(ALLO)IMMUNE-MONITORING IN KIDNEY TRANSPLANTATION

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PRESENT OF NOVEL THERAPEUTICS IN TRANSPLANTATION

Search for BIOMARKERS allowing IS individualisation

• “Sufficient” long-term kidney allograft survival using current IS (1990-2000)

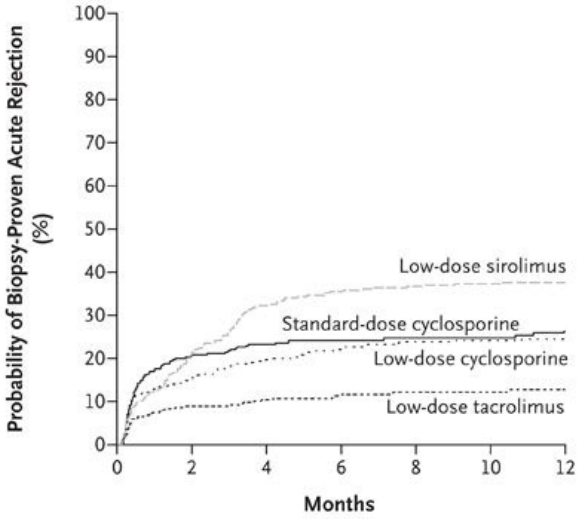
→ IS-related adverse events (Cancer, C-V, Nephrtoxicity) are “well-accepted” related to current graft/ patient survival rates

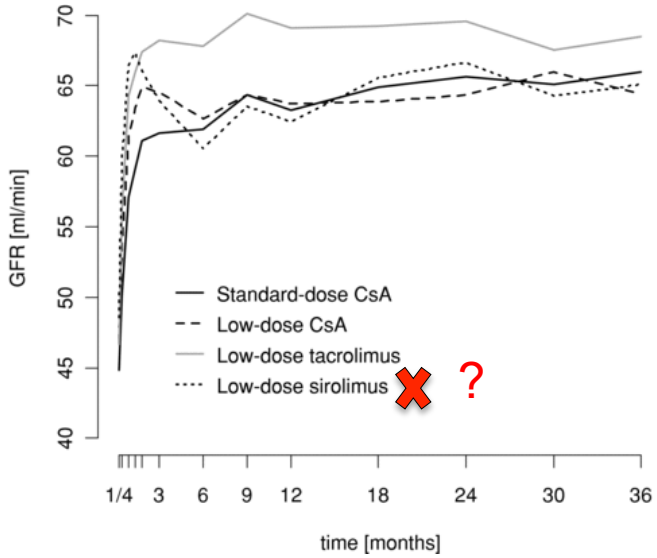
• Stringent hurdles of regulatory agencies for new drug approval as compared to other fields of Medicine (Oncology, Rheumatology)

• Lack of investment on new targets/drugs for Organ TX by Pharma Industry

Short vs Long-term follow-up studies → Biomarker success

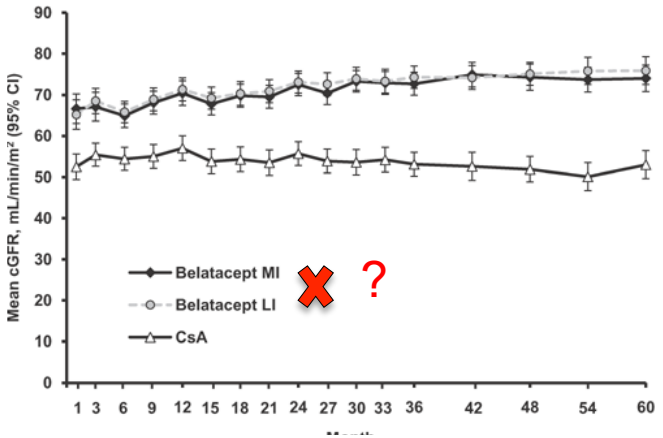
Symphony Trial (CNI vs mTor-i)





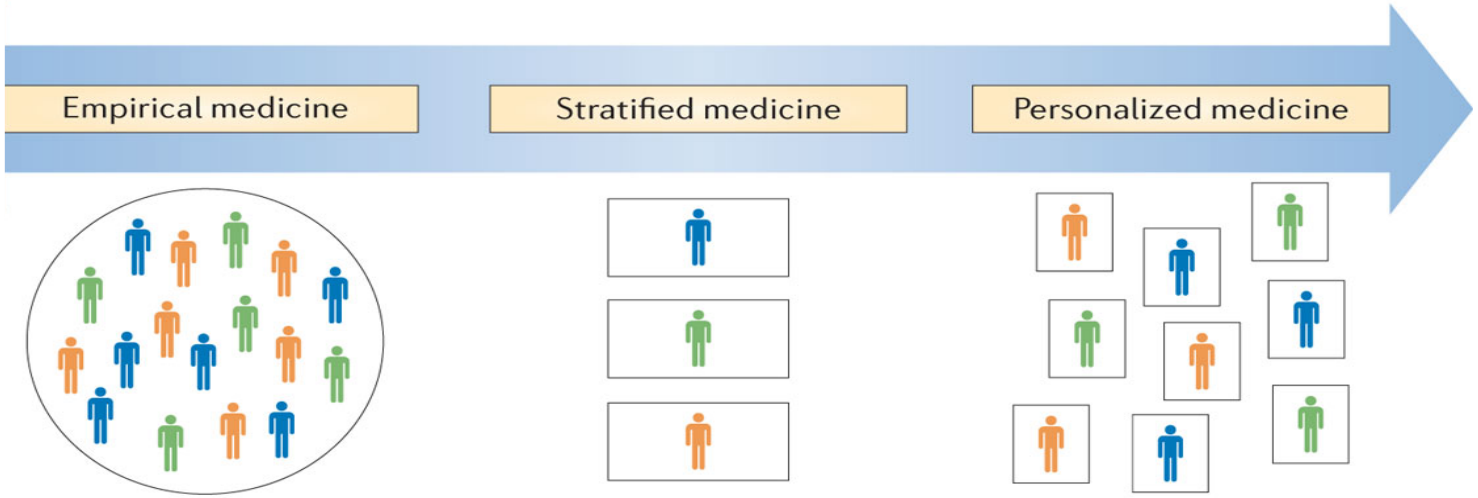
Beneffit Trial (Belatacept vs CNI)

Month 12 endpoints	Belatacept MI (n = 219)	Belatacept LI (n = 226)	Cyclosporine (n = 221)
Acute rejection			
Acute rejection, n (%)	49 (22)	39 (17)	16 (7)
95% CI	16.9–27.9	12.3–22.2	3.8–10.7
Difference from CsA (97.3% CI)	15.1 (7.9, 22.7)	10.0 (3.3, 17.1)	–
Banff grade, n (%)			
Mild acute (IA)	7 (3)	4 (2)	3 (1)
Mild acute (IB)	3 (1)	8 (4)	5 (2)
Moderate acute (IIA)	17 (8)	16 (7)	6 (3)
Moderate acute (IIB)	20 (9)	10 (4)	2 (1)
Severe acute (III)	2 (1)	1 (<1)	0



Ekberg H et al, NEJM 2008; Vincenti F et al AJT 2010

BIOMARKER-driven Strategies



A **generalized** treatment

Evidence based

Different treatments for **groups**

Evidence based

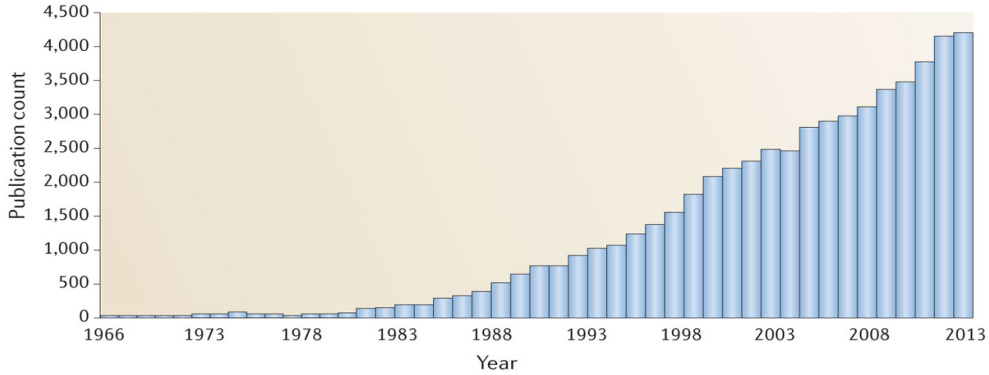
Biomarker led

Individual treatments for **each**

Evidence based

Biomarker led

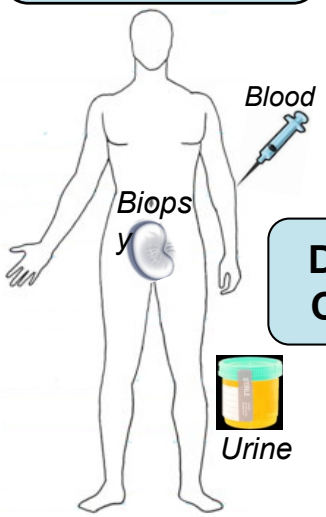
Volum of “Immune biomarkers” puiblications in Transplantation



Lord G et al Nat Immuol Rev 2015

Transplant Patient Biomaterial

- PBMC
- Serum
- Plasma
- Tissue
- Sedimentary cells
- Supernatant
- BAL
- Saliva



Demographic Clinical Data

High-Throughput Discovery

Genome DNA
(Epigenetics, SNPs)

Transcriptome
mRNA, miRNA, siRNA
(Gene regulation)

Proteome / Metabolome
Proteins
(Gain-/loss of function)

Informatics Statistics

Biology

Clinical Phenotype

Biomarker Panel I

VALIDATION

Initial Validation

INTERNAL

- **Meta-analyses**
  (independent sample sets from public databases)
- **Confounder Analyses**
  (sample bias, technology bias, patient bias)
- **Pathway analyses**
- **Gene-set enrichment**

Refinement

Unique / Combined Biomarker Panel

EXTERNAL

Cross Validation

- **Independent samples**
- Cross Organ
- Integrative
- Intertechnological
- **MA back validation**

Prospective Validation

- independent, serial samples
- **clinical settings**
- process optimization

Clinical Application

Regulation

- FDA
- MicroArray Quality Control (MAQC)

Valid Biomarker

Clinical Trial

- cohort studies
  (healthy vs sick)
- causality test

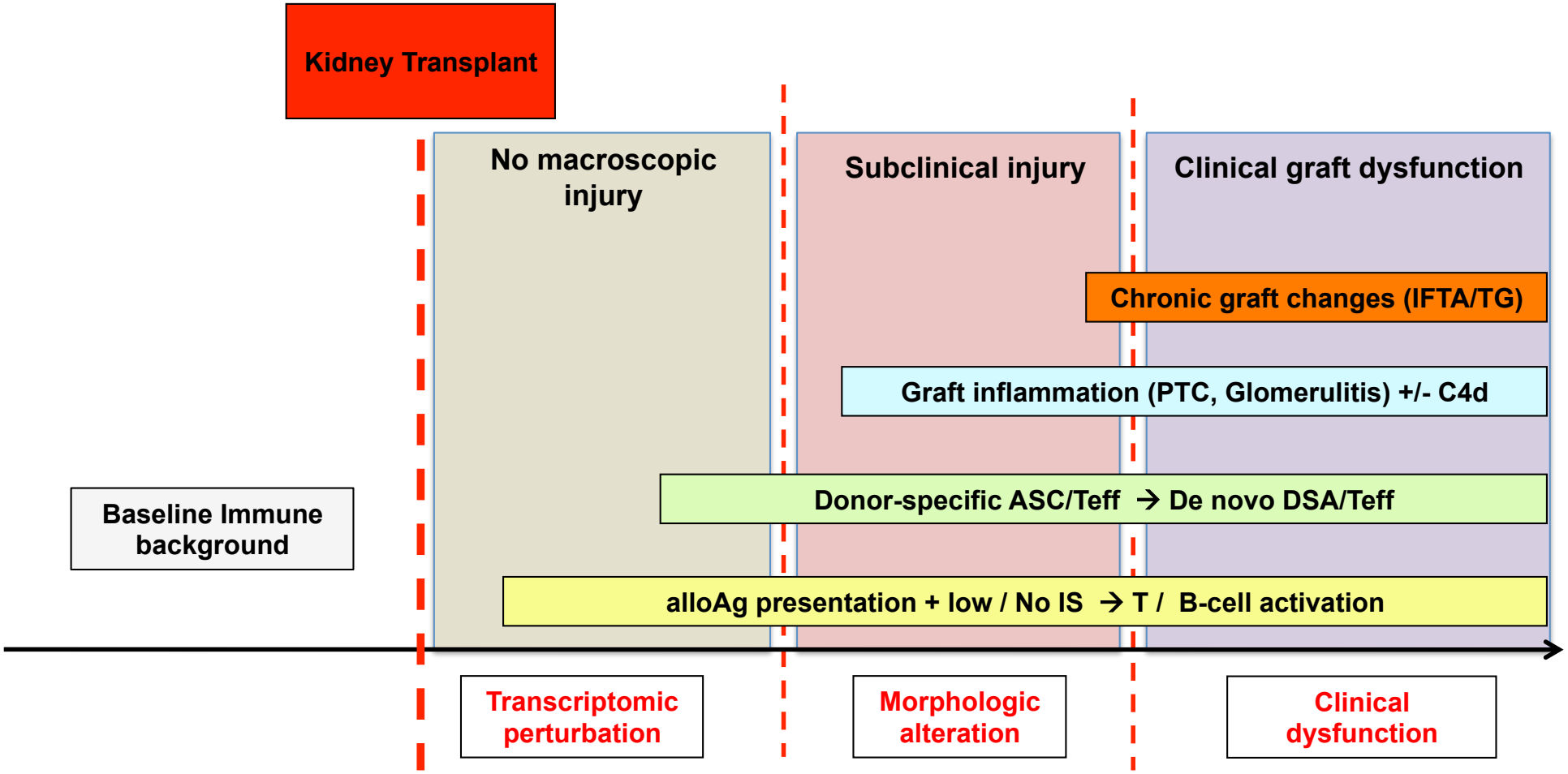
Assay-Establishment

- sensitive, specific, reliable
- reproducible
- feasible for clinical application

Bestard & Sarwal 2015 In press

Chronic Rejection is driven by ongoing, subclinical "pre-AR"

Window for Biomarkers to evaluate pathogenic processess in response to Therapeutic intervention



Bestard & Sarwal. Ped Nephrol 2015

OMICS in Transplantation

Genomics

Gene Polimorphisms
→ Change in Phenotypes

Transcriptomics
"functional genomics"

Single/Multiple Gene transcripts
→ Gene function

Proteomics

Proteins encoded by the genome
→ Cell process

Metabolomics

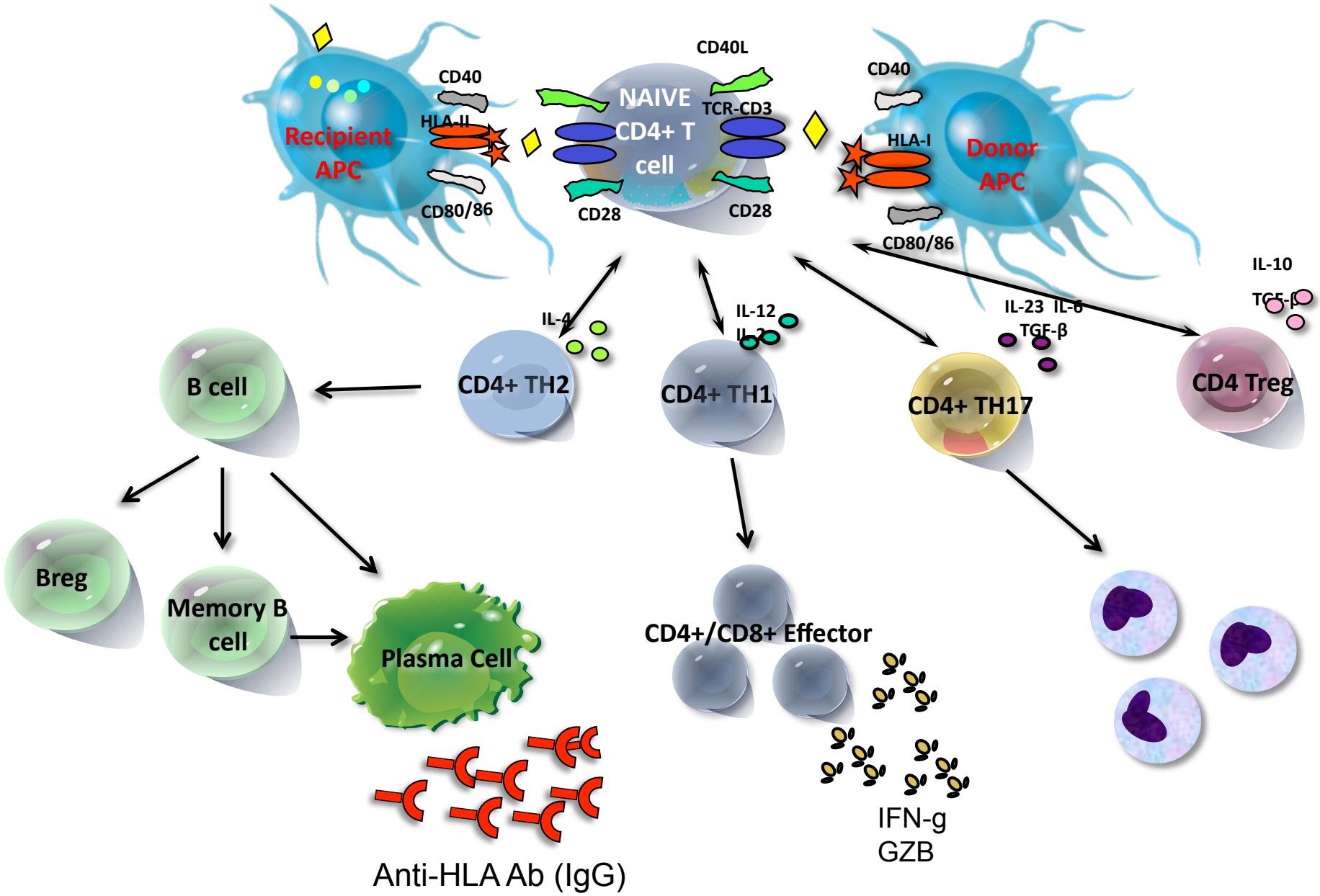
Metabolite/panels
→ Cell process in disease states

Immune function
(cell-based assays)

