CLUB
DES JEUNES
NÉPHROLOGUES

Complement Activating anti-HLA DSA and Solid Organ Transplant Survival

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www.paristransplantgroup.org

Paris Translational Research Center
for Organ Transplantation

THE PREVALENT ORGAN TRANSPLANT UNIVERSE

1,000,000

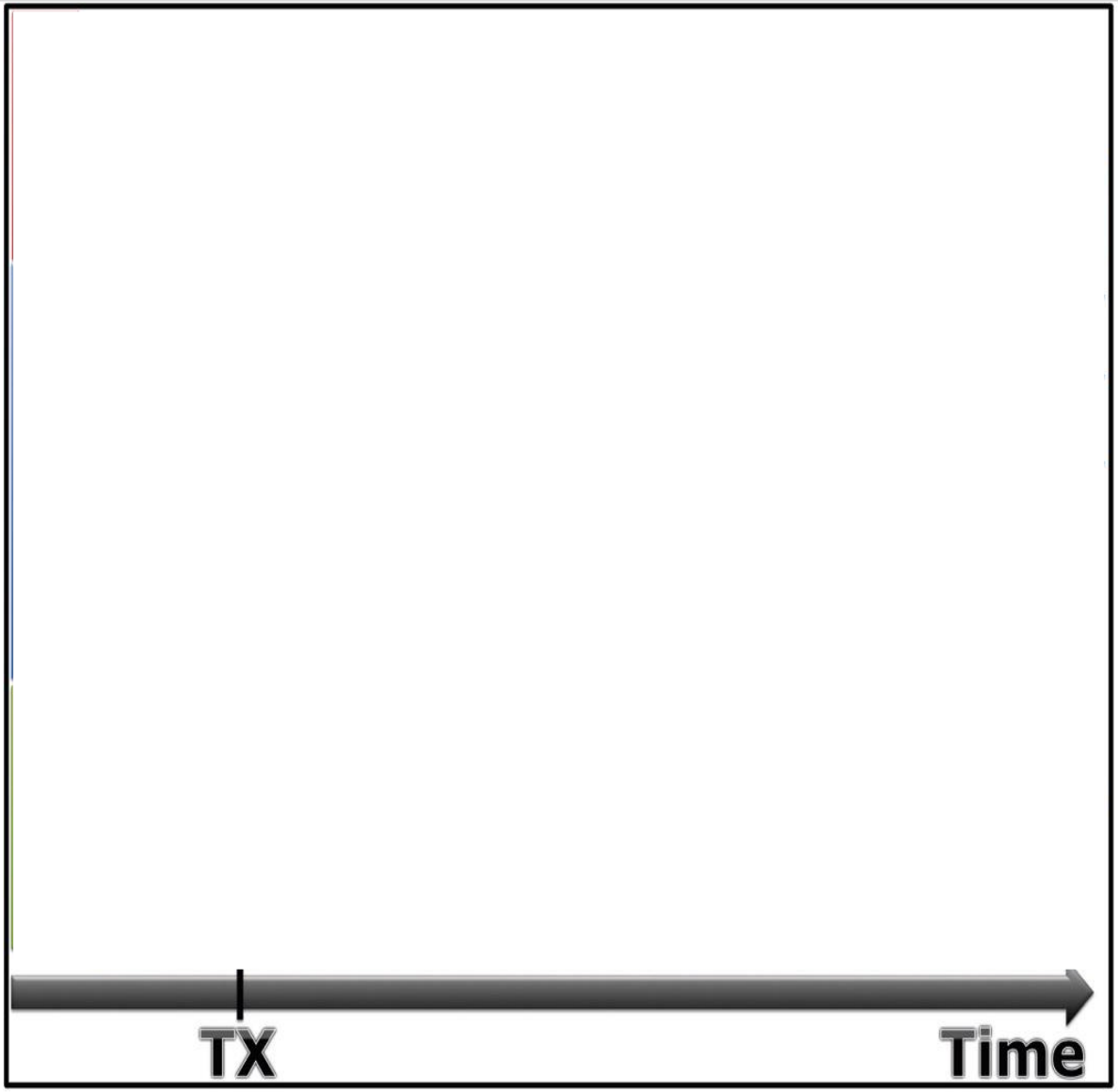
(700,000 kidneys)

80,000 *new* kidney transplants per year

About 15% have DSA: 150,000

About half have ABMR: 75,000

NATUREL HISTORY OF DSA MEDIATED KIDNEY ALLOGRAFT INJURY



Loupy, Hill & Jordan, Nat reviews 2012

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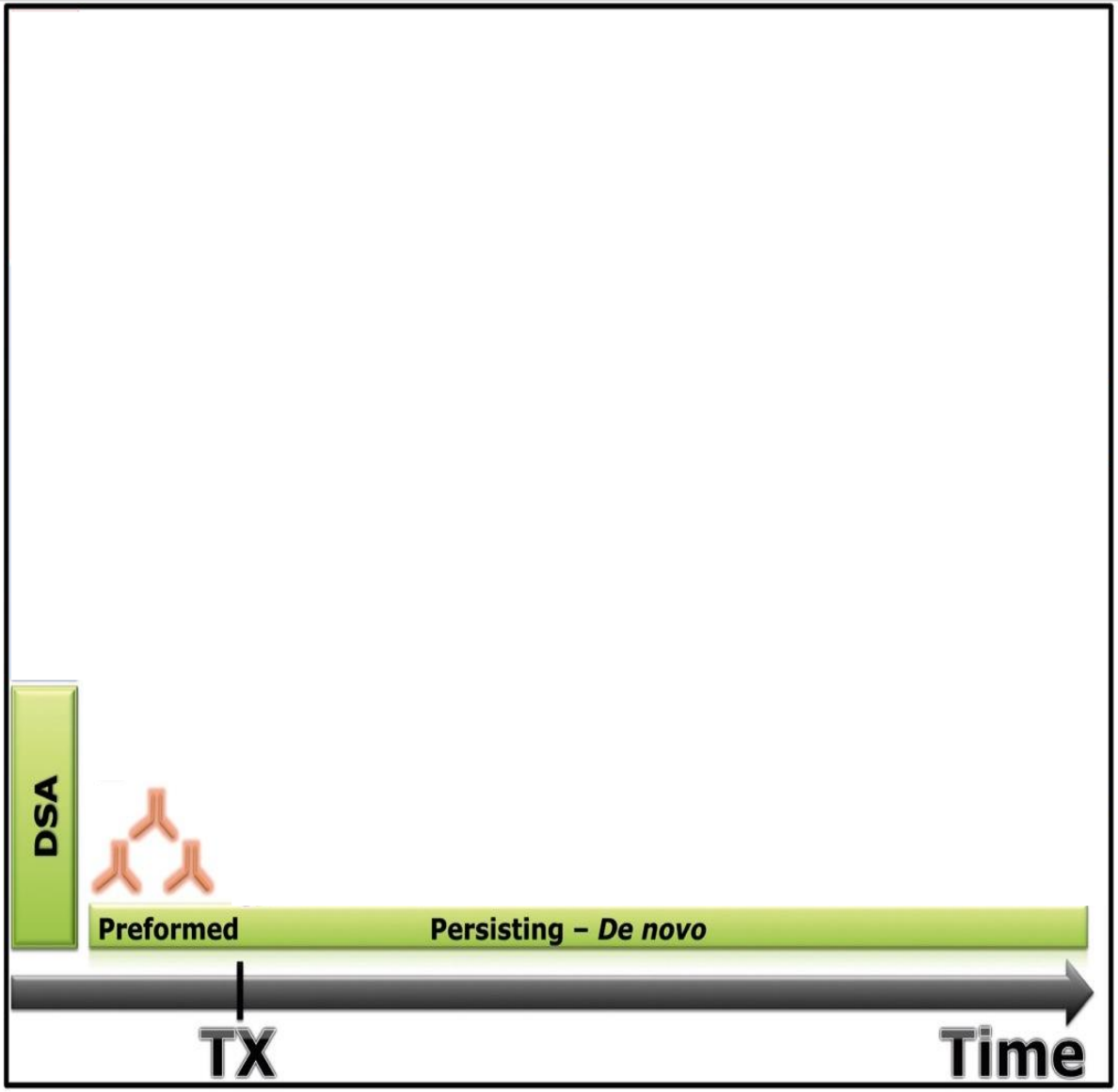

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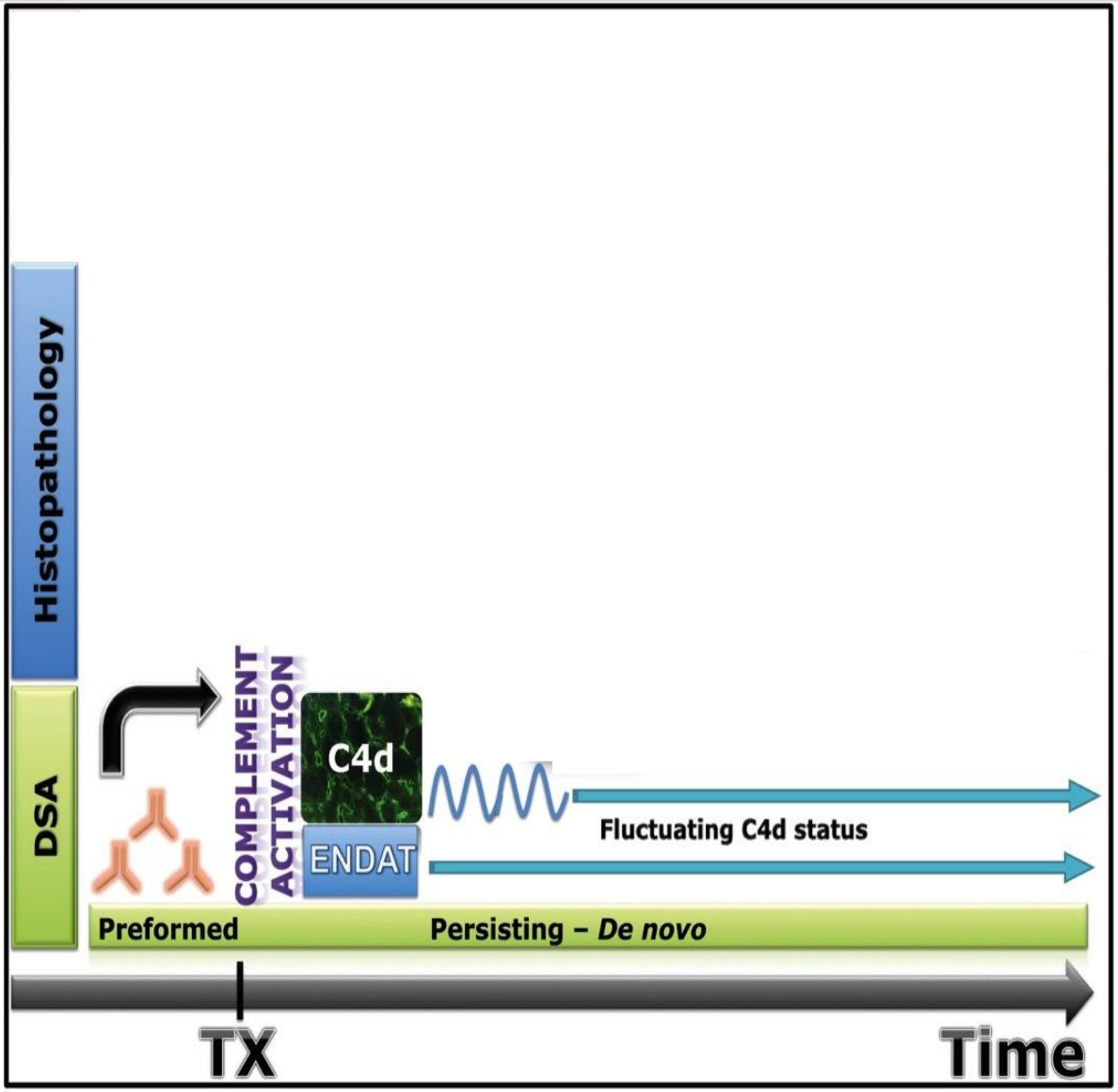

