
In the name of
God

New Biomarkers in the Solid Allograft Rejection

Hassan Argani MD.
Professor of Nephrology, Tehran ,Iran

17th International Congress of Nephrology, Dialysis, and Transplantation
Tabriz , Iran 19-22 November 2019

Definitions

Conventional Biomarkers

New biomarkers in Serum, urine, and tissue

Summary & Conclusion

Definitions

Conventional Biomarkers

New biomarkers in Serum urine and tissue

Summary & Conclusion

Incidence of ABMR

- Acute
  - Heart-10-30%
  - Lung: unknown
  - Kidney: 20-30%
- Chronic AMR
  - Heart: 20-40%
  - Lung: unknown
  - Kidney: 30-40%

MONITORING FOR AMR

- Clinical Parameters
  - Heart: Diminished ventricular ejection fraction
  - Lung: Diminished FEV1
  - Kidney: Diminished GFR
- Histology
  - Ab and/or Complement deposition (C3d, C4d)
- Serum Markers
  - Donor specific antibody (DSA) to HLA
  - Antibodies to self antigens

Definition of Biomarker based on the NIH

"A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"

World J Transplant 2017 June 24; 7(3): 161-178

Principal applications of biomarkers

(1) Diagnosis or identification of patients affected by a disease or an abnormal condition

(2) Staging of the severity or extent of a disease

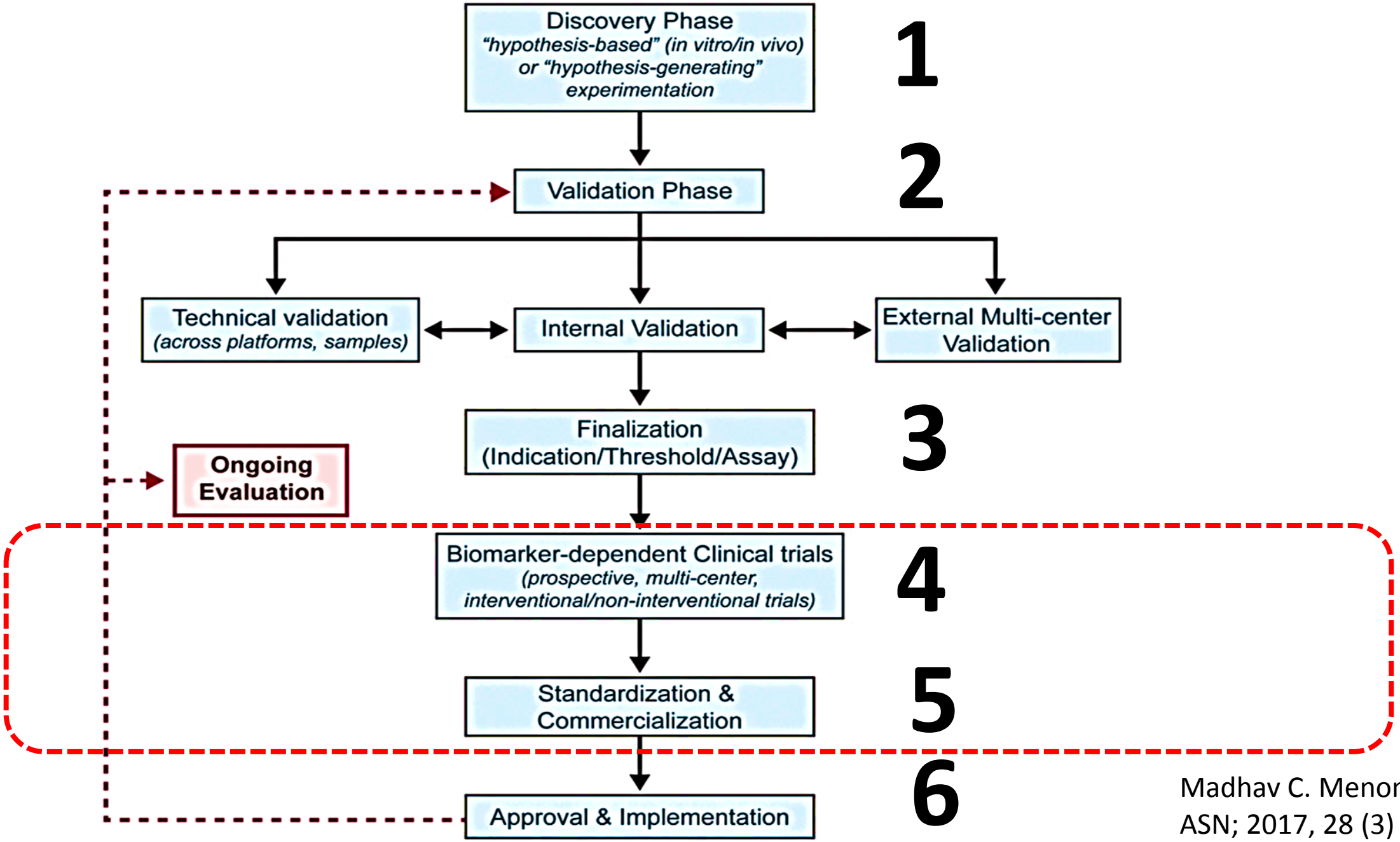
(3) Prognosis of a disease

(4) Prediction and monitoring of a clinical response to an intervention

Identification of an ideal biomarker that predicts patients at risk of shorter kidney allograft survival

Elusive of a Tansplantologist

Biomarkers development should proceed through a lifecycle



Madhav C. Menon etal.
ASN; 2017, 28 (3) 735-747

Definitions

Conventional Biomarkers

New biomarkers in Serum urine and tissue

Summary & Conclusion

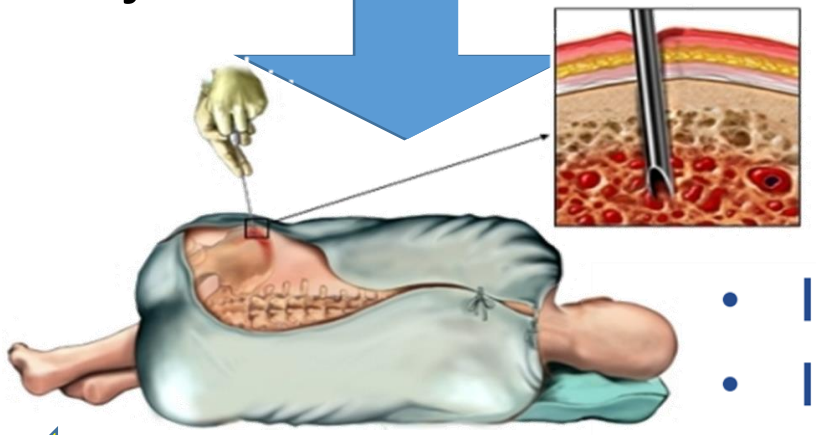
Traditional markers	It is worthwhile when	It is worthless because
Increased serum cr	❖ Increasing serum cr. ≥25% ❖ Stops decreasing of cr. ❖ Any incremental increase in serum creatinine in patients with increased risk for ABMR (eg, highly sensitized patients, recipients of ABO-incompatible renal allografts, patients with donor-specific antibodies [DSAs], and patients with inadequate immunosuppression).	❑ Is neither sensitive nor specific. ❑ Subclinical rejection develops in the absence of increased cr.
Proteinuria >1 g/day	❖ is an important and independent predictor of graft failure	❑ Is a nonspecific marker of graft injury. ❑ An association between proteinuria and specific pathologic processes in the renal allograft has not been well described

Increased serum cr

Proteinuria >1 g/day

Non specific

Suspicion of acute rejection



Renal Bx. Still is the Golden standard for Dx of Rejection

Protocol Bx.?

Complications:

Sampling errors
Costly
Labor intensive
Invasive

- Inconvenient
- Invasive
- Potentially harmful
- Expensive
- Banff is always changing
- Poor concordance between pathologists
- Too late

An ideal marker in kidney transplantation

- ❑ To Identify incipient allograft injury
- ❑ To discern the type of injury
- ❑ Preferably to predict the outcome