Cellular assays
→ Functionality of alloimmune response

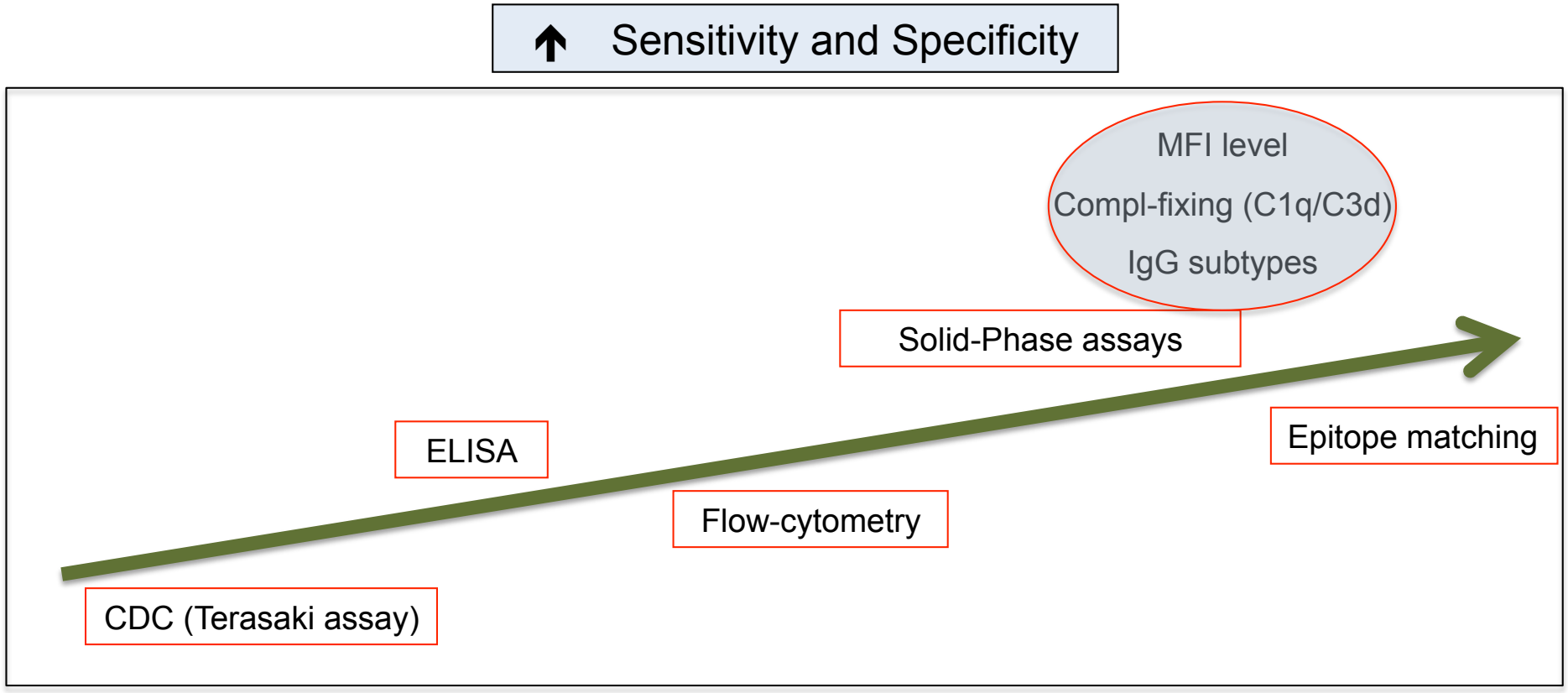
Bestard & Sarwal 2015 In press

Immune-monitoring Alloimmune responses



Bestard O Nefrología Clínica. Ed Panamericana

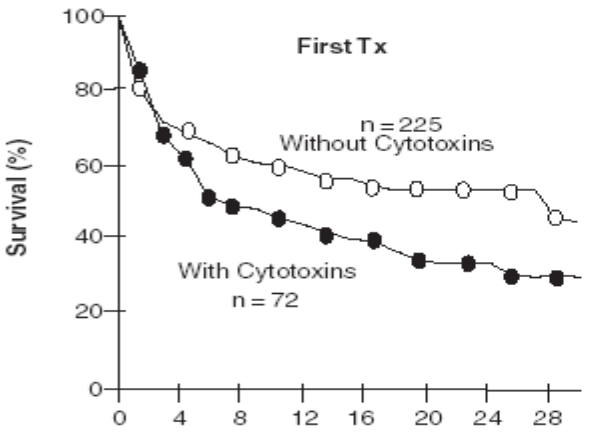
Assessment of anti-donor humoral alloresponses



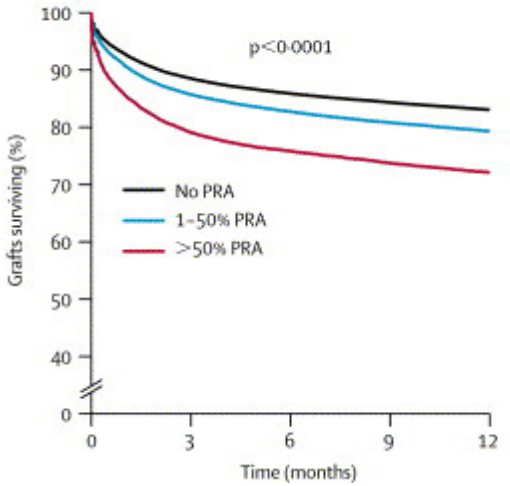
Improvement of Ab detection systems

Challenge of clinical interpretation

Pre-TX CDC-XM+ and worse GS

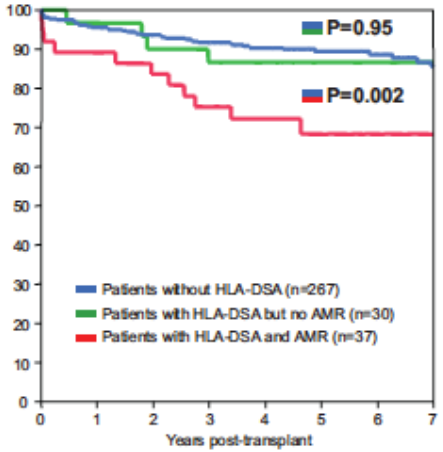


Terasaki et al NEJM 1969

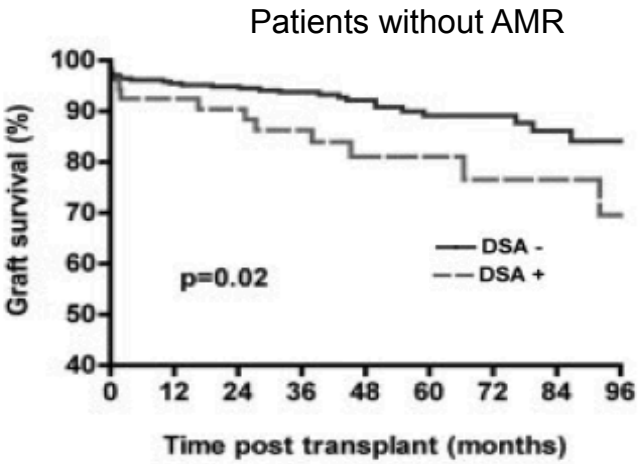


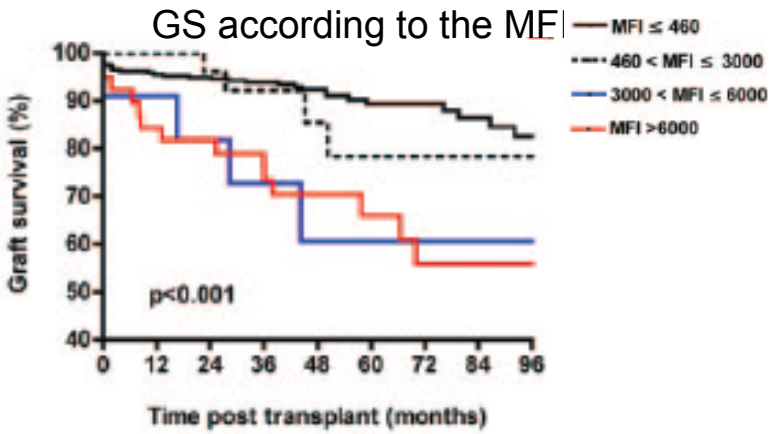
Opelz G, Lancet 2005

Pre-TX Solid-Phase assays and DSA in presence of CDC and FCM XM-



Amico et al Transplant 2009

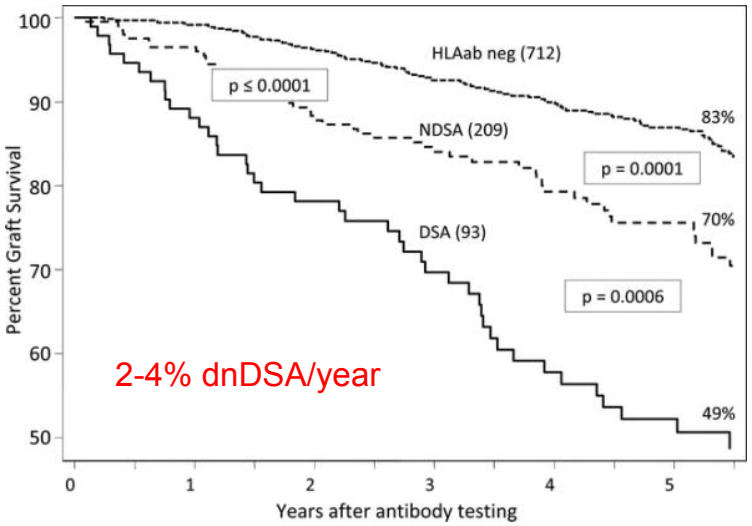




Lefacheur C et al JASN 2010

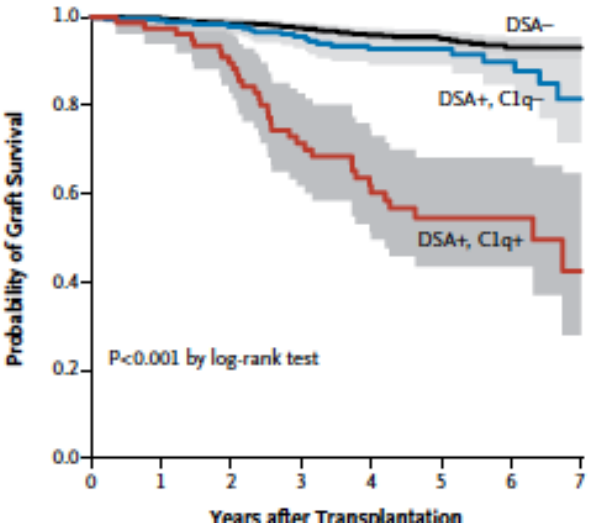
Post-TX Complement-fixing Ab effect

De novo DSA



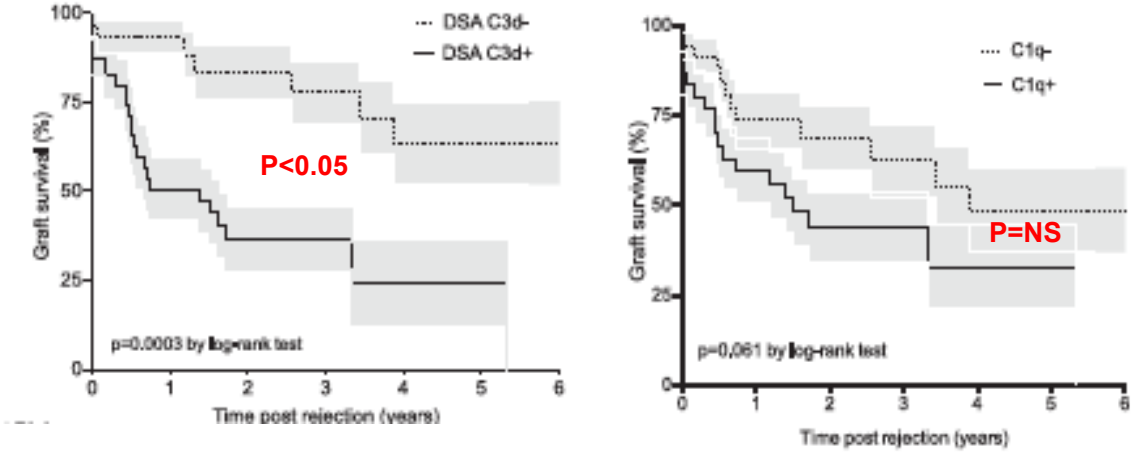
Lachmann, Nils et al Transplant 2009

De novo C1q-binding DSA

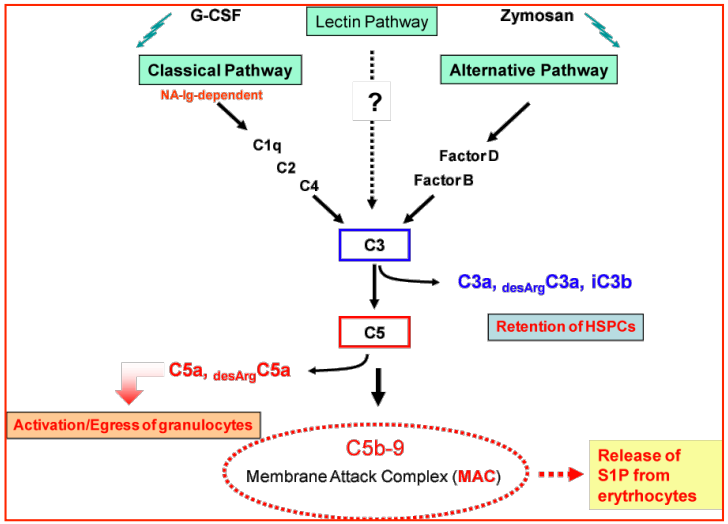


Loupy A et al NEJM

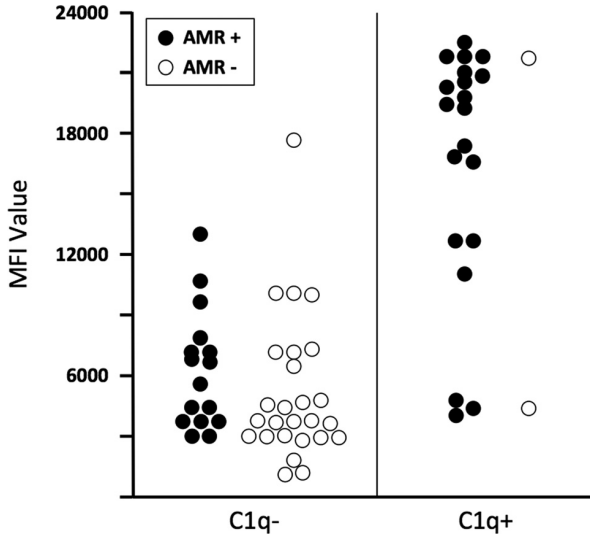
DSA C3d vs C1q at **ABMR**



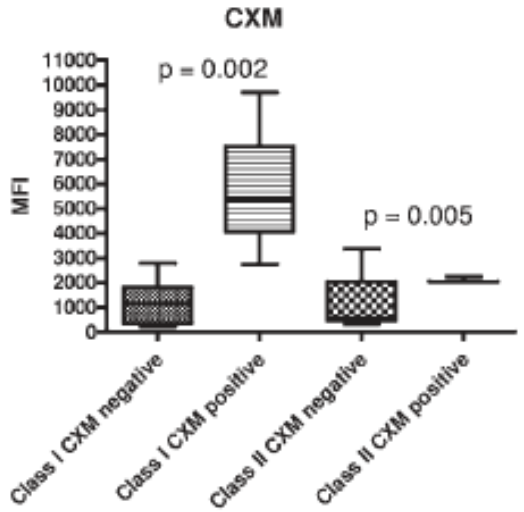
Thaunat O et al JASN 2015



Complement-fixing DSA and Ab strength



Maggie Yell et al Transplant 2014



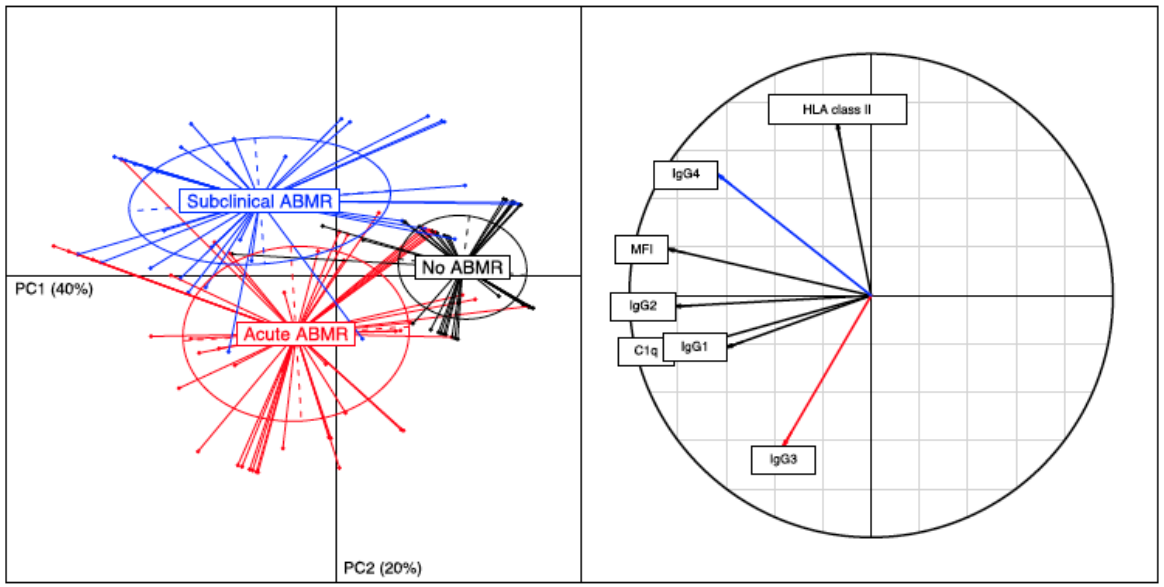
Morris GP et al Hum Immunol 2009

Low MFI DSA, non-Compl-fixing Ab

→ Neg FCM and CDC XM

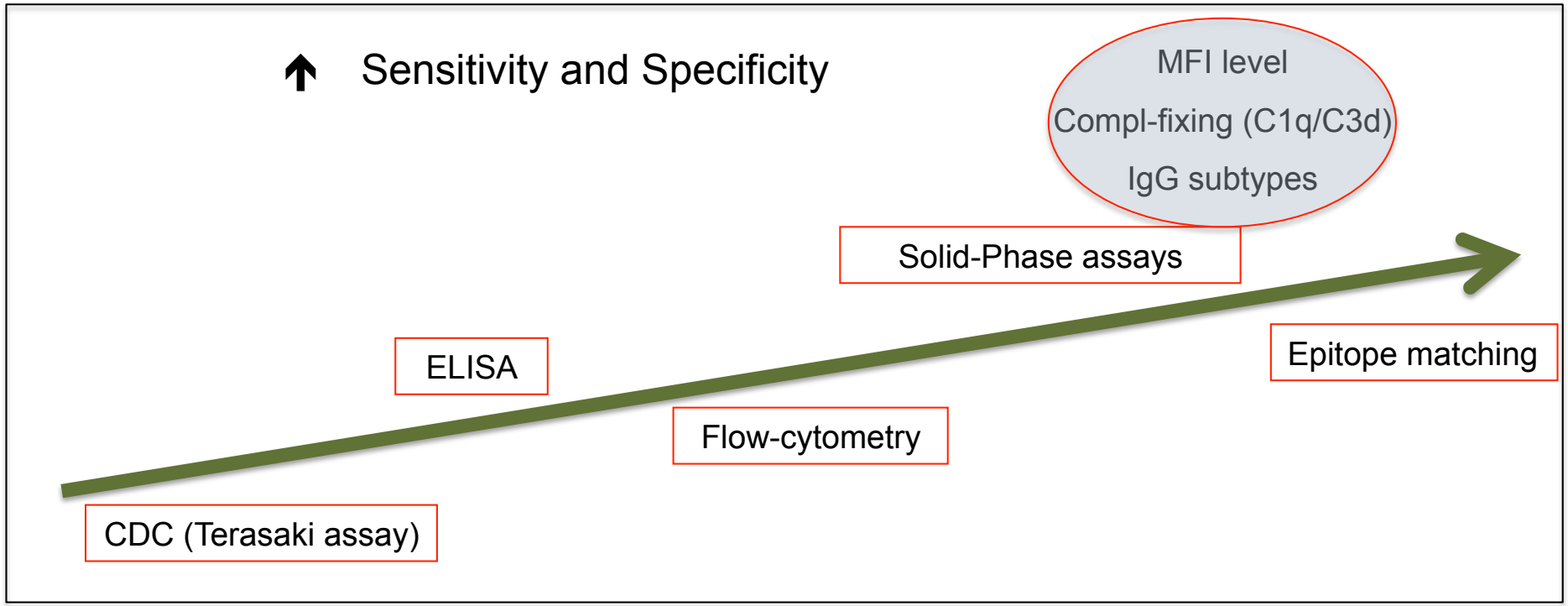
→ Acceptable DSA ?

