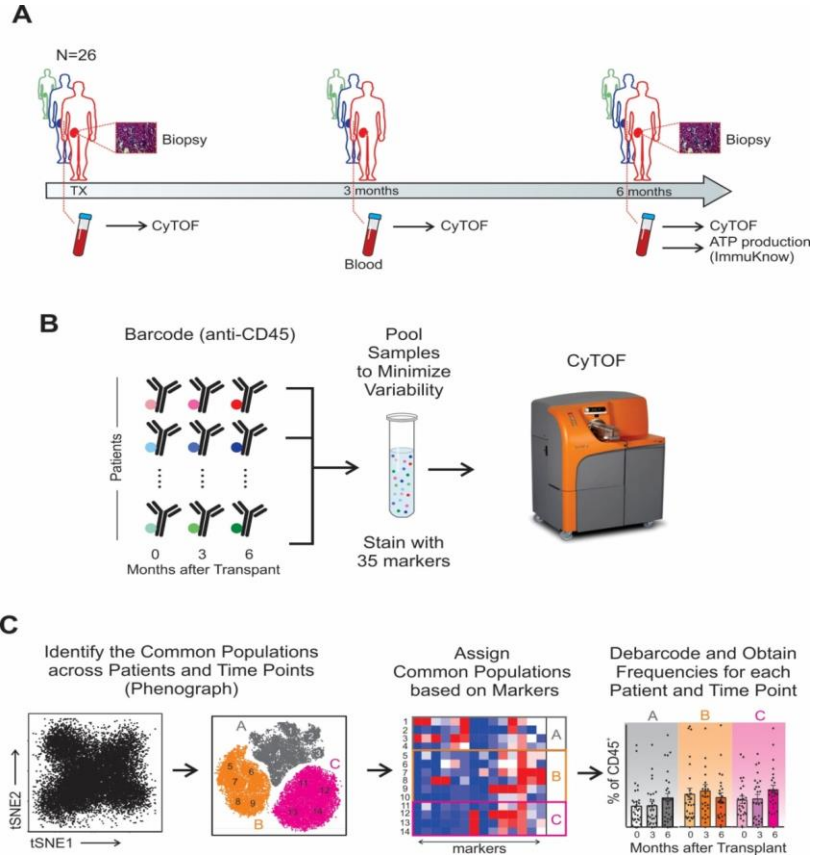
1 Department of Pediatrics, Stanford University, Palo Alto, California, United States of America, 2 Division of Cardiovascular Medicine, Department of Medicine, Stanford University, Palo Alto, California, United States of America, 3 California Pacific Medical Center Research Institute, San Francisco, California, United States of America

Peripheral blood T cell exhaustion as a biomarker for posttransplant outcome

- A differentiation state that prevents immunopathology in situations of persistently high antigen load and inflammation
- Exploited by pathogens and tumors to dampen or silence potentially protective immunity
- Associated with PD1 expression (target of checkpoint blockade)
- Functionally: progressively decreased proliferative capacity and interleukin-2 (IL-2) production followed by a reduced ability to secrete tumor necrosis factor a (TNFα) and interferon γ (IFNγ)
- Role in transplantation is unclear but more exhaustion is hypothesized to be associated with better outcomes (opposite of tumors)