rapidly, accurately, inexpensively and non-invasively

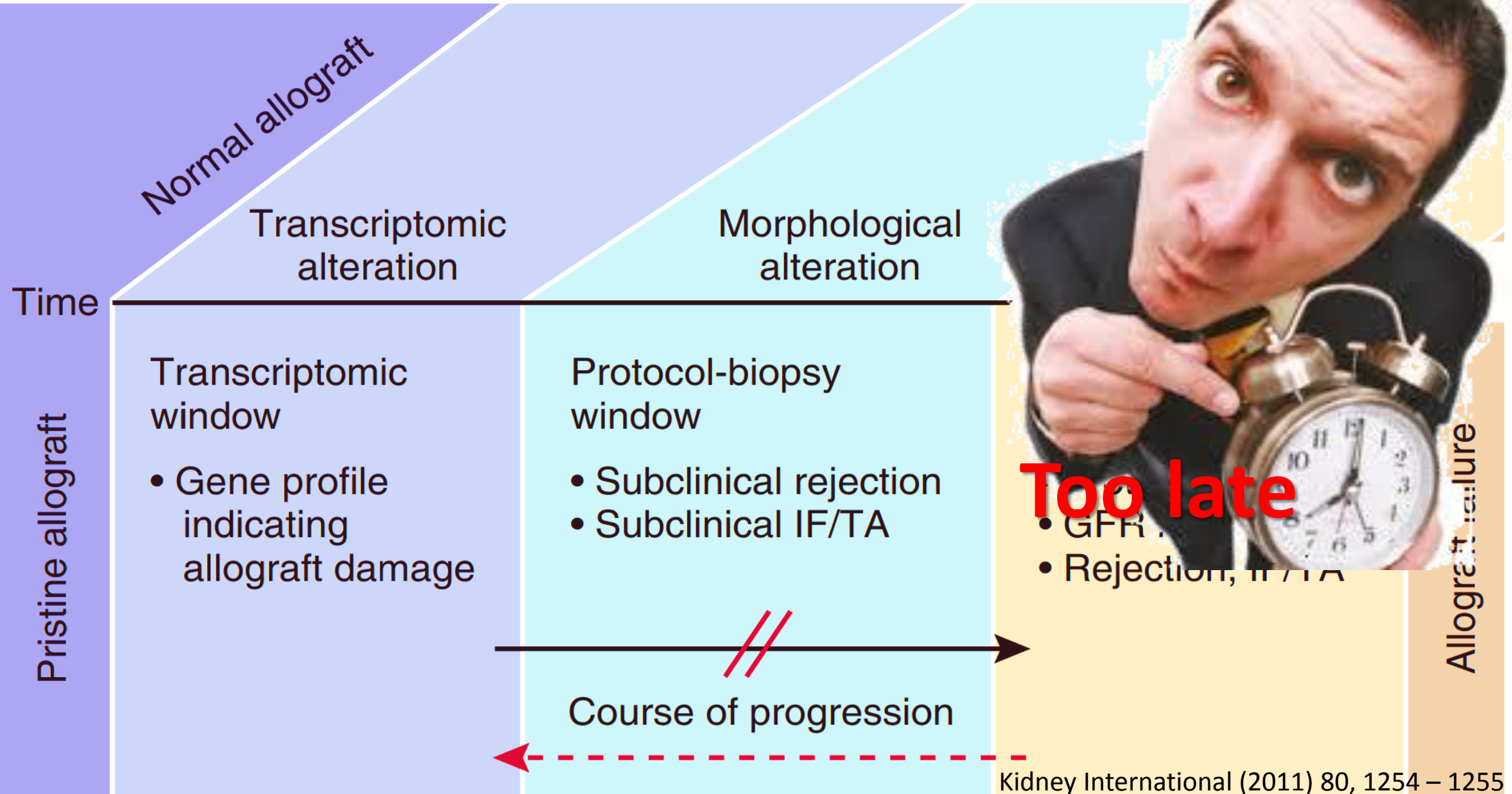
Definitions

Conventional Biomarkers

New biomarkers in Serum urine and tissue

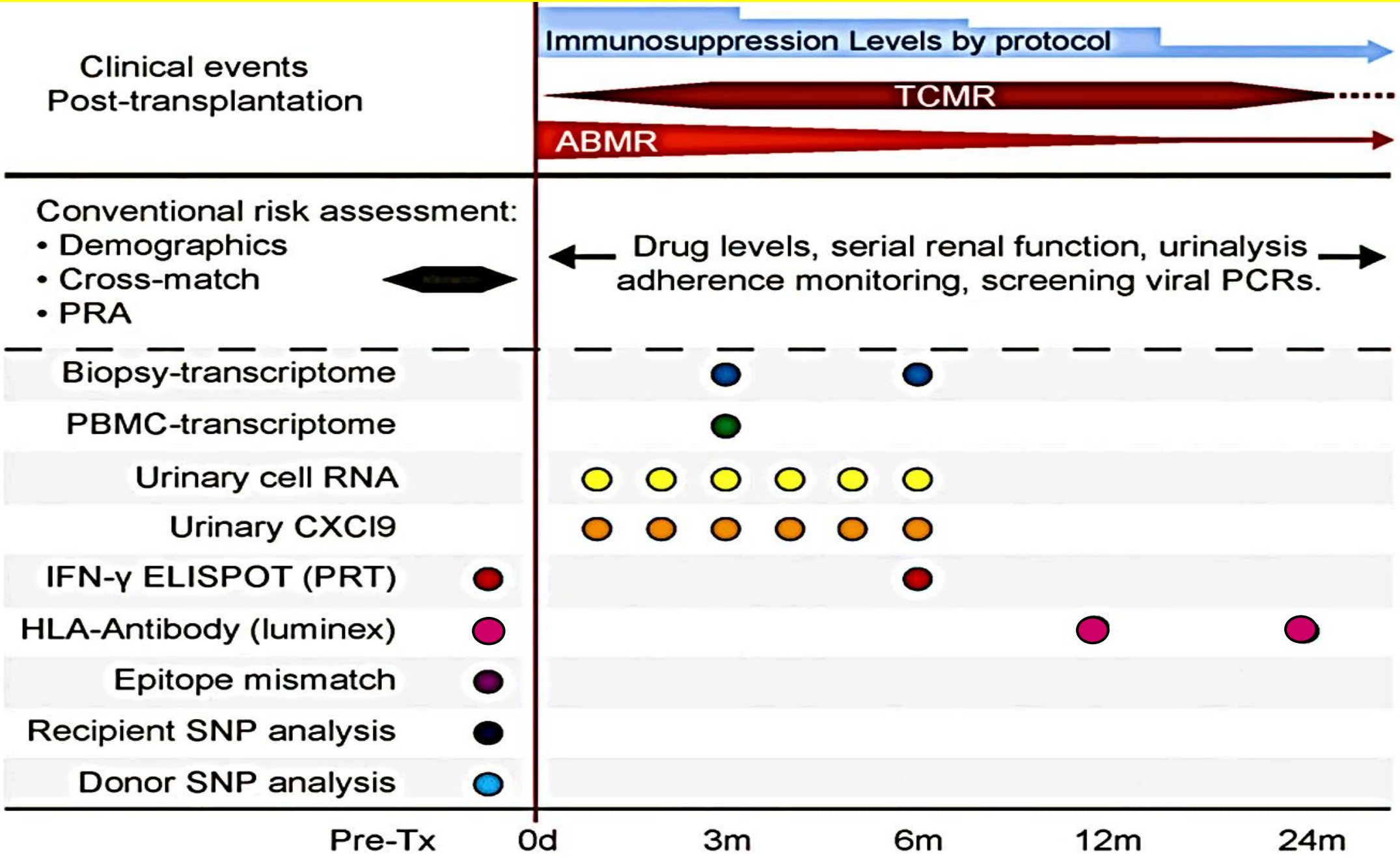
Summary & Conclusion

Progression of chronic allograft damage



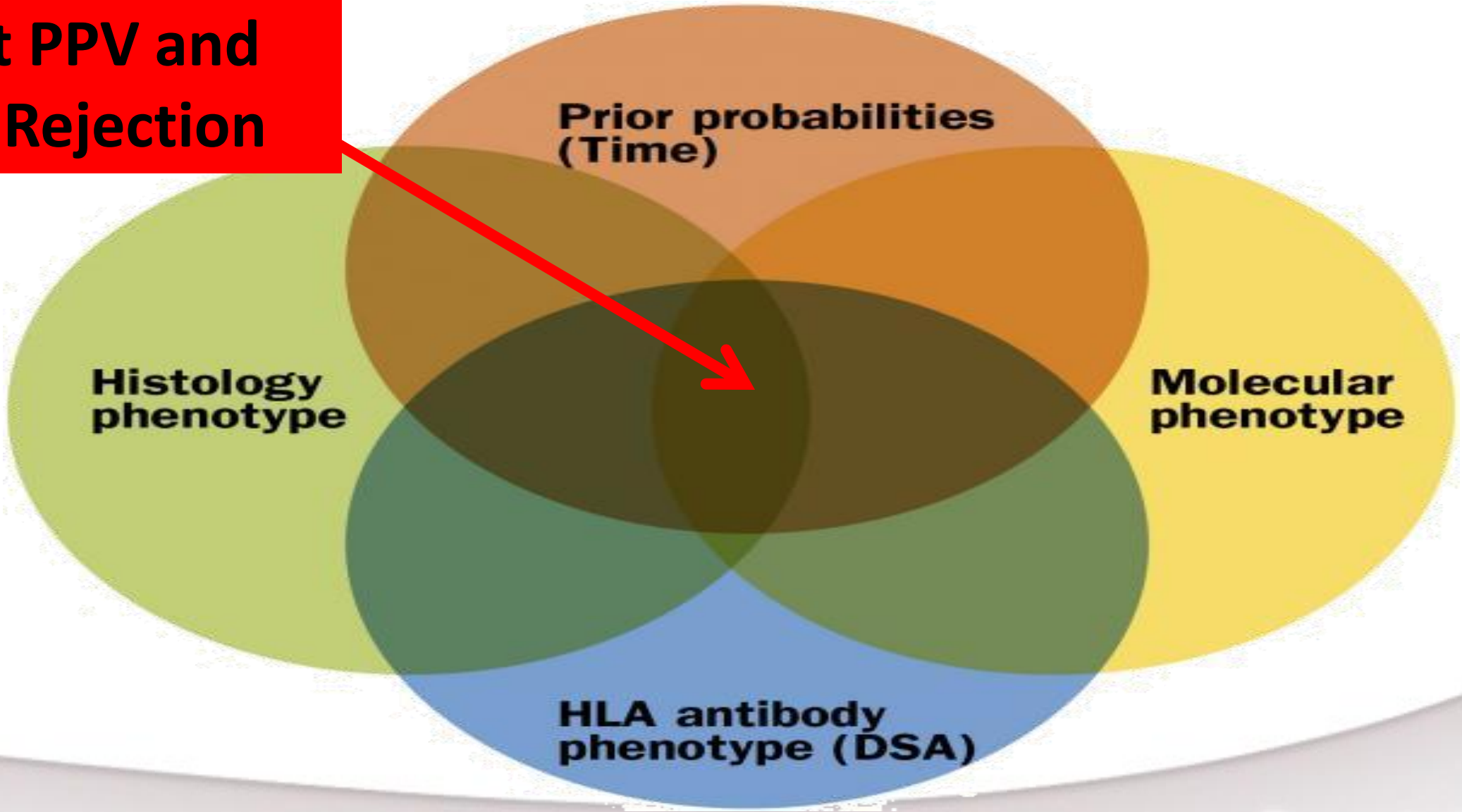
Kidney International (2011) 80, 1254 – 1255

Diagnosis of Tx rejection based on biomarkers will require use of multiple testing strategies

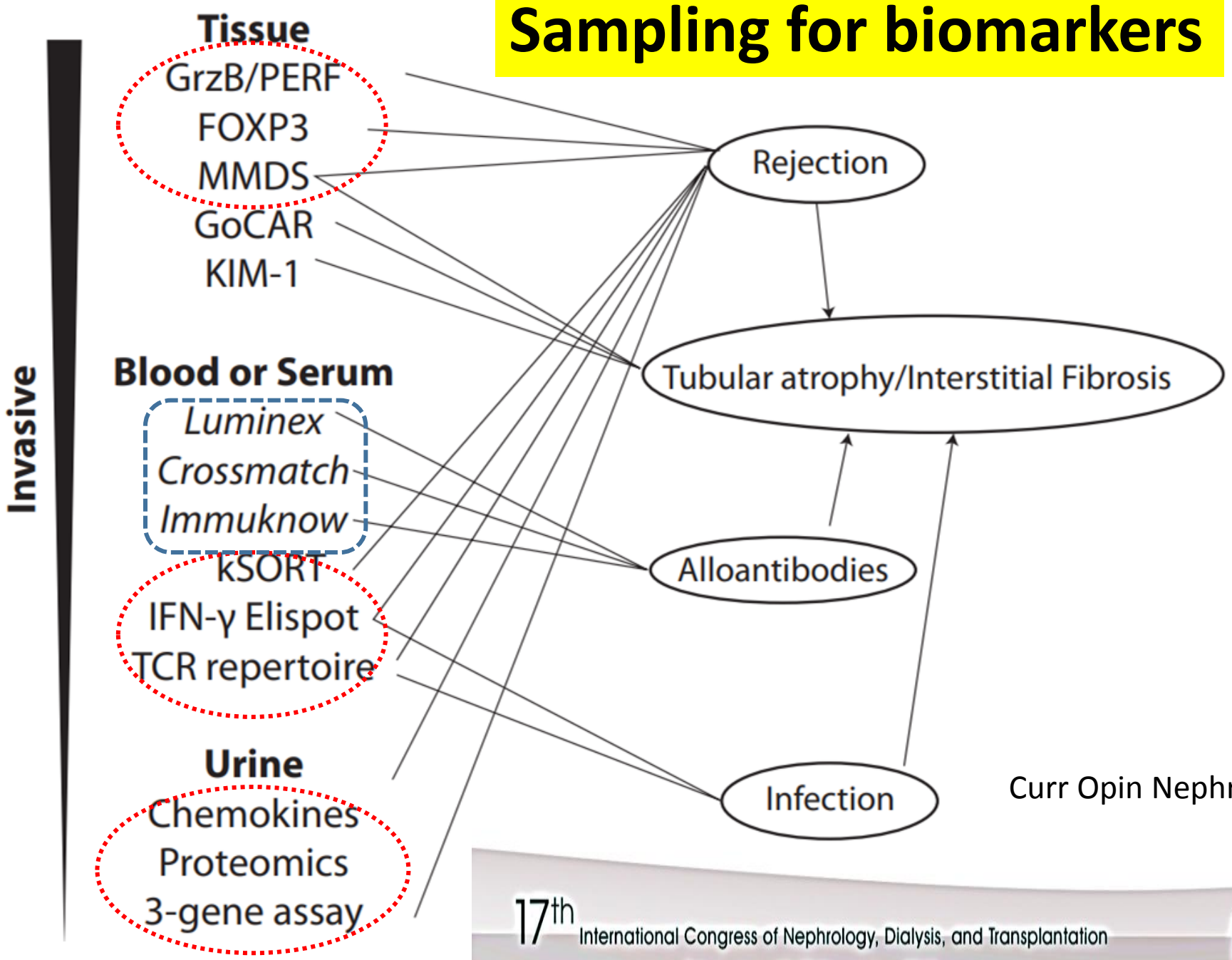


MC. Menon etal.
ASN; 2017, 28 (3)
735-747

The Best PPV and NPV for Rejection

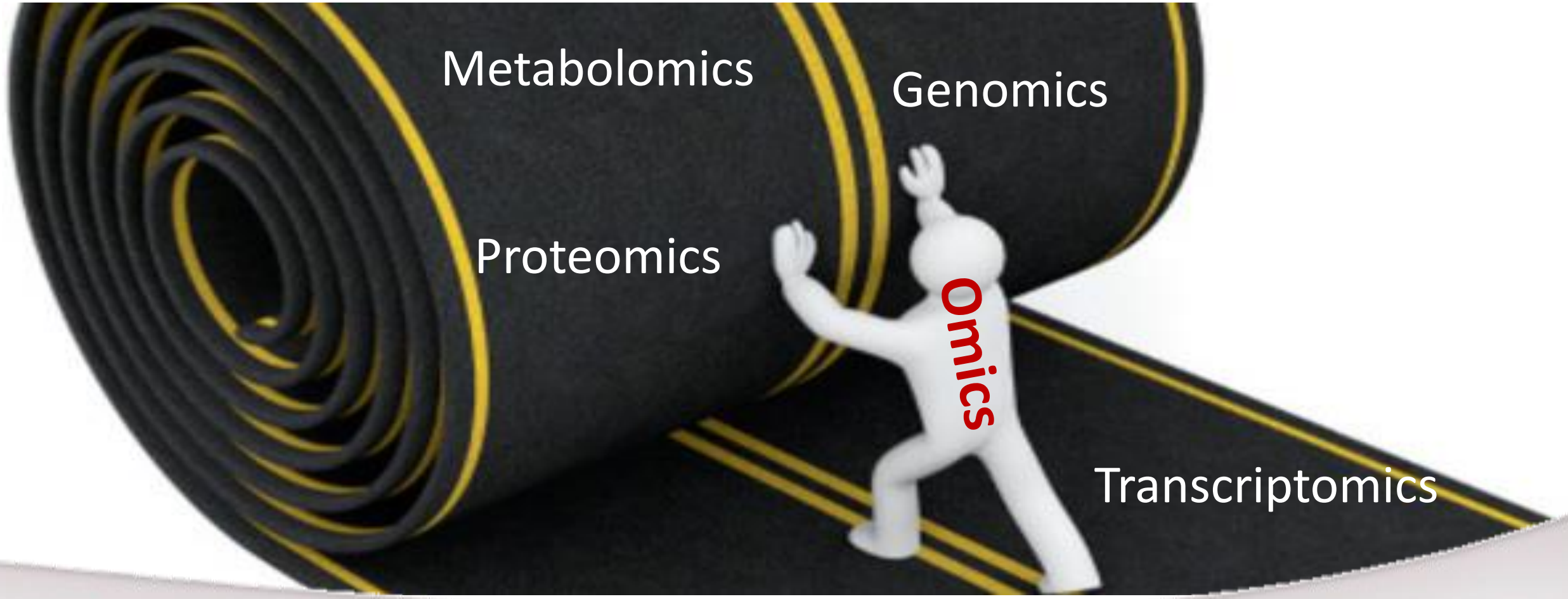


Sampling for biomarkers



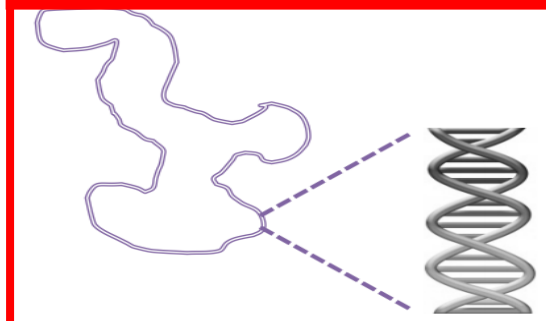
Curr Opin Nephrol Hypertens 2017, 26:509–515

Development of "omics" methods in the field of transplantation has paved the way for the development of several candidate biomarkers



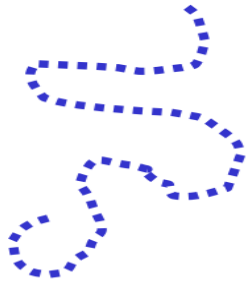
Characteristics of the Omics biomarkers

Genomics:
The study of genome for estimating the risk for an individual to develop a disease



GENOME

Transcriptomics:
The study of expression patterns of all gene transcript



mRNA (Transcript)

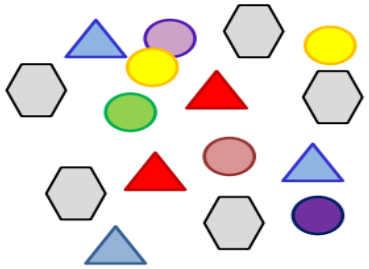
TRANSCRIPTOME

Proteomics:
The systematic analysis of proteins for their identity, quantity and function

Proteins

PROTEOME

Metabolomics:
The quantitative analysis of all the metabolites of a specific biological sample



Metabolites

METABOLOME

Blood/Urine

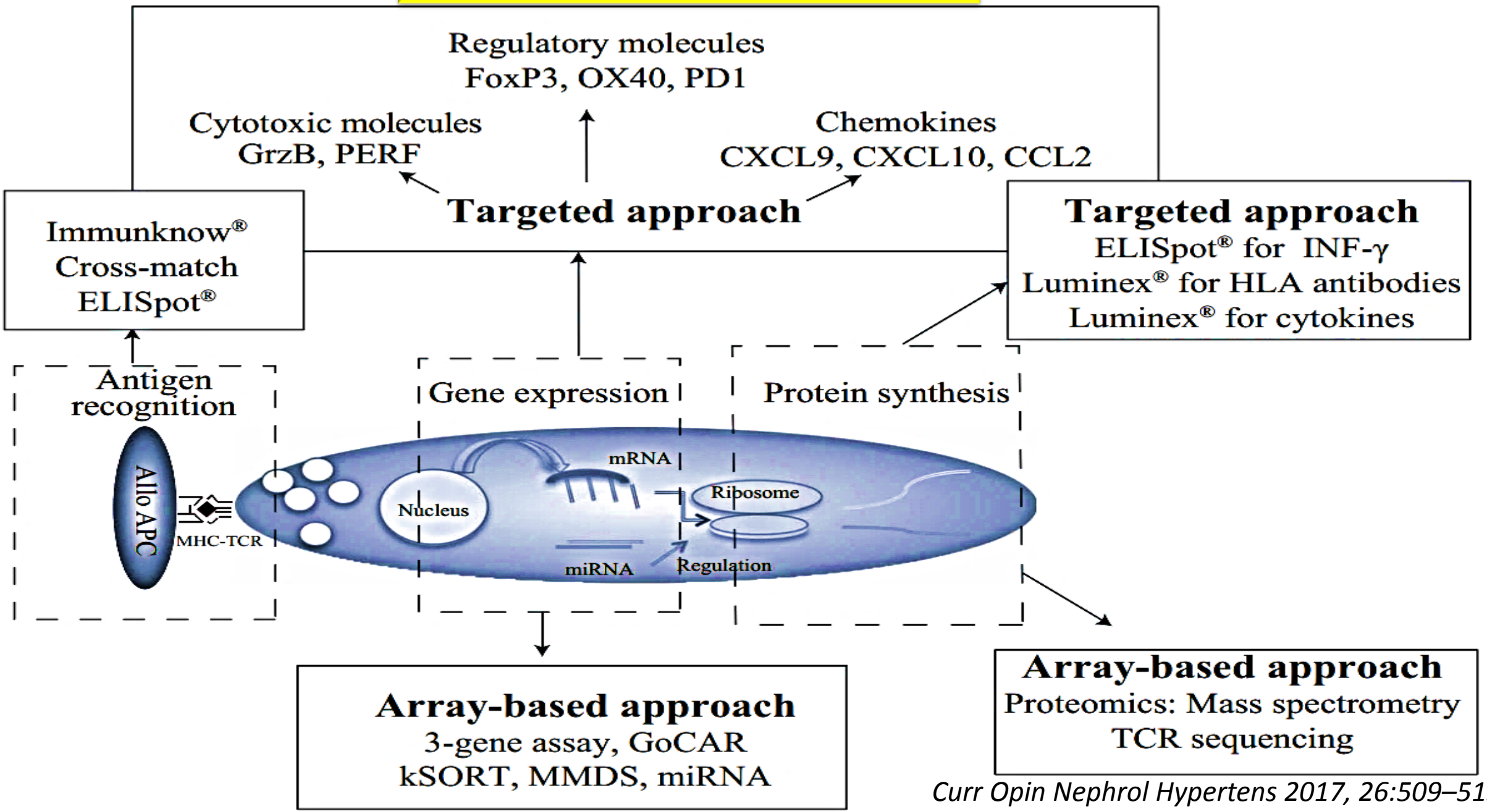
World J Transplant 2017 June 24; 7(3): 161-178