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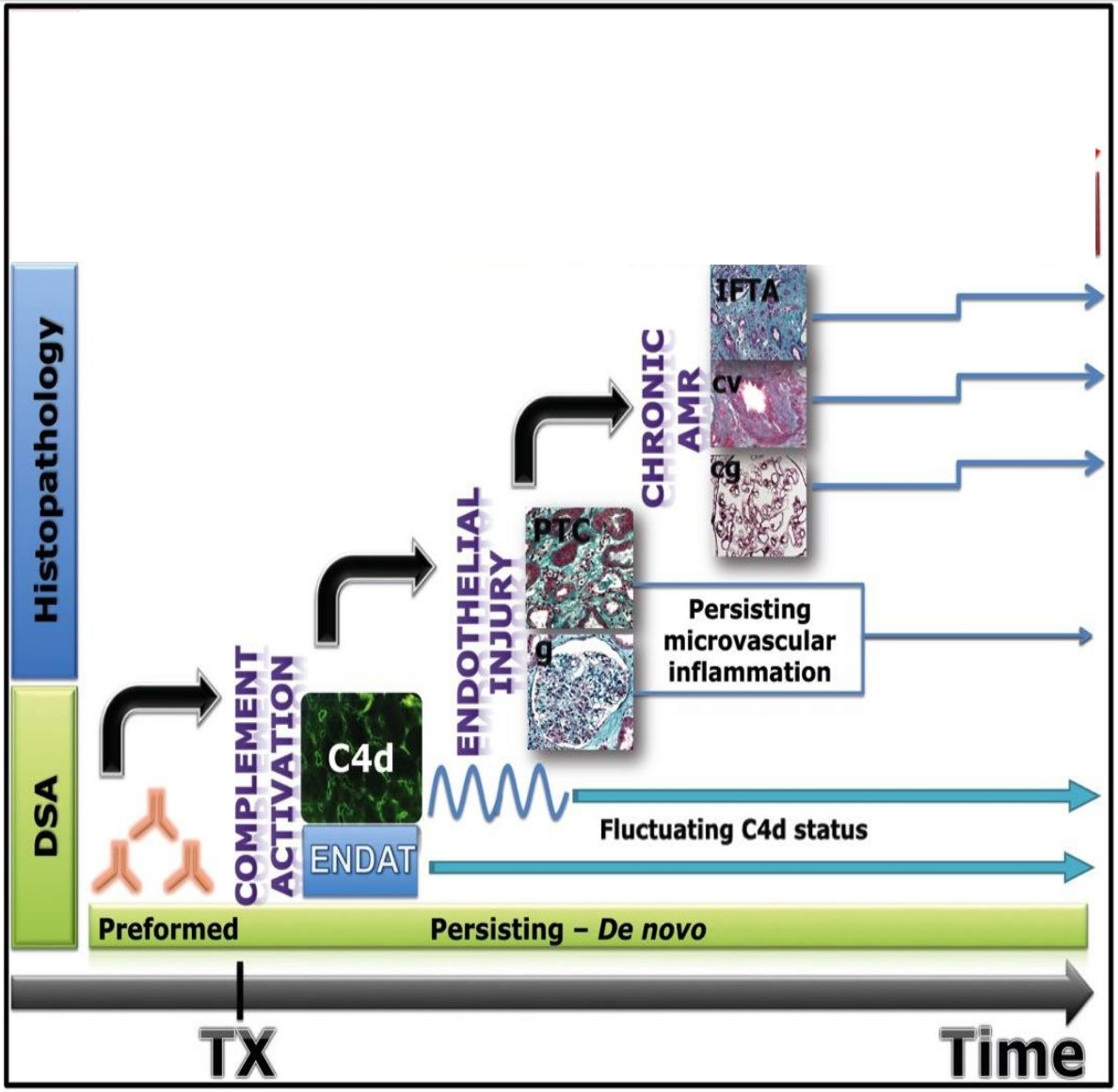

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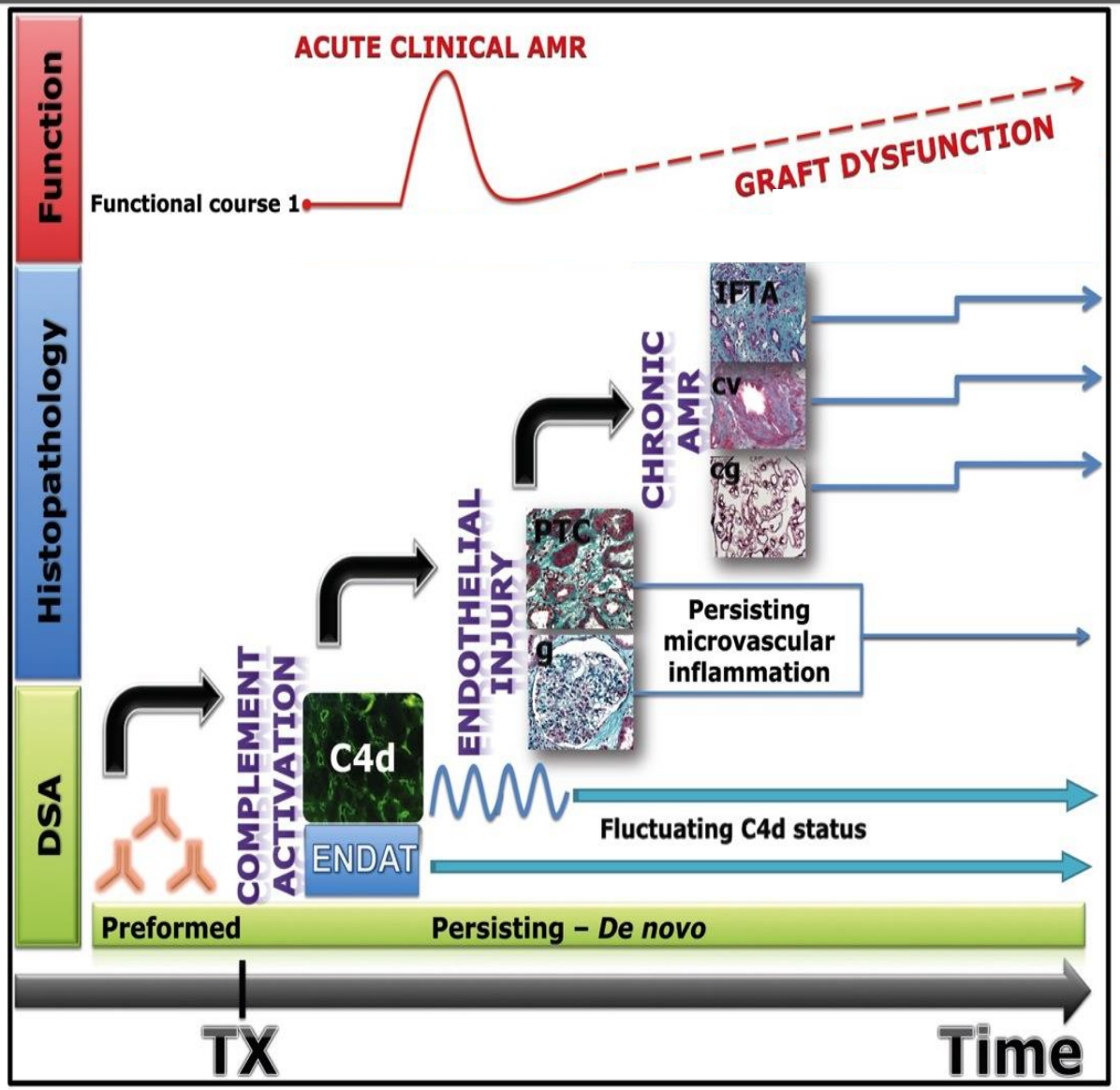