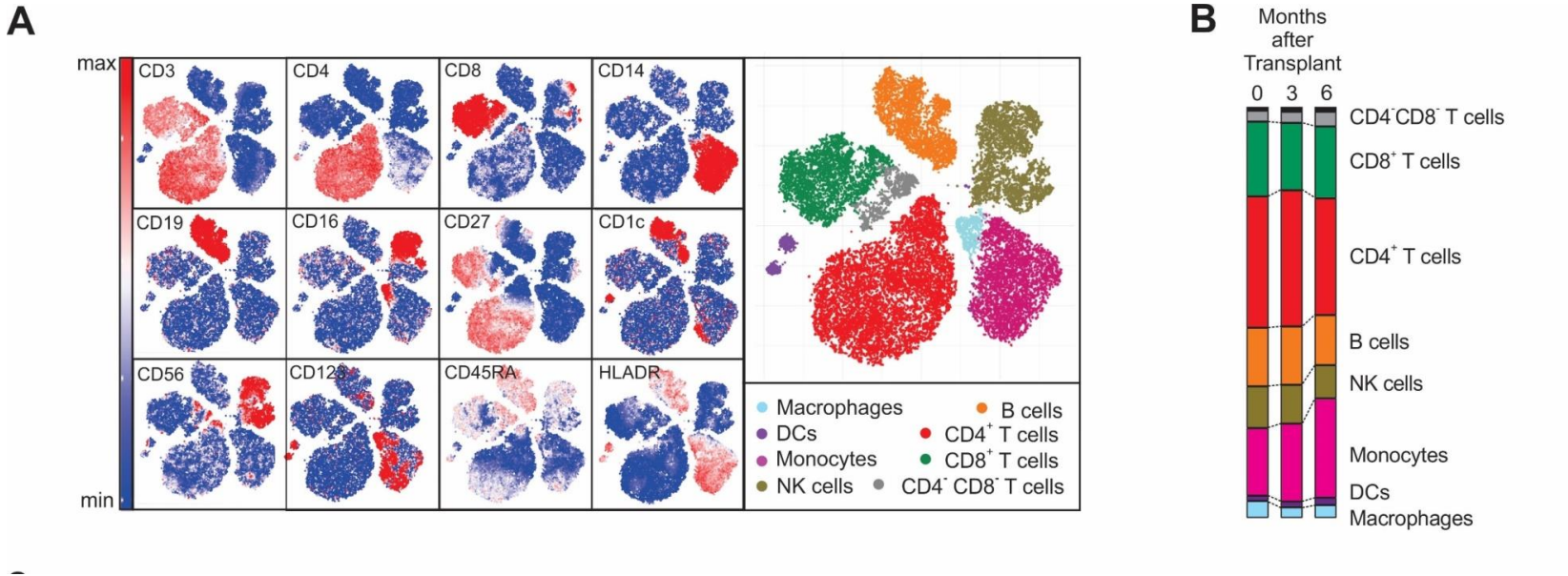
Exhaustion and kidney transplant outcomes

Paolo Cravedi and Miguel Fribourg



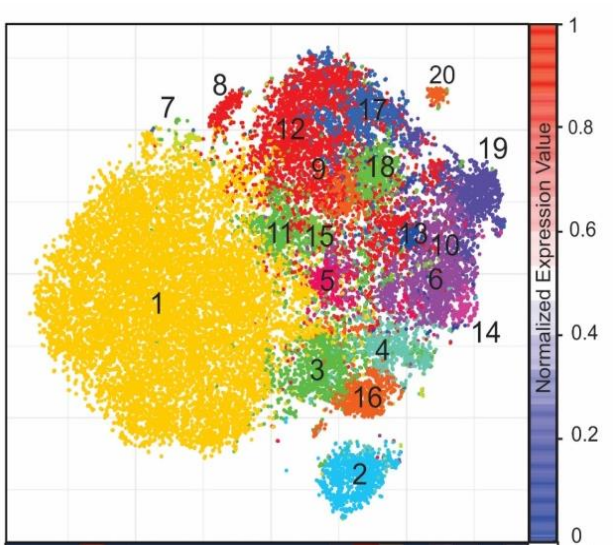
Kidney International 2019, in press
In revision