dnDSA IgG subtypes and clinical phenotypes



Lefacheur C et al JASN 2015

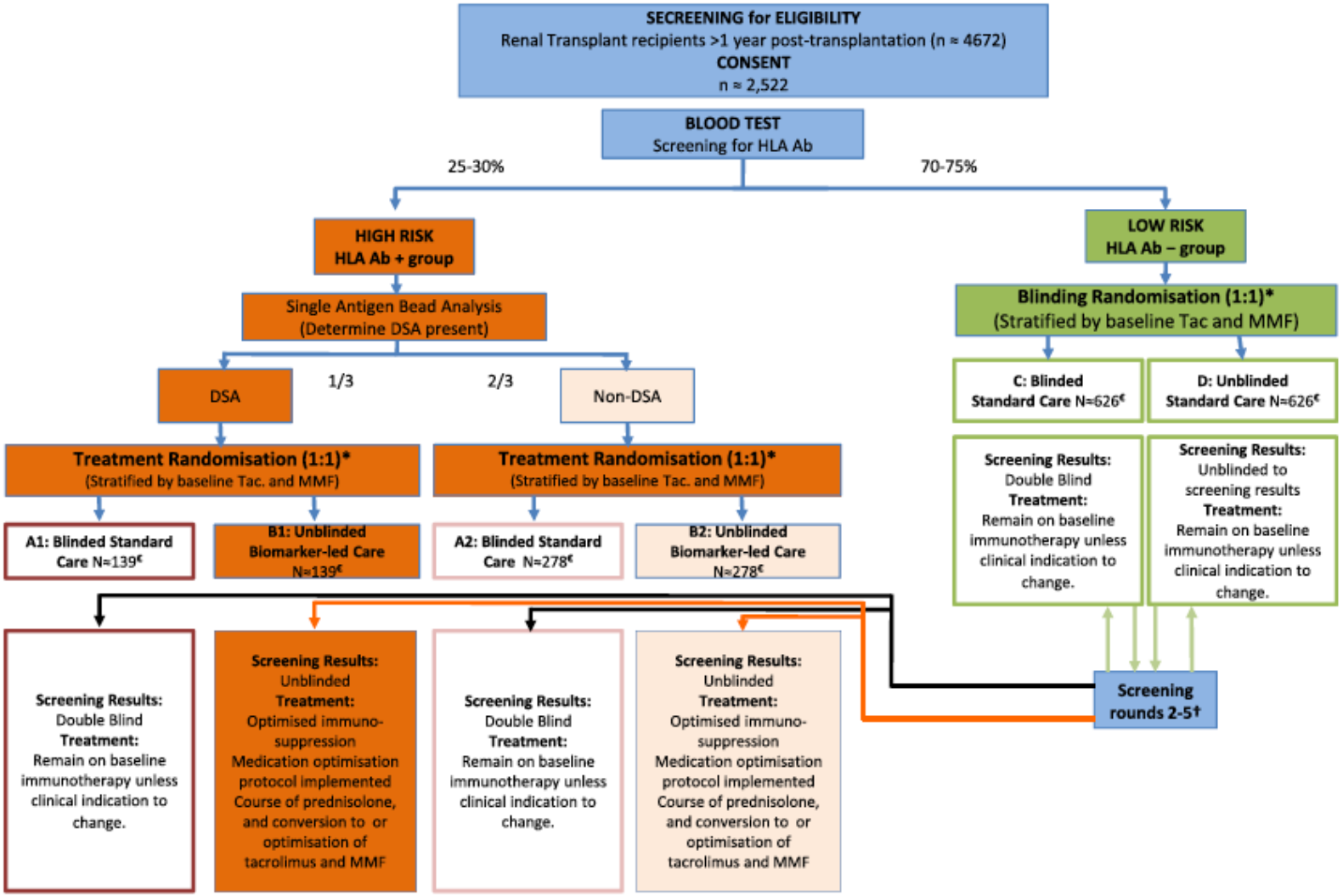
Assessment of effector anti-donor humoral alloresponses



- Significance of presence of anti-HLA Ab
- Significance of the specificity of DSA (class I and II)
- Significance of the strength (titer/MFI) and function (Comp-Fixing/IgG subtype) of DSA
- Significance of the "time" (*preformed* or *de novo*) and phenotype (clinical or subclinical)
- **Clinical Management ??**

Interventional Biomarker-driven Trials

OutSMART Trial



Dorlin A et al Trials 2014

Commentary

Detecting the Humoral Alloimmune Response: We Need More Than Serum Antibody Screening

Gonca E. Karahan,[1] Frans HJ. Claas,[1] and Sebastiaan Heidt[1]

Abstract: Whereas many techniques exist to detect HLA antibodies in the sera of immunized individuals, assays to detect and quantify HLA-specific B cells are only just emerging. The need for such assays is becoming clear, as in some patients, HLA-specific memory B cells have been shown to be present in the absence of the accompanying serum HLA antibodies. Because HLA-specific B cells in the peripheral blood of immunized individuals are present at only a very low frequency, assays with high sensitivity are required. In this review, we discuss the currently available methods to detect and/or quantify HLA-specific B cells, as well as their promises and limitations. We also discuss scenarios in which quantification of HLA-specific B cells may be of additional value, besides classical serum HLA antibody detection.

(*Transplantation* 2015;00: 00–00)

LITERATURE Watch

Implications for transplantation

Identifying Diversity Within Memory B Cell Populations

CITATION **Zuccarino-Catania GV, Sadanand S, Weisel FJ, Tomayko MM, Meng H, Kleinstein SH, et al. CD80 and PD-L2 define functionally distinct memory B cell subsets that are independent of antibody isotype. Nat Immunol 2014; 15: 631-617.**

Review

Memory B Cells in Transplantation

Anita S. Chong[1] and Roger Sciammas[2]

Abstract: Much of the research on the humoral response to allografts has focused on circulating serum antibodies and the long-lived plasma cells that produce these antibodies. In contrast, the interrogation of the quiescent memory B cell compartment is technically more challenging and thus has not been incorporated into the clinical diagnostic or prognostic toolkit. In this review, we discuss new technologies that have allowed this heretofore enigmatic subset of B cells to be identified at quiescence and during a recall response. These technologies in experimental models are providing new insights into memory B cell heterogeneity with respect to their phenotype, cellular function, and the antibodies they produce. Similar technologies are also allowing for the identification of comparable memory alloreactive B cells in transplant recipients. Although much of the focus in transplant immunology has been on controlling the alloreactive B cell population, long-term transplant patient survival is also critically dependent on protection by pathogen-specific memory B cells. Techniques are available that allow the interrogation of memory B cell response to pathogen re-encounter. Thus, we are poised in our ability to investigate how immunosuppression affects allospecific and pathogen-specific memory B cells, and reason that these investigations can yield new insights that will be beneficial for graft and patient survival.

(*Transplantation* 2015;99: 21–28)

Up-dated 2013 Banff classification of kidney allograft Biopsies

Table 2: Revised (Banff 2013) classification of antibody-mediated rejection (ABMR) in renal allografts

Acute/active ABMR; all three features must be present for diagnosis[1,2]

1. Histologic evidence of acute tissue injury, including one or more of the following:
   - Microvascular inflammation ($g > 0$[3] and/or $ptc > 0$)
   - Intimal or transmural arteritis ($v > 0$)[4]
   - Acute thrombotic microangiopathy, in the absence of any other cause
   - Acute tubular injury, in the absence of any other apparent cause
2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or $C4d > 0$ by IHC on paraffin sections)
   - At least moderate microvascular inflammation ($[g + ptc] \geq 2$)[5]
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated[6]
3. Serologic evidence of donor-specific antibodies (DSAs) (HLA or other antigens)

Chronic, active ABMR; all three features must be present for diagnosis[1,7]

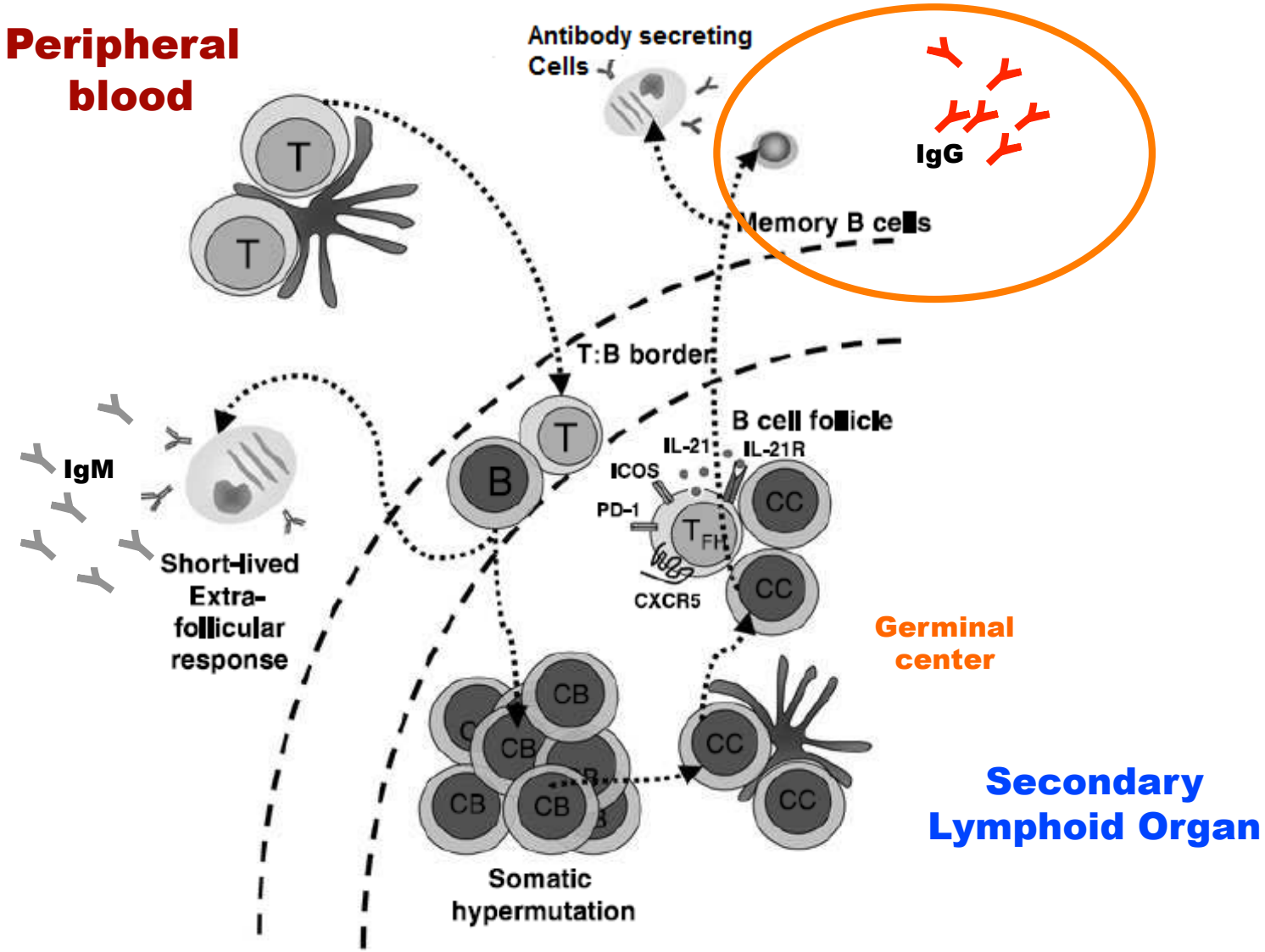
1. Morphologic evidence of chronic tissue injury, including one or more of the following:
   - Transplant glomerulopathy (TG) ($cg > 0$)[8], if no evidence of chronic thrombotic microangiopathy
   - Severe peritubular capillary basement membrane multilayering (requires EM)[9]
   - Arterial intimal fibrosis of new onset, excluding other causes[10]
2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or $C4d > 0$ by IHC on paraffin sections)
   - At least moderate microvascular inflammation ($[g + ptc] \geq 2$)[5]
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated[6]
3. Serologic evidence of DSAs (HLA or other antigens)

C4d staining without evidence of rejection; all three features must be present for diagnosis[11]

1. Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or $C4d > 0$ by IHC on paraffin sections)
2. $g = 0$, $ptc = 0$, $cg = 0$ (by light microscopy and by EM if available), $v = 0$; no TMA, no peritubular capillary basement membrane multilayering, no acute tubular injury (in the absence of another apparent cause for this)
3. No acute cell-mediated rejection (Banff 97 type 1A or greater) or borderline changes

[7]Lesions of chronic, active ABMR can range from primarily active lesions with early TG evident only by EM (cg1a) to those with advanced TG and other chronic changes in addition to active microvascular inflammation. In the absence of evidence of current/recent antibody interaction with the endothelium (those features in the Second Section), the term active should be omitted; in such cases DSA may be present at the time of biopsy or at any previous time posttransplantation.

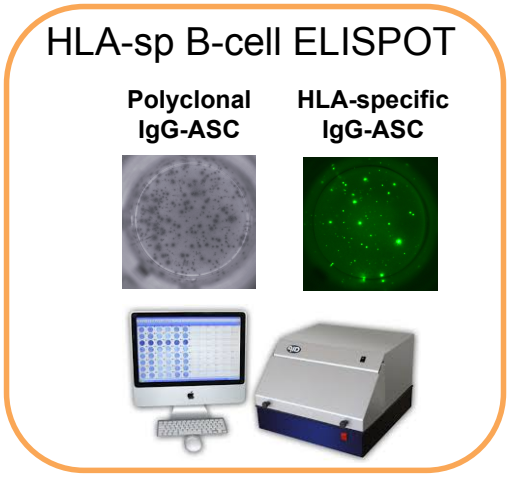
Generation of memory B-cell responses

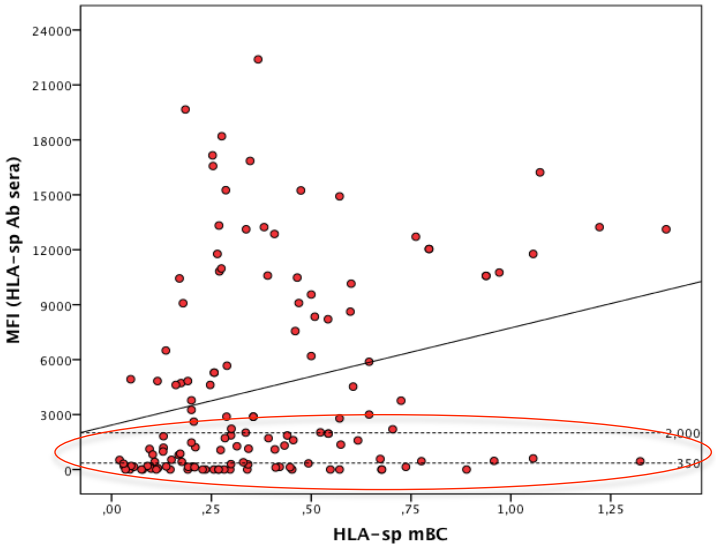


After encounter of a previous Ag, mBc rapidly differentiate into **ASC**
(already class switched and somatic hypermutation done)

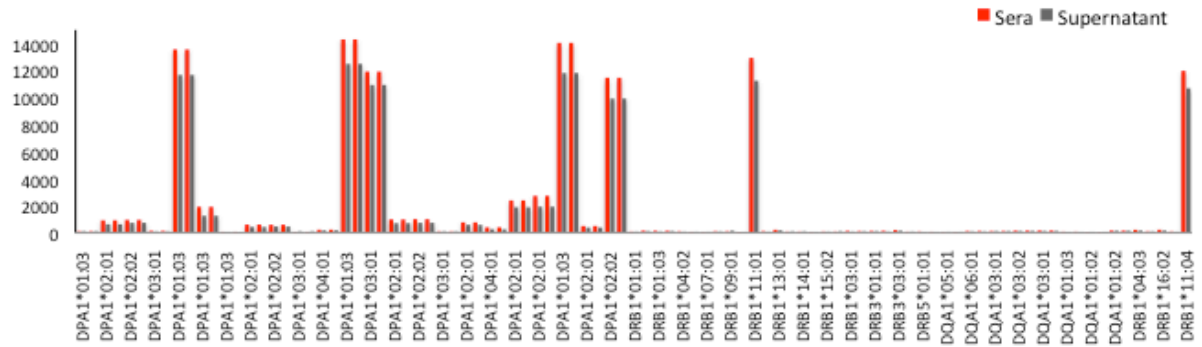
Lanzavecchia et al Nat rev 2010

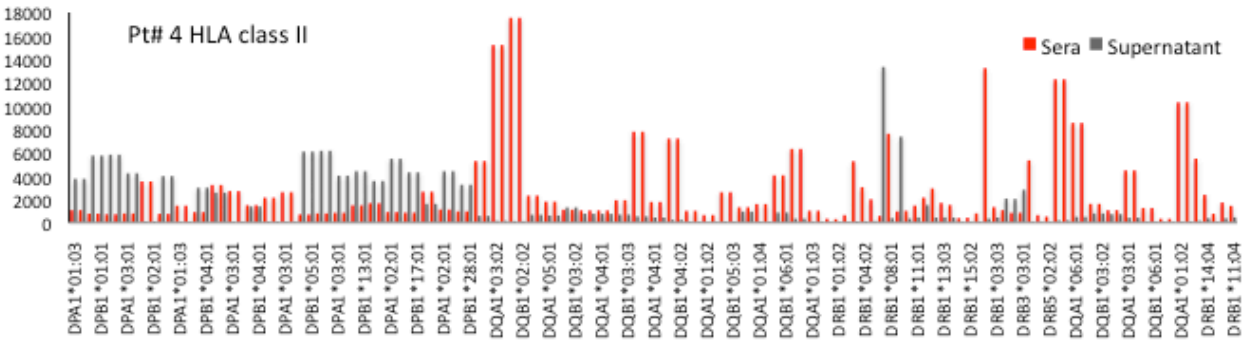
Preformed donor-specific mBC and risk of ABMR