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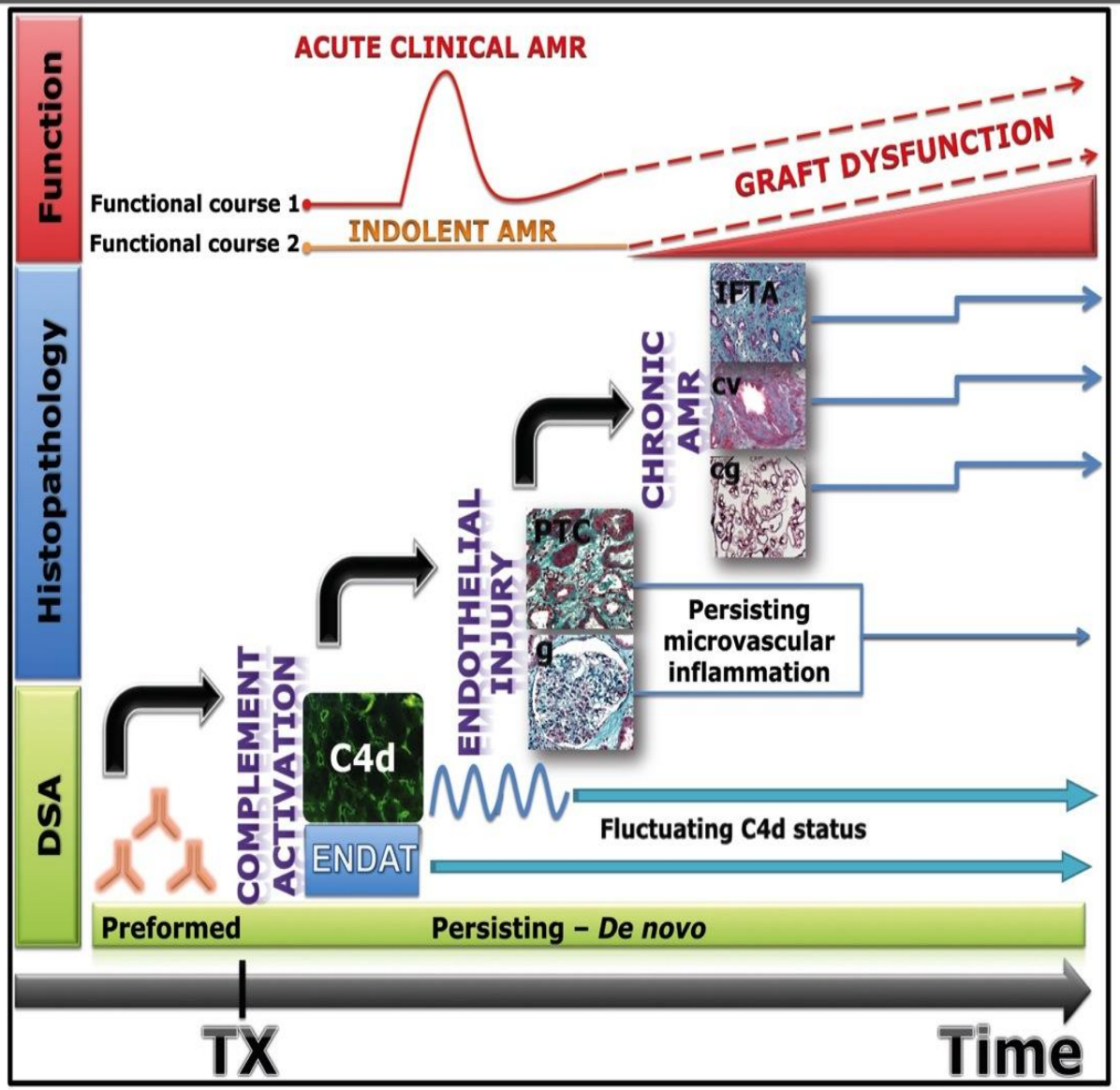
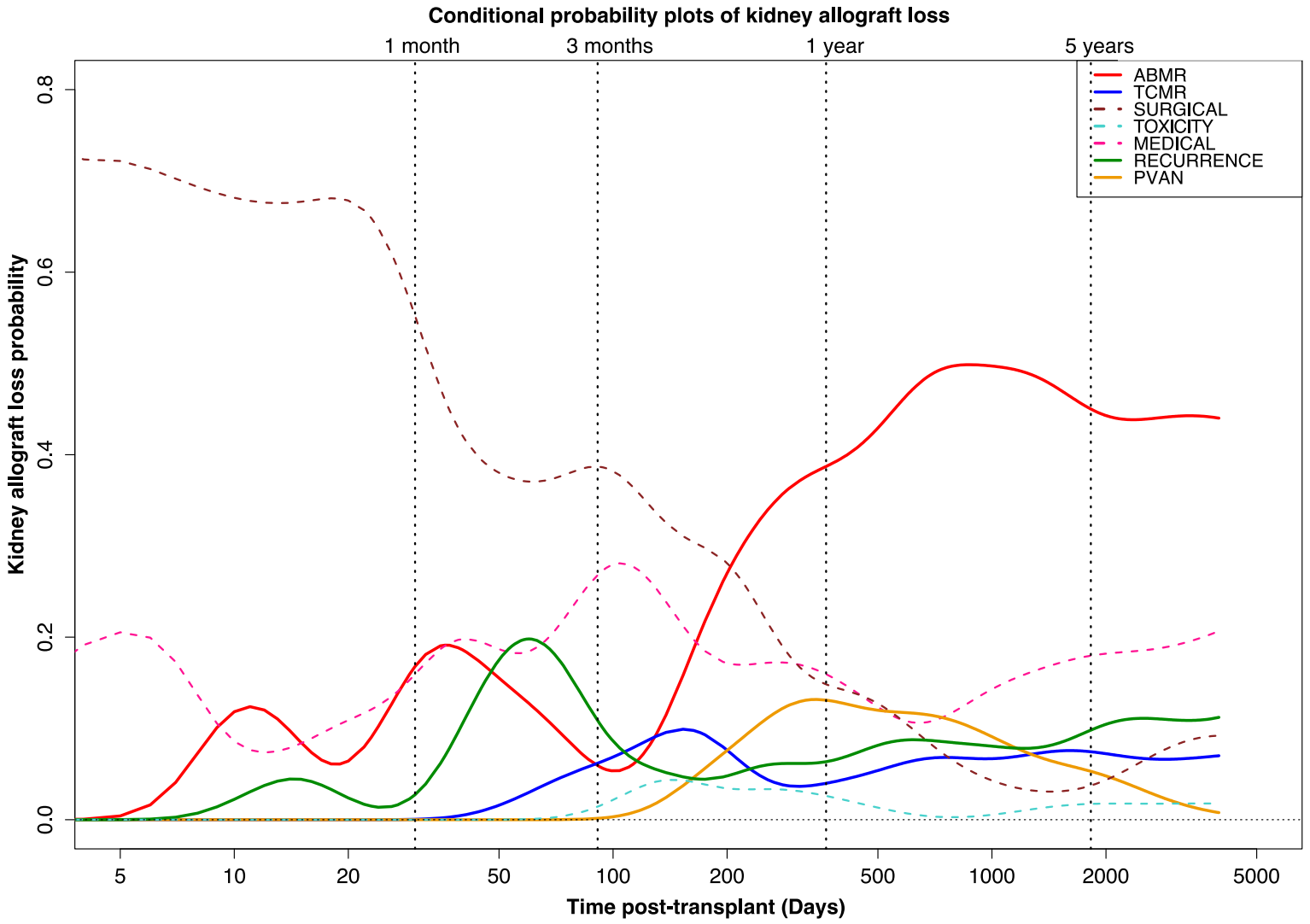


Loupy, Hill & Jordan, Nat reviews 2012

ABMR IS THE MAIN CAUSE OF LATE ALLOGRAFT LOSS



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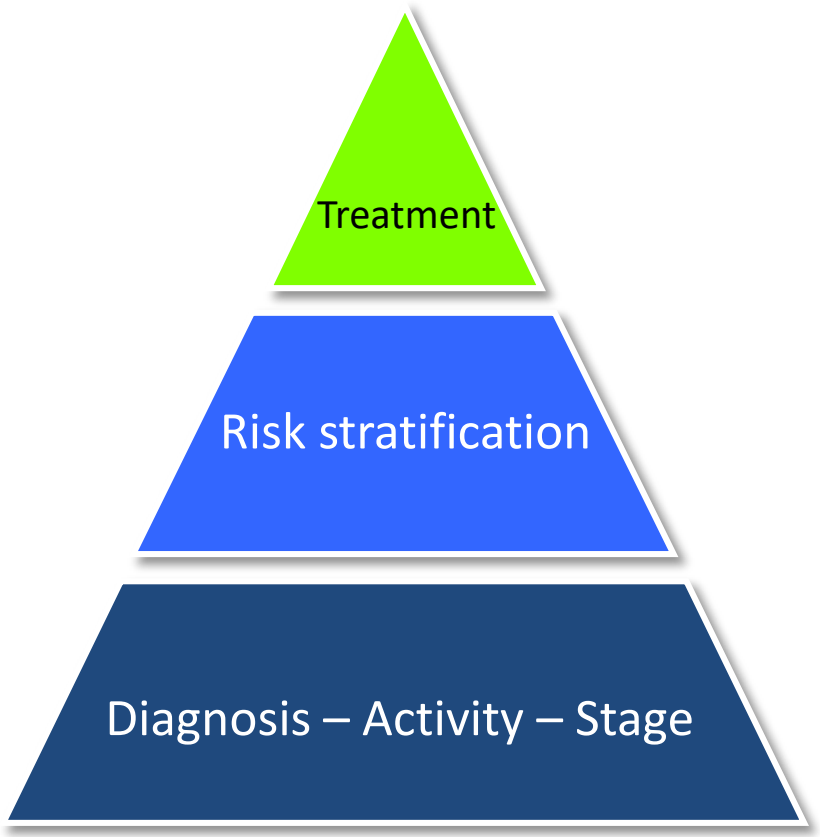
Loheac C & Aubert O et al. Unpublished data

WHAT NEED TO BE ACHIEVED



Washington, Sept 28th 2015

- ✓ ABMR = current outstanding matter of concern
- ✓ Reponse to therapy in ABMR is unknown
- ✓ PHENOTYPES ARE MANDATORY
- ✓ Precision composite end point is needed

