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Sharktank

The Force is in the Urine

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CTOT Clinical Trials in Organ Transplantation

UTTING 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

I have no relevant financial relationships.

Characteristics of an Ideal biomarker

- It should
  - be positive prior to histopathological changes, and should be indicative of active damage.
  - be sensitive and also correlate with the severity of damage.
  - provide specificity- differentiating various types of injury
  - be highly reproducible
  - be accessible in the peripheral tissue, e.g. In the blood or the urine.
  - be analytically stable so it can be measured after some time has passed
  - be within the pathway of a known mechanism of disease.
  - be cheap, easy to perform, and ideally be applicable in point of care settings

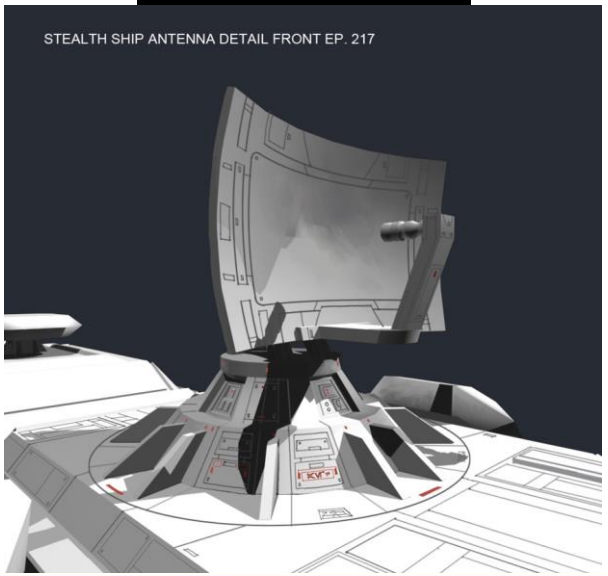
Simpler has its advantages for a biomarker

Biomarkers in transplantation-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 of graft injury
- Predict DGF
- Detect Immune tolerance

Biomarkers in transplantation-Noninvasive diagnosis of graft injury

STAR WARS RADAR



- Prevent morbidity of biopsy
- Differentiate rejection from other causes of acute transplant dysfunction
- Assess response to anti-rejection therapy
- Detect subclinical or incipient injury and or fibrosis with stable graft function

Biomarkers that can detect subclinical injury would be helpful

Urine: the window to the kidney's soul

- **Molecular analysis of transplant rejection: marching onward JEM 2013** Fadi G. Lakkis, Timothy R. Billiar
- M. Suthanthiran
- (note that no one has ever made that analogy for blood or biopsy samples)

Using urine biomarkers to diagnose rejection (and differentiating it from other diagnoses) in transplant recipients with an increased serum creatinine over baseline

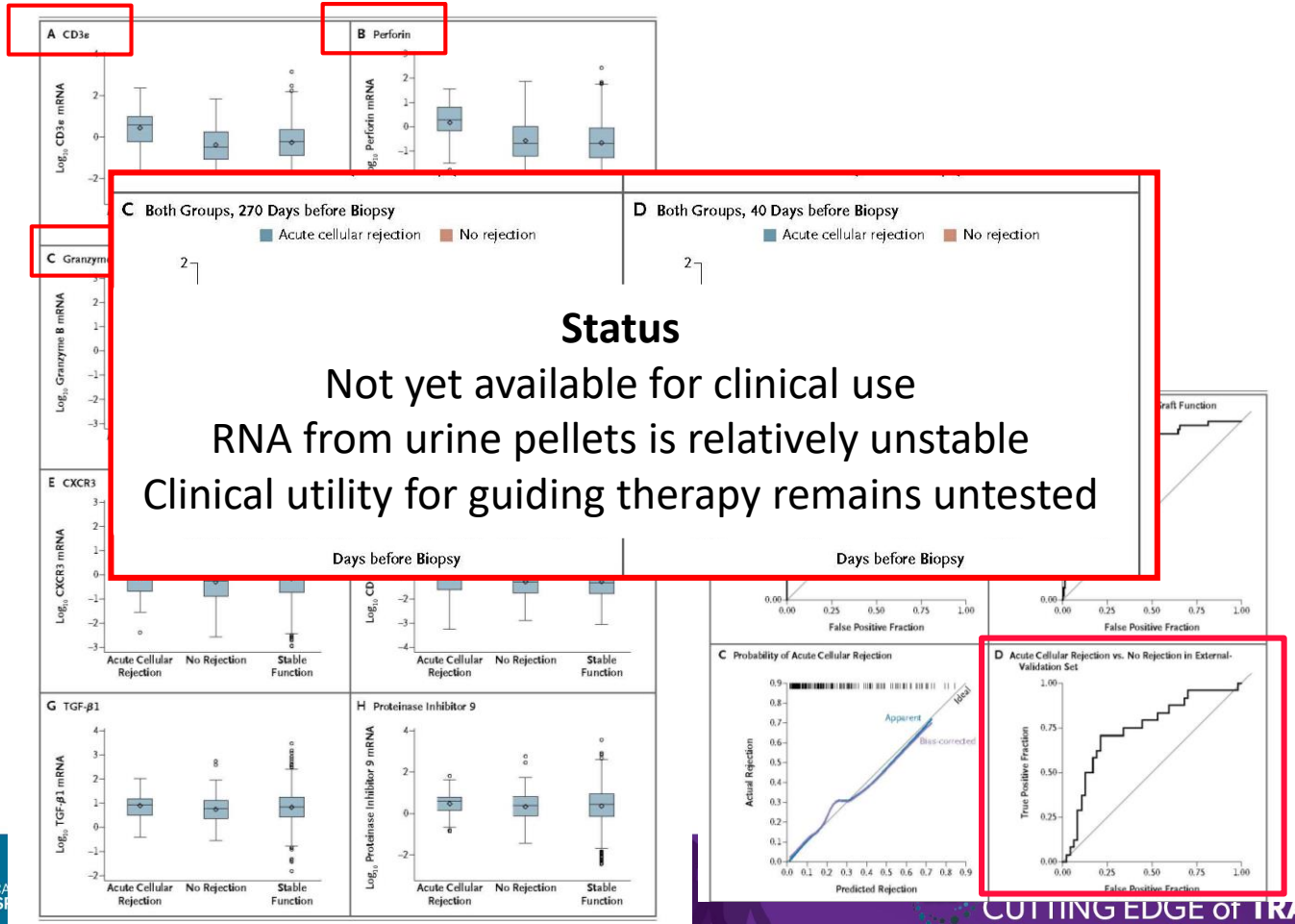
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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