42/136 (30.8%)

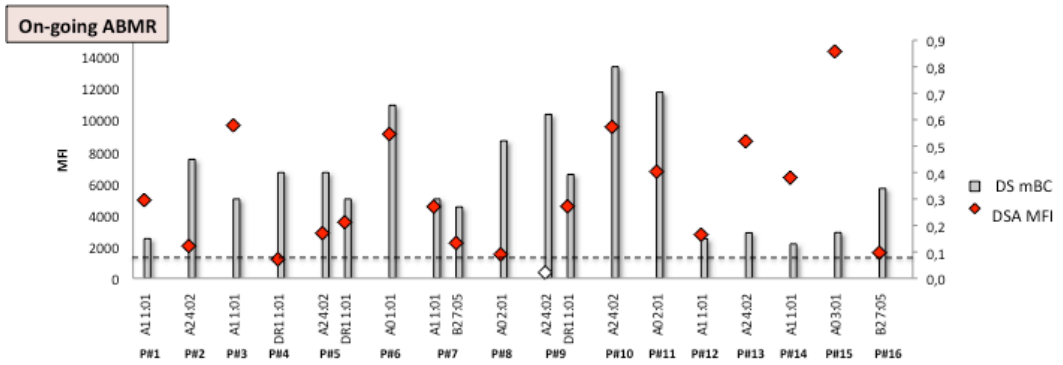


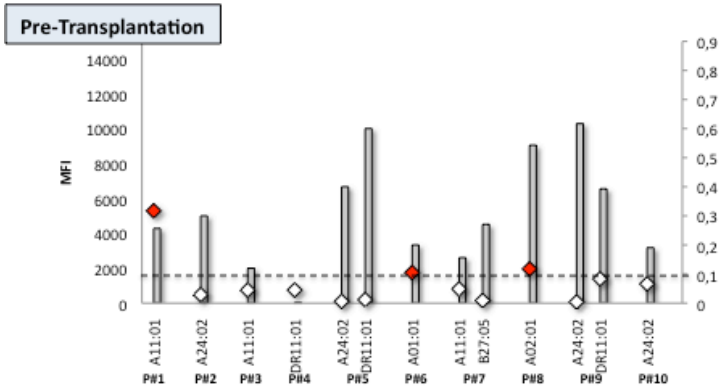


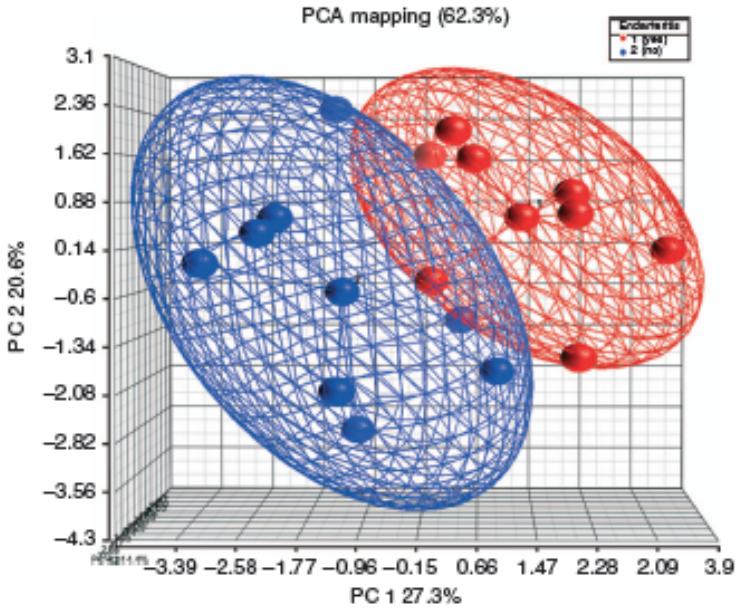
Different Ag-sp repertoirs between mBC and circulating HLA-Ab

Lúcia & Bestard Kidney Int 2015
Heidt S et al AJT 2011

Preformed donor-specific mBC and risk of ABMR



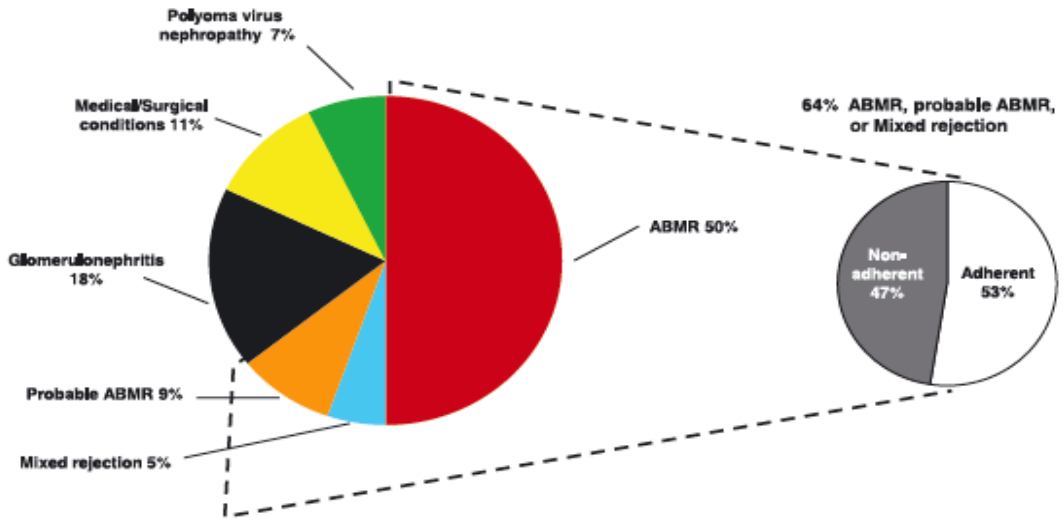


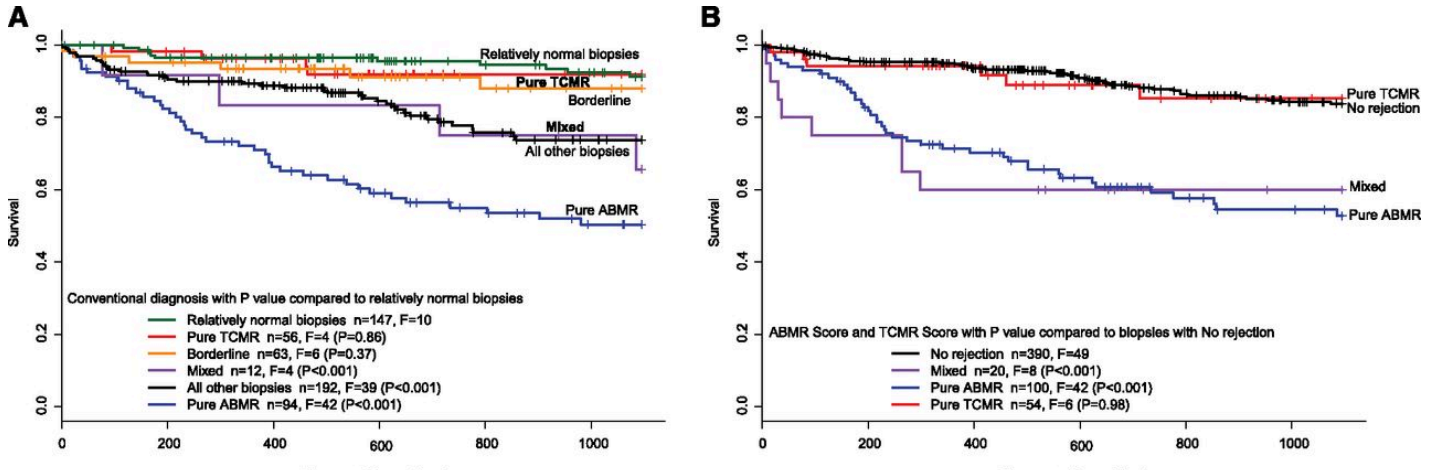


High frequency of d-s mBC and ABMR + **Endarteritis**

Lúcia & Bestard Kidney Int 2015

Humoral rejection as main cause of Chronic rejection and late graft loss



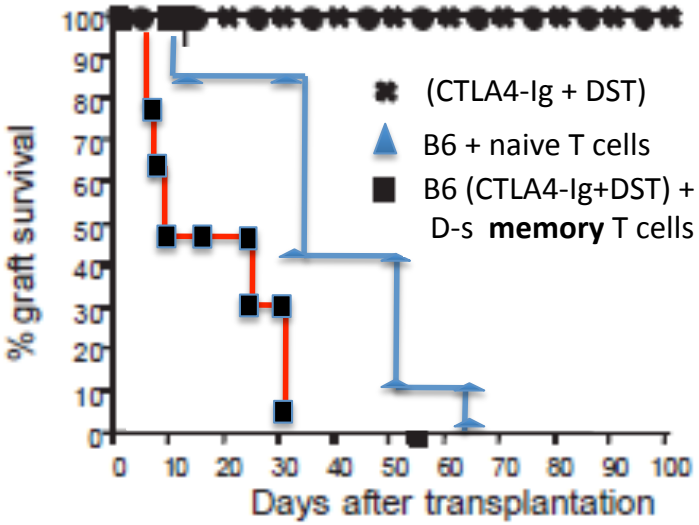


Sellares J et al. Am J Transplant 2012
Halloran P JASN 2015

Impact of **Cellular Immunity** in Anima models

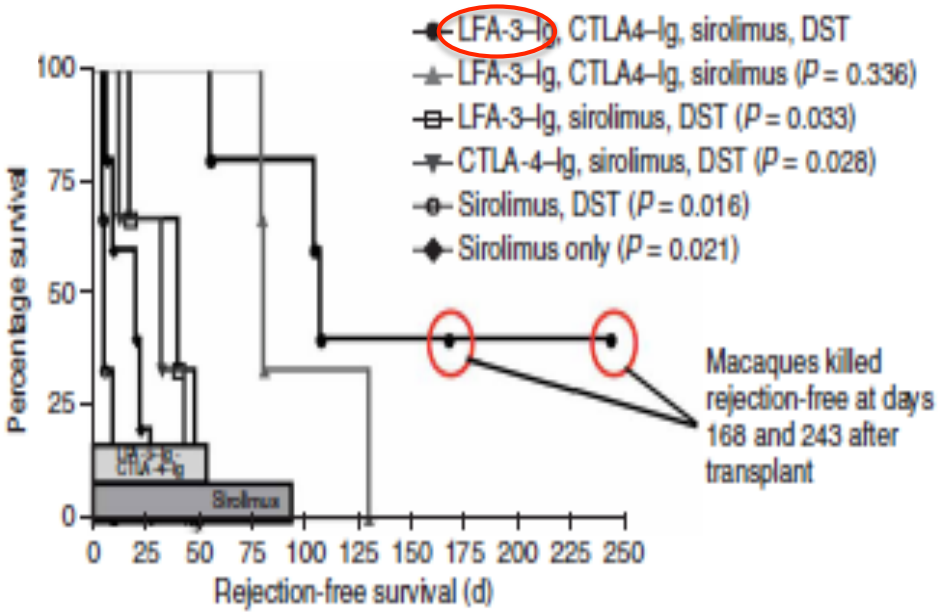
Abbrogation of allograft Tolerance/Survival in Presence of anti-donor T-cell alloreactivity

Murine Models of Transplant Tolerance
(anti-CD28 / anti-CD40) + DST



Sayegh M et al, J Immunol 1996

Transplant Tolerance in **Non-human primate**
(anti-CD28/SRL) + DST



Weaver T et al. Nat Med 2009

Impact of cellular immunity in Humans

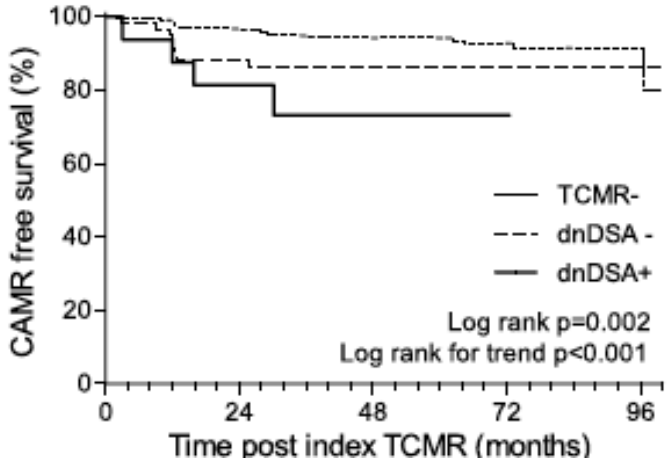
TMCR in “Non-sensitized individuals”

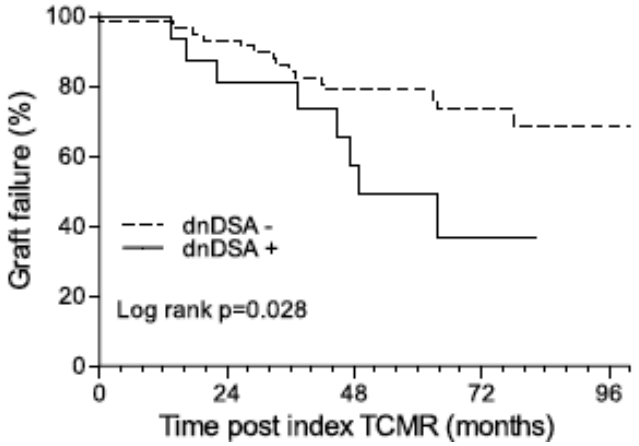
SYMPHONY STUDY	Standard-Dose Cyclosporine (N=390)	Low-Dose Cyclosporine (N=399)	Low-Dose Tacrolimus (N=401)	Low-Dose Sirolimus (N=399)	P Value†
Acute rejection					
At 6 mo					
Biopsy-proven (excluding borderline values) — %	24.0	21.9	11.3	35.3	<0.001
P value for comparison with tacrolimus	<0.001	<0.001	Reference	<0.001	
At 12 mo					
Suspected and treated — %	32.8	29.5	17.2	43.5	<0.001
P value for comparison with tacrolimus	<0.001	<0.001	Reference	<0.001	
Biopsy-proven (including borderline values) — %	30.1	27.2	15.4	40.2	<0.001

BENEFIT STUDY	Belatacept MI (n = 219)	Belatacept LI (n = 226)	Cyclosporine (n = 221)
Acute rejection			
Acute rejection, n (%)	49 (22)	39 (17)	16 (7)
95% CI	16.9–27.9	12.3–22.2	3.8–10.7
Difference from CsA (97.3% CI)	15.1 (7.9, 22.7)	10.0 (3.3, 17.1)	–
Banff grade, n (%)			
Mild acute (IA)	7 (3)	4 (2)	3 (1)
Mild acute (IB)	3 (1)	8 (4)	5 (2)
Moderate acute (IIA)	17 (8)	16 (7)	6 (3)
Moderate acute (IIB)	20 (9)	10 (4)	2 (1)
Severe acute (III)	2 (1)	1 (<1)	0

Ekberg H et al, NEJM 2008
Vincenti F et al AJT 2010

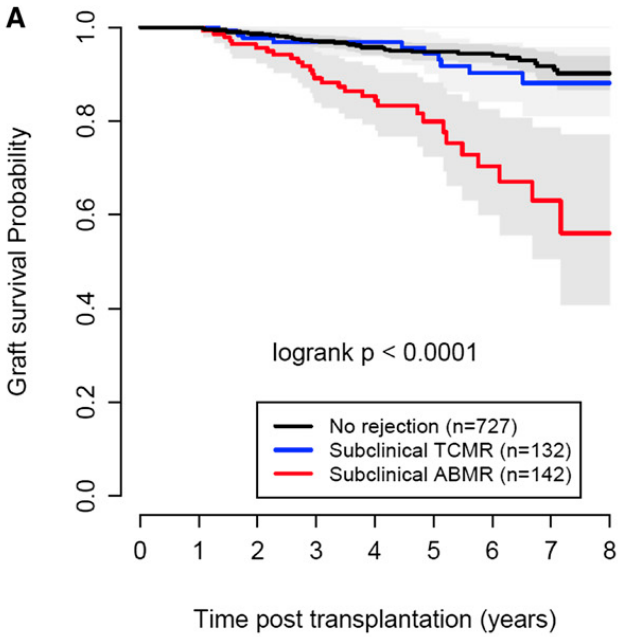
Development of dnDSA after TCMR and worse outcome