Presence/absence of anti-HLA antibody is not enough

Lefaucheur, AJT, 2008

Thresholds of HLA-DSA

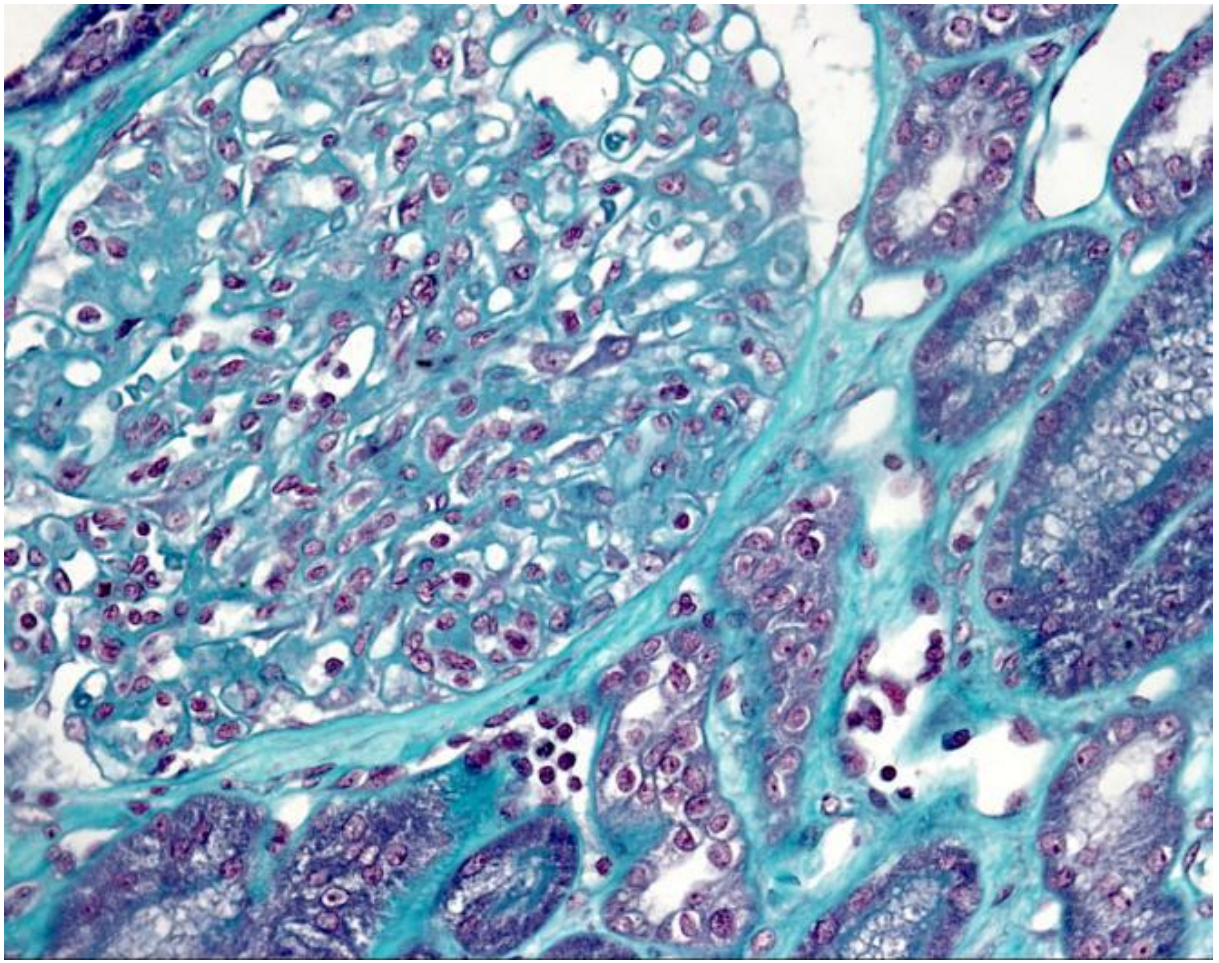
Lefaucheur & Loupy, JASN, 2010

ARE DSA EQUAL?

Miss C.
3rd graft

DSA DQ2

MFI 2460

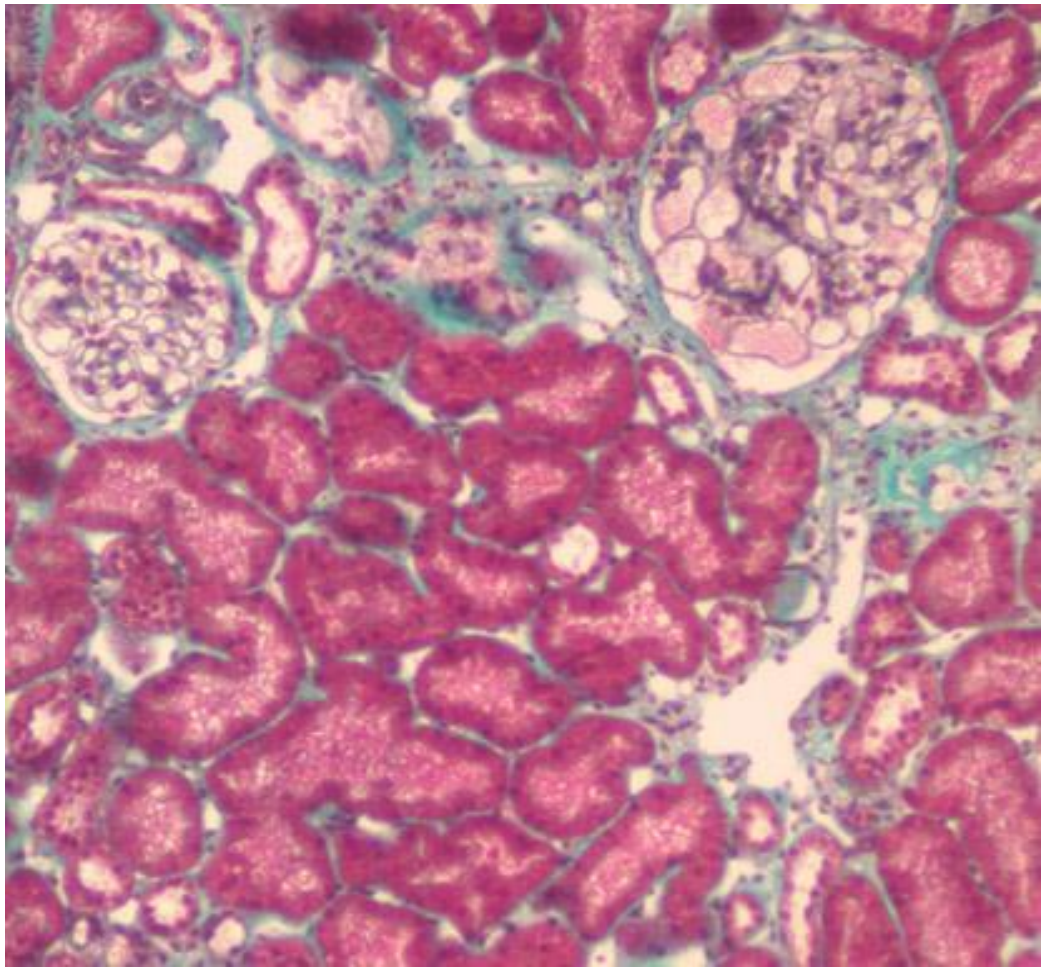


ARE DSA EQUAL?

Mr D
1st graft

DSA DR 7

MFI 3800



Presence/absence of anti-HLA Ab:
Not enough

↓

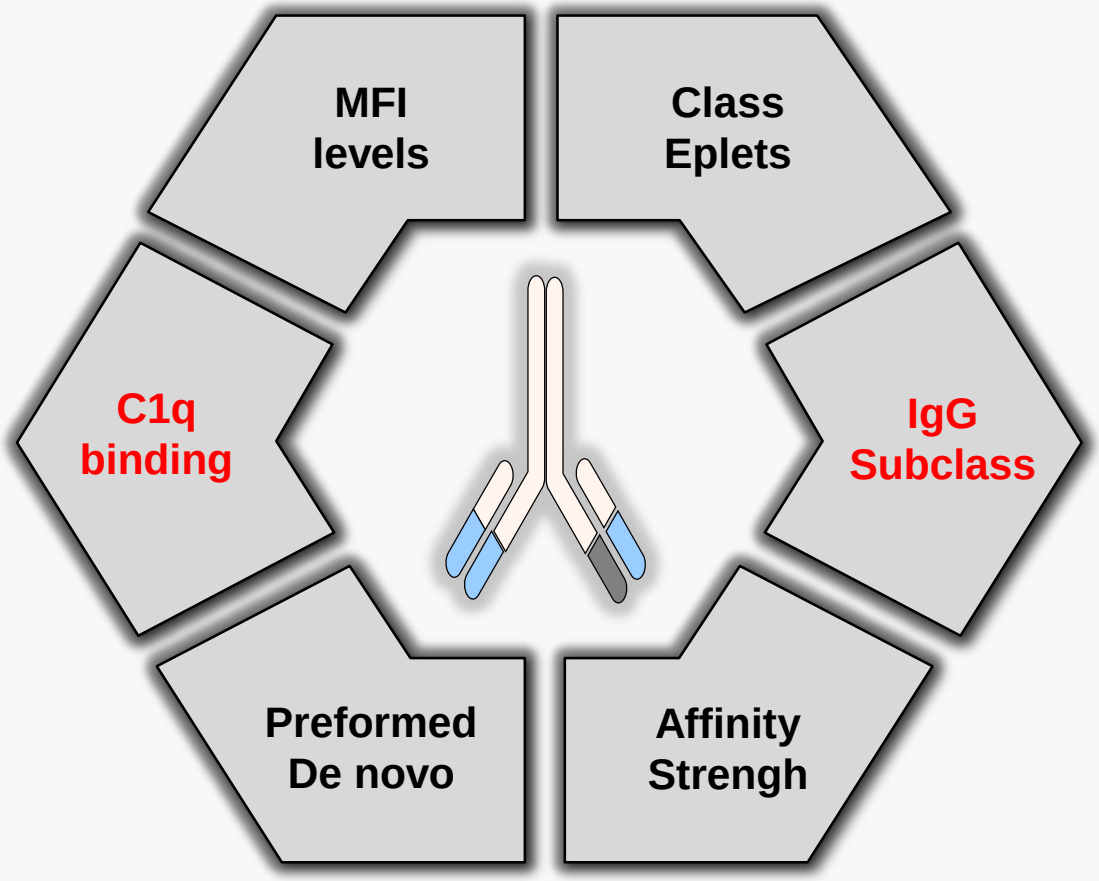
Thresholds of HLA-DSA:
Not enough

↓

We need new tools for risk stratification !