Manikkam Suthanthiran, M.D., Joseph E. Schwartz, Ph.D., Ruchuang Ding, M.D., Michael Abecassis, M.D., Darshana Dadhania, M.D., Benjamin Samstein, M.D., Stuart J. Knechtle, M.D., John Friedewald, M.D., Yolanda T. Becker, M.D., Vijay K. Sharma, Ph.D., Nikki M. Williams, B.S., Christina S. Chang, B.S., Christine Hoang, B.S., Thangamani Muthukumar, M.D., Phyllis August, M.D., M.P.H., Karen S. Keslar, M.S., Robert L. Fairchild, Ph.D., Donald E. Hricik, M.D., Peter S. Heeger, M.D., Leiya Han, M.D., M.P.H., Jun Liu, Ph.D., Michael Riggs, Ph.D., M.P.H., David N. Ikle, Ph.D., Nancy D. Bridges, M.D., and Abraham Shaked, M.D., Ph.D., for the Clinical Trials in Organ Transplantation 04 (CTOT-04) Study Investigators

N ENGL J MED 369;1 NEJM.ORG JULY 4, 2013



Status

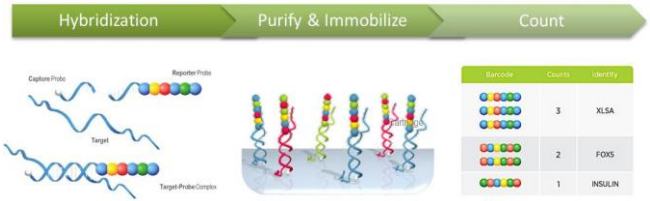
Not yet available for clinical use

RNA from urine pellets is relatively unstable

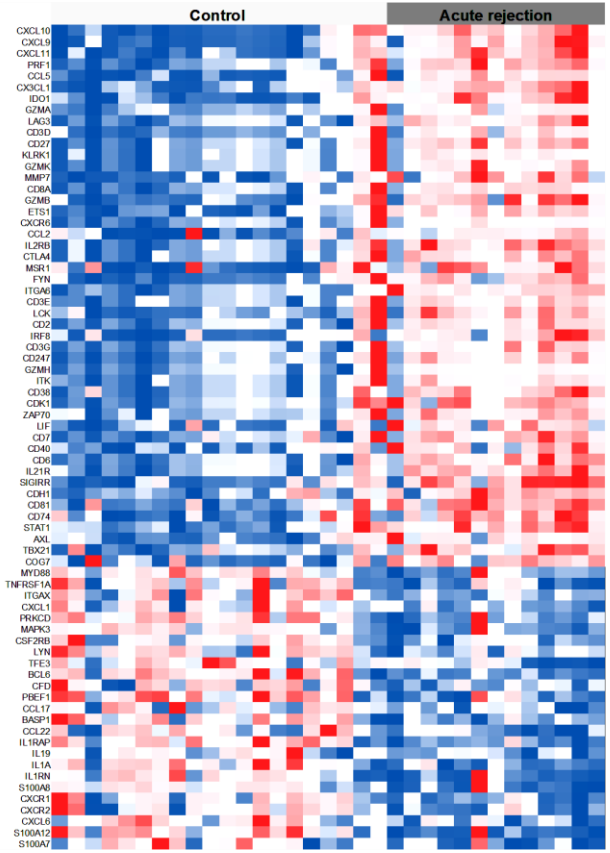
Clinical utility for guiding therapy remains untested

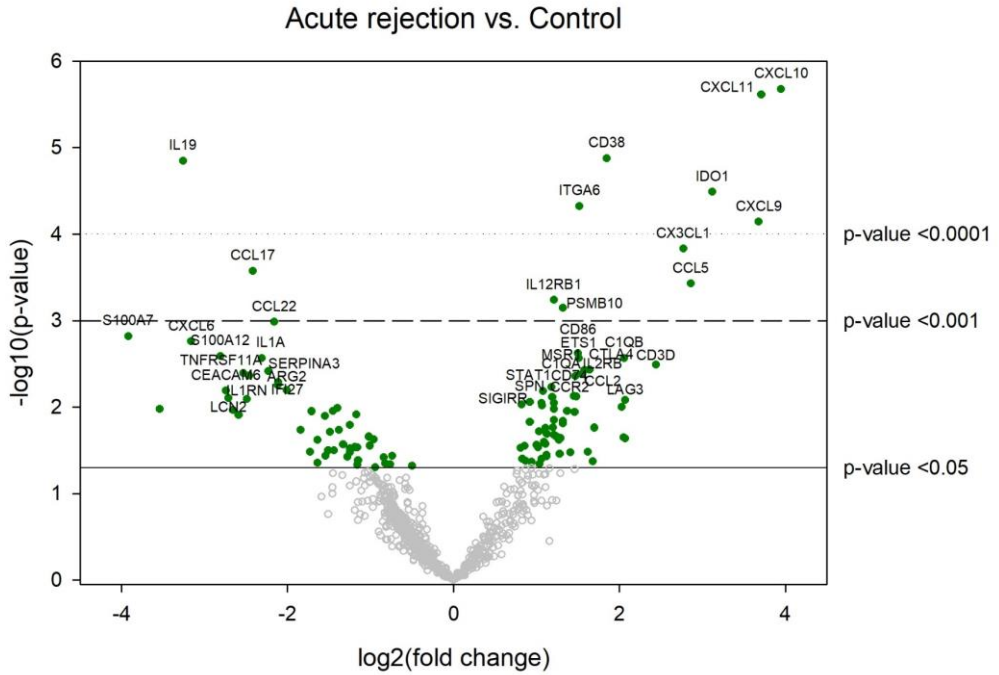
Nanostring assessments of urinary gene expression profiles