Proteomic studies for acute rejection

Ref.	Biomarker candidate	Sample type	Sample numbers	Outcome
Freue *et al*[69]	TTN, LBP, CFD, MBL2, SERPINA10, AFM, KNG1, LCAT, SHBG	Plasma	32	AR
Sigdel *et al*[70]	UMOD, PEDF, CD44	Urine	60	AR
Wu *et al*[71]	NF-κB, STAT1, STAT3 and 63 other proteins	Plasma	13	AR
Loftheim *et al*[72]	IGFBP7, VASN, EGF, LG3BP	Urine	12	AR
Sigdel *et al*[73]	HLA-DRB1, FGB, FGA, KRT14, HIST1H4B, ACTB, KRT7, DPP4	Urine	154	AR

World J Transplant 2017 June 24; 7(3): 161-178

Biomarkers identification



Curr Opin Nephrol Hypertens 2017, 26:509–515

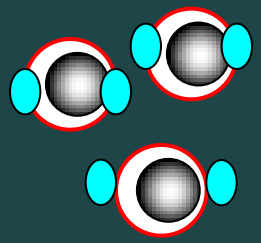
Cylex Method

Lymphocyte separation

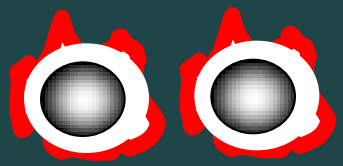
Lymphocyte stimulation by PHA

Magnetic separation

wash



ATP



Cell lysis to release ATP

ATP Detection Reagents

Luminometer measures light intensity

LIVER TRANSPLANTATION 18:1245-1253, 2012

ORIGINAL ARTICLE

ImmuKnow as a Diagnostic Tool for Predicting Infection and Acute Rejection in Adult Liver Transplant Recipients: A Systematic Review and Meta-Analysis

Emilio Rodrigo,[1] Marcos López-Hoyos,[2] Mario Corral,[3] Emilio Fábrega,[4] Gema Fernández-Fresnedo,[1] David San Segundo,[2] Celestino Piñera,[1] and Manuel Arias[1]

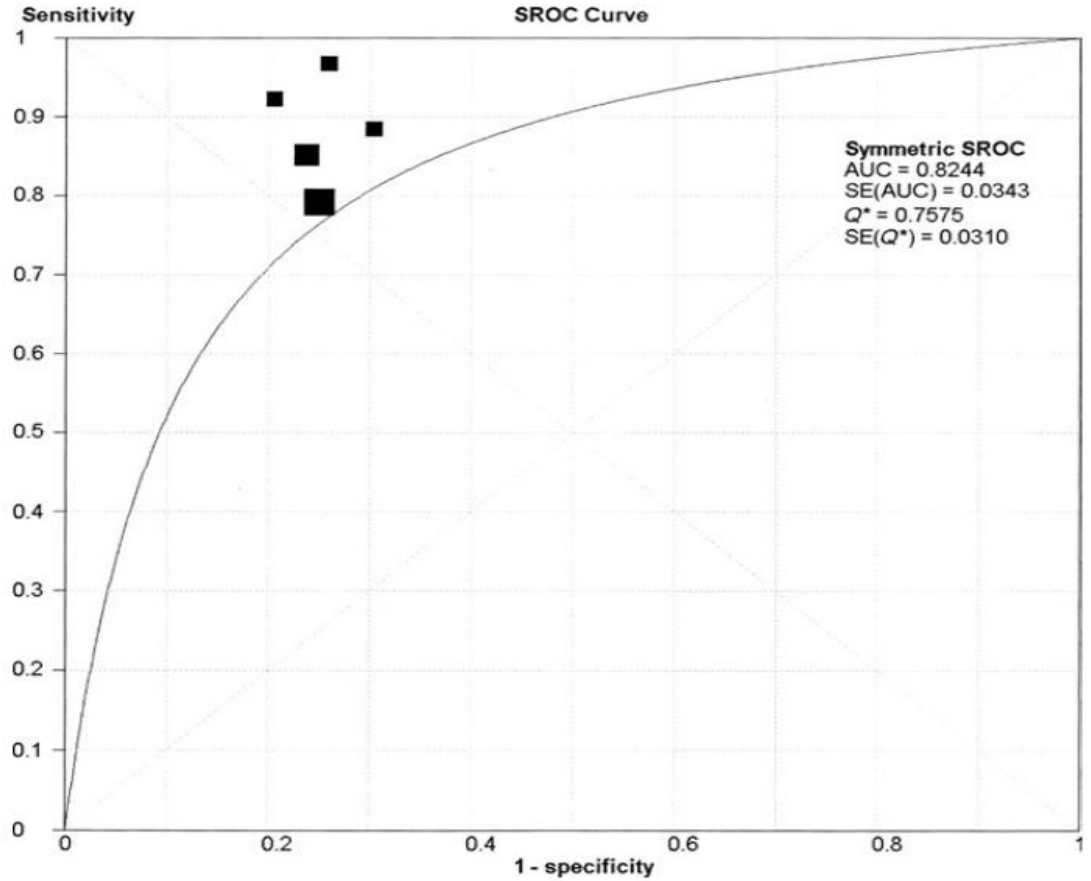


Figure 5. SROC curve based on all studies included in the systematic review of ImmuKnow for the prediction of infection in liver transplant recipients.

Additional studies are required to accurately assess the specific role of ImmuKnow monitoring for rejection in liver transplantation.

ERYTHROCYTE BOUND C4d (E-C4d)

- Increased cell bound complement activation product, C4d, detected on the surface of erythrocytes.
- Have been shown to correlate with
  - Disease activity in systemic lupus erythematous (SLE)
  - Acute rejection after cardiac transplantation
- E-C4d have increased half-life compared to serum C3 and C4d
- More reliable tool compared to serum/biopsy C3d or C4d.

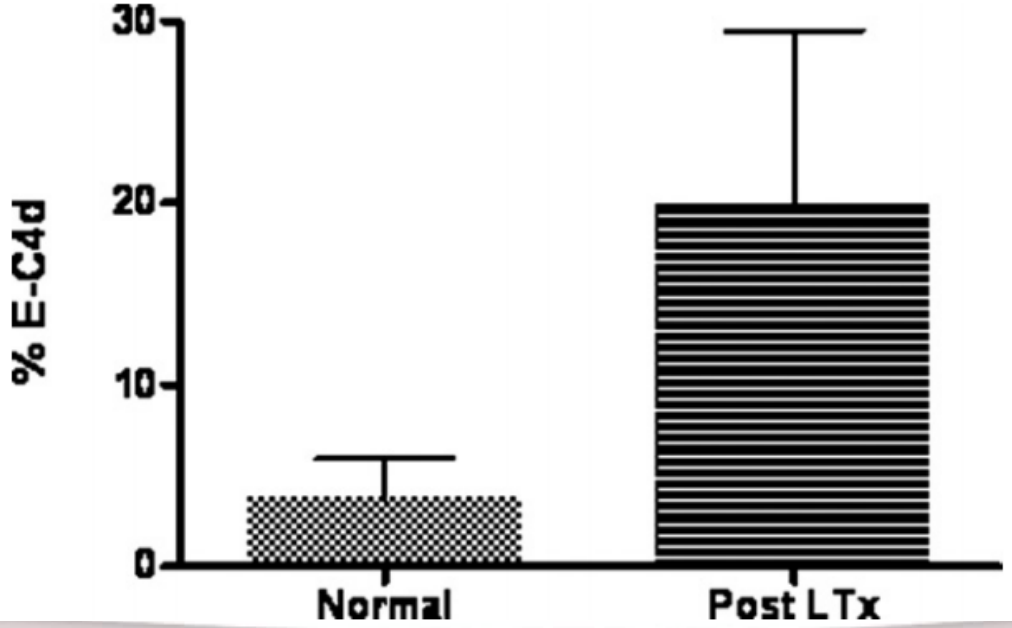
J Heart Lung Transplant 2010;29:410 – 416

ELSEVIER

The Journal of Heart and Lung Transplantation

http://www.ihltonline.org

Increased erythrocyte C4D is associated with known alloantibody and autoantibody markers of antibody-mediated rejection in human lung transplant recipients



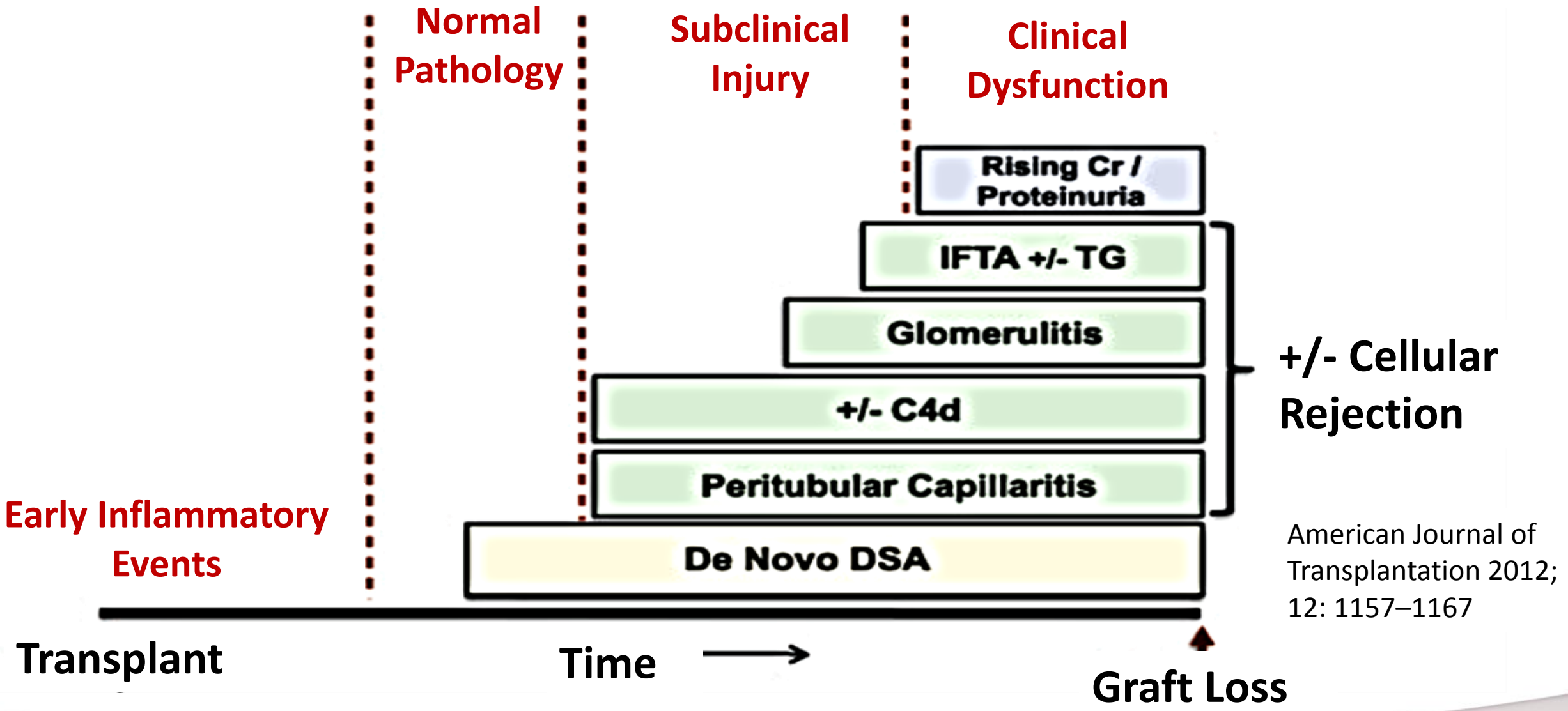
DSA monitoring

- High levels of DSAs at the time of transplantation➔ Contraindication for Tx.

- Low levels of DSA➔ is not a contraindication to transplant➔ Require individual risk assessment.

- DSA should be considered as a risk factor for rather than diagnostic of ABMR.

Proposed natural history of dnDSA



American Journal of Transplantation 2012; 12: 1157–1167

Pre TX survey of DSA in 402 Renal TX. Recipients for subsequent ABMR

	Positive Crossmatched by CDC	Positive Crossmatched by Luminex
Sensitivity	41%	91%
Specificity	97%	85%
PPV	54%	35%

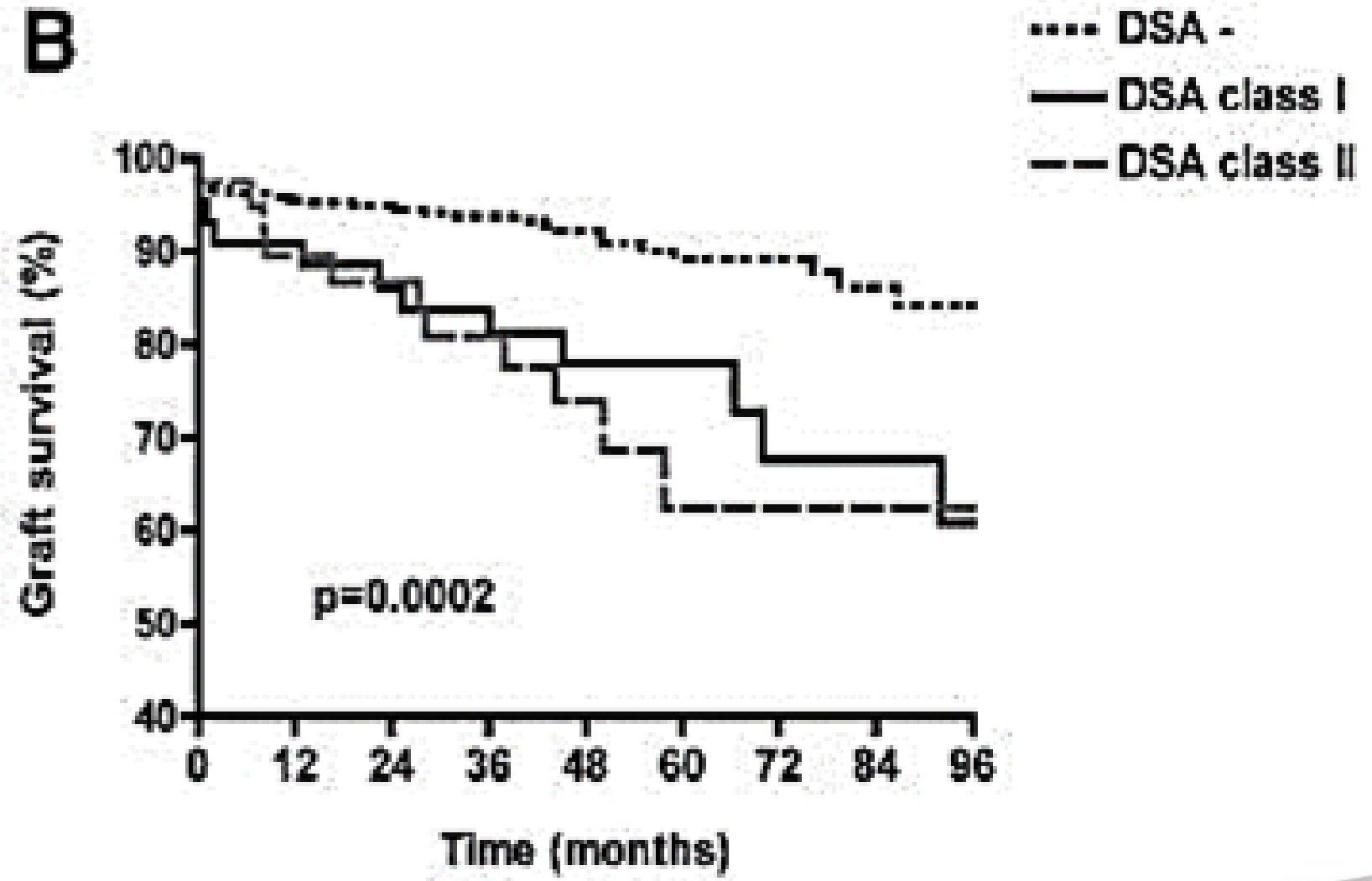
Lefaucheur C. etal. J Am Soc Nephrol. 2010;21(8):1398–1406.