Chemouny JM et al Transplant 2015

Sc-ABMR and Sc-TCM developing dnDSA show worse outcome



Sc-TCMR developing DSA and TXGP

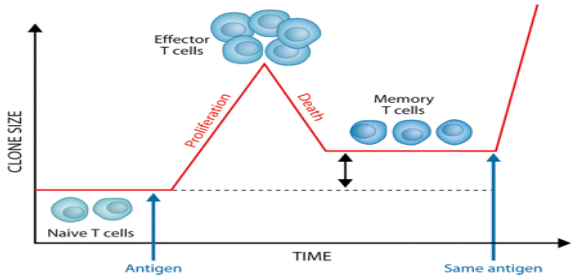
→ Worse allograft outcome

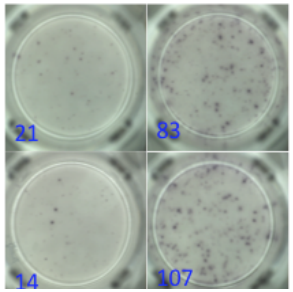
Loupy A et al JASN 2015

Immune assays to monitor anti-donor T-cell alloimmune responses

Assay	Readout	Description	Advantages	Disadvantages
MLR and CFSE	Proliferation	Donor and recipient cells are co-cultured. Dilution of CFSE is measured to determine recipients T-cell proliferation by flow cytometry.	Relatively easy to perform Inexpensive	Time-consuming (days of culture) Poorly reproducible Only measures proliferation
Immunknow®	Intracellular ATP production	Measures lymphocyte activity (intracellular ATP production), monitoring early responses to stimulation	Highly reproducible Relatively inexpensive Rapid	Non-antigen-specific Poor sensitivity and specificity
Intracellular cytokine staining	Cytokine production	Detects intracellular cytokine production by flow cytometry, after stimulation followed by the addition of secretion inhibitors	Functional readout Analysis of different cellular subsets Only few hours of culture	Detectable frequencies > 1/10,000 Expensive
ELISPOT	Cytokine secretion	Recipient cells are cultured with T-cell depleted donor cells and cytokines are captured by coating antibody of the plates. After development each spot represents a single cell secreting cytokine.	Functional readout Able to detect low frequencies (<1/10,000) Only 16–24 h of culture	Labor-intensive Expensive
Tetramer/peptide staining	Peptide-MHC binding	MHC tetramers identify and label specific T-cells by epitope specific binding and are analyzed by flow cytometry.	Stimulation not needed Possible to sort and recover cells Easy and fast	No functional readout Instability of the tetramers Peptide/tetramer pools are limited

IFN-γ Elispot assay → Frequency of Ag-specific T-cell sensitization/Recall responses

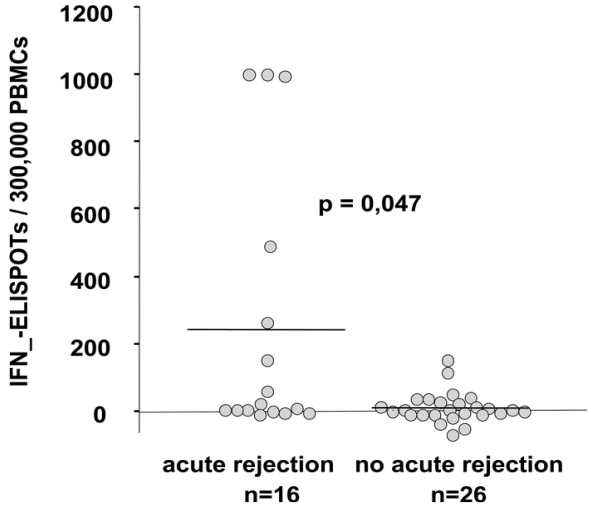




Crespo & Bestard Clin Biochem 2015
Cravedi P Transplant Rev 2015

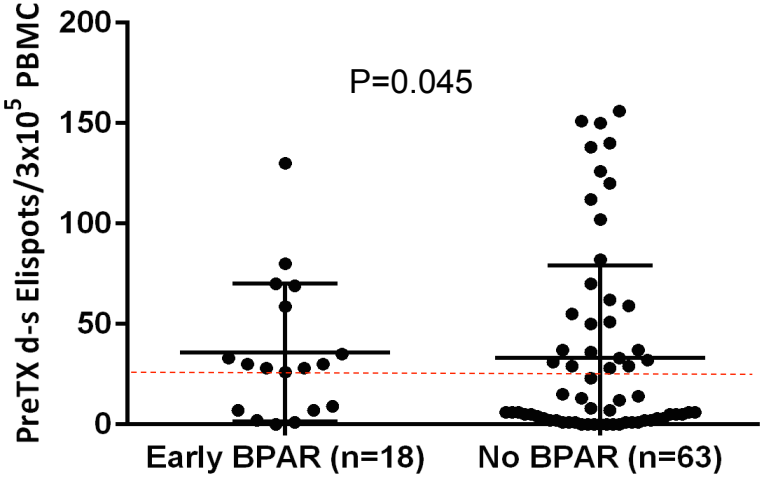
Pre-transplant donor-specific or PRT ELISPOT

Pre-Transplant dsELISPOT and **TCMR**



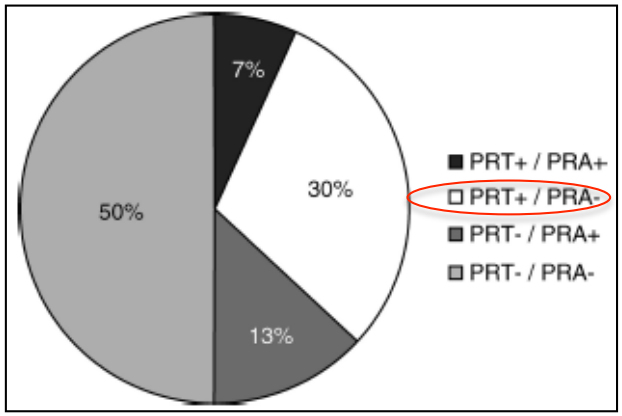
Nickel P et al Transpl 2004

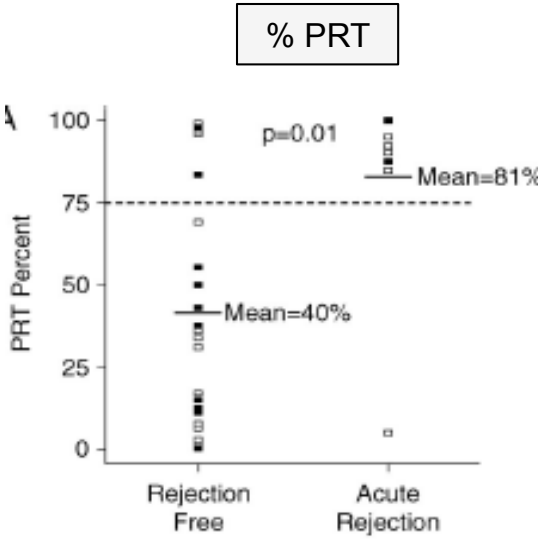
Pre-Transplant dsELISPOT and (early) **TCMR**



Crespo E et al Plos One 2015

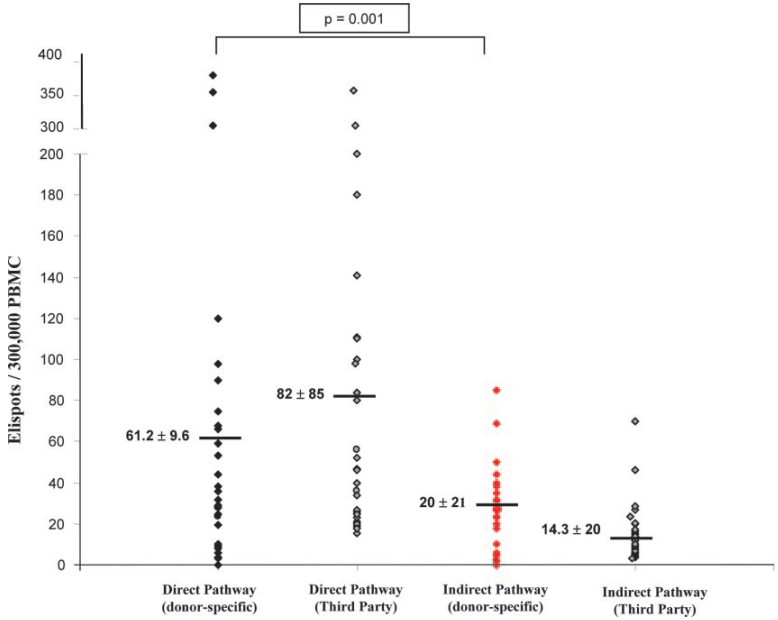
Panel of alloreactive memory/effector T cells (**PRT**) Pre-Transplantation



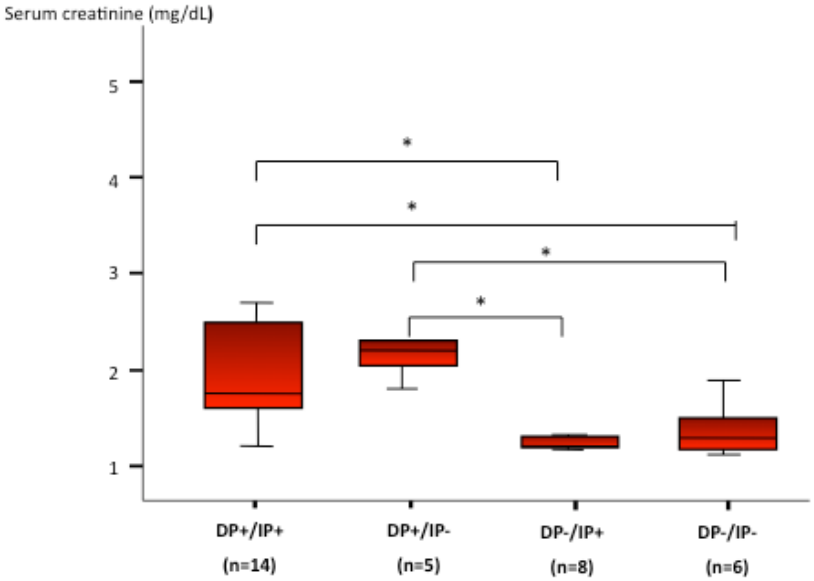


Nickel P et al J Am Soc Nephrol 2006
Heeger P et al. J Am Soc Nephrol 2006
Poggio ED et al. Transplant 2007

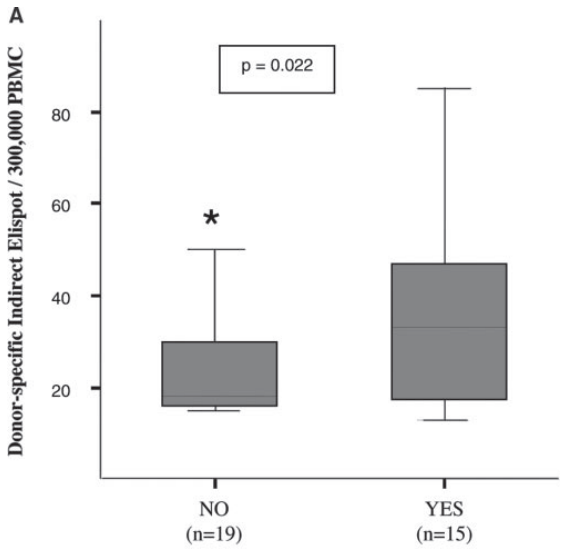
Post-Transplant Direct and Indirectly alloreactive T cells in renal Transplantation



Graft function and d-s T-cell alloreactivity



Proteinuira, DSA and Indirect T-cell alloreactivity



Bestard O et al. J Am Soc Nephrol 2008

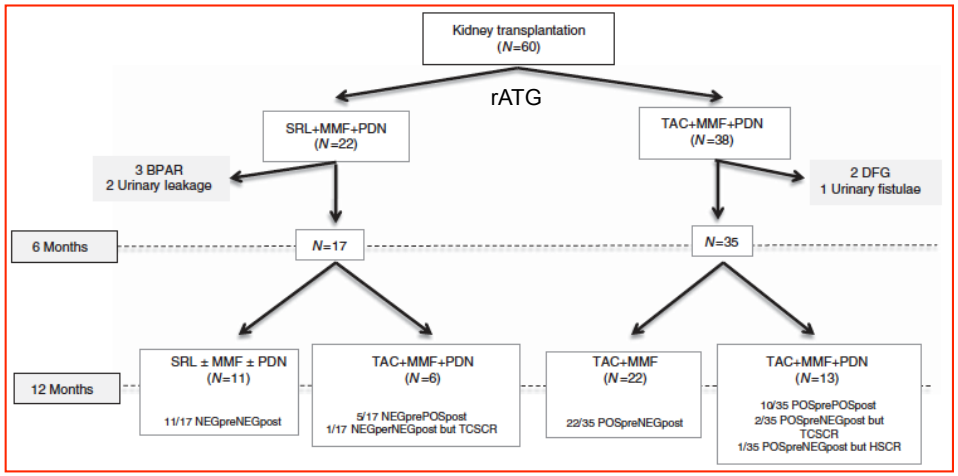
Prospective Pilot Biomarker-driven Trial

clinical investigation http://www.kidney-international.org

see commentary on page 1074

Prospective assessment of antidonor cellular alloreactivity is a tool for guidance of immunosuppression in kidney transplantation

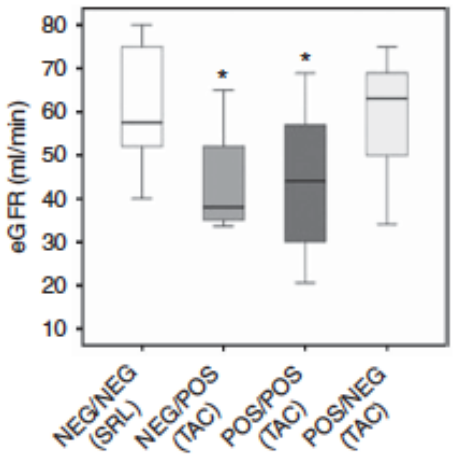
Oriol Bestard[1,2], Josep M. Cruzado[1,2], Marc Lucia[2], Elena Crespo[2], Linda Casis[2], Birgit Sawitzki[3], Katrin Vogt[3], Carme Cantarell[4], Joan Torras[1,2], Edoardo Melilli[1], Richard Mast[5], Alberto Martinez-Castelao[1], Montse Gomà[6], Petra Reinke[3], Hans-Dieter Volk[3] and Josep M. Grinyó[1,2]

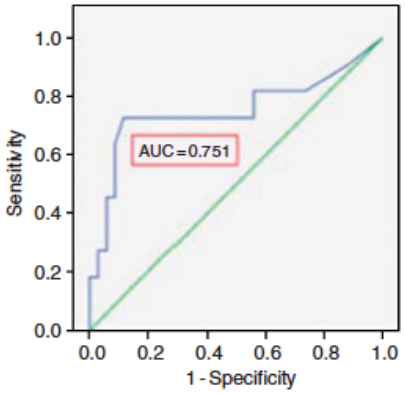


Low Incidence of BPAR in CNI-free regimens

12-month adverse events	Group A (NEG) SRL (N=22)	Group B (POS) TAC (N=38)
Opportunistic Infections - CMV (disease) - BK-virus - *Pneumocistis Jiroveci* - Herpes Zoster - Toxoplasmosis chorioretinitis	0 (0%) 0 0 0 0 0	*11 (29%) 6 2 1 1 1
Urological complications	8 (36%)	8 (21%)
DGF (ATN)	0 (0%)	1 (26%)
Profound vein thrombosis	2 (9%)	0 (0%)
Non-skin Malignancies	0 (0%)	1 (26%)
Biopsy proven acute rejection (BPAR) Type	3 (13,5%) 8% TCMR IA, IB, ABMR	0 (0%)
6-m Acute histological lesions (n=46) TCSCR (Yes/No)	(n=15) 4(26,6%) / 11(73,4%)	(n=31) 7(22,5%) / 24(77,5%)
6-m Chronic histological lesions (n=46) IF/TA (<I, II, III)	(n=15) 12(80%)/2(13%)/1(7%)	(n=31) 23(74%)/5(16%)/3(10%)
Graft survival	21/22 (95,5%)	35/38 (92,1%)
Patient survival	22/22 (100%)	36/38 (94,7%)

Post-TX ELISPOT and long-term graft function and SCR





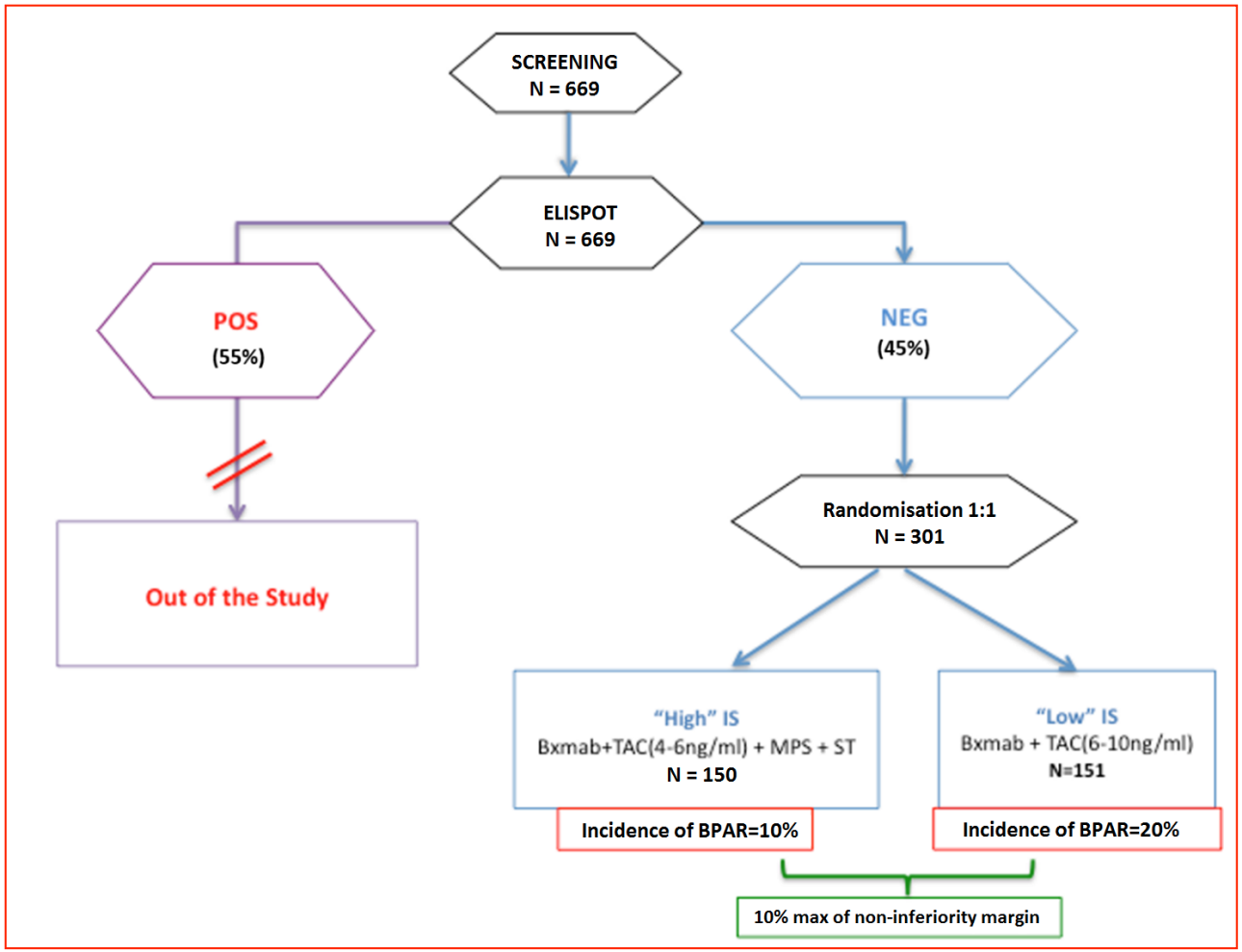
Variable	Predictive value		
	Specificity	Sensitivity	NPV
6-Month d-s IFN-γ Elispot (TCSCR)	80%	50%	91.4%