- Collaboration through CTOT with Rob Fairchild, Cleveland Clinic and Rosalind Mannon, UAB
- Diagnosing rejection in context of acute graft dysfunction
- Nanostring rapidly quantifies hundreds of RNA species without need for amplification
- FDA approved biomarkers have emerged from this technology in cancer

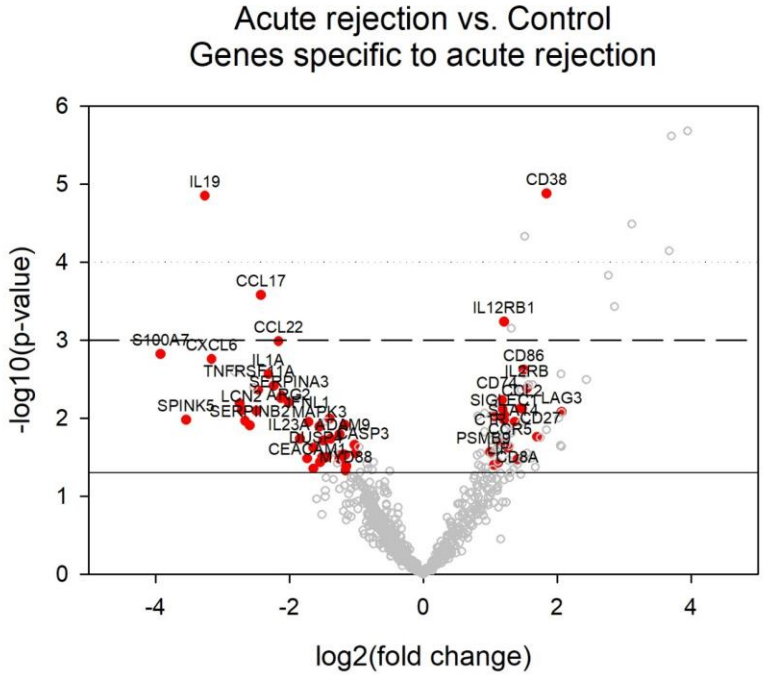


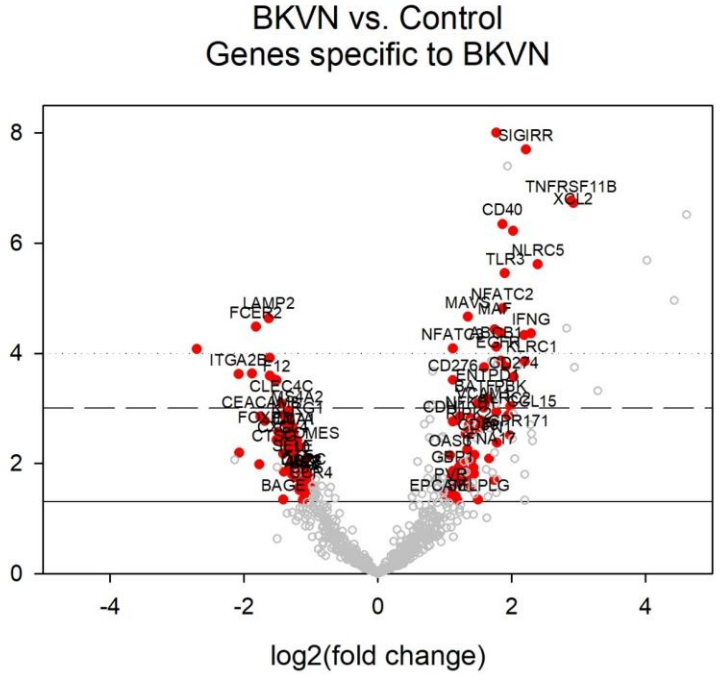
Gene expression at the time of biopsy-proven acute rejection



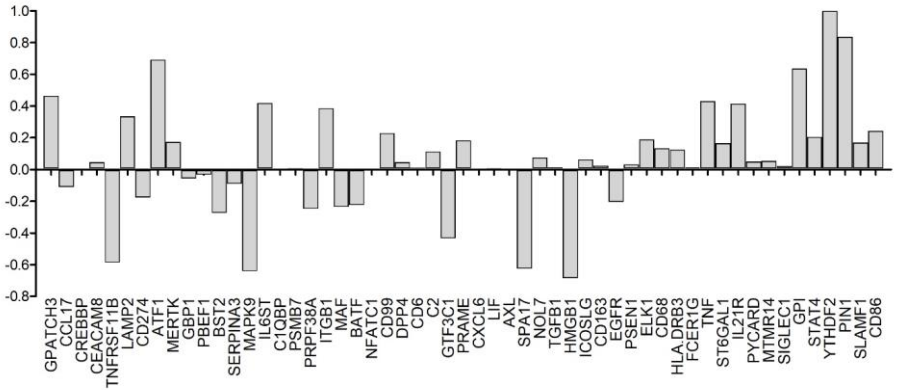


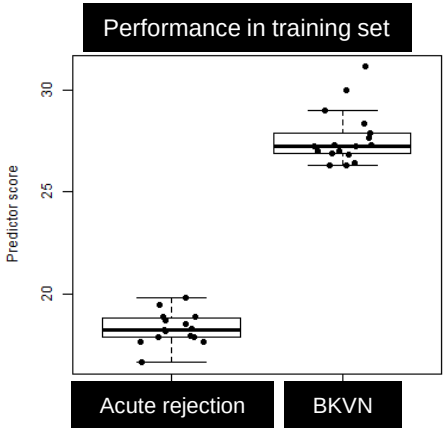
Gene expression changes unique to AR and BKVN