Pre-TX.
DSA monitoring
Post-TX.

Preexisting DSA predict outcome in kidney transplantation

Pre-TX.

DSA monitoring



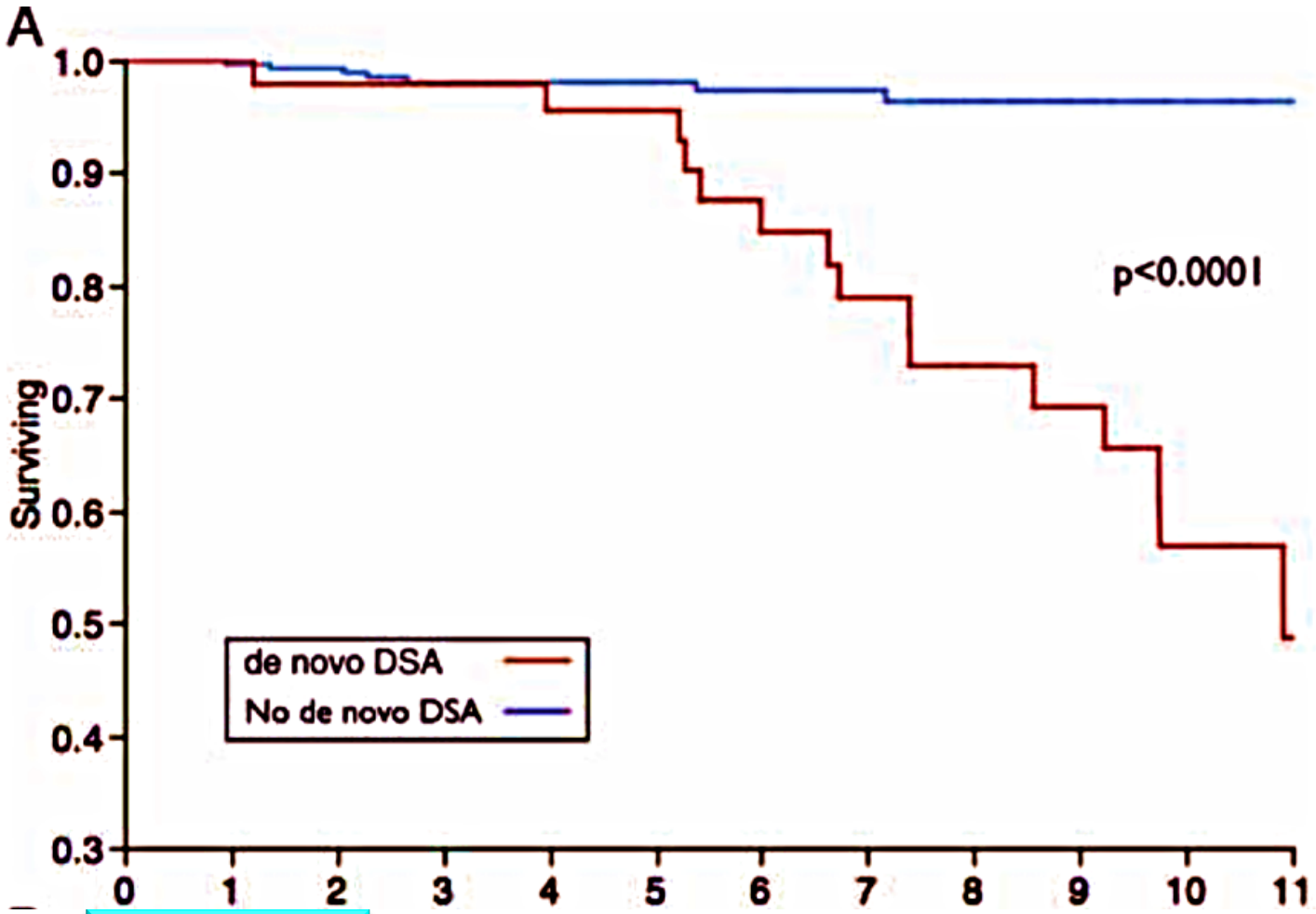
Lefaucheur C. etal. J Am Soc Nephrol. 2010;21(8):1398–1406.

The graft survival of patients of dnDSA+ compared with dnDSA-

DSA monitoring

Post-TX.

dn-DSA

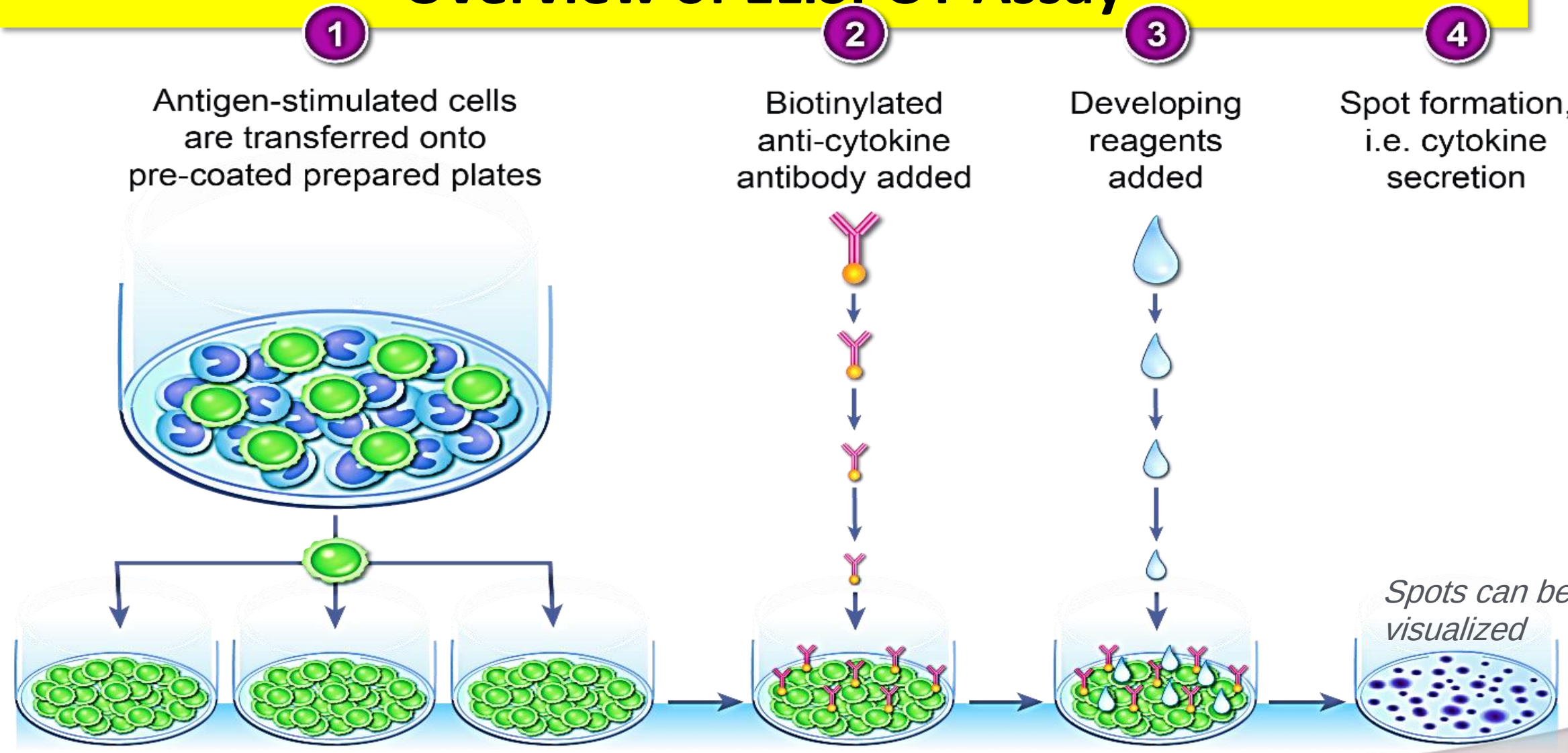


American Journal of Transplantation 2012; 12: 1157–1167

ELISPOT

- Monitoring of the memory T cells
- measures IFN-gamma secretion by recipient T cells in response to donor antigens
- Enumerates Cytokine secreting cells quantitatively and qualitatively

Overview of ELISPOT Assay



Drawbacks of Elispot

- Laborious
- Time-consuming
- Impractical use in clinical practice.

Körber et al. J Transl Med (2016) 14:175
DOI 10.1186/s12967-016-0932-7

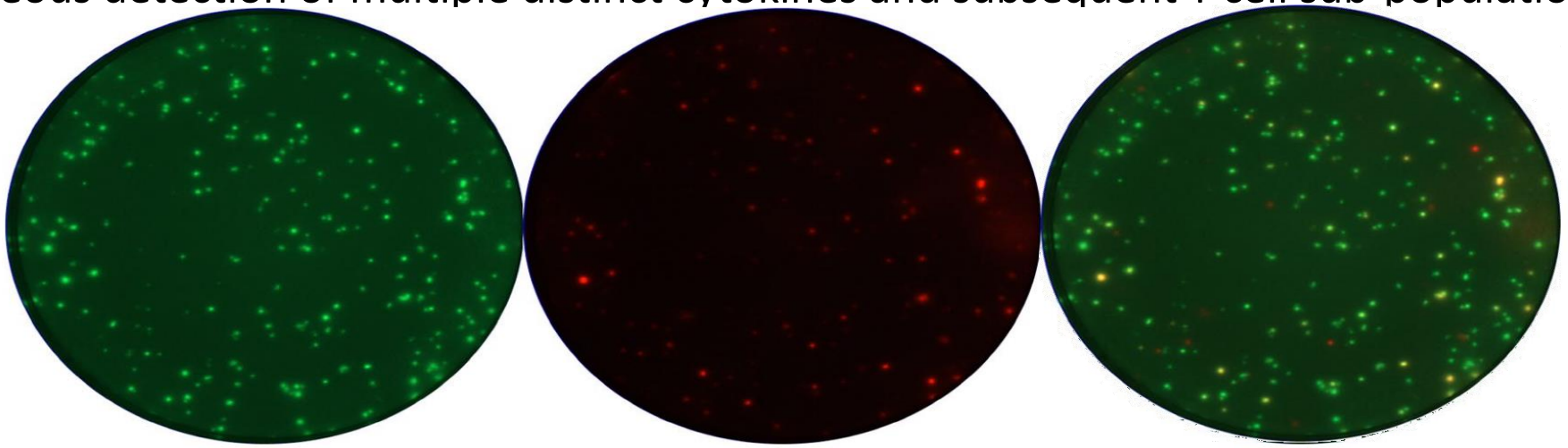
Journal of Translational Medicine

METHODOLOGY Open Access

Validation of an IFNγ/IL2 FluoroSpot assay for clinical trial monitoring

Nina Körber[1], Uta Behrends[3,4], Alexander Hapfelmeier[5], Ulrike Protzer[1,2,4] and Tanja Bauer[1,2,4*]

More recently FluoroSpot assay utilizes fluorochrome-conjugated detection antibodies. Thereby allowing the simultaneous detection of multiple distinct cytokines and subsequent T cell sub-population analysis



IFNγ secreting T cells **IL2 secreting T cells** **IFNγ + IL2 secreting T cells**

Kidney Solid Organ Response Test (KSORT)

Is a microarray-based assay to detect patients at high risk for acute rejection. It Employs quantitative PCR to measure the relative mRNA expression levels of 17 genes known to be associated with acute rejection with 93% sensitivity and specificity.