- **Affinity/Avidity of Anti-HLA antibodies**
- **Complement activation: C1q**
- **Subclasses: IgG1-4**
- **Pre-existing vs. *de novo* DSA**

Integrative and multiplex assessment of DSA



Graft failures
Disease progression
Risk prediction
Validation

- Tyan et al; **Human Immunol 2011**
- Sicard et al; **JASN 2014**
- Taupin et al; **JASN 2015**
- Viglietti et al**: JASN 2015**
- Akalin et al; **KI 2015**
- Canër et al; **Transplantation 2015**
- Aubert O et al, **JASN 2017**

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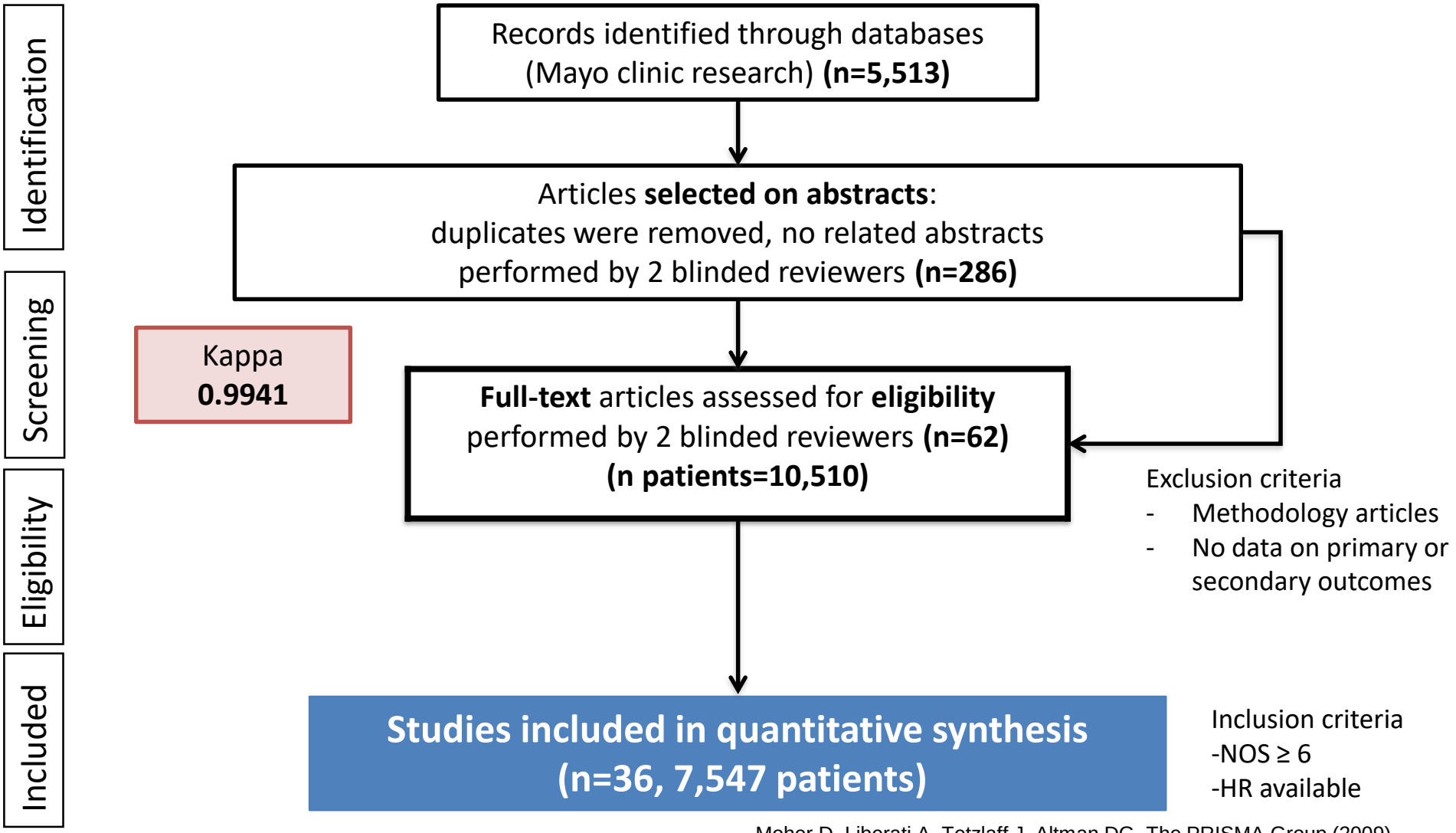
Anti-HLA DSA characteristics associated with risk of allograft loss

- **Complement binding capacity**
- **IgG subclass composition**

Meta-Analysis: Clinical Significance of Complement Activating Anti-HLA DSA in Kidney Transplantation



Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009)

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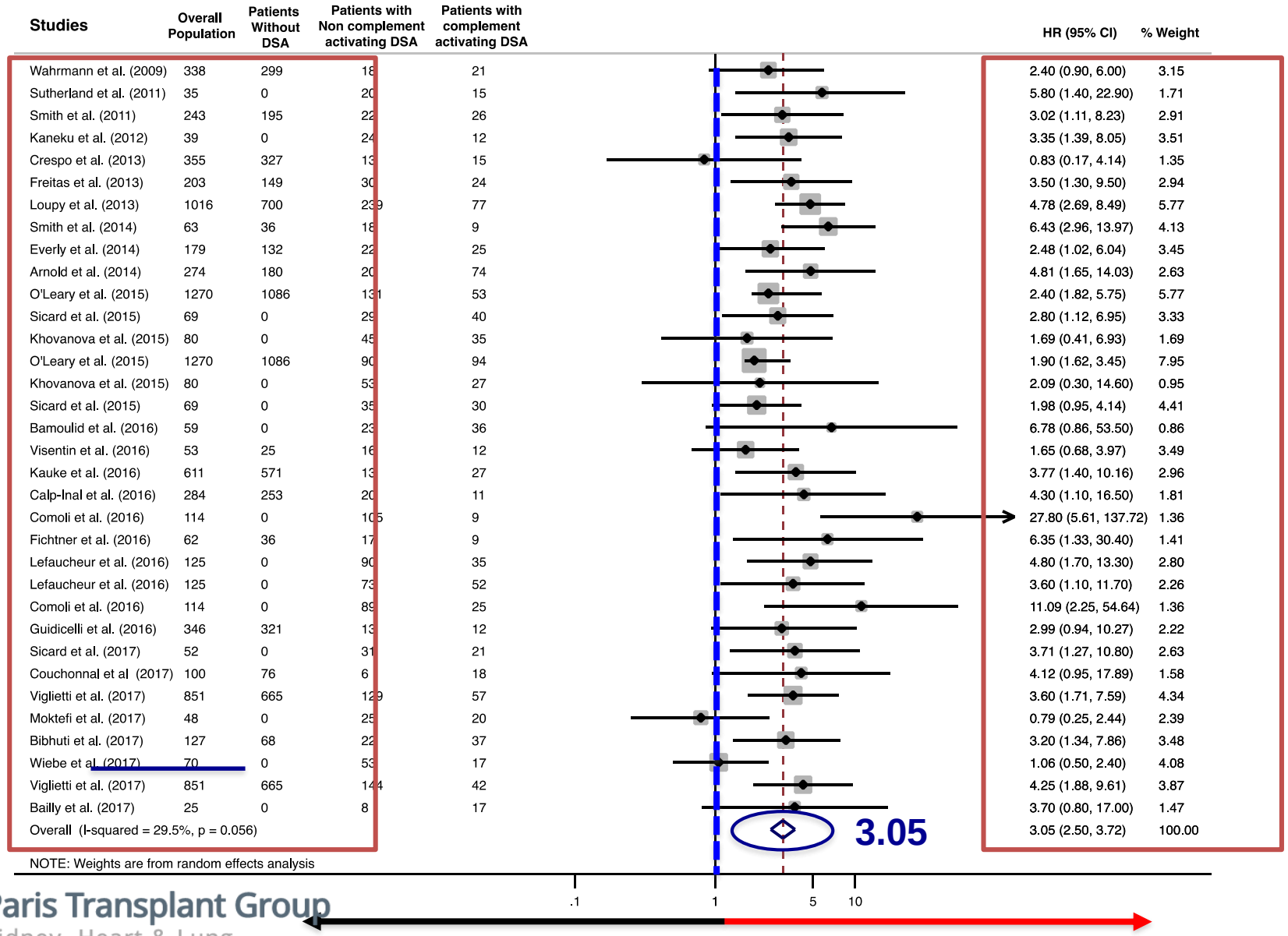
Loheac C, Aubert O et al PLOS MEDICINE IN PRESS

Meta-Analysis: Clinical Significance of Complement Activating Anti-HLA DSA in Kidney Transplantation