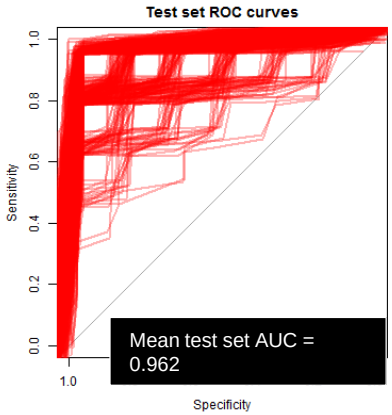




Gene expression changes in the urine distinguish injury caused by acute rejection from injury caused by BK virus nephropathy

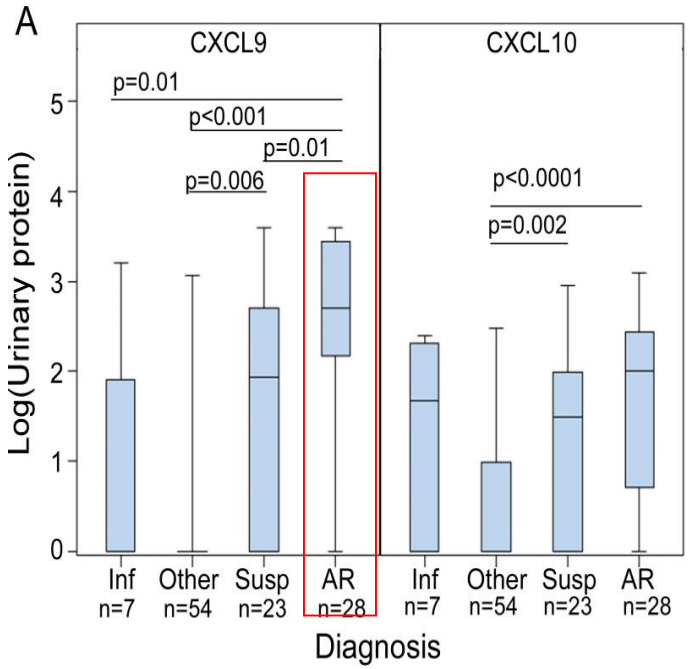






Urinary chemokines (CXCL9): is simpler good enough?

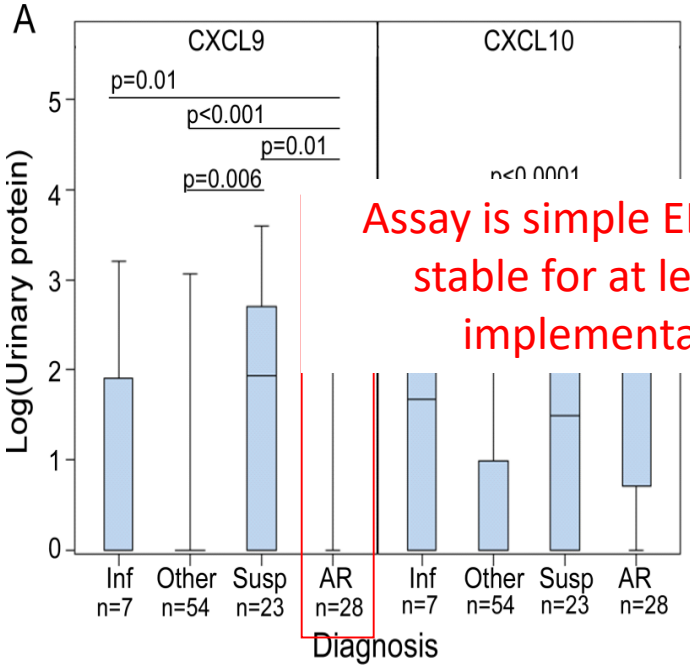
Urinary chemokine protein (ELISA) to diagnose AR

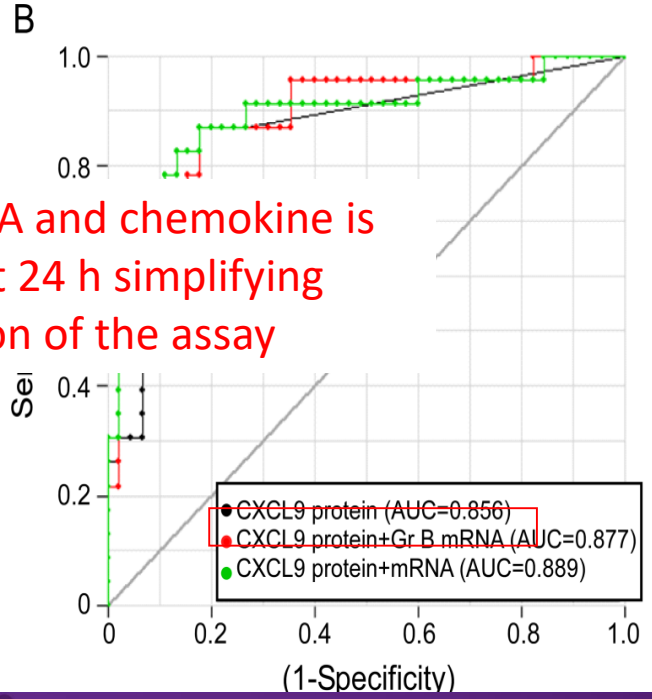


- CTOT01 patients
- Observational cohort 280 subjects

Hricik et al Am J Transplantation 2013

Urinary chemokine protein (ELISA) to diagnose AR





Assay is simple ELISA and chemokine is stable for at least 24 h simplifying implementation of the assay