World J Transplant 2017 June 24; 7(3): 161-178

Symbol	Gene name	Cytoband
Genes derived from the NIH SNSO1 study		
DUSP1	Dual-specificity phosphatase 1	*5q35.1*
NAMPT	Nicotinamide phosphoribosyltransferase	*7q22.3*
PSEN1	Presenilin 1	*14q24.2*
MAPK9	Mitogen-activated protein kinase 9	*5q35.3*
NKTR	Natural killer cell triggering receptor	*3p22.1*
CFLAR	CASP8 and FADD like apoptosis regulator gene	*2q33.1*
IFNGR1	Ligand binding chain of the gamma interferon receptor gene	*6q23.3*
ITGAX	Integrin alphaXchain protein encoding gene	*16p11.2*
RNF130	Ring finger motif encoding gene	*5q35.3*
RYBP	RING1 and YY1 binding protein encoding gene	*3p13*
Genes added to improve the accuracy of kSORT		
CEACAM4	Carcinoembryonic antigen related cell adhesion molecule 4	*19q13.2*
EPOR	Erythropoietin receptor encoding gene	*19p13.2*
GZMK	Granzyme K encoding gene	*5q11.2*
RARA	Retinoic acid receptor encoding gene	*17q21.2*
RHEB	Ras homolog enriched in brain encoding gene	*7q36.1*
RXRA	Retinoic X receptor alpha encoding gene	*9q34.2*
SLC25A37	Solute carrier family 25 number 37 encoding gene	*8p21.2*

PLOS Medicine; November 2014, Issue 11, 1-15

OPEN ACCESS Freely available online

PLOS | MEDICINE

The kSORT Assay to Detect Renal Transplant Patients at High Risk for Acute Rejection: Results of the Multicenter AART Study

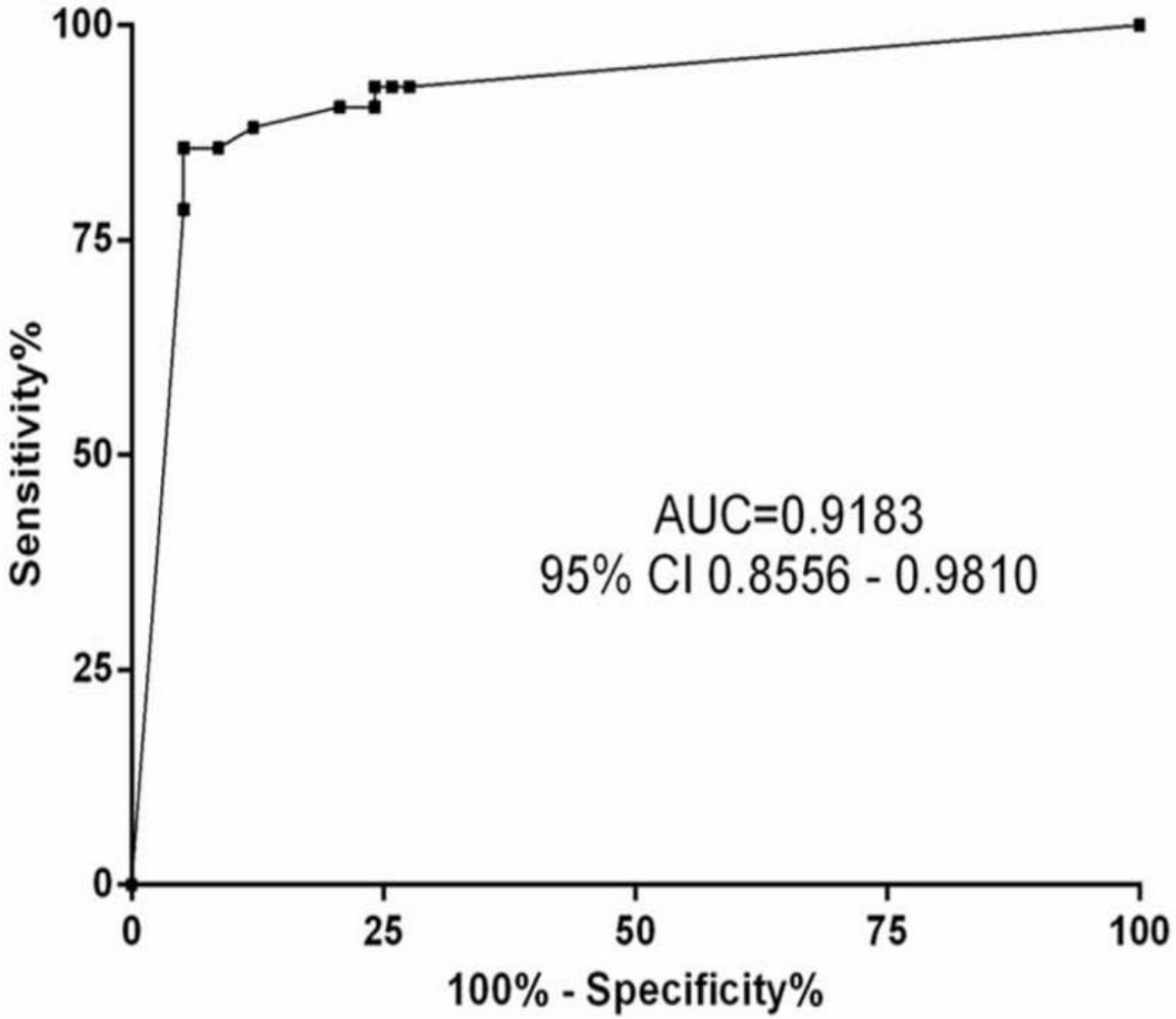
Silke Roedder[1☯], Tara Sigdel[1☯], Nathan Salomonis[2☯], Sue Hsieh[1], Hong Dai[3¤a], Oriol Bestard[4], Diana Metes[5], Andrea Zeevi[5], Albin Gritsch[6], Jennifer Cheeseman[7], Camila Macedo[5], Ram Peddy[3], Mara Medeiros[8], Flavio Vincenti[1], Nancy Asher[1], Oscar Salvatierra[9], Ron Shapiro[5], Allan Kirk[7¤b], Elaine Reed[6], Minnie M. Sarwal[1]*

1 Department of Surgery, University of California San Francisco, San Francisco, California, United States of America, **2** Biomedical Informatics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States of America, **3** California Pacific Medical Center, San Francisco, California, United States of America, **4** Renal Transplant Unit, Bellvitge University Hospital, Barcelona, Spain, **5** Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, United States of America, **6** Immunogenetics Center, University of California Los Angeles, Los Angeles, California, United States of America, **7** Department of Surgery, Emory University, Atlanta, Georgia, United States of America, **8** Laboratorio de Investigacion en Nefrologia, Hospital Infantil de México Federico Gómez, Mexico City, Mexico, **9** Stanford University, Stanford, California, United States of America

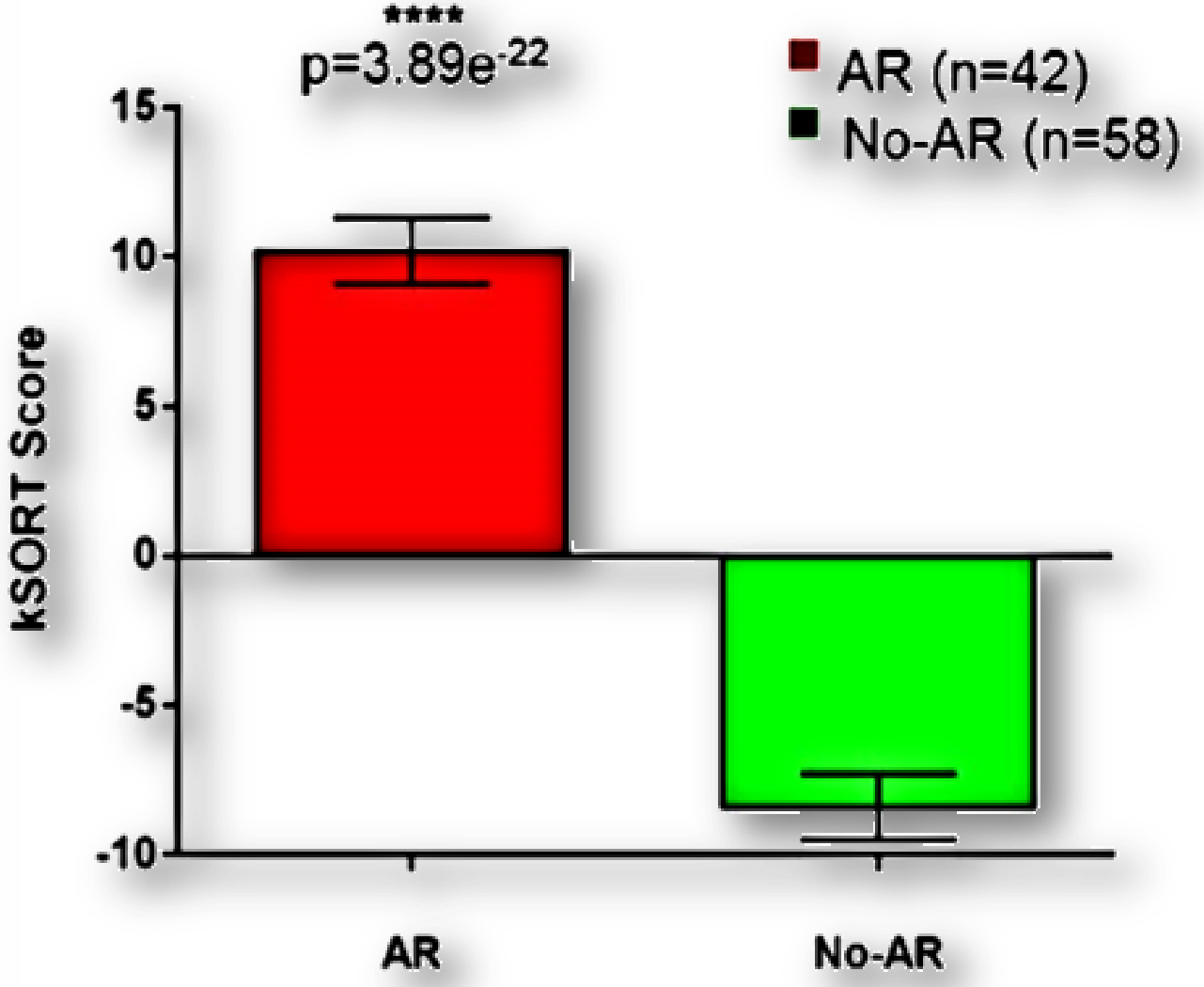
Abstract

Background: Development of noninvasive molecular assays to improve disease diagnosis and patient monitoring is a critical need. In renal transplantation, acute rejection (AR) increases the risk for chronic graft injury and failure. Noninvasive diagnostic assays to improve current late and nonspecific diagnosis of rejection are needed. We sought to develop a test using a simple blood gene expression assay to detect patients at high risk for AR.

Methods and Findings: We developed a novel correlation-based algorithm by step-wise analysis of gene expression data in 558 blood samples from 436 renal transplant patients collected across eight transplant centers in the US, Mexico, and Spain between 5 February 2005 and 15 December 2012 in the Assessment of Acute Rejection in Renal Transplantation (AART) study. Gene expression was assessed by quantitative real-time PCR (QPCR) in one center. A 17-gene set—the Kidney Solid Organ Response Test (kSORT)—was selected in 143 samples for AR classification using discriminant analysis (area under the



ROC analysis demonstrated high sensitivity and specificity for the kSORT assay



Mean kSORT scores were significantly higher in all true AR samples than in all true No-AR samples

- ❑ was able to identify subclinical rejection.
- ❑ was unable to distinguish between acute TCMR and ABMR.

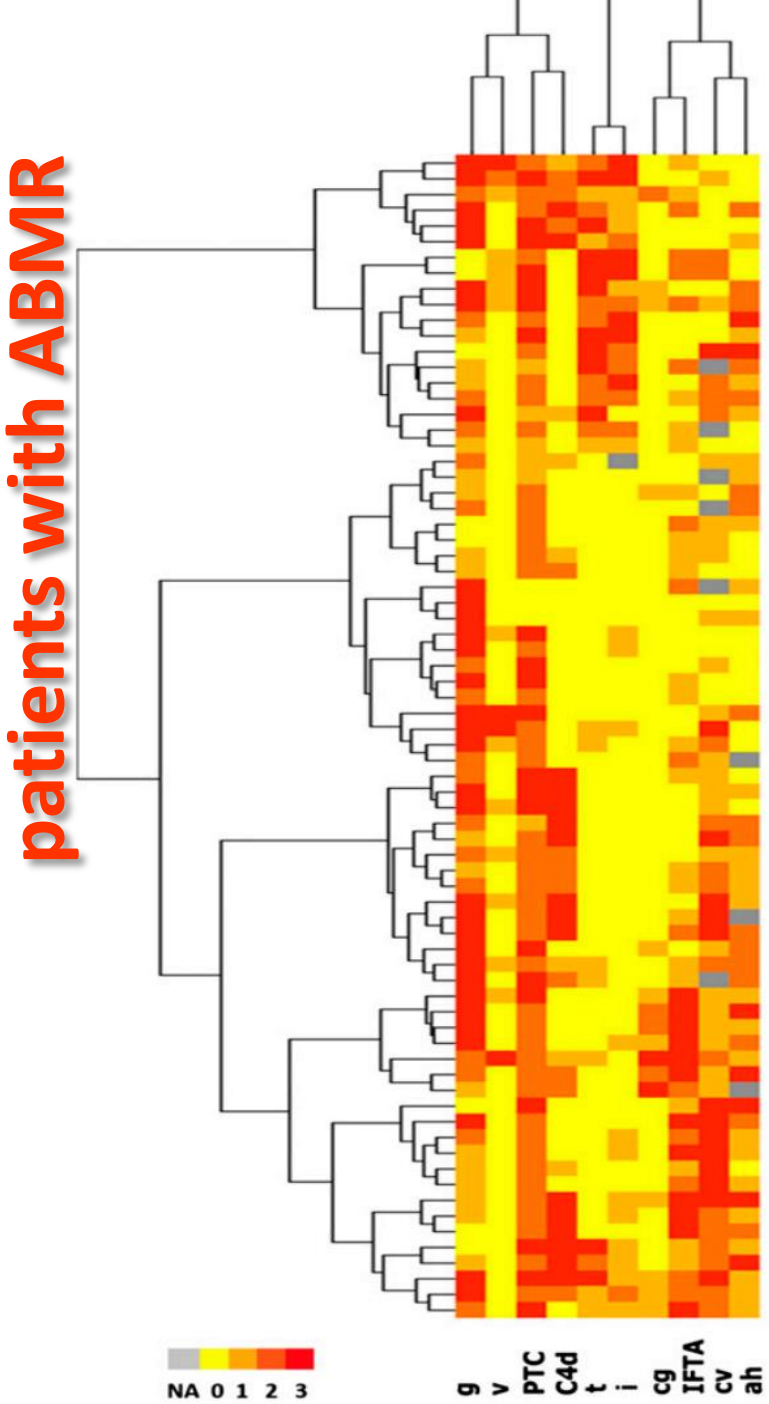
PLOS Medicine; November 2014, Issue 11, 1-15

BASIC RESEARCH www.jasn.org

Molecular Microscope Strategy to Improve Risk Stratification in Early Antibody-Mediated Kidney Allograft Rejection

Alexandre Loupy,*† Carmen Lefaucheur,†‡ Dewi Vernerey,†§ Jessica Chang,‖ Luis G. Hidalgo,‖¶ Thibaut Beuscart,† Jerome Verine,** Olivier Aubert,† Sébastien Dubleumortier,†† Jean-Paul Duong van Huyen,*†‡‡ Xavier Jouven,† Denis Glotz,†‡ Christophe Legendre,*† and Philip F. Halloran‖§§

939 kidney recipients at Necker Hospital (2004–2010; principal cohort) and 321 kidney recipients at Saint Louis Hospital (2006–2010; validation cohort) and assessed patients with ABMR in the first 1 year post-transplant



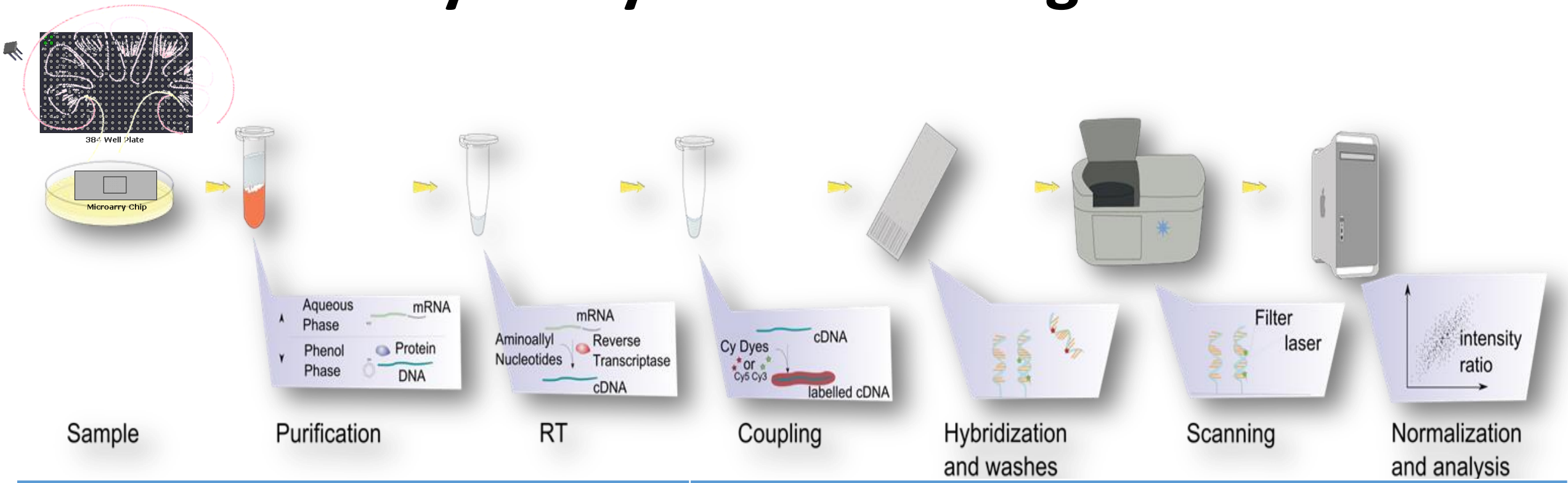
ABMR patients with similar histopathology may show different levels of molecular signals, reflecting distinct activity and disease state

Conclusion

- The MMS provides insight better than the classic, histology-based approach.
- It guides clinical management and clinical trials in transplant medicine.