Bestard O et al Kidney Int 2013

Prospective Randomized Biomarker-driven Trial

CELLIMIN Trial

Prospective donor-specific **Cell**ular alloresponse assessment for **Im**munosuppression **Min**imization in de novo renal transplantation



Transcriptional diagnosis of pathologic phenotypes in allograft biopsies

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‖§§

*Paris Descartes University and Hôpital Necker and ‡Hôpital Saint Louis, Assistance Publique–Hôpitaux de Paris, Paris, France; †Paris Translational Research Center for Organ Transplantation, Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche-S970, Paris, France; §Unit 3181, University Hospital of Besançon, France; ‖Alberta Transplant Applied Genomics Centre, Edmonton, Alberta, Canada; ¶Department of Laboratory Medicine and Pathology and §§Department of Medicine, Division of Nephrology and Transplant Immunology, University of Alberta, Edmonton, Alberta, Canada; **Department of Pathology, Saint Louis Hospital, Paris, France; ††Pateforme Centres de Resources Biologiques, Institut Gustave Roussy, Villejuif, France; and ‡‡Department of Pathology, Necker Hospital, Paris, France

http://www.kidney-international.org original article

Progressive histological damage in renal allografts is associated with expression of innate and adaptive immunity genes

Maarten Naesens[1,2], Purvesh Khatri[1,3], Li Li[1], Tara K. Sigdel[1], Matthew J. Vitalone[1], Rong Chen[3], Atul J. Butte[3], Oscar Salvatierra[4] and Minnie M. Sarwal[1]

[1]Division of Nephrology, Department of Pediatrics, Stanford University School of Medicine, Stanford, California, USA; [2]Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium; [3]Division of Systems Medicine, Department of Pediatrics, Stanford University School of Medicine, Stanford, California, USA and [4]Department of Surgery, Stanford University School of Medicine, Stanford, California, USA

Banff 2013 Meeting Report

Table 2: Revised (Banff 2013) classification of antibody-mediated rejection (ABMR) in renal allografts

Acute/active ABMR; all three features must be present for diagnosis[1,2]

1. Histologic evidence of acute tissue injury, including one or more of the following:
   - Microvascular inflammation ($g > 0$[3] and/or $ptc > 0$)
   - Intimal or transmural arteritis ($v > 0$)[4]
   - Acute thrombotic microangiopathy, in the absence of any other cause
   - Acute tubular injury, in the absence of any other apparent cause
2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
   - ~~At least moderate microvascular inflammation ($[g + ptc] \geq 2$)~~[5]
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated[6]
3. Serologic evidence of donor-specific antibodies (DSAs) (HLA or other antigens)

Chronic, active ABMR; all three features must be present for diagnosis[1,7]

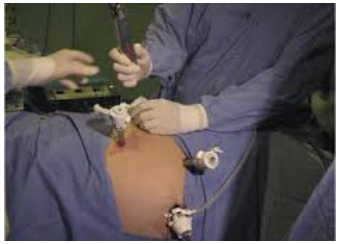
1. Morphologic evidence of chronic tissue injury, including one or more of the following:
   - Transplant glomerulopathy (TG) ($cg > 0$)[8], if no evidence of chronic thrombotic microangiopathy
   - Severe peritubular capillary basement membrane multilayering (requires EM)[9]
   - Arterial intimal fibrosis of new onset, excluding other causes[10]
2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
   - At least moderate microvascular inflammation ($[g + ptc] \geq 2$)[5]
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated[6]
3. Serologic evidence of DSAs (HLA or other antigens)

C4d staining without evidence of rejection; all three features must be present for diagnosis[11]

1. Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
2. $g = 0$, $ptc = 0$, $cg = 0$ (by light microscopy and by EM if available), $v = 0$; no TMA, no peritubular capillary basement membrane multilayering, no acute tubular injury (in the absence of another apparent cause for this)
3. No acute cell-mediated rejection (Banff 97 type 1A or greater) or borderline changes

A **Transcriptomic Window in BLOOD** predates clinical and histological injury

Uninvasive approach
kSORT Test



No macroscopic injury

Subclinical injury

Clinical graft dysfunction

Chronic graft changes (IFTA/TG)

Kidney Transpalnt

Graft inflammation (PTC, Glomerulitis) +/- C4d

Baseline Immune background

Donor-specific ASC / Teff → De novo DSA / Teff

alloAg presentation + low / No IS → T / B-cell activation

Transcriptomic perturbation

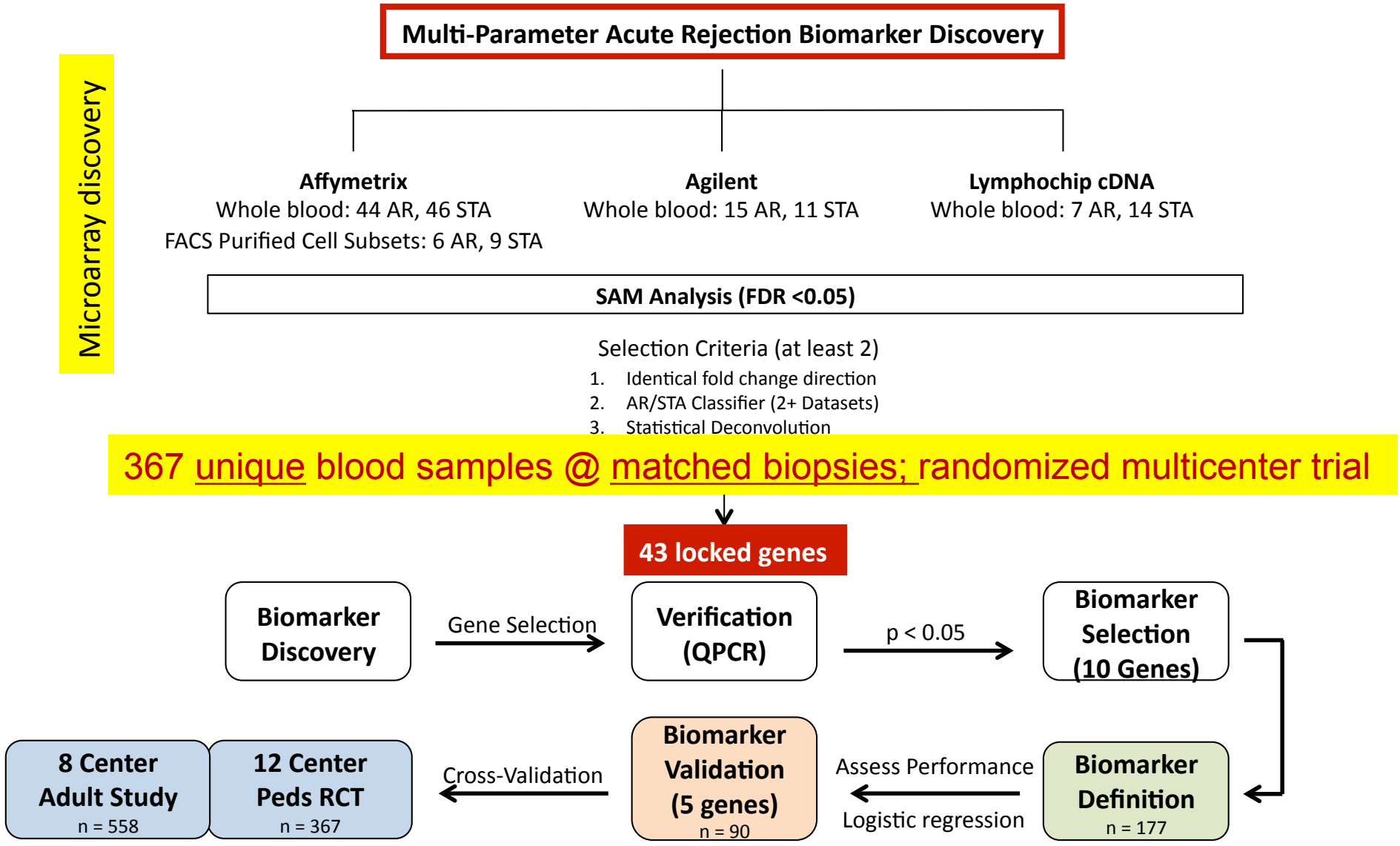
Morphologic alteration

Clinical alteration

Bestard & Sarwal In Press 2015

Unbiased discovery of AR specific genes in peripheral blood:

Controlled for clinical, demographic and bx confounders



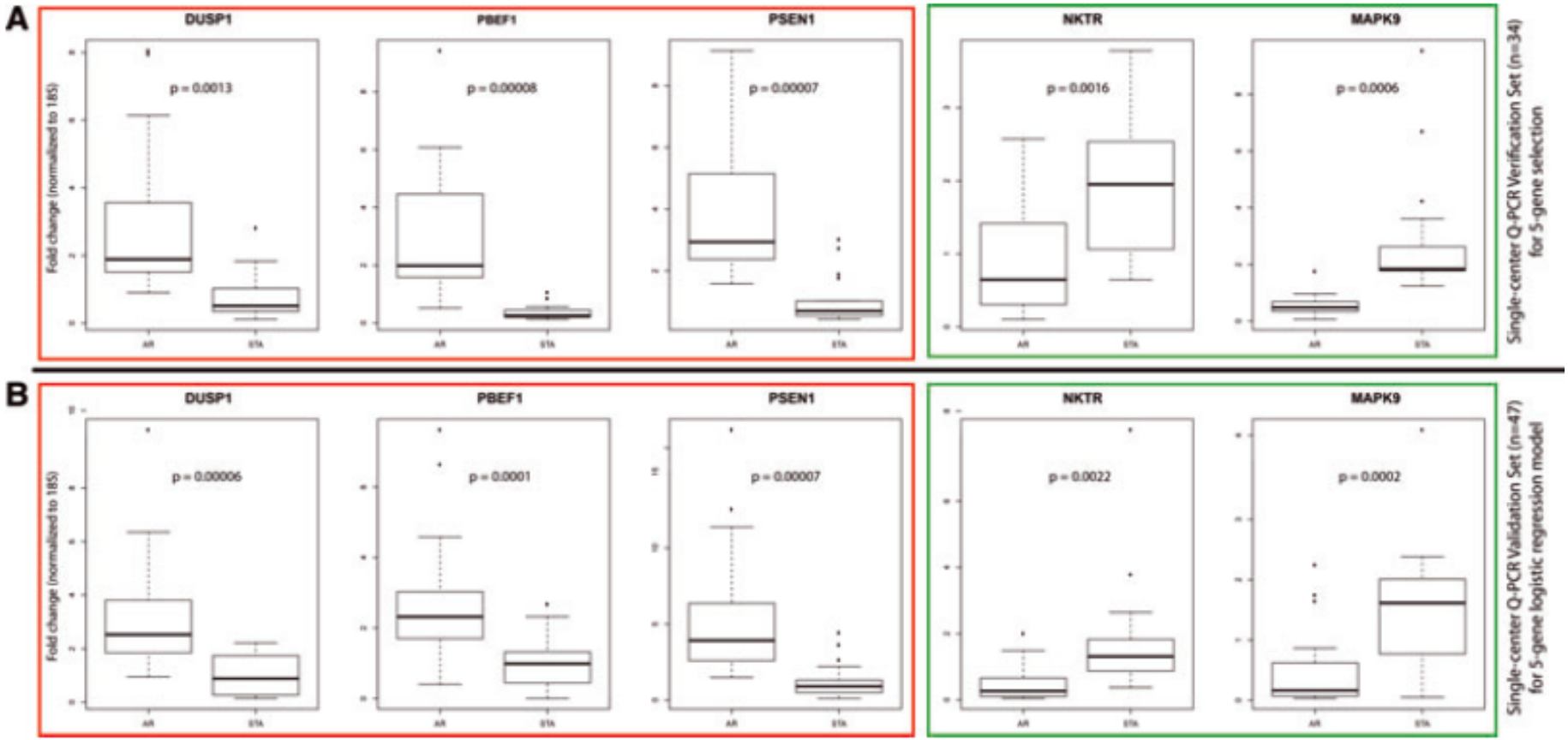
Li & Sarwal et al, AJT, 2012

Validation of Blood Genes in Kidney TX

Chi-square score for logistic regression models using all 10 or only **5 genes** did not significantly increase the Chi-square score → thus a 5-gene model was used to built the prediction model

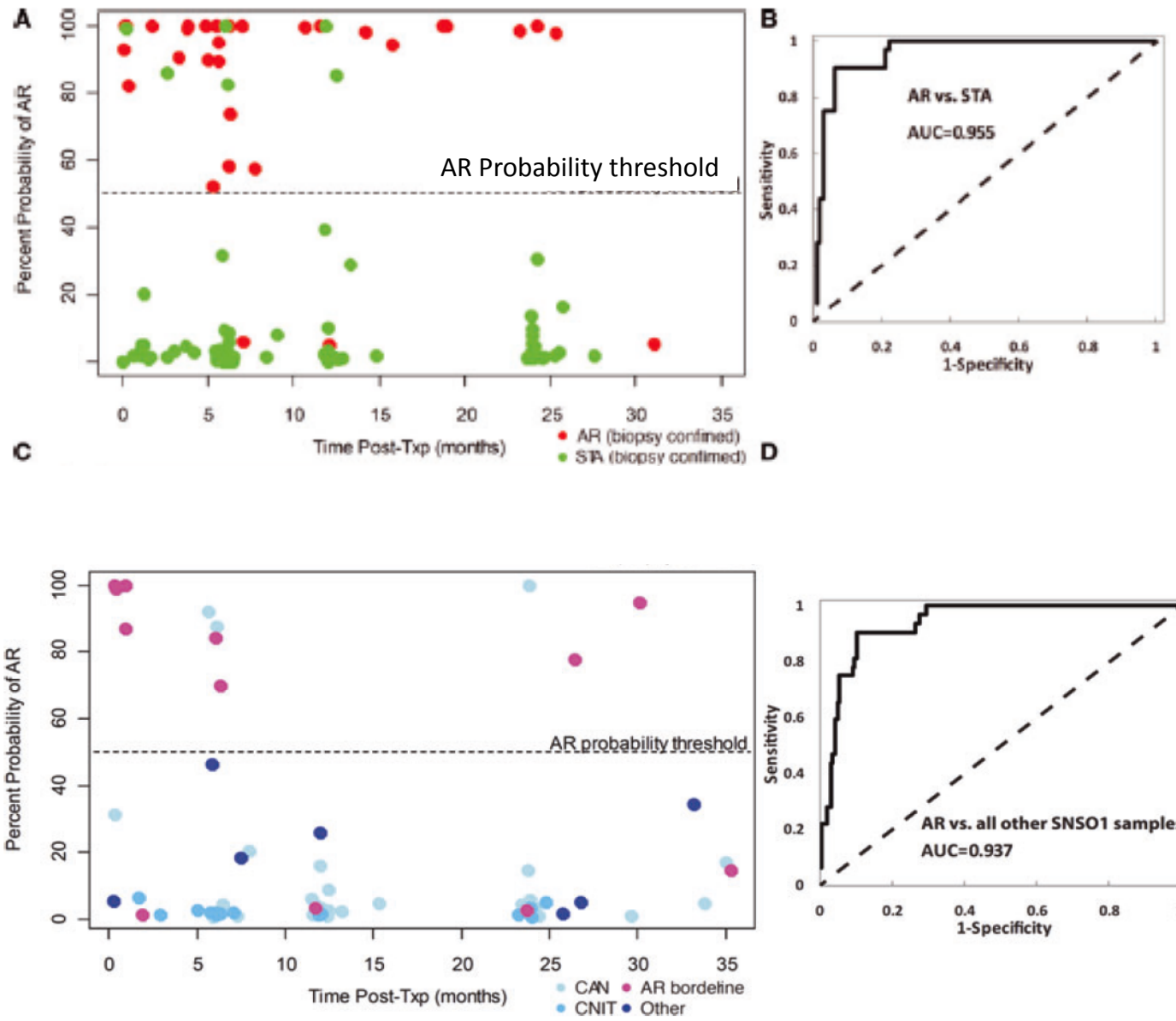
Up-regulated in AR

Down-regulated in AR



Li & Sarwal et al, AJT, 2012

Multicenter Validation of qPCR Blood Genes for AR by the 5-gene set



NIH SNSO1- RCT, 12 US centers
Independent Multicenter Validation in a randomized control trial

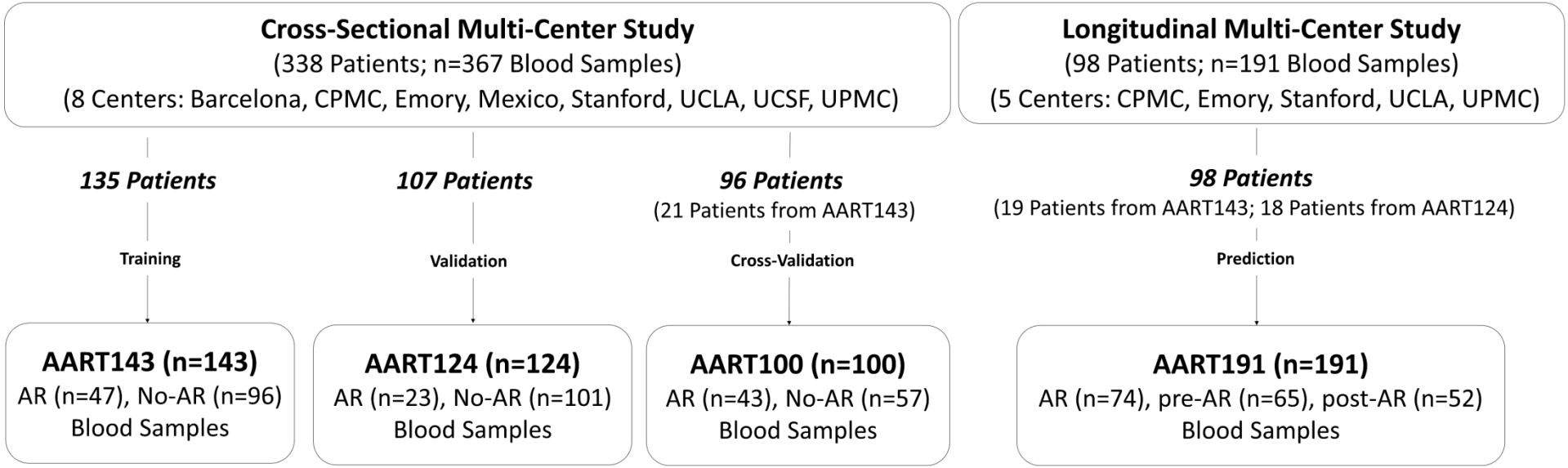
Li & Sarwal et al, AJT, 2012

The Assessment of Acute Rejection in Transplantation (**AART**) Multicenter Study