Hricik et al Am J Transplantation 2013

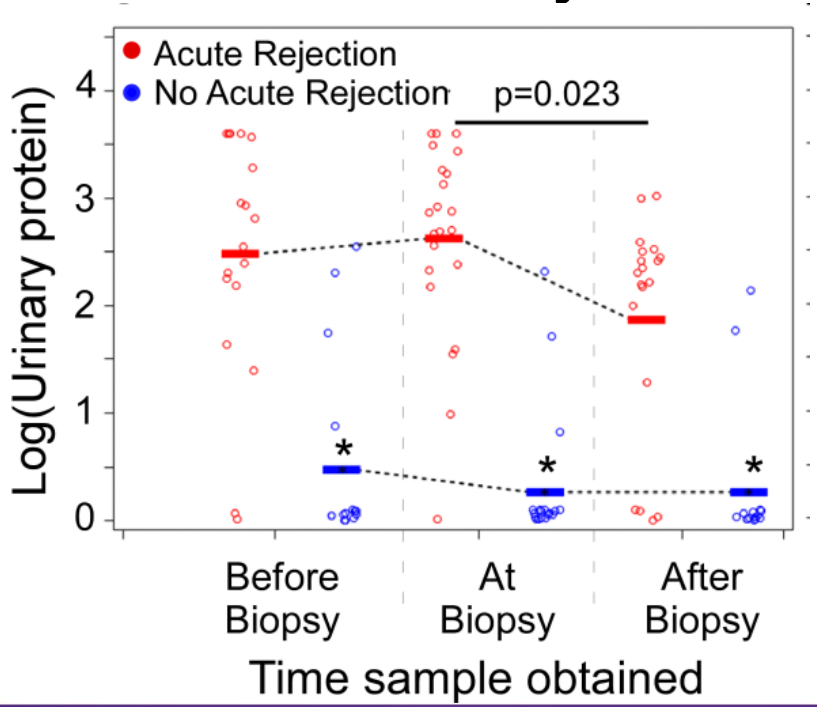
Logistic Regression and Bootstrap Validation of urinary markers for diagnosing Banff ≥1A acute rejection*

Parameter Estimates and tests			ROC-based Discrimination Measures			Positive/Negative Predictive Value	
Model Predictors	**OR(95% CI)**	**P-value**	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Univariate Models							
Granzyme B mRNA	2.26(1.30,3.92)	0.0039	0.730	70.8	81.6	65.4	85.1
CXCL9 mRNA	2.77(1.59,4.80)	0.0003	0.788	66.7	79.6	61.5	83.0
CXCL9 Protein	3.40(2.12,5.47)	<0.0001	0.856	85.2	80.7	67.6	92.0
CXCL10 Protein	3.25(1.89,5.57)	<0.0001	0.768	74.1	86.0	71.4	87.5

"False" positive results are infections including BK

Can we detect injury with the biomarker before it is clinically apparent?

Urinary CXCL9 is elevated 30 d prior to clinically detectable rejection

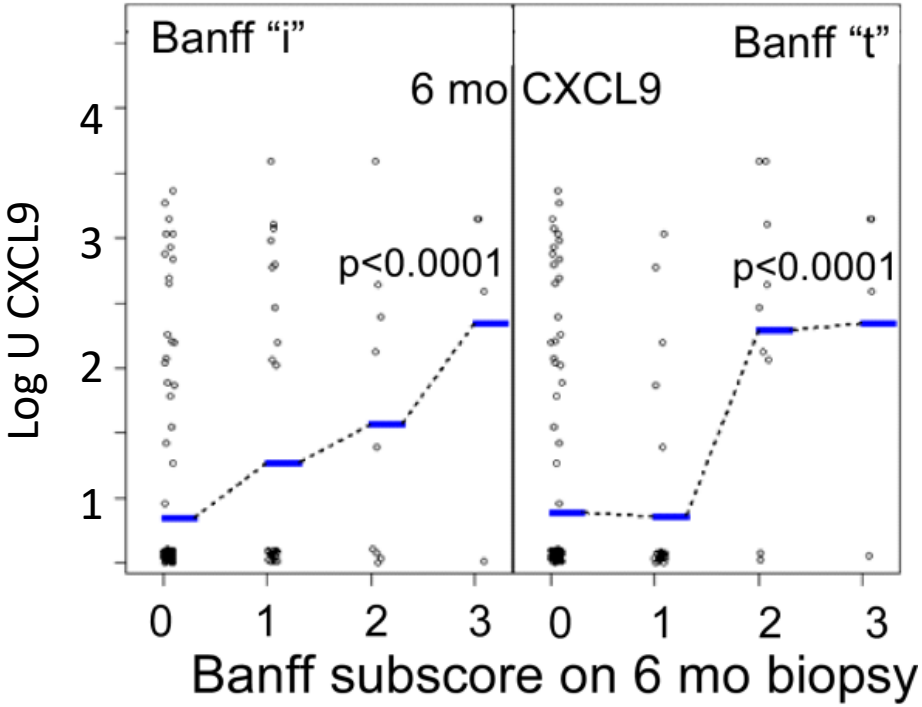


Hricik et al
Am J Transplantation
2013

Does urinary CXCL9 detect subclinical inflammation?

- We had 170 protocol biopsies at 6 mo posttransplant
- We correlated urinary CXCL9 with biopsy pathology scores (done blinded to the knowledge of the CXCL9 values)