J Am Soc Nephrol 25: 2267–2277, 2014

Microarray analysis of the allograft tissue



Advantages of Microarray	Disadvantages of Microarray
Small sample requirement	The need for a kidney biopsy to obtain a sample for analysis
Reproducibility of the results	

Future applications of the MM

- (Microarray costs are falling)
- Every biopsy will be read with histology and MM
- Strong potential for automation
- Results interpreted by comparison with archive
- Estimates of uncertainty to guide clinical decisions
- Guidance in use of highly expensive drugs
- Integrated assessment with histopathology
- International objective standards
- Support for next generation clinical trials

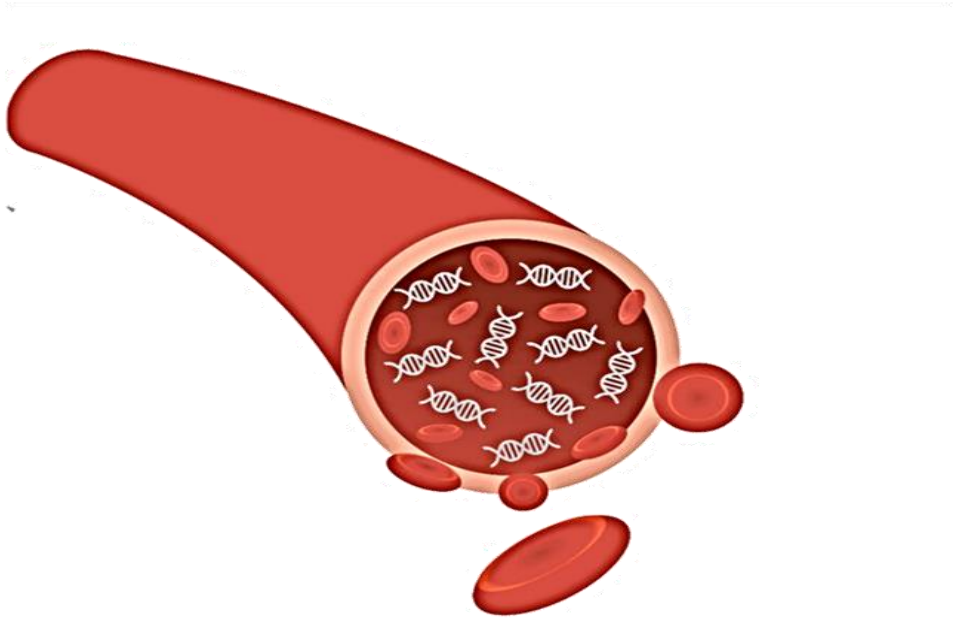
donor-derived cell-free DNA (dd-cfDNA) analysis

Molecular blood biomarkers

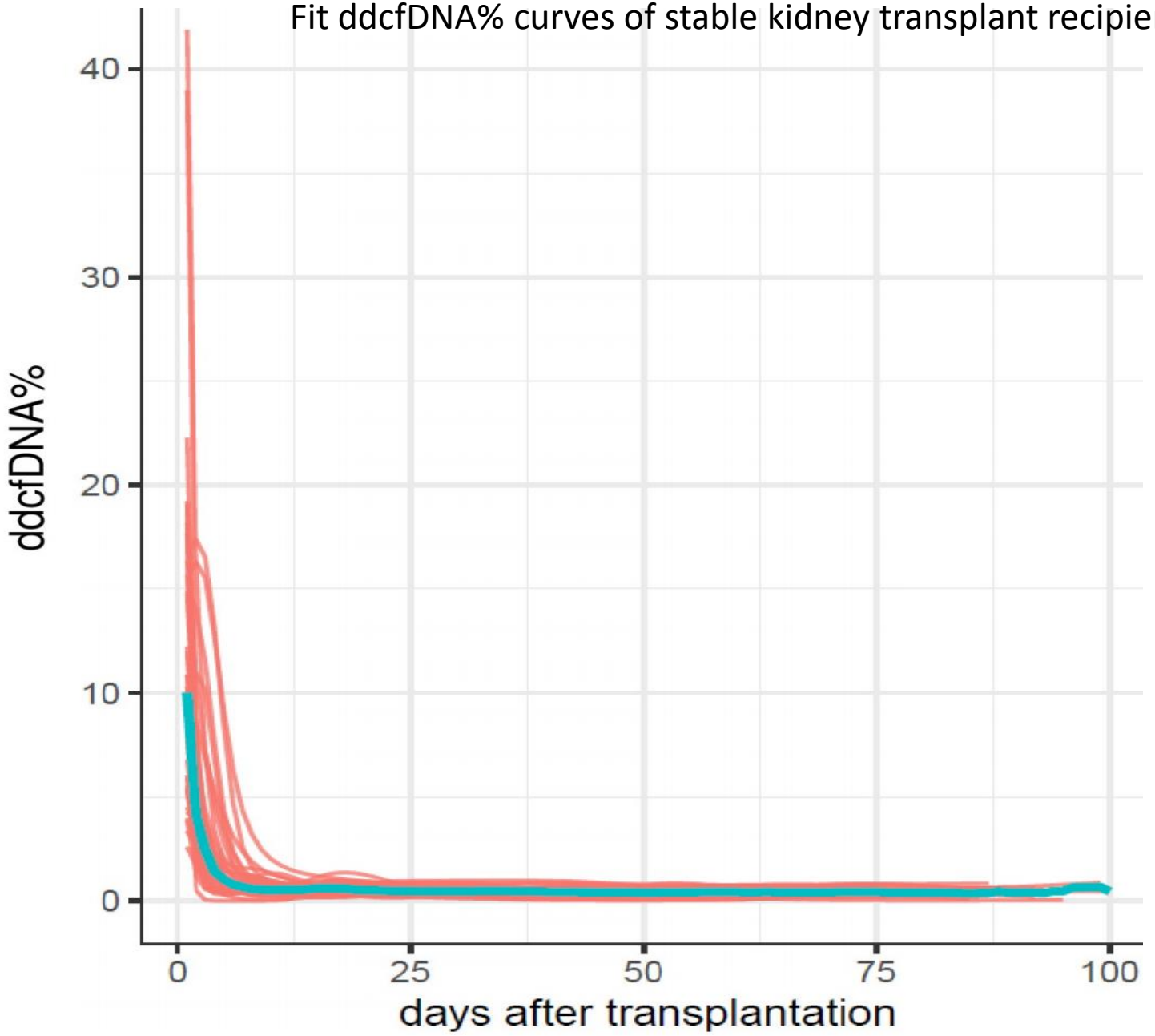
What Is Cell-Free DNA (cfDNA)?

JOHNS HOPKINS MEDICINE

- Cell-free DNA refers to fragments of DNA in the bloodstream that originate from cells undergoing cell injury and death
- DNA degrades into nucleosomal units consisting of ~166 bases
- cfDNA is cleared from the blood by the liver and kidney, and has a half-life of ~30 minutes

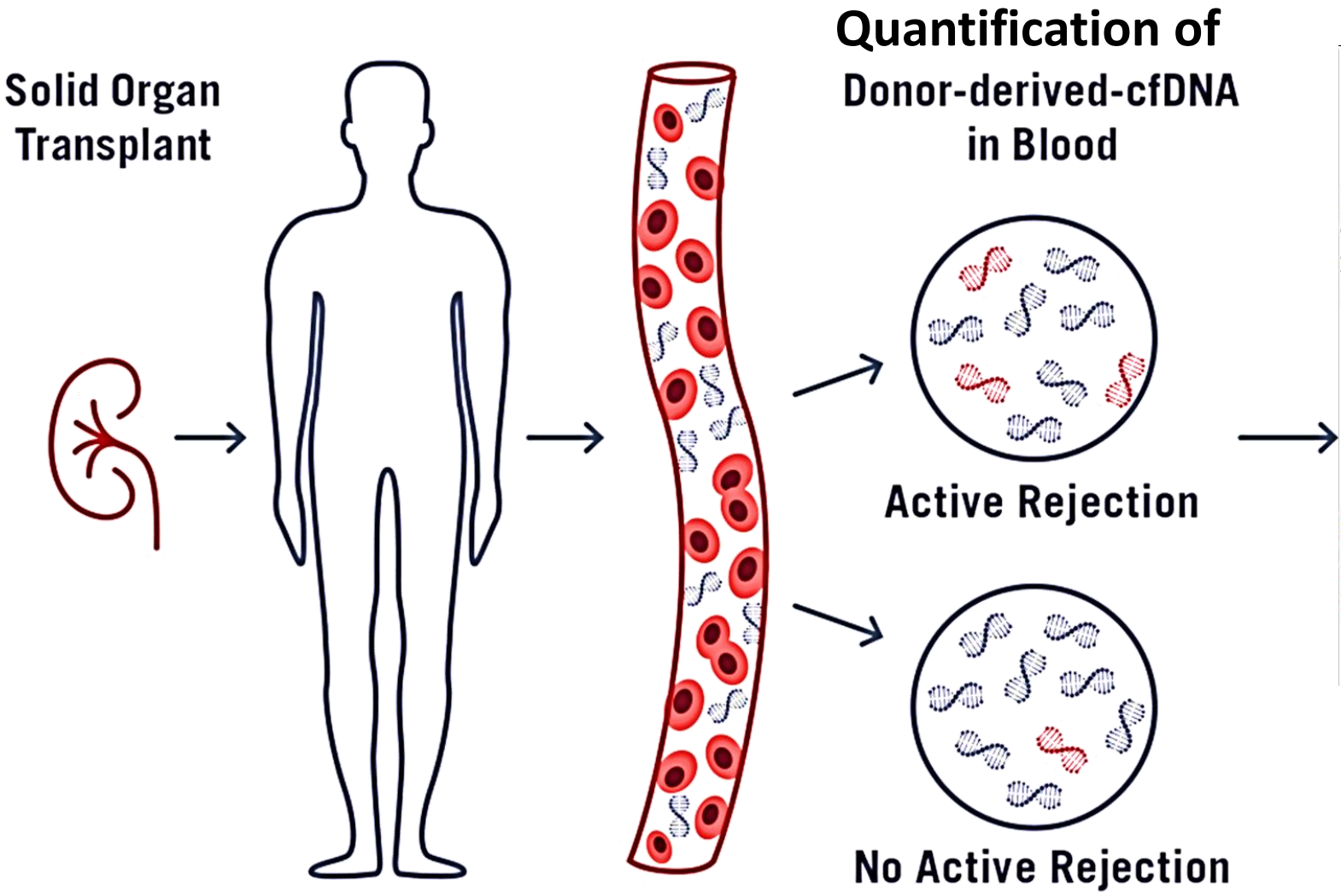


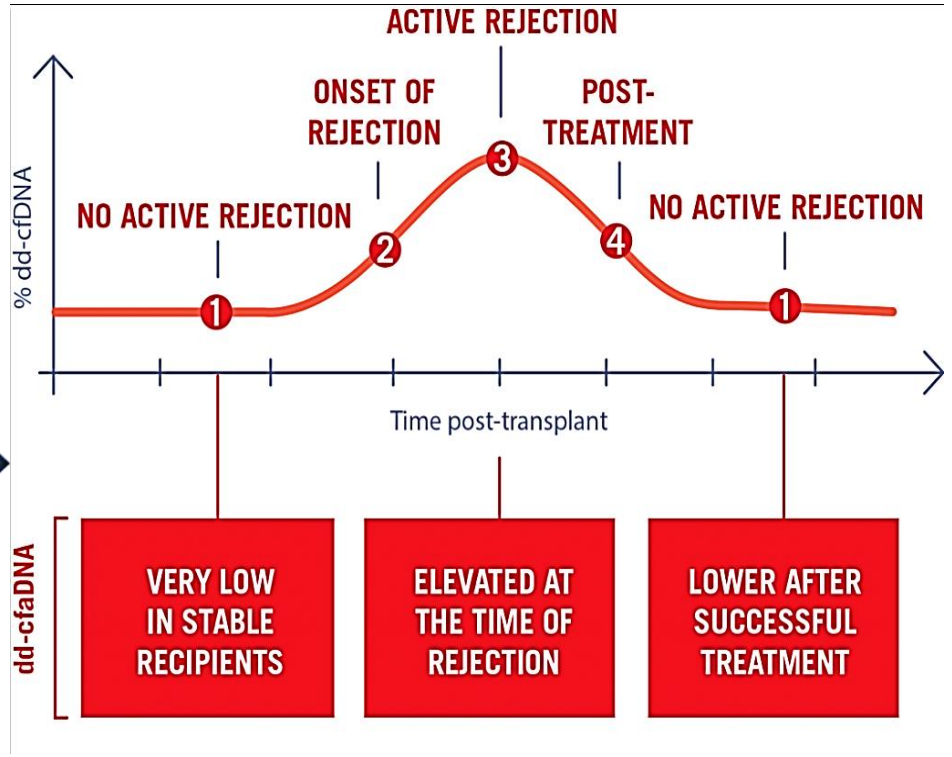
Fit ddcfDNA% curves of stable kidney transplant recipients