Development of a novel correlation-based algorithm by step-wise analysis of gene expression data in *8 Transplant programs in the US, SPAIN, Mexico*

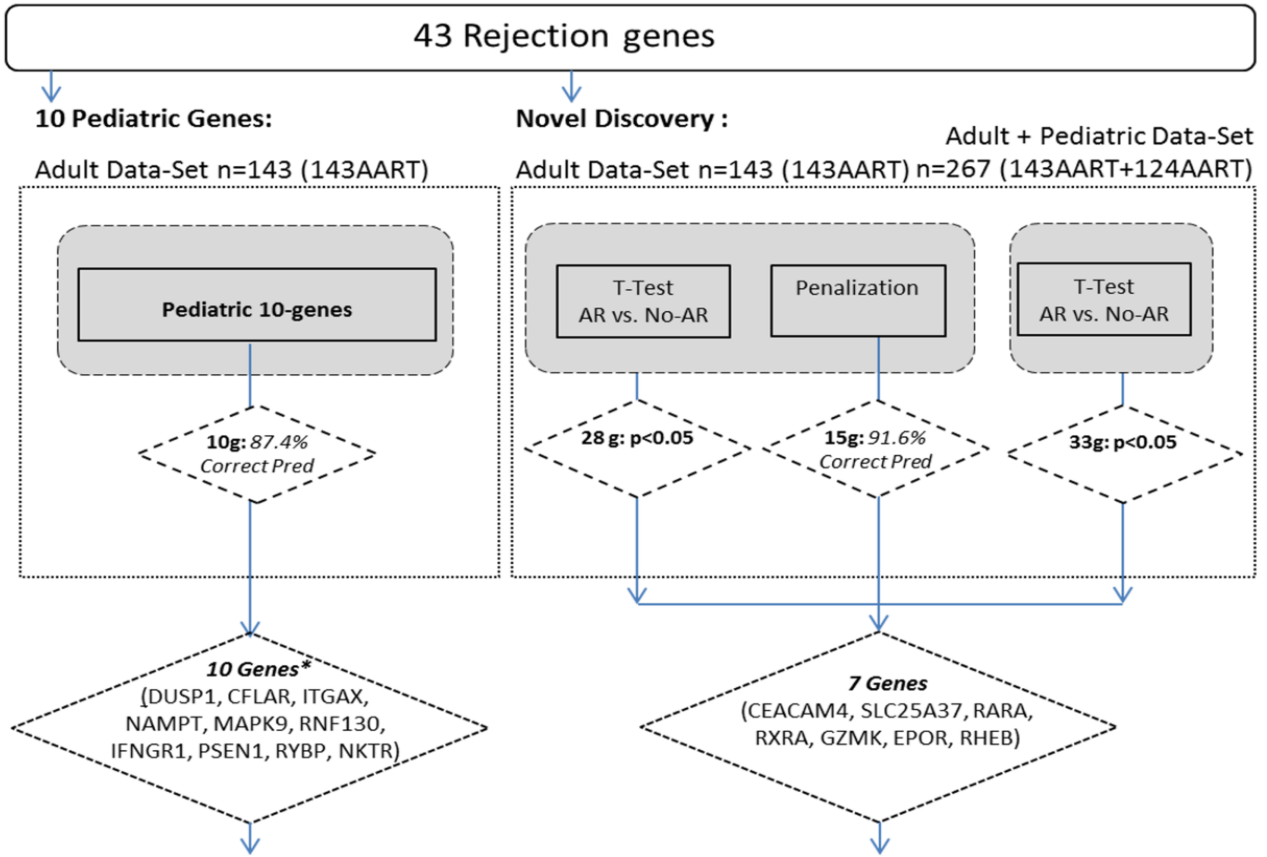
43 gene Fluidigm/ABI QPCR → 17 genes:
7 additional genes selected for inclusion with the original 10 gene-set from *Li et al, AJT, 2012*

Assessment of 558 Samples from 436 Patients in the **AART Study** (Patient/Sample Flow):



Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

Selection of the final 17 Genes for the KSORT set up analysis

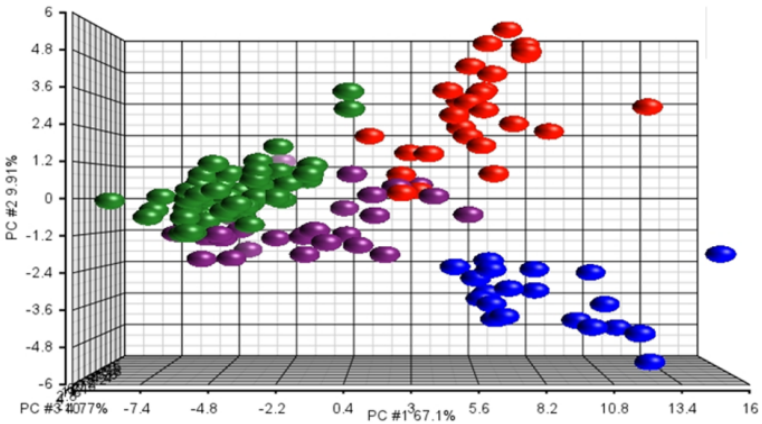


Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

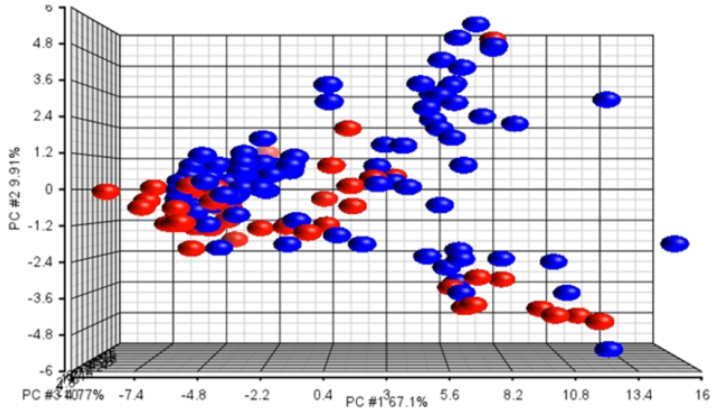
PCA for segregation by sample collection/sites/clinical Phenotypes

Normalization of QPCR data by mixed ANOVA corrected for the dominant effect of sample collection site on gene expression (1C) and resulted in segregation of samples into AR and No-AR (1D)

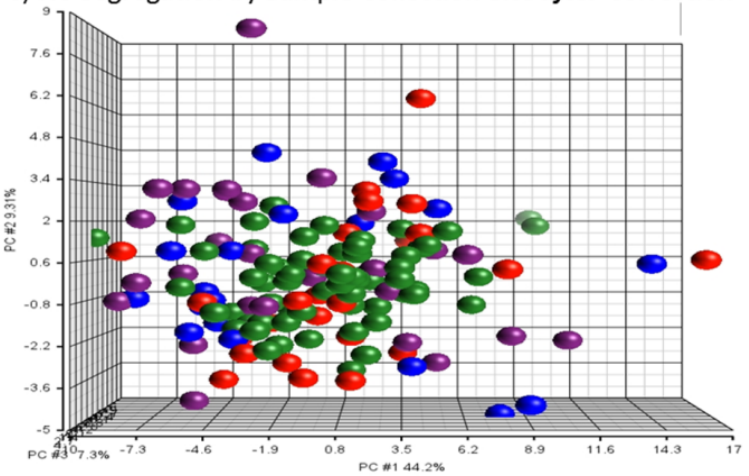
A) Segregation by Sample Collection site ***before*** Correction



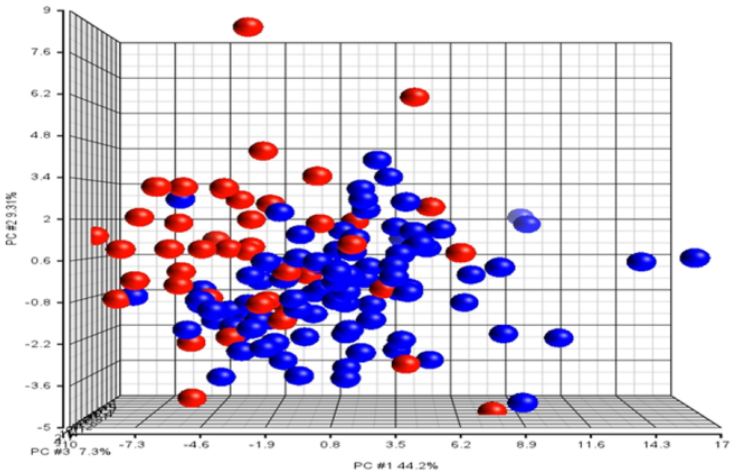
B) No Segregation by Phenotype ***before*** Correction



C) No Segregation by Sample Collection Site ***after*** Correction



D) Segregation by Phenotype ***after*** Correction



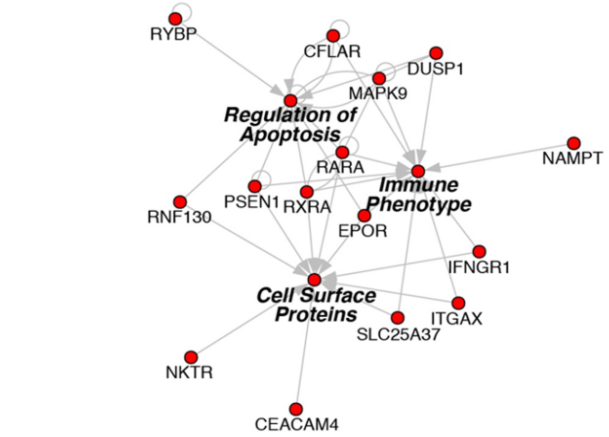
Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

The biological basis of the 17 genes

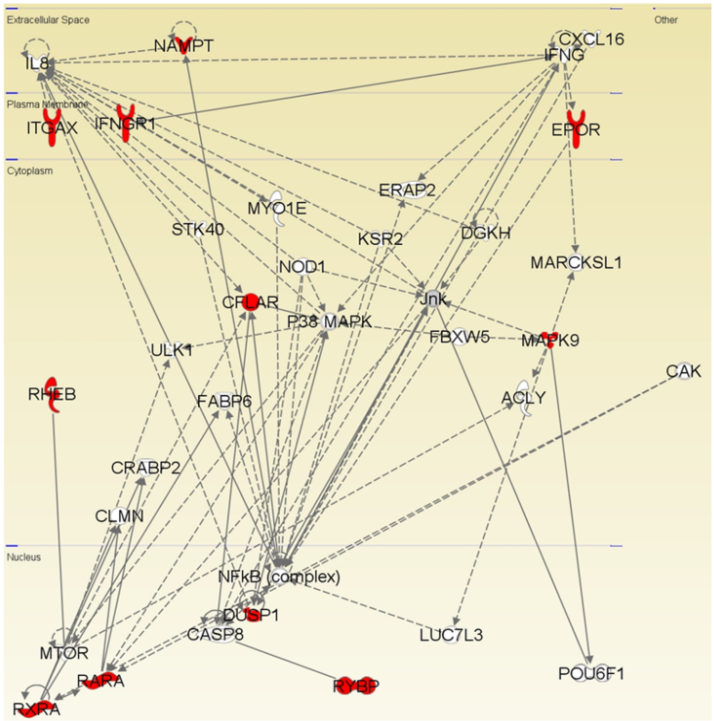
Pathway and Network analyses demonstrated strong biological correlation of genes supporting correlation seen in gene expression across AR and No-AR samples by QPCR

→ **Apoptosis regulation, Immune Phenotype and Cell surface proteins**

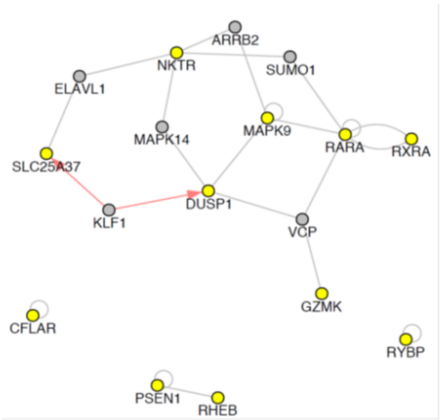
5A



5B



5C

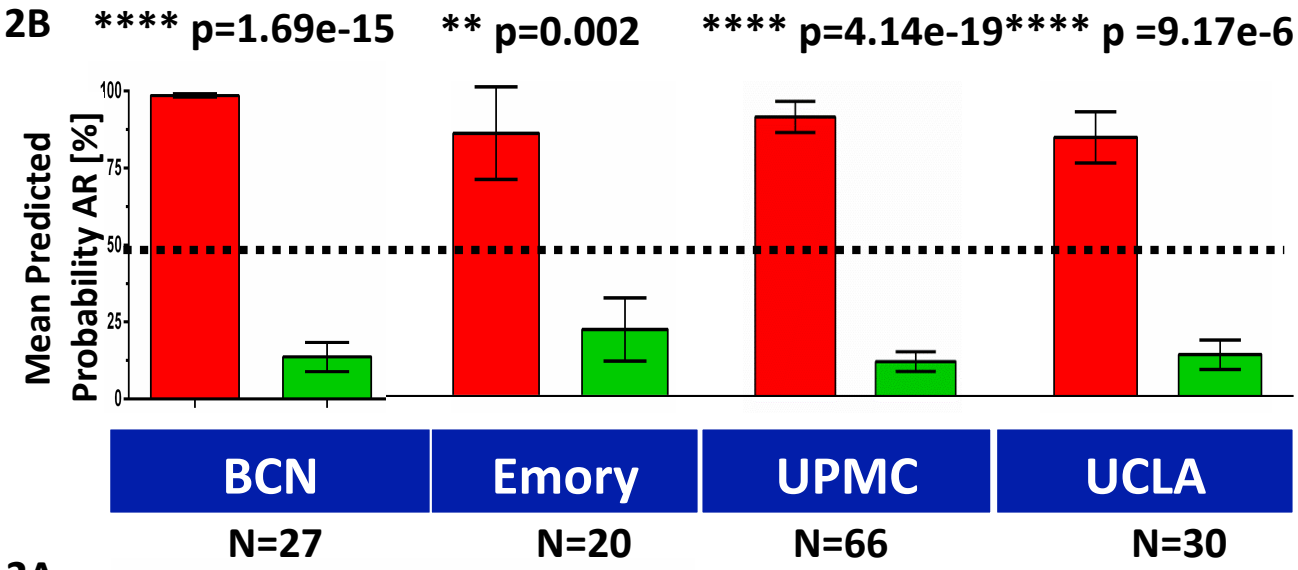


Ingenuity Pathways analysis (IPA) → common role of 11/17Genes in cancer, cell death and cell survival

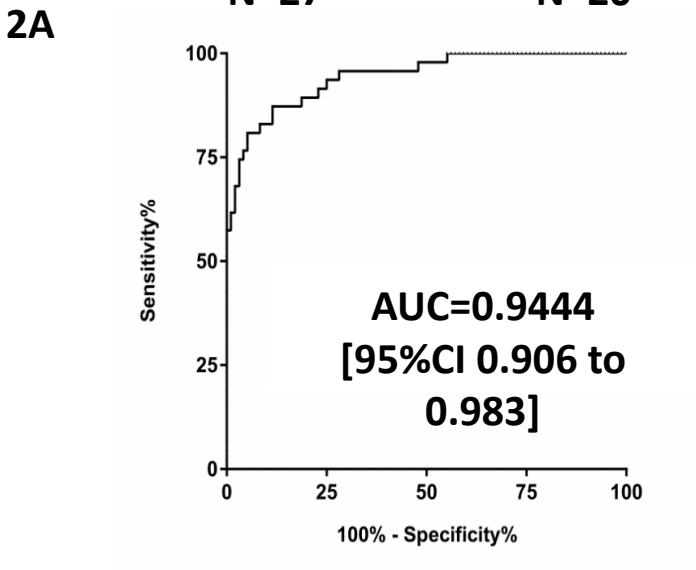
Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

The Kidney Solid Organ Response Test (KSORT)

A 17 gene-set modelled by Logistic regression classifies Acute Rejection



AART143; adults

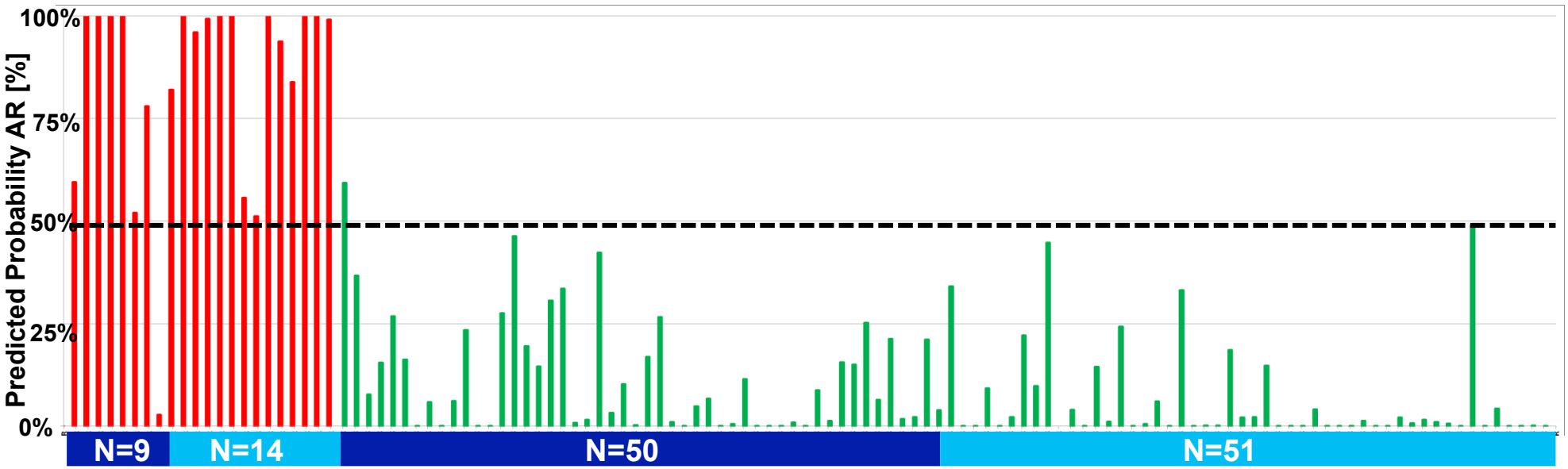


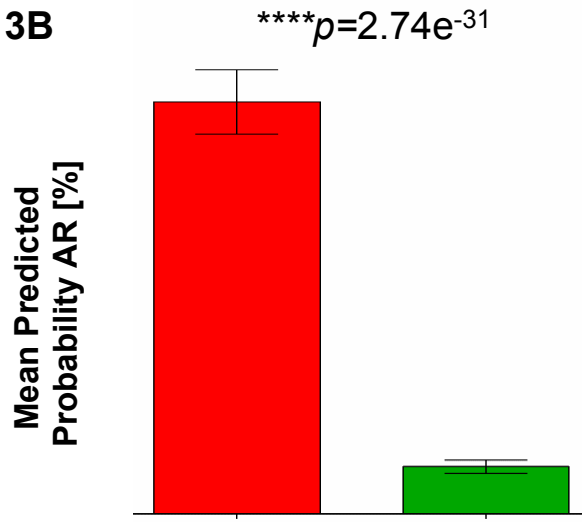
- AR (n=47)
- No-AR (n=96)
- Adults (n=143)
- -- Θ Threshold for AR