Urinary CXCL9 correlates with “i” and “t” subscores on biopsies at 6 mo

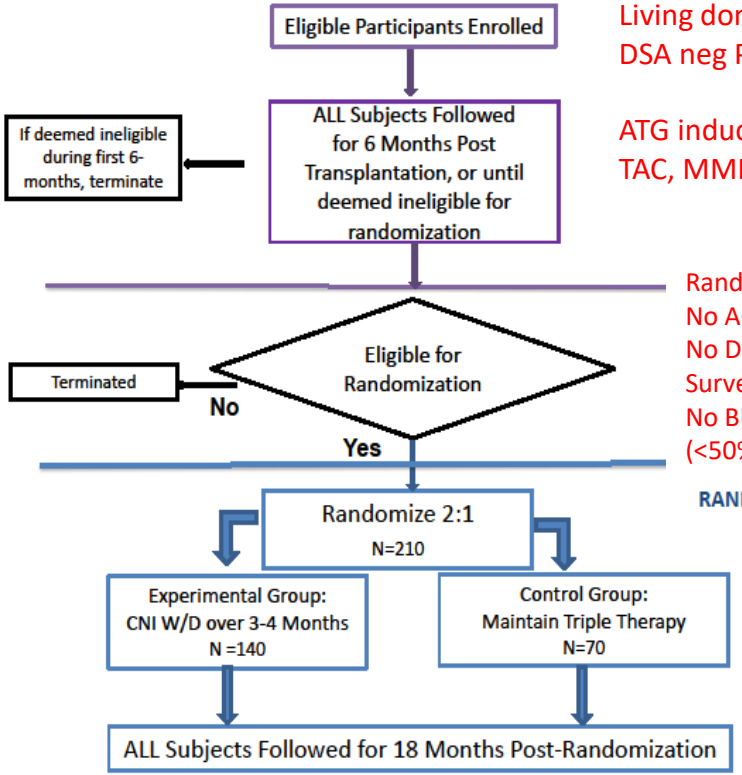


Can urinary CXCL9 detect incipient rejection in subjects undergoing decreases in immunosuppression

(maybe we don't need the big guns)?

CTOT09

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



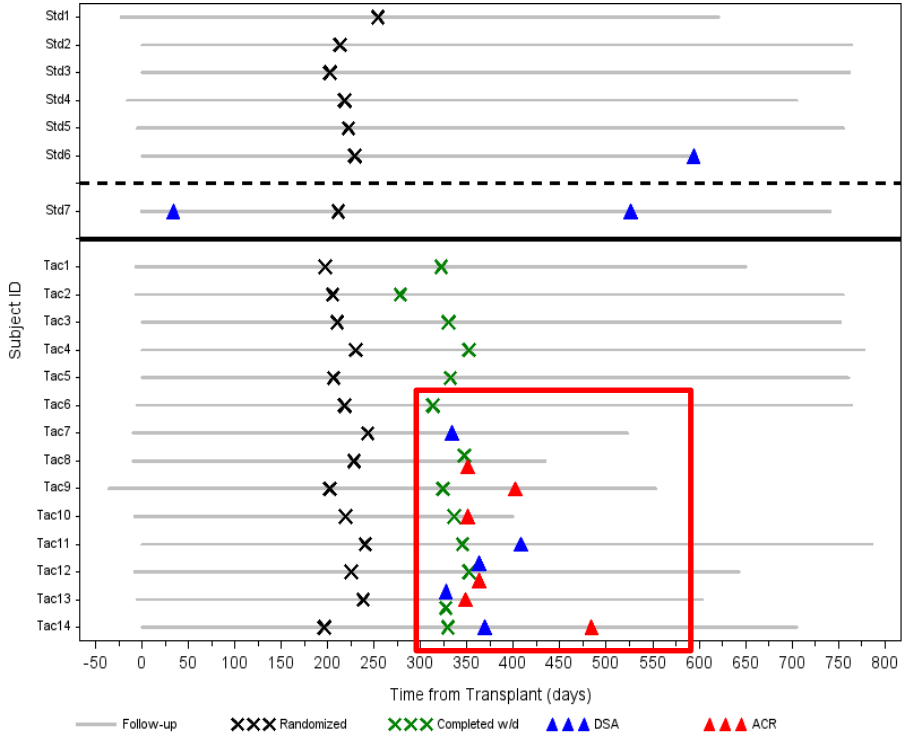
Living donor transplants
DSA neg PRA<30%

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

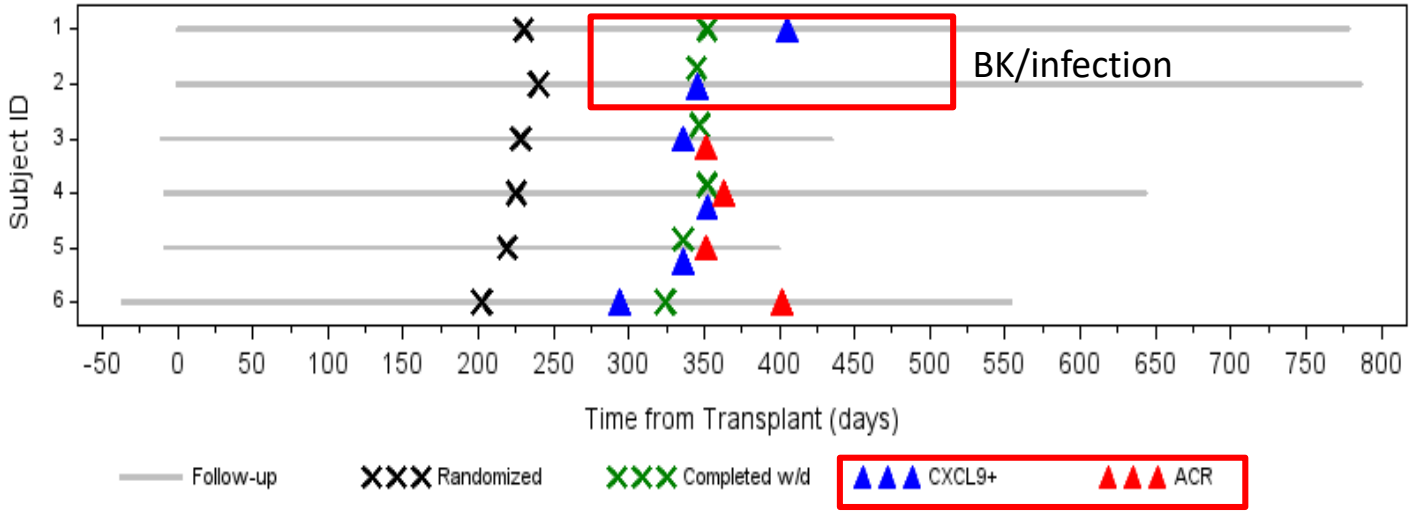
Study terminated by DSMB based on pre-defined endpoints after 21 randomized due to absence of equipoise



Is urinary CXCL9 informative?