Gielis EM. etal. PLoS One. (2018) 13 (12); 1-16

Donor-derived cell-free DNA





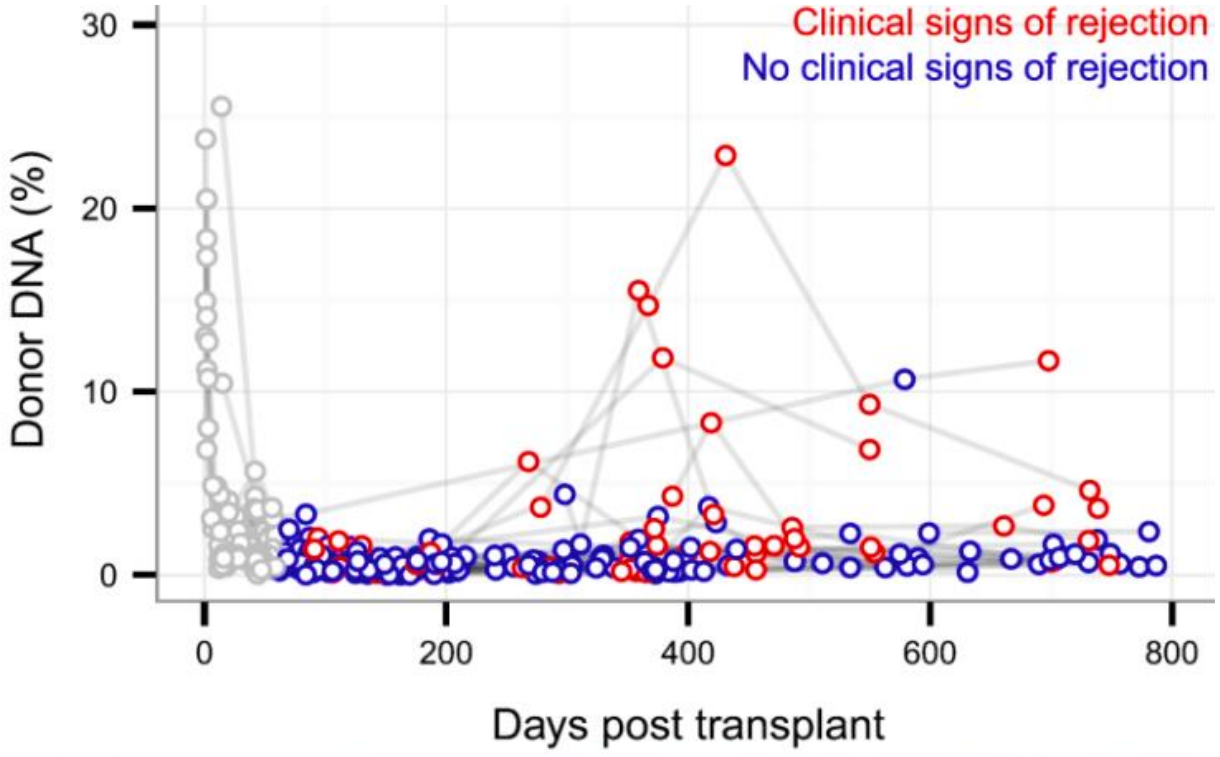
Methods for measuring dd-cfDNA:

- RT-PCR,
- Droplet Digital PCR
- SNP

During allograft rejection, large amounts of dd-cfDNA are released from the injured allograft into the bloodstream

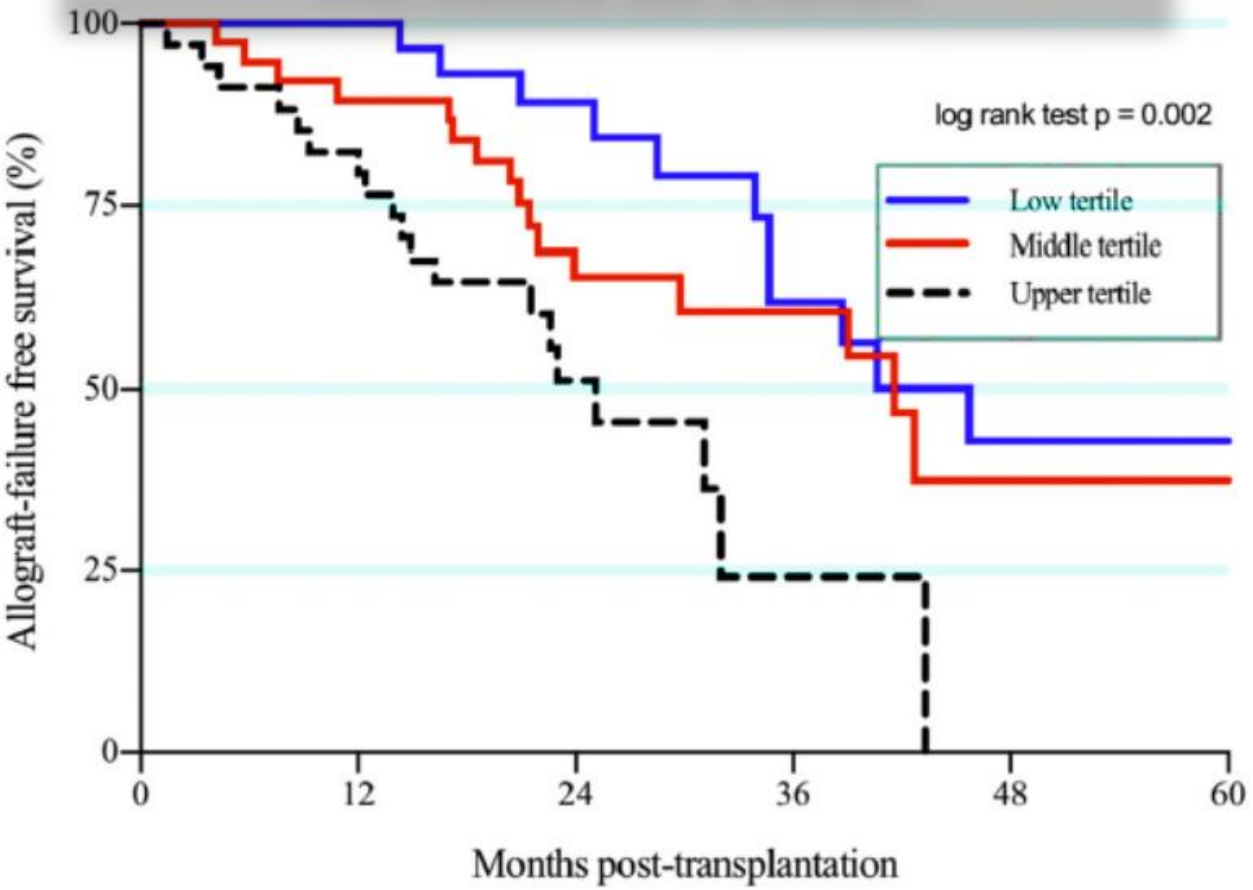
Donor-derived cell-free DNA

Increased Sensitivity of ABMR with increased dd-cfDNA



De Vlaminck et al, PNAS 2015; 112: 13336-41

Decreased graft survival with increased dd-cfDNA



Agbor-Enob etal; E-biomedicine, 2019

Multiple Studies Describe the Ability of dd-cfDNA to Identify Rejection in Organ Transplantation

Year	Organ	Publication	Technology
1998	Kidney & Liver	Lo et al, Lancet 351 (9112):1329	PCR, gender mis-match
2006	Pancreas-kidney	Gadi et al, Clin Chem 52:379	HLA qPCR
2009	Kidney	Moriera et al., Clin Chem 55:1958	PCR, gender mis-match
2011	Heart	Snyder et al, PNAS 108(15):6229	NGS shotgun, SNP detection
2013	Heart, Kidney, Liver	Beck et al, Clin Chem 59:12	Digital PCR, SNP detection
2014	Heart	Hiddestrand et al, JACC 63:1224	Targeted NGS
2014	Heart	DeVlaminck et al, Sci Transl Med. 6(241):241	NGS shotgun, SNP detection
2014	Liver	Macher et al, PLOS One 9(12):e113987	PCR, gender mis-match
2015	Lung	DeVlaminck et al, PNAS 112 (43): 13336	NGS shotgun, SNP detection
2016	Heart	Grskovic et al, J Mol Diag 18(6):890-902	AlloSure (SNP targeted NGS)
2017	Kidney	Bloom/Brennan et al , JASN 28(7):2221	AlloSure (SNP targeted NGS)
2017	Kidney	Bromberg et al, JALM 2(3): 309-321	AlloSure (SNPs targeted NGS)
2017	Liver	Schütz et al , PLoS Medicine 14(4):e1002286	Digital PCR, SNP detection
2018	Lung	Khush et al, J Heart Lung Transplant, online	NGS shotgun, SNP detection

Overview of the DART Clinical Validation Study

The Circulating Donor-Derived Cell-Free **D**NA in Blood for Diagnosing **A**cute **R**ejection in Kidney **T**ransplant Recipients (DART) is the clinical validation study for AlloSure

14 DART Centers Nationwide (US)

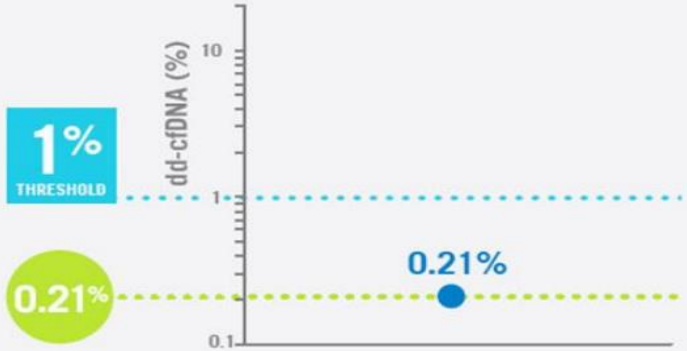
Bloom/Brennan et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034.

dd-cfDNA has very high NPV for “Active Rejection” greater than Banff 1A

96% of AlloSure results for samples from DART healthy stable recipients are below the 1% threshold

50% of AlloSure results for samples from DART healthy stable recipients are below 0.21%

ALLOSURE CAN RULE OUT REJECTION

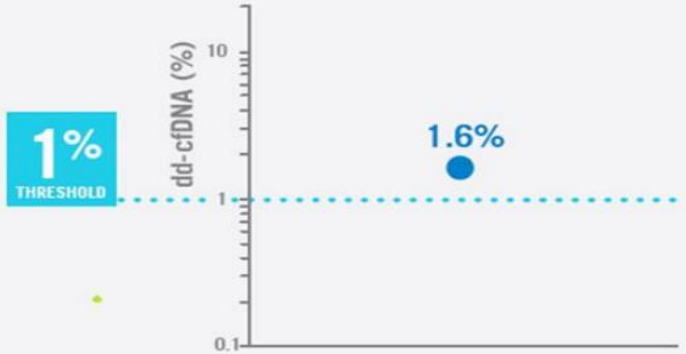


95% NPV for Active Rejection*

Sensitivity: 85%
Specificity: 33%
} at 0.21% dd-cfDNA
Prevalence: 10%[3†]

0.21% is the median from DART healthy stable recipients

ALLOSURE HAS HIGH SPECIFICITY FOR REJECTION DETECTION



44% PPV for Active Rejection*

Sensitivity: 52%
Specificity: 93%
} at 1.6% dd-cfDNA
Prevalence: 10%[3†]

1.6% is the median from DART active rejection

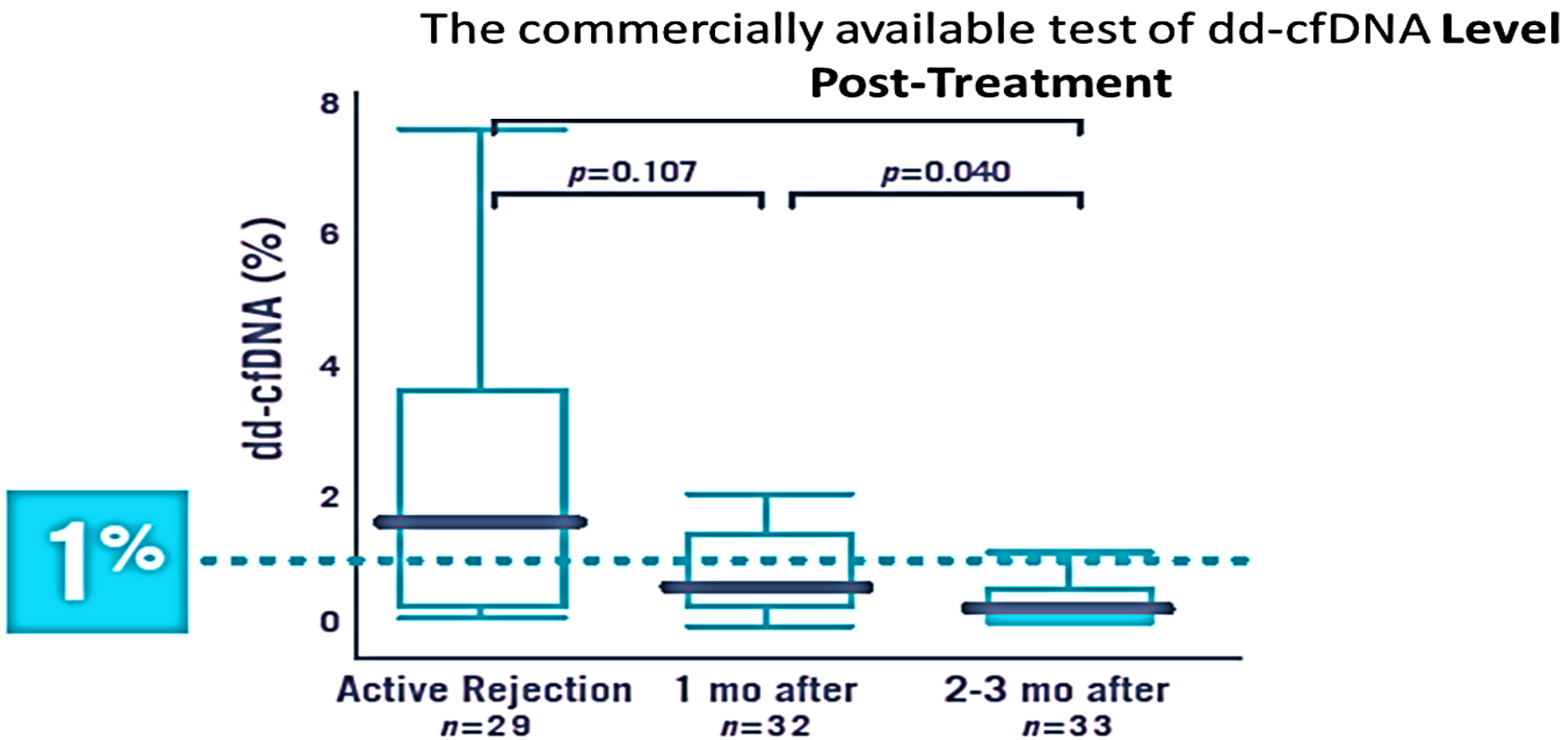
*Active Rejection = Acute/active ABMR; Chronic, active ABMR; and TCMR IA and greater
† Prevalence of rejection within the first year post-transplant

JOHNS HOPKINS MEDICINE

Brennan ATC 2017 Data Suggest that dd-cfDNA Can Be Used to Monitor Therapy

JOHNS HOPKINS MEDICINE

- The commercially available test of dd-cfDNA levels decrease following rejection treatment