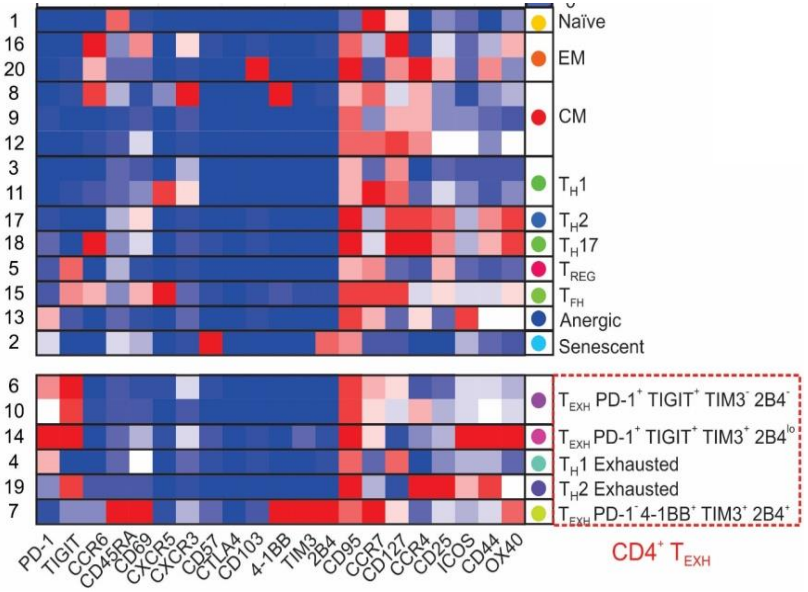
26 CTOT01 subject samples (frozen) studied at 0, 3 and 6 mo post-transplant



Kidney International 2019, in press

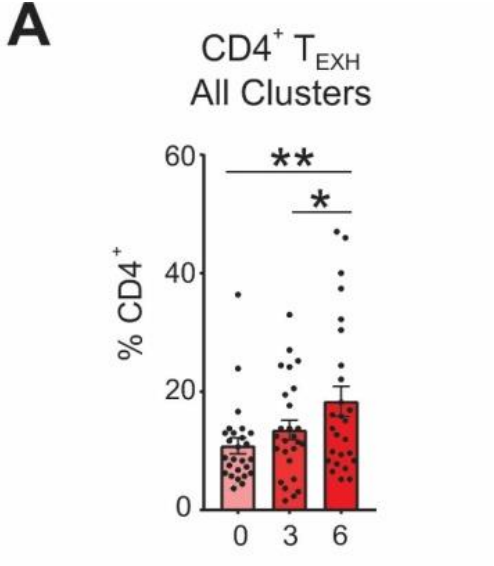
Identification of T cell exhaustion subsets





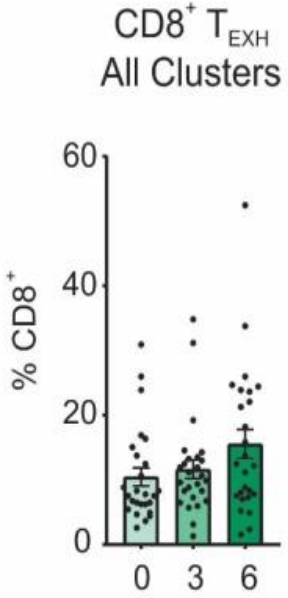
Kidney International 2019, in press

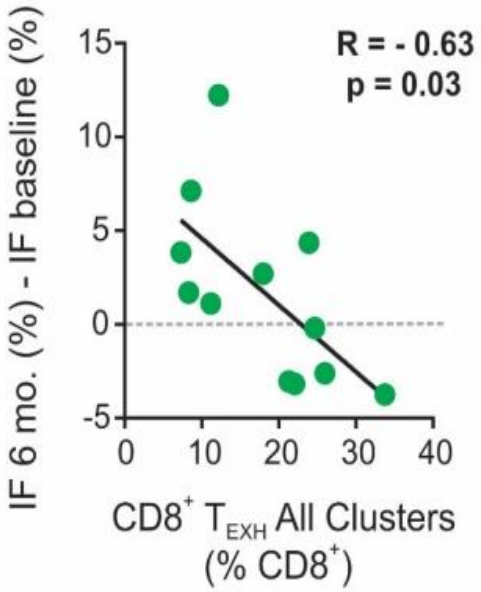
Increased CD4$^+$ T_{EXH} cells post-transplant, inverse correlation with ATP production and association with graft fibrosis