Results

Timeline of events: CXCL9 positivity predates diagnosis of ACR during TAC withdrawal



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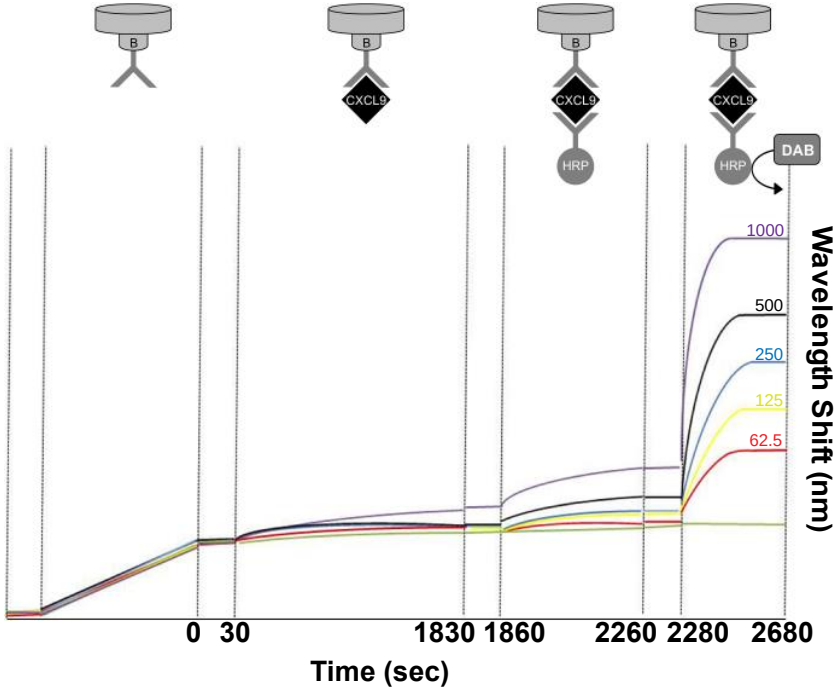
Hricik et al J Am Soc Nephrology 2015 (in press)

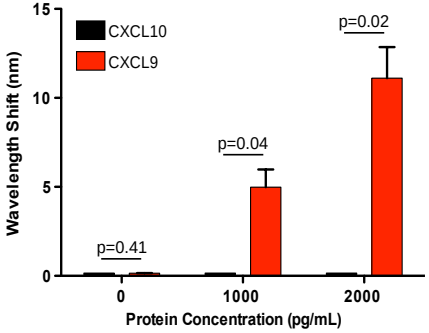
Can CXCL9 measurements be performed rapidly as a potential "point of care" test?

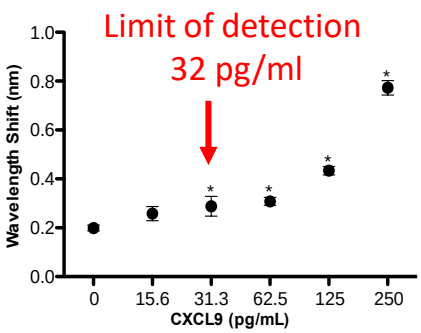
Biolayer interferometry (BLI)