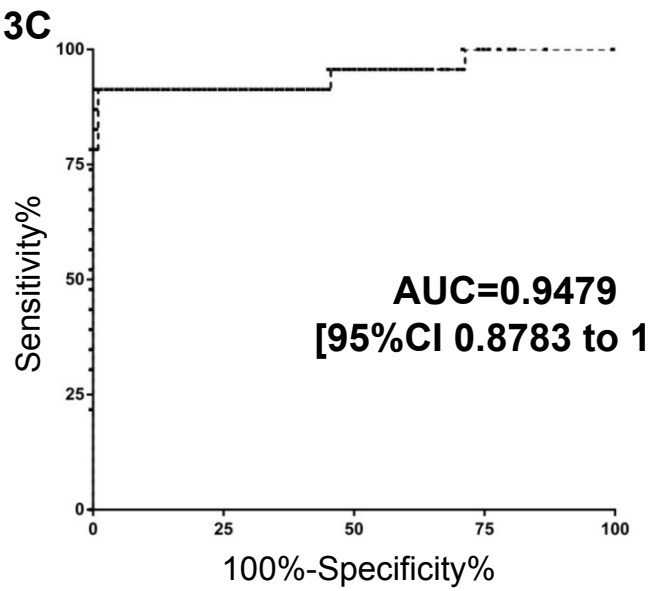
Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

Validation of kSORT in 124 independent samples across different ages and settings

AART124; adult and pediatrics





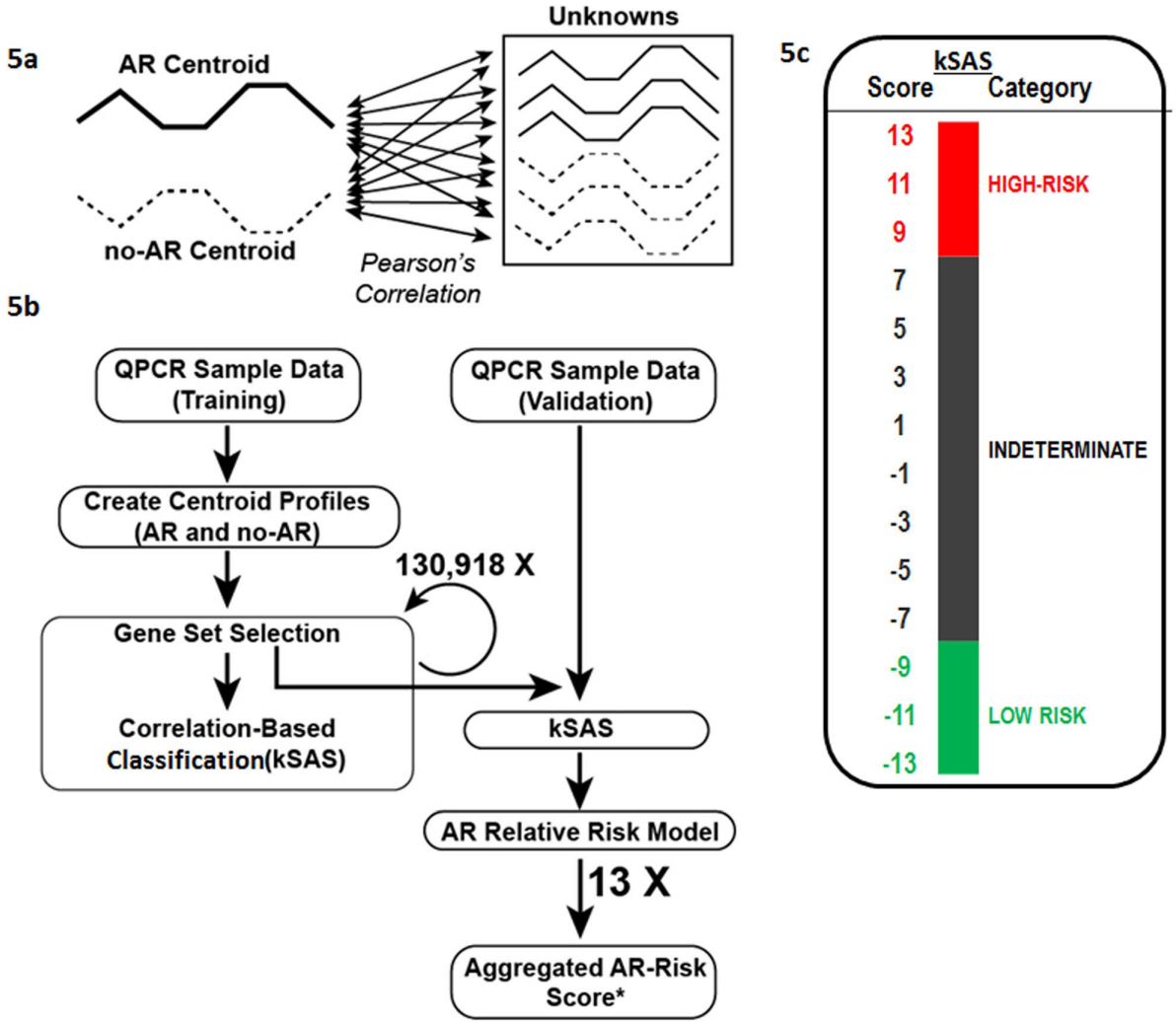


AR (n=23)
No-AR (n=101)
Adults (n=59)
Pediatrics (n=65)
--- Θ Threshold for AR

Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

The Solid Organ Response Test (SORT) → KSAS algorithm

*A novel reference-based algorithm (using 13 12-gene models) (KSAS) was developed in 100 independent samples to provide a numerical AR risk score, to classify patients as **high** risk versus **low** risk for AR*

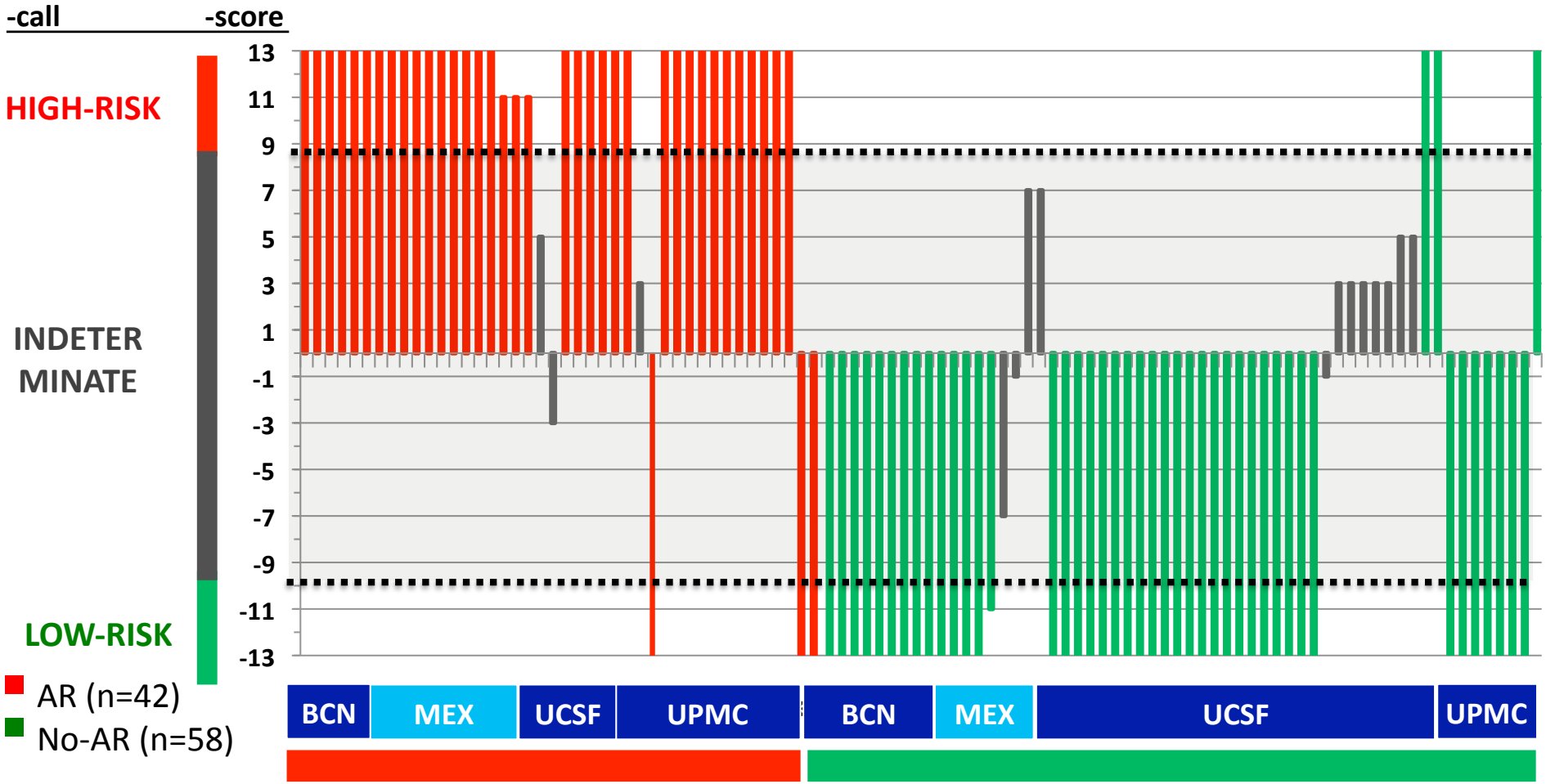


Expression values of the 17-gene kSORT model in unknown samples were correlated to corresponding AR and No-AR reference values (centroids) by Pearson correlation. (B) For kSORT assay development, QPCR data from 100 samples were divided into training (n = 32) and independent validation sets (n = 68). (C) 13 12-gene models from the 17-gene kSORT model generated numerically aggregated AR risk scores for each sample and categorized them into three groups: high risk for AR (aggregated AR risk score $9), low risk for AR (aggregated AR risk score #29), and indeterminate (aggregated AR risk score ,9 and .29) category

Performance of the kSORT assay

The blood 17 gene kSORT assay defines high vs low immune risk in kidney TX

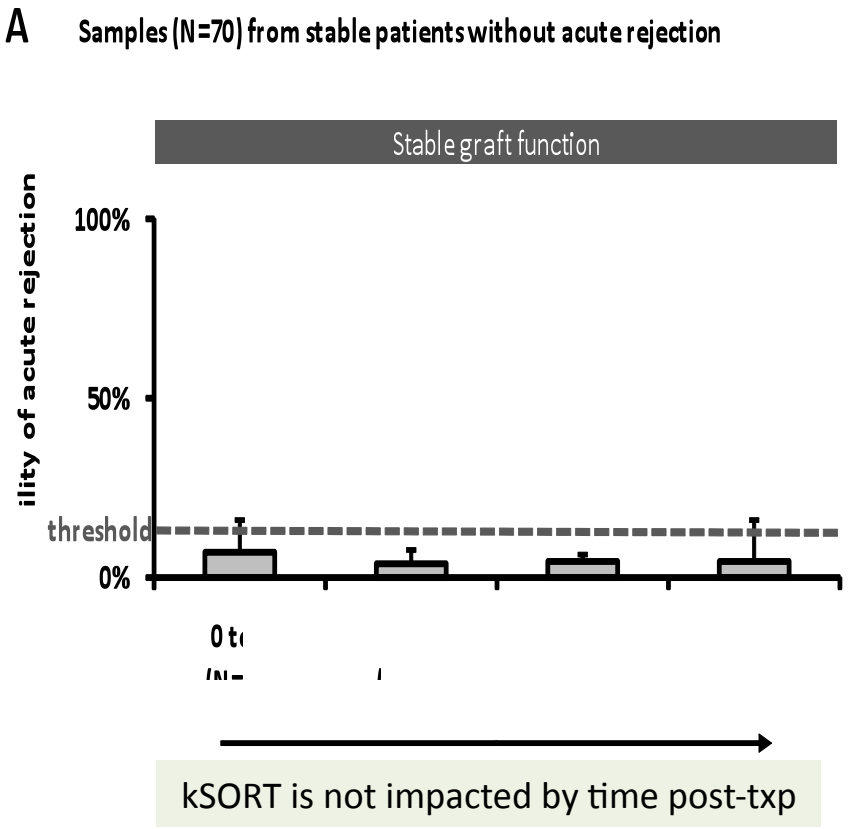
kSORT-RISK SCORE in 100 samples from SNS01 study (32 trainning set vs 68 validation set)



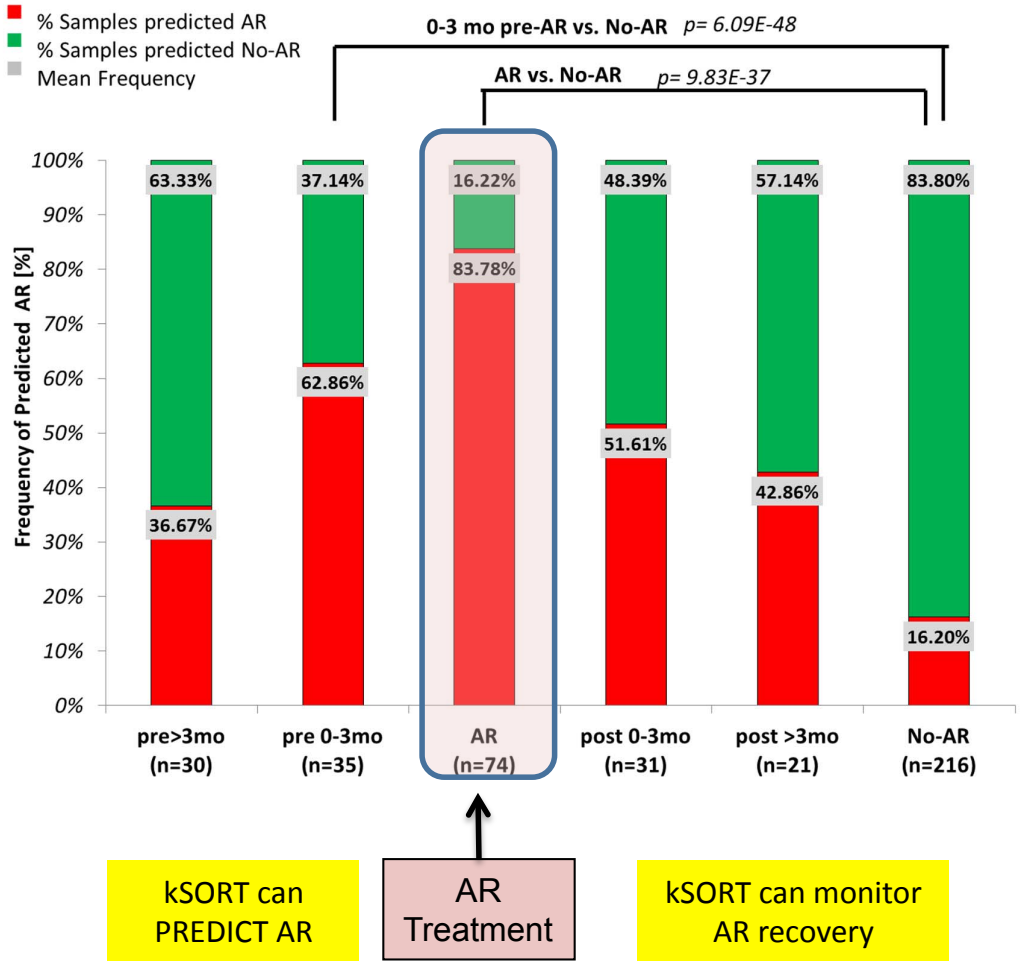
Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

The Transcriptomic Window in Blood

kSORT detects pre-AR 3-4 months before rise in Screatinine

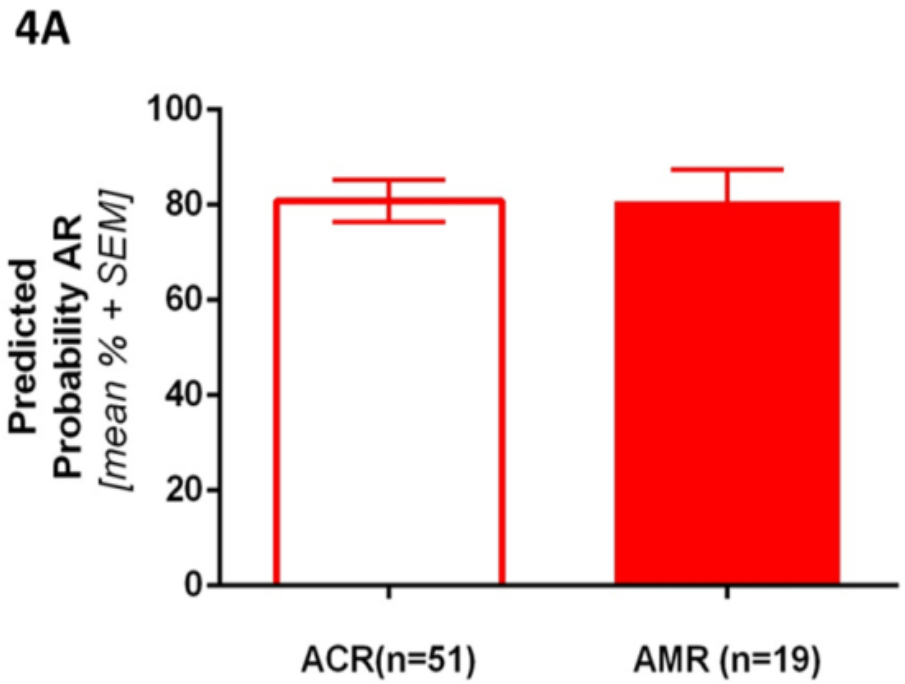


kSORT dynamics in 191 serially collected samples



Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

TCMR vs ABMR?



Different therapeutic approaches

kSORT was designed to pick up both TCMR and ABMR

→Hypothesis of a common rejection Immunologic origin of allograft rejection

→Functional Immune assays may provide more insight of the immune effector mechanisms

Roedder, Sigdel, Bestard, et al, Plos Medicine 2014

PRESENT / FUTURE OF ORGAN TRANSPLANTATION

Validation of
BIOMARKERS allowing IS individualisation/optimization