Kidney International 2019, in press

26 CTOT01 subject samples (frozen) studied at 0, 3 and 6 mo post transplant: $CD8^+$ T cell subsets

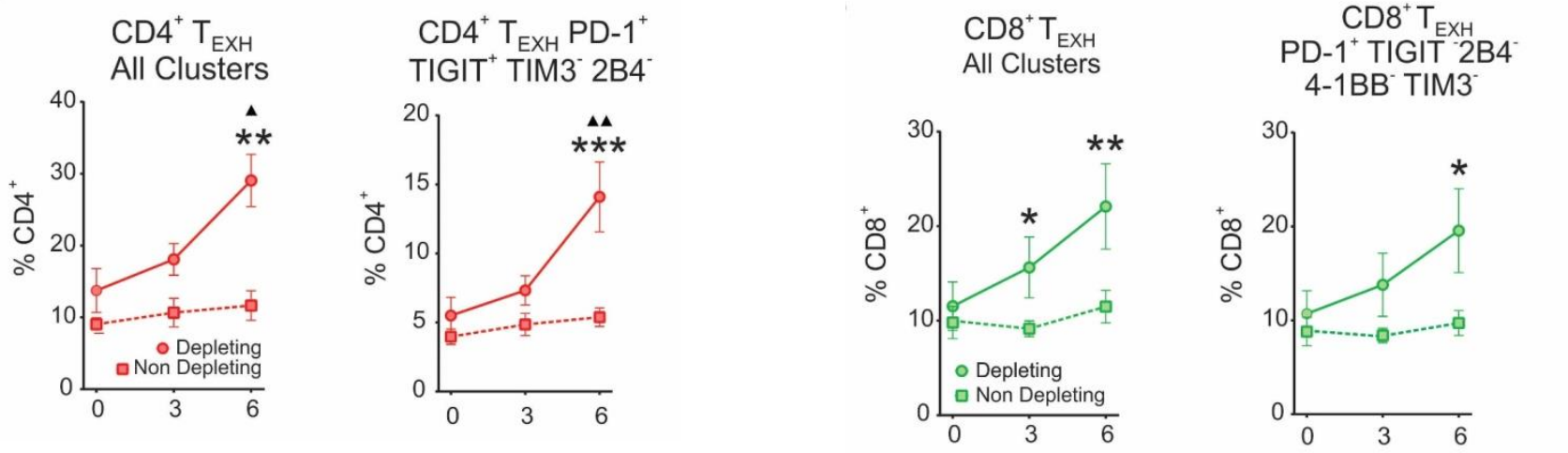




Kidney International 2019, in press

T cell exhaustion phenotype associates with ATG induction

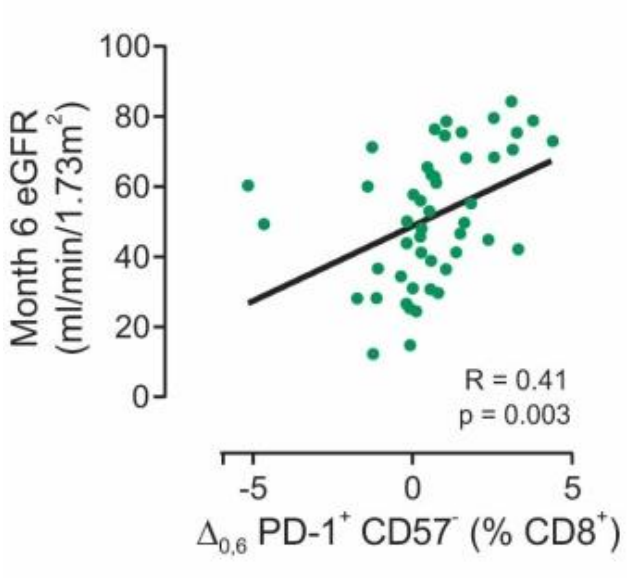
CTOT01 was an observational study and ATG given at discretion of investigator