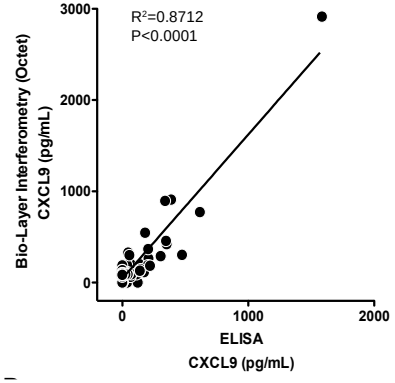


Rogue one "rapid fire" imperial Walker

BLI detection of CXCL9 is sensitive, specific and results agree with ELISAs

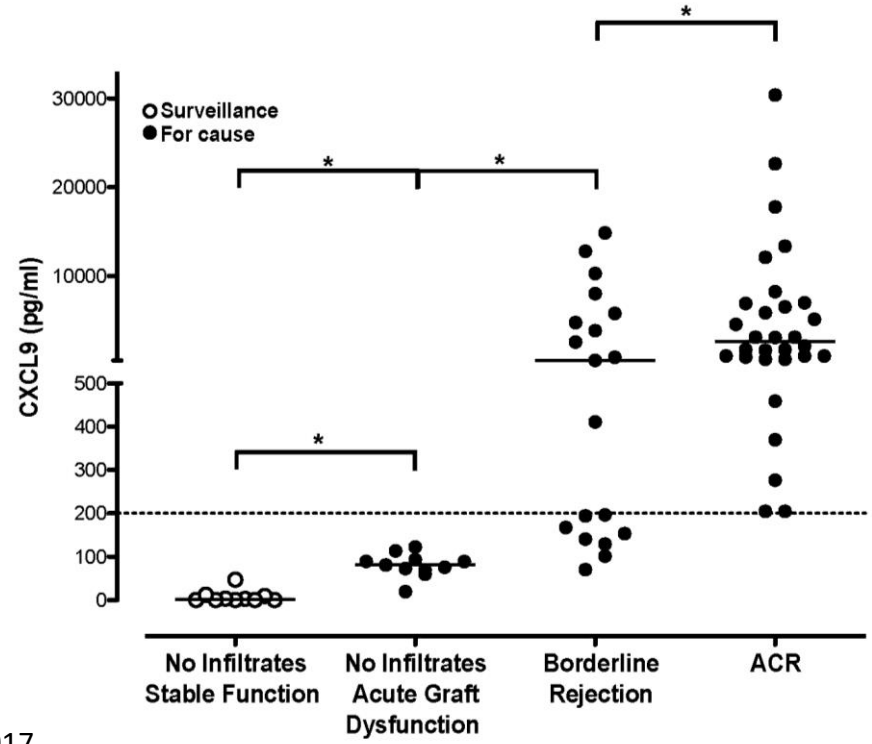






Gandolfini et al Kid Int Reports 2017

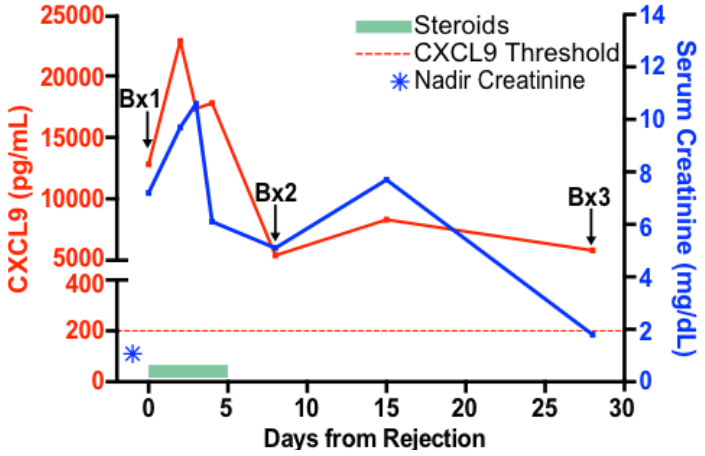
CXCL9 by BLI can diagnose ACR in BKV-neg subjects



Gandolfini et al Kid Int Reports 2017

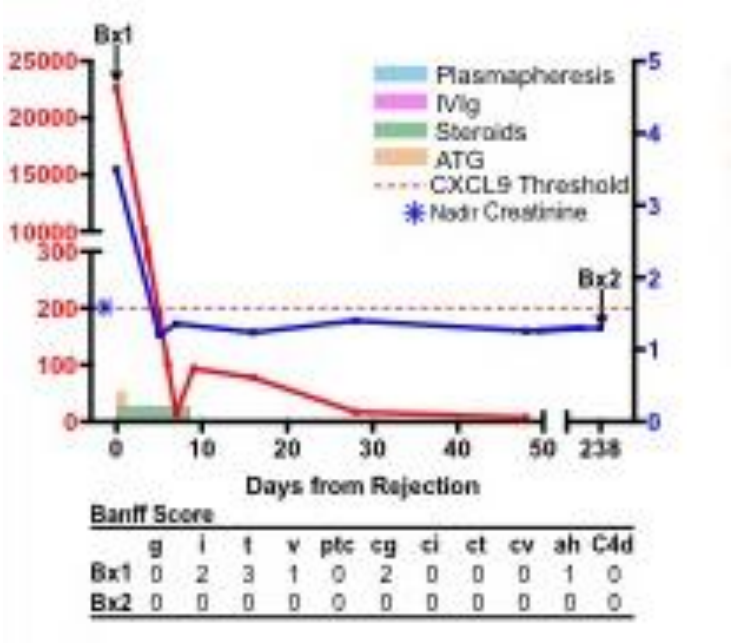
Can serial, rapid monitoring of urinary CXCL9 provide insight regarding effectiveness of anti-rejection therapy in BKV-neg subjects treated for ACR?

Serial U CXCL9 monitoring can detect persistent rejection



Banff Score

	g	i	t	v	ptc	cg	ci	ct	cv	ah	C4d
Bx1	0	2	1	0	0	0	0	0	1	0	0
Bx1	0	1	0	0	0	0	0	0	0	0	0
Bx3	0	1	1	0	0	0	1	1	0	0	



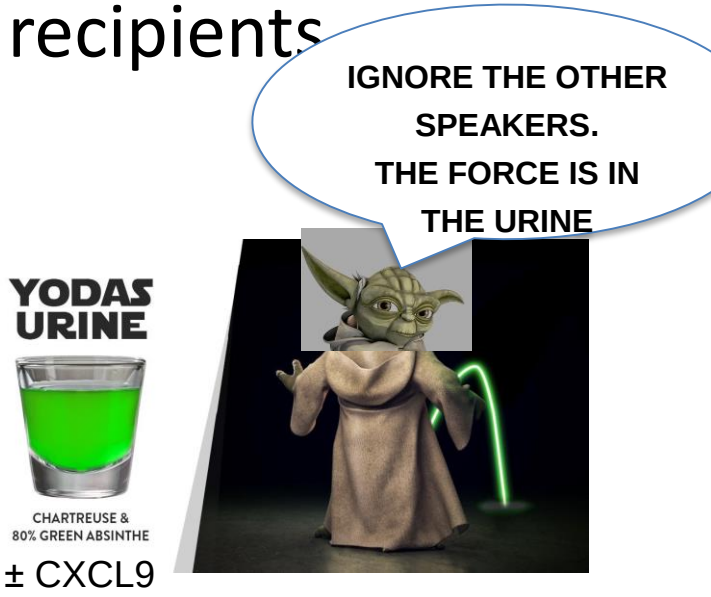
Banff Score

	g	i	t	v	ptc	cg	ci	ct	cv	ah	C4d
Bx1	0	2	3	1	0	2	0	0	0	1	0
Bx2	0	0	0	0	0	0	0	0	0	0	0

Gandolfini et al Kid Int Reports 2017

Urinary CXCL9 and urinary nanostring analyses can impact care of transplant recipients

- Diagnose rejection (may differentiate from infection)
- Detect inflammation prior to clinically evidence graft dysfunction
- Inform regarding effectiveness of therapy
- Relatively easy to perform, potential for point of care use and commercialization
- Needs to be more widely used and examined in the clinical arena
- Clinical trials need to be done to determine if therapy based on the biomarker influences outcome



Heeger Consortium CTOT Collaborators

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D Foley, U Wisc	*Madison, United States*
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Thank you