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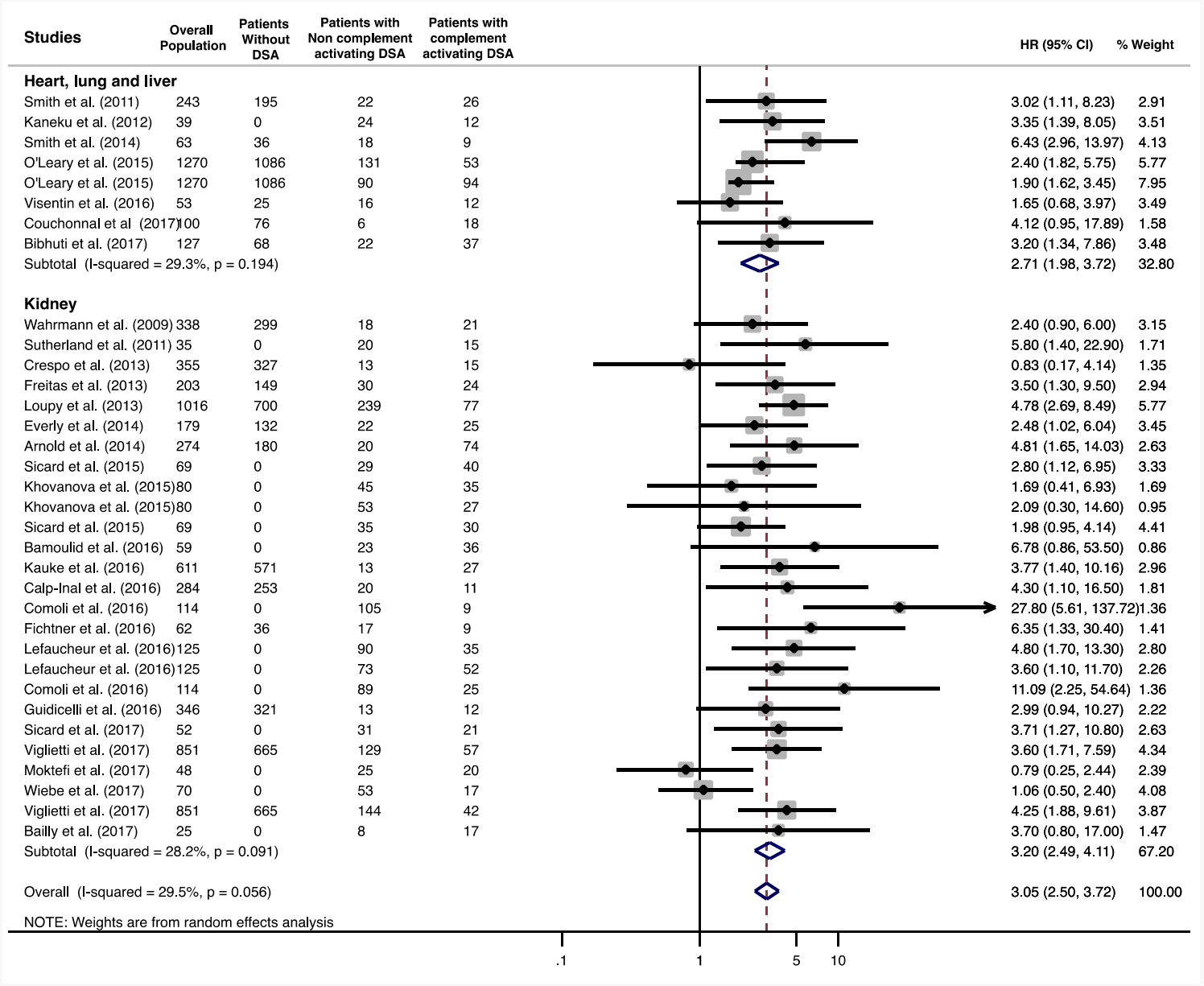
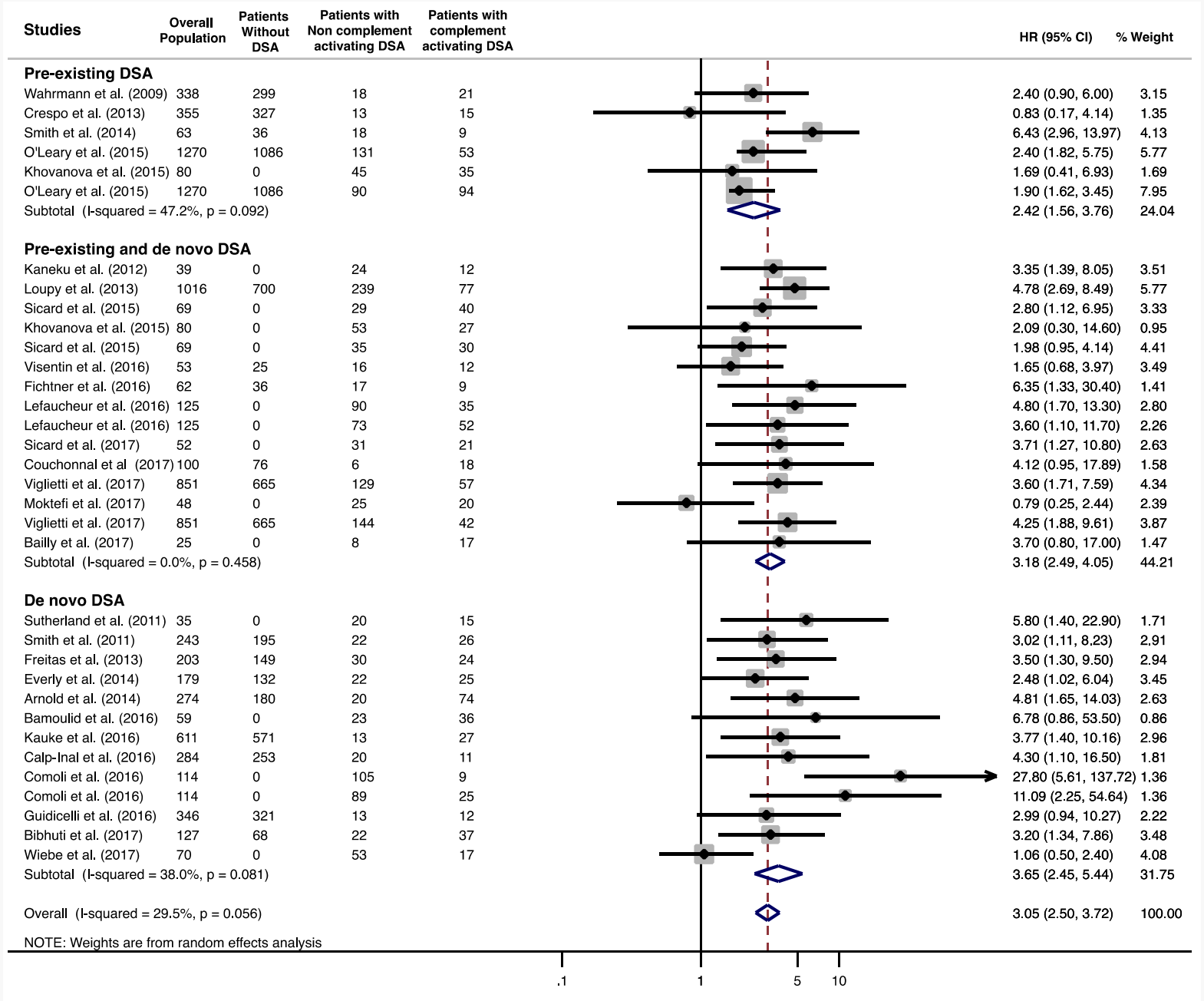
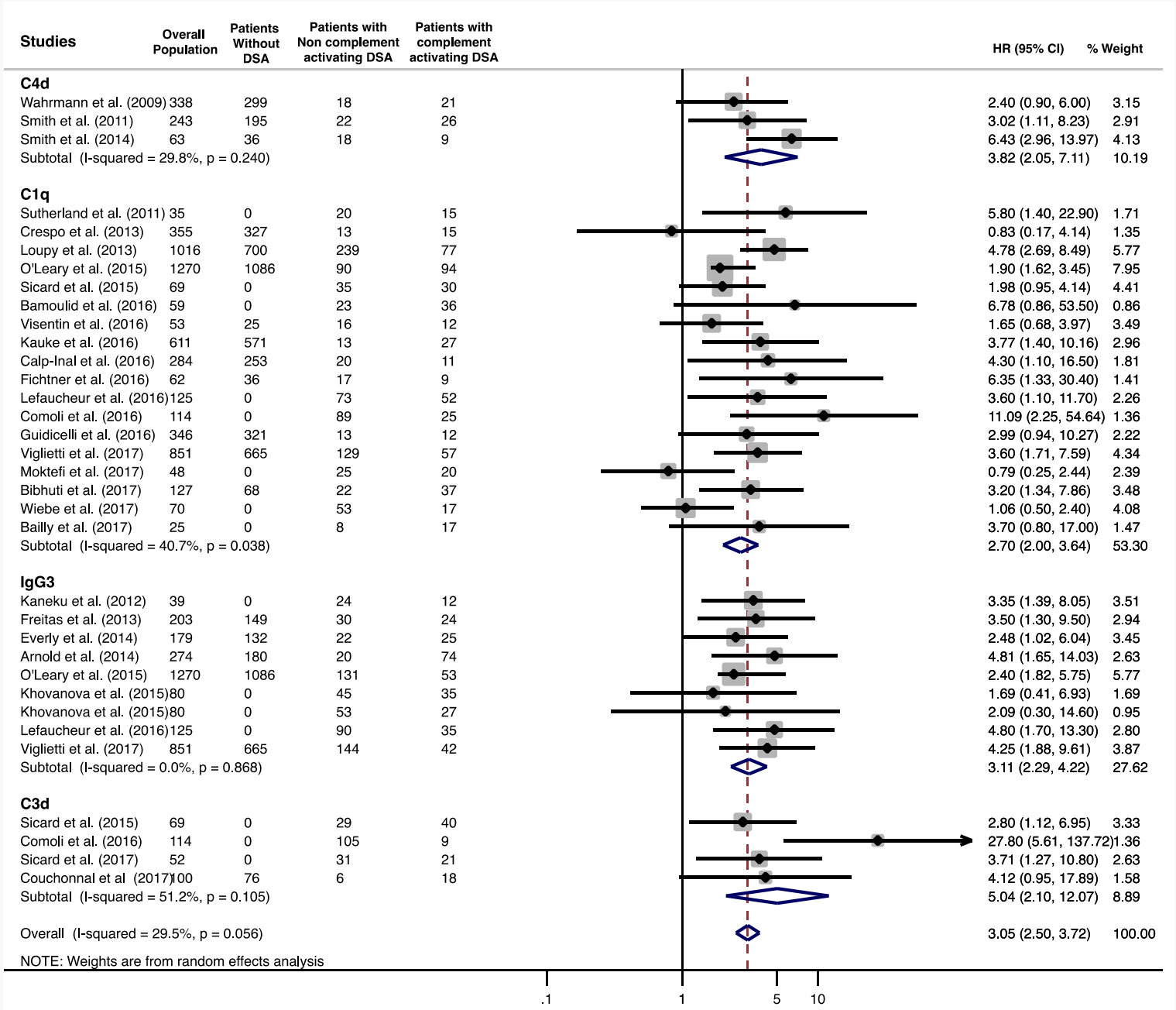
C' activating Ab: multiorgan relevance