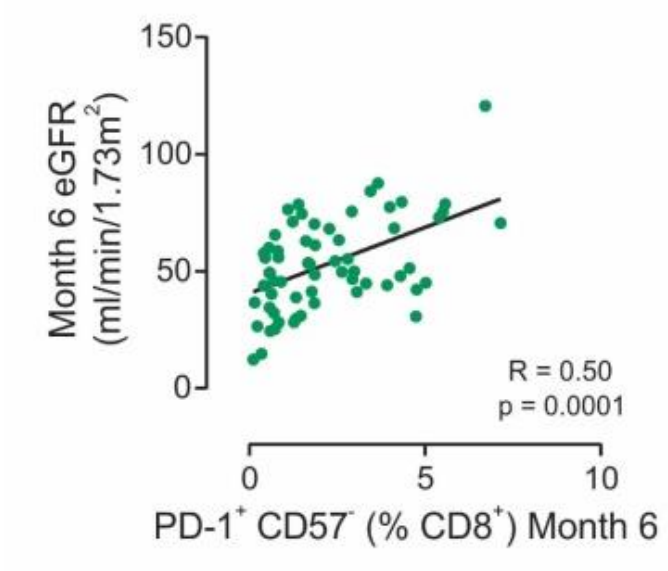


Months post-transplant

Kidney International 2019, in press

50 CTOT19 subjects: flow cytometry using PD1 and CD57
Texh at 6 mo associates with better eGFR

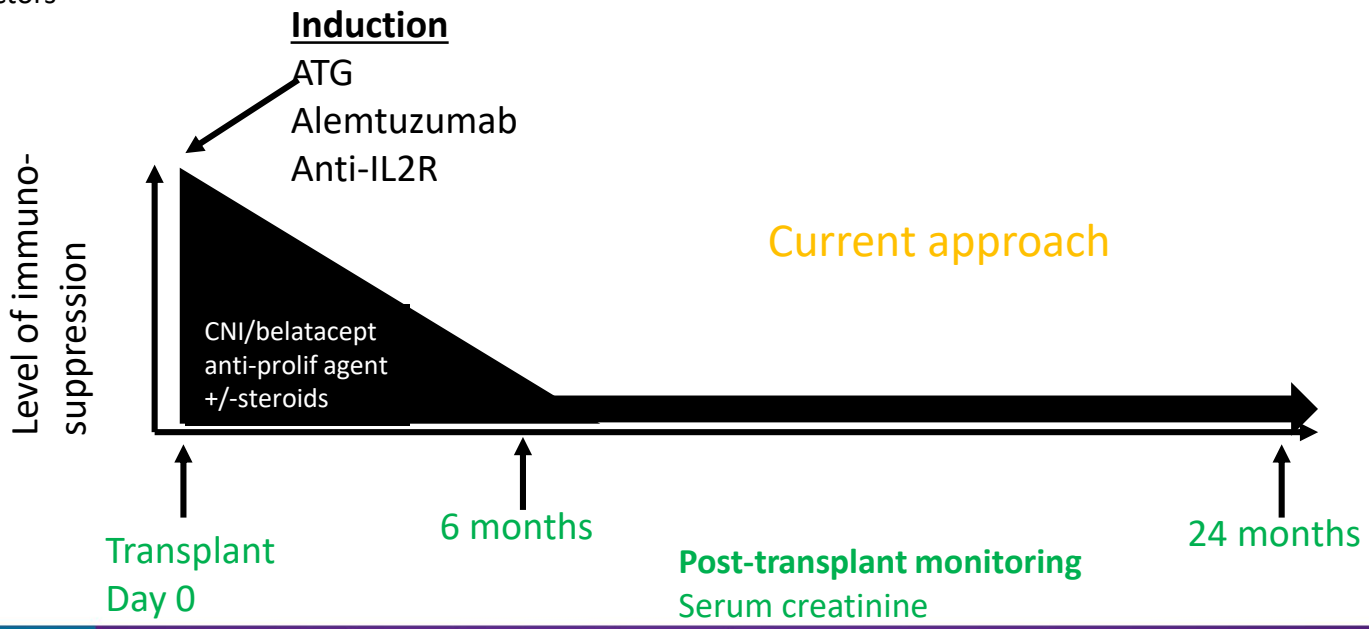




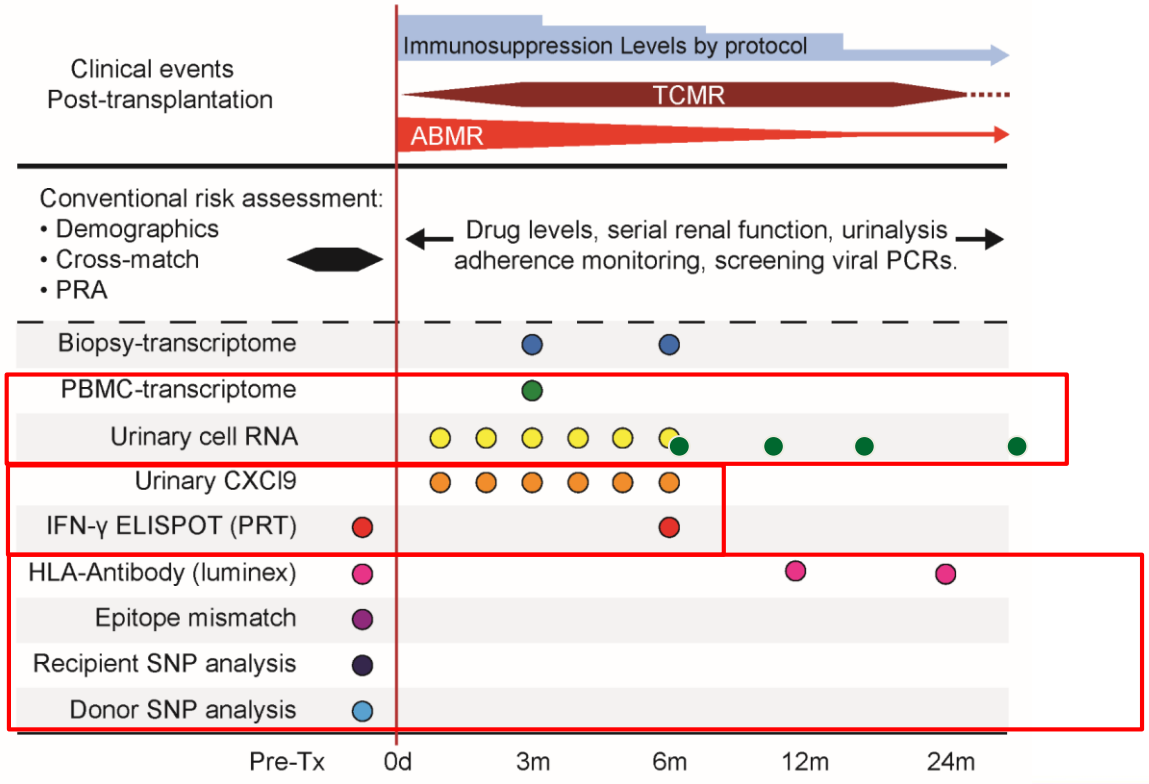
Kidney International 2019, in press

What do we do now?

Pre-transplant
Risk assessment
HLA typing
Cross matching
Implantation biopsy
Clinical risk factors



Precision/Individualized Care



Menon et al JASN 2017

Need to perform controlled trials to assess utility of biomarker-directed changes in therapy

- Incorporate validated biomarkers into clinical trial designs
  - Test whether biomarker based changes in therapy including during drug withdrawal detect subclinical injury and improve outcomes
  - Randomized controlled trials
    - One arm standard of care
    - One arm treat based on biomarker status
    - Is outcome better in the biomarker guided group?

Heeger Consortium CTOT Collaborators

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R Fairchild, E Poggio -- Cleveland Clinic	*Cleveland, United States*
K Tinckam -- Toronto General Hospital	*Toronto, Canada*
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Thank you

Funded By NIAID