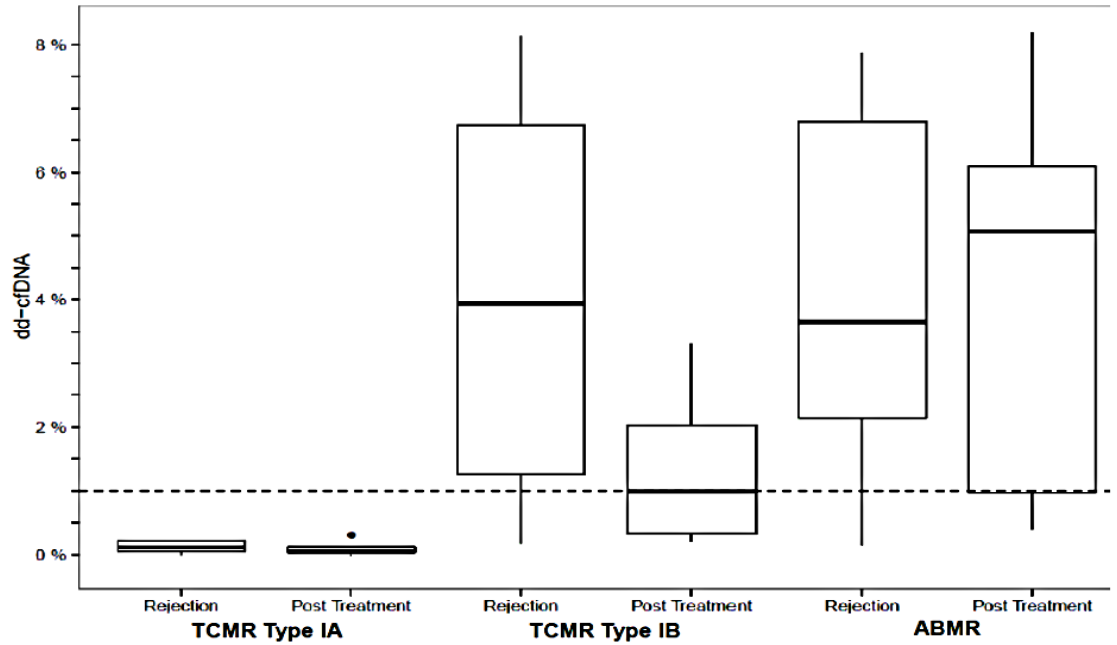


Brennan et al., Am J Transplant 17:536 (suppl 3), 2017.

dd-cfDNA is elevated in TCMR IB and ABMR and Reduced After Treatment for TCMR but not AMR

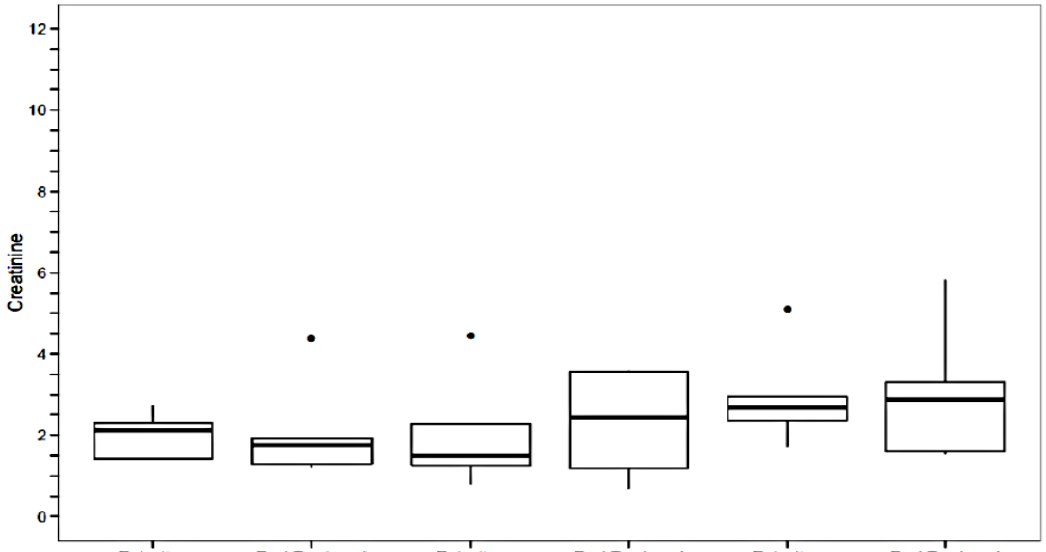
JOHNS HOPKINS MEDICINE

dd-cfDNA (%)



	TCMR Type IA		TCMR Type IB		ABMR	
Median:	0.1%	0.1%	3.9%	1.0%	3.7%	5.1%
Number:	5		4		5	

Creatinine (mg/dL)



	TCMR Type IA		TCMR Type IB		ABMR	
	2.1	1.8	1.5	2.4	2.7	2.9
	5		4		5	

Key Messages from DART

JOHNS HOPKINS MEDICINE

- dd-cfDNA discriminates **Active Rejection** (Acute/active ABMR; Chronic, active ABMR; or TCMR) from **No Active Rejection** with high accuracy*
- dd-cfDNA is more accurate than **Serum Creatinine** in diagnosis of **Active Rejection***
- dd-cfDNA is highly sensitive in distinguishing **ABMR** from **No ABMR***
- dd-cfDNA levels decrease following **Rejection Treatment**

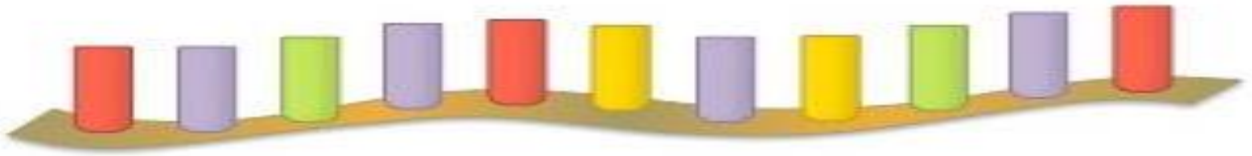
"dd-cfDNA is a non-invasive test of allograft injury that may enable more frequent, quantitative, and safer assessment of allograft rejection and injury status"

* In patients with clinical suspicion of active rejection, the most common cause for the clinical suspicion of active rejection was elevated serum creatinine

Bloom/Brennan et al. Cell-free DNA and active rejection in kidney allografts. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034

Urine biomarkers

mRNAs, miRNAs, and proteins and peptides



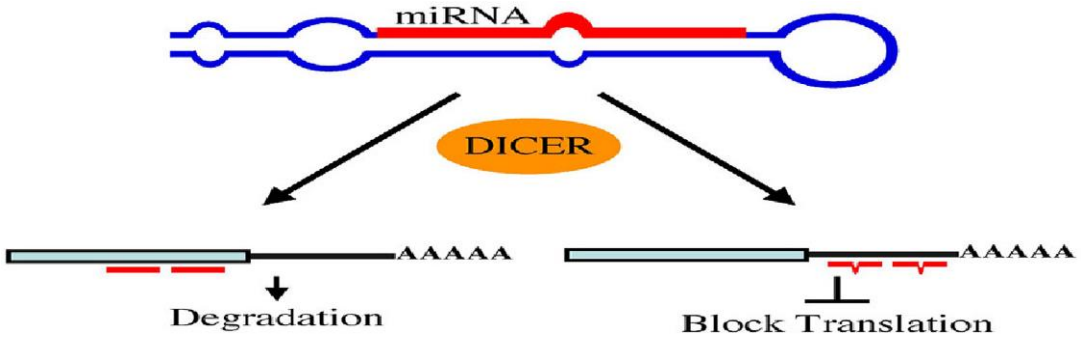
Messenger RNA in the Urine

Urinary mRNA could serve as a noninvasive method to diagnose acute TCMR

- Several potential mRNAs (eg, perforin, granzyme B, IFN-inducible protein-10, CD3), could distinguish or even predict the diagnosis of acute TCMR more than 75%.

- The potential for extensive degradation of mRNAs in urine is one important limitation of this assay.

Urine microRNAs



Quantification of miRNA in urine samples has emerged as an alternative method to assess allograft status

- miR-10a, miR-10b, and miR-210 were strongly deregulated in the urine of patients with acute TCMR
- The combination of miRNA profiling of biopsy and urine samples could be used to monitor graft function and predict progression to chronic allograft dysfunction.

Urine proteins (proteomics)

A number of urinary immune-related proteins have been identified as biomarkers of acute rejection of the renal allograft

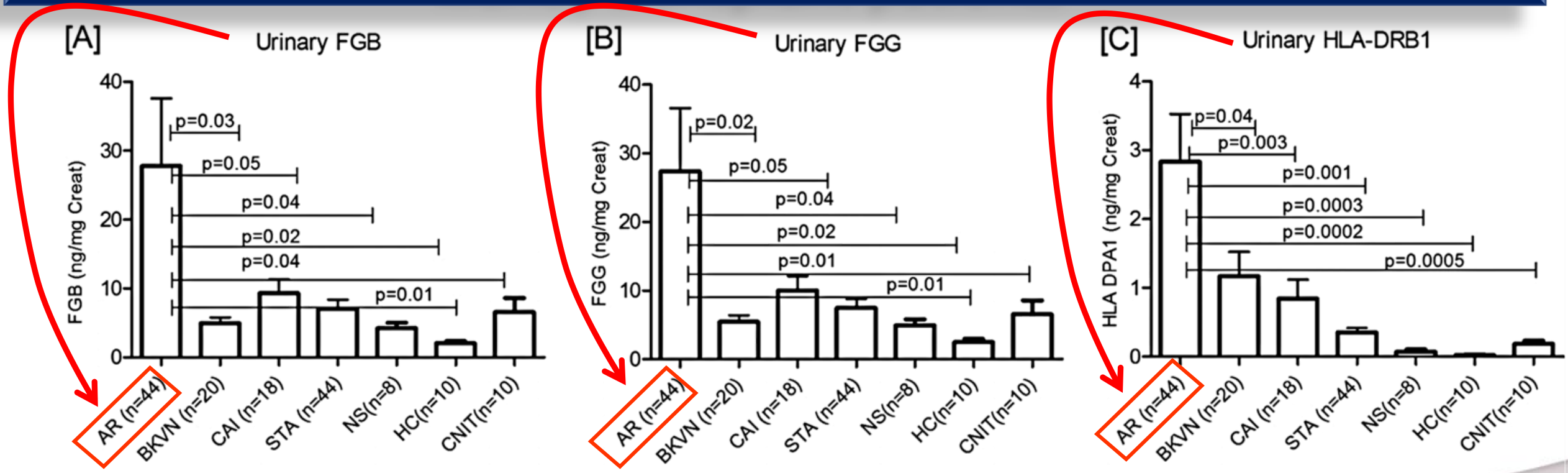
- Elevated urinary levels of chemokine (C-X-C motif) ligands 9 and 10 (CXCL9 and CXCL10) have been associated with acute TCMR
- monocyte chemoattractant protein 1 at six months posttransplant was a predictor of severe IF/TA and graft dysfunction at two years posttransplant
- Low urinary CXCL10 levels were associated with improved rejection-free allograft survival at one year posttransplant
- Low Urinary CXCL10➔97% NPV for TC rejection➔ to find optimal candidates for immunosuppressive drug weaning.
- However, increased urinary CXCL9 and CXCL10 levels have also been detected in patients with BK virus (BKV) infection➔ PPV is low

The Identification of Novel Potential Injury Mechanisms and Candidate Biomarkers in Renal Allograft Rejection by Quantitative Proteomics*[S]

Tara K. Sigdel‡, Nathan Salomonis‡, Carrie D. Nicora§, Soyoung Ryu¶, Jintang He§, Van Dinh‡, Daniel J. Orton§, Ronald J. Moore§, Szu-Chuan Hsieh‡, Hong Dai‡, Minh Thien-Vu‡, Wenzhong Xiao¶, Richard D. Smith§, Wei-Jun Qian§, David G. Camp 2nd§, and Minnie M. Sarwal‡‖

Molecular & Cellular Proteomics 13: 10, 621–631,2014

Urine proteomics provide an effective strategy for biomarker discovery with the goal of monitoring transplant injury in kidney transplant patients



Molecular & Cellular Proteomics 13: 10, 621–631,2014

Definitions

Conventional Biomarkers

New biomarkers in Serum urine and tissue

Summary & Conclusion

Selected biomarkers for outcomes in kidney transplantation: summary of current status

Authors	Assay Name	Assay Type	Timing Post-Transplant	Outcome
FDA-approved assays				
Patel and Terasaki[19]	CDC crossmatch	Microcytotoxicity assay	Pretransplant	Hyper-AR/early graft loss
Mahoney *et al.*[149]	Flow crossmatch	Flow cytometry	Pretransplant	Early graft loss (<2 mo)
Pei *et al.*[150]	Lumine	HLA beads; flow cytometry	Variable pre-/post-transplant	Anti-HLA Ab
Ashokkumar *et al.*[61]	Pleximmune	T cytotoxic memory cell assay	Rejection episodes	Biopsy-proven AR
He *et al.*[63]	Cylex-Immuknow	Lymphocyte ATP generation assay	Serial <30 mo	CD4-T cell function
Loupy *et ald.*[32]	C1q binding assay	Flow cytometric C1q binding[b]	Baseline, 1 yr, rejection episode	TCMR/ABMR/ graft loss
Selected externally validated assays in kidney transplantation (pending FDA approval)				
Hricik *et al.*[47]	IFN-γ ELISPOT	Donor-reactive memory T cell	Preatransplant	*De novo* DSA and or rejection
Hricik *et al.*[70]	Urine CXCL9	Urine ELISA	Serial <6 mo, rejection episode	TCMR
Suthanthiran *et al.*[86]	Urine three-gene signature	Urinary RNA by qPCR	Serial <12 mo, rejection episode	AR
Roedder *et al.*[119]	KSORT	Peripheral blood RNA by qPCR	Serial <24 mo, rejection episode	Biopsy-proven AR
Halloran *et al.*[112]	ENDAT	Graft biopsy RNA by microarray	Variable (1 wk to 31 yr)	ABMR
O'Connell *et al.*[18]	GoCAR score	Graft biopsy RNA by microarray (3-mo protocol biopsies)	3 mo biopsy	Prediction of fibrosis/ histologic progression

Madhav C. Menon etal.
ASN; 2017, 28 (3) 735-747

Overview of the diagnostic performance of biomarkers in detecting kidney transplant rejection

Authors	Biomarkers	Sample size	Rejection type	AUC	Sensitivity%	Specificity%	PPV%	NPV%
Suthanthiran et al. (28)	Three-gene signature in urine cell pellets	$N = 485$ kidney transplant patients $N = 4{,}300$ urine samples	Acute TCMR	0.74	71	72		
Roedder et al. (29)	kSORT	$N = 436$ kidney transplant patients $N = 558$ blood samples	Acute rejection (both TCMR and ABMR)	0.94	83.0	90.6	93.2	
Hricik et al. (30)	CXCL9 protein	$N = 255$ kidney transplant patients	Banff $\geq$ 1 rejection	0.86	85.2	80.7	67.6	92
Rabant et al. (31)	CXCL9	$N = 247$ kidney transplant patients $N = 290$ matched kidney biopsies and urine samples	TCMR	0.86	80	87	23.5	98.9
Rabant et al. (31)	CXCL10	$N = 247$ kidney transplant patients $N = 290$ matched kidney biopsies and urine samples	ABMR	0.70	73	61.6	25.7	92.6
Rabant et al. (31)	CXCL10	$N = 247$ kidney transplant patients $N = 290$ matched kidney biopsies and urine samples	Mixed rejections	0.80	74.2	83.3	40.4	95.5
Bloom et al. (32)	dd-cfDNA	$N = 102$ kidney transplant patients $N = 107$ plasma samples matched with a biopsy.	Active rejection	0.74	59	85	61	84

Eikmans M. etal, Frontiers in Medicine, 08 January 2019; 1-7

Conclusion

- Implementation of the new biomarkers into standard clinical practice remain challenging.
- Most new biomarkers have high NPV for rejection, but not enough PPV
- Dedicated, prospective, interventional trials are required to demonstrate that the use of these biomarkers improves patient or transplant outcomes.
- Significant limitations should be solved before regular usage in clinical practice such as: generalizability, cost, ease of interpretation, and identification of patient populations who may benefit from more than standard-of-care surveillance.
- The definitive Dx. of renal allograft dysfunction still requires an allograft biopsy, it remains the gold standard for the assessment of graft status.

Thank you for your attention