C' activating Ab: Timing of Ab detection



C' activating Ab: type of test

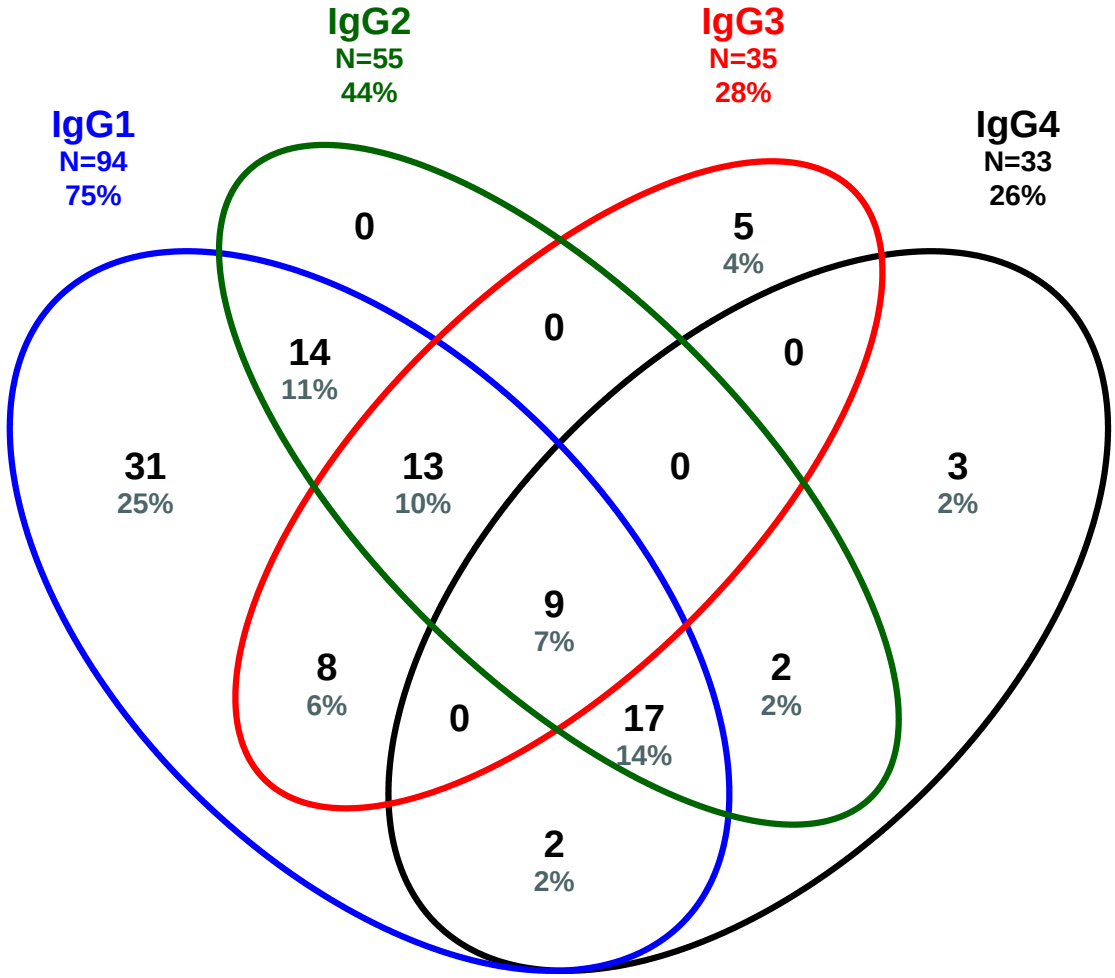


Factors influencing complement fixing (CF) HLA-Ab in vitro (C1q reactivity)

1. Presence of complement fixing (CF) IgG subtypes (IgG1/IgG3)
2. Level of IgG subtypes (weak /strong MFI)
3. Mixture of CF and non CF (NCF) (C1q reactivity)
4. Impact of antibody removal therapy:

   Loss of C1q reactivity – diminished IgG subtype reactivity- NOT Switch of IgG subtype

DSA IgG subclass distribution



IgG1	N=94	75%
IgG2	N=55	44%
IgG3	N=35	28%
IgG4	N=33	26%

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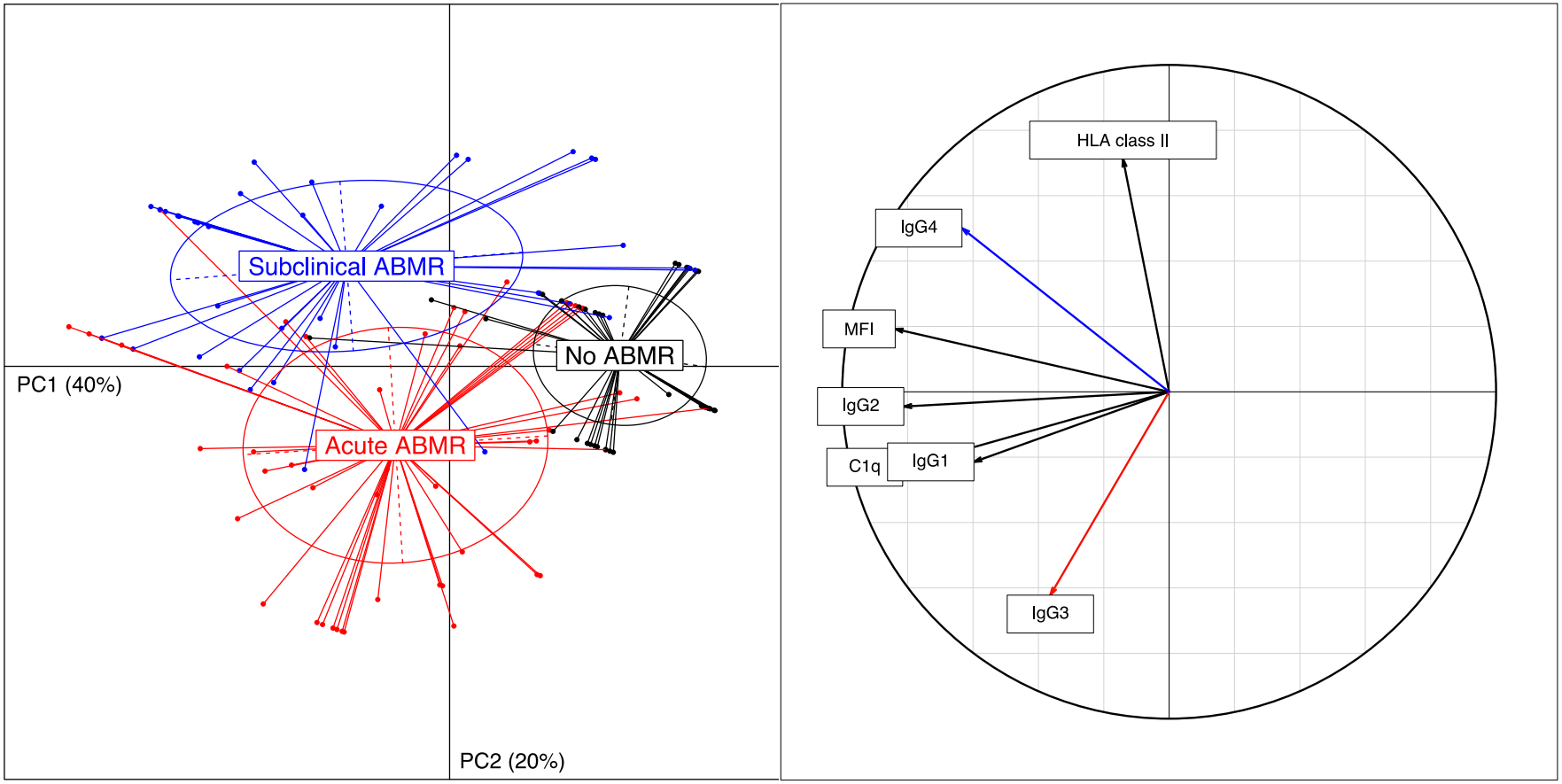
DSA IgG SUBCLASS COMPOSITION ACCORDING TO C1q STATUS

IgG subclass composition	All patients N=157	C1q- DSA N=113	C1q+ DSA N=44
None	24 (15)	24 (21)	0
1	28 (18)	21 (19)	7 (16)
2	6 (4)	6 (5)	0
3	6 (4)	4 (4)	2 (5)
4	5 (3)	5 (4)	0
1+2	24 (15)	21 (19)	3 (7)
1+3	28 (18)	13 (12)	15 (34)
1+4	5 (3)	3 (3)	2 (5)
2+4	4 (3)	4 (4)	0
1+2+3	8 (5)	2 (2)	6 (14)
1+2+4	17 (11)	10 (9)	7 (16)
1+2+3+4	2 (1)	0	2 (5)

All C1q-binding DSAs were positive for IgG1 and/or IgG3 subtypes

C1q negative DSA does not indicate absence of IgG1 or IgG3

Identification of distinct patterns of injury according to DSA characteristics



1) IgG1-4, C1q and pan-IgG MFI segregate presence/absence of ABMR

2) IgG3 and IgG4 segragate ABMR phenotype

Lefaucheur C, JASN, 2015

IMMUNOLOGIC DETERMINANTS OF C1q POSITIVITY: MULTIVARIATE MODEL

DSA: N=157

C1q DSA: N=44

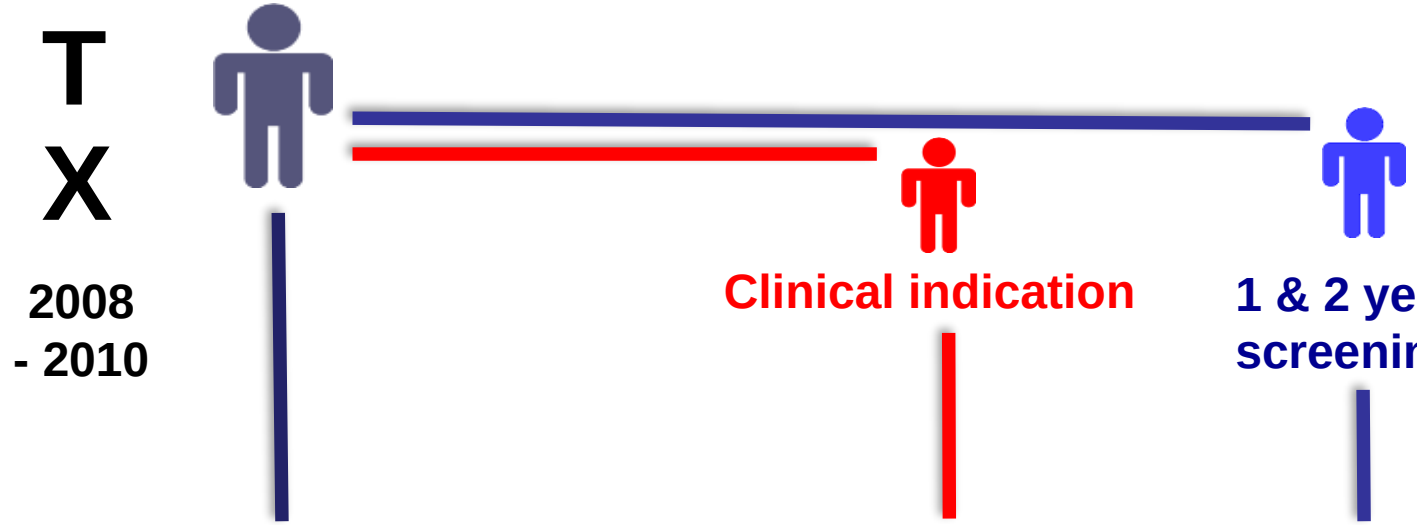
	OR	95%CI	P
MFI level	1	[1.00-1.00]	<0.001
IgG1			
No	1		
Yes	5.59	[0.90-34.60]	0.064
IgG3			
No	1		
Yes	3.66	[1.40-9.54]	0.008

Univariate analysis considered: MFI level, HLA class, IgG1, IgG2, IgG3 and IgG4 subclasses

Systematic monitoring and characterization of DSA could add to the predictive value for allograft loss of the conventional approach based on their detection and strength

STUDY DESIGN

Prospective DSA monitoring strategy



Day-0 DSA

- HLA class, specificity, MFI
- C1q-binding
- IgG1-4 subclasses

Post-Tx DSA

- HLA class, specificity, MFI
- C1q-binding
- IgG1-4 subclasses

Graft Biopsy
GFR
Prot U

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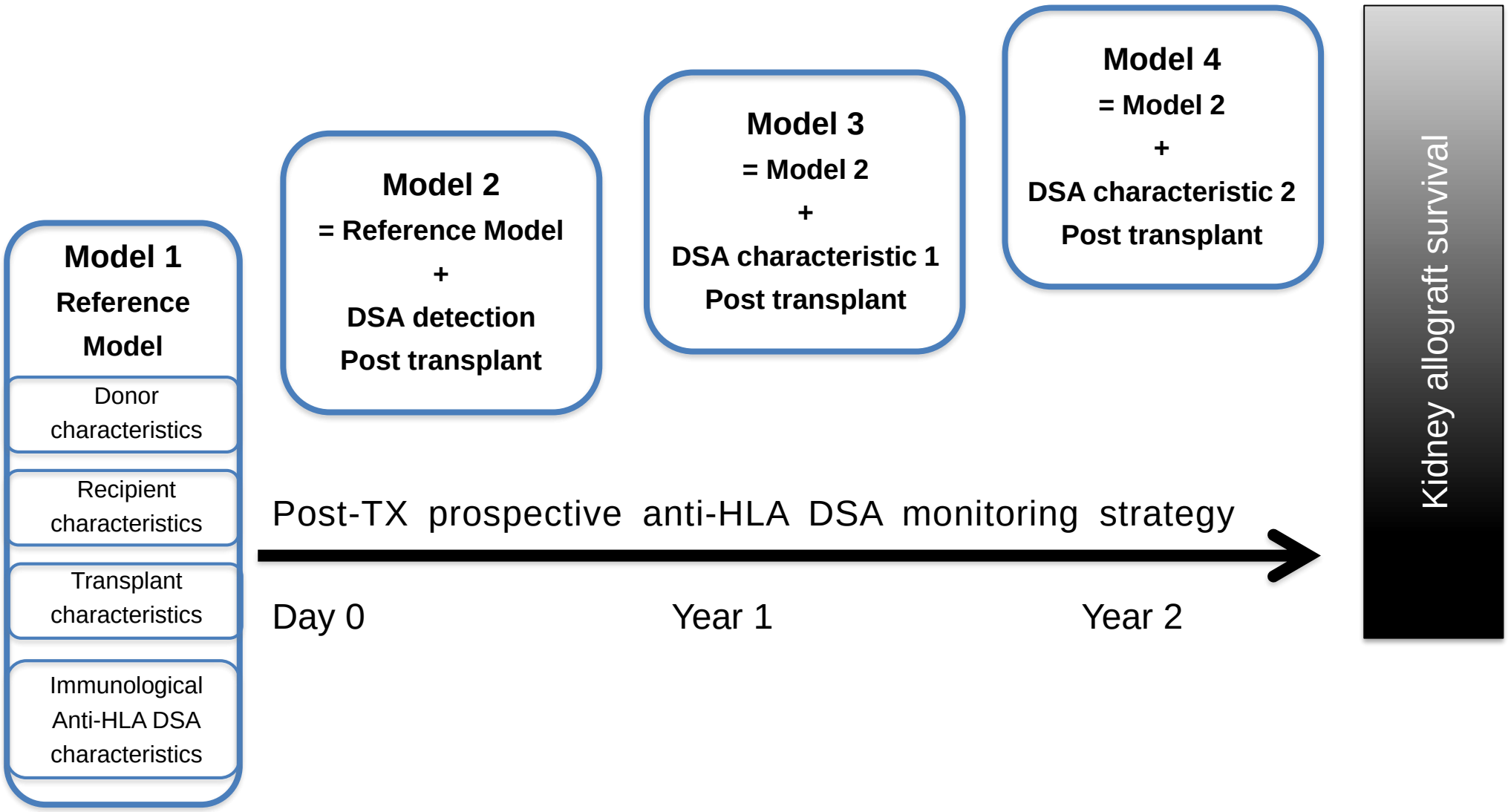
DYNAMIC MODELING TO ASSESS IMPROVEMENT IN RISK PREDICTION ACCORDING TO DSA MONITORING AND CHARACTERIZATION



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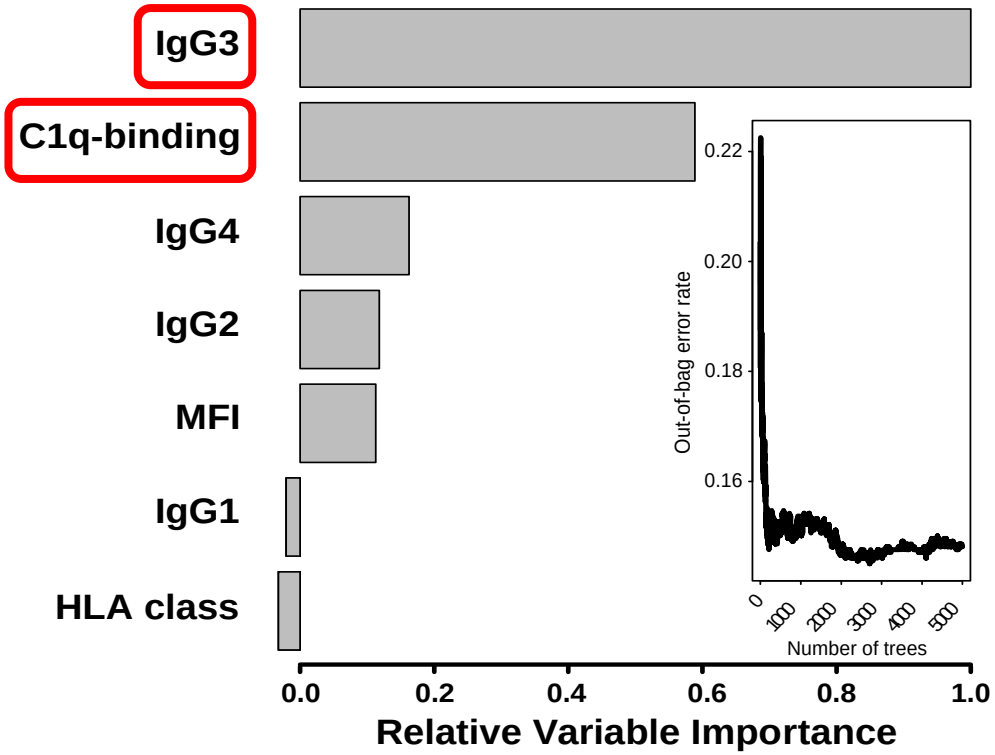
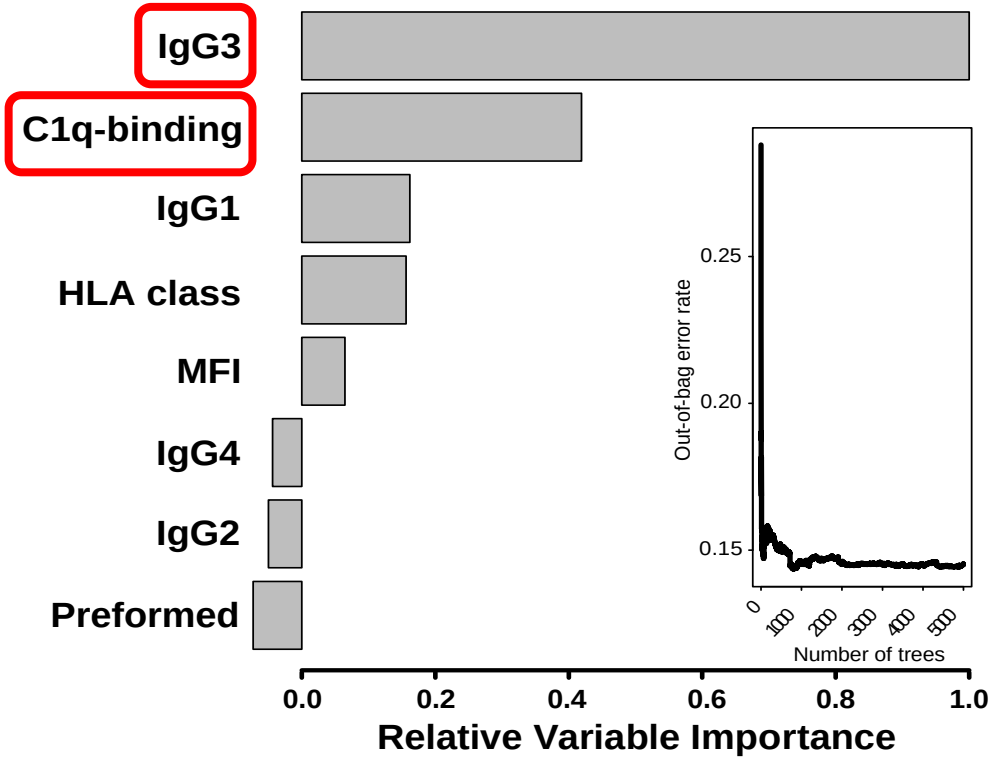
DSA CHARACTERISTICS AND ABILITY TO CLASSIFY ALLOGRAFT LOSS

Hierarchical ranking by multivariate survival random forest modeling

Day-0 (N=110)



Post-Tx (N=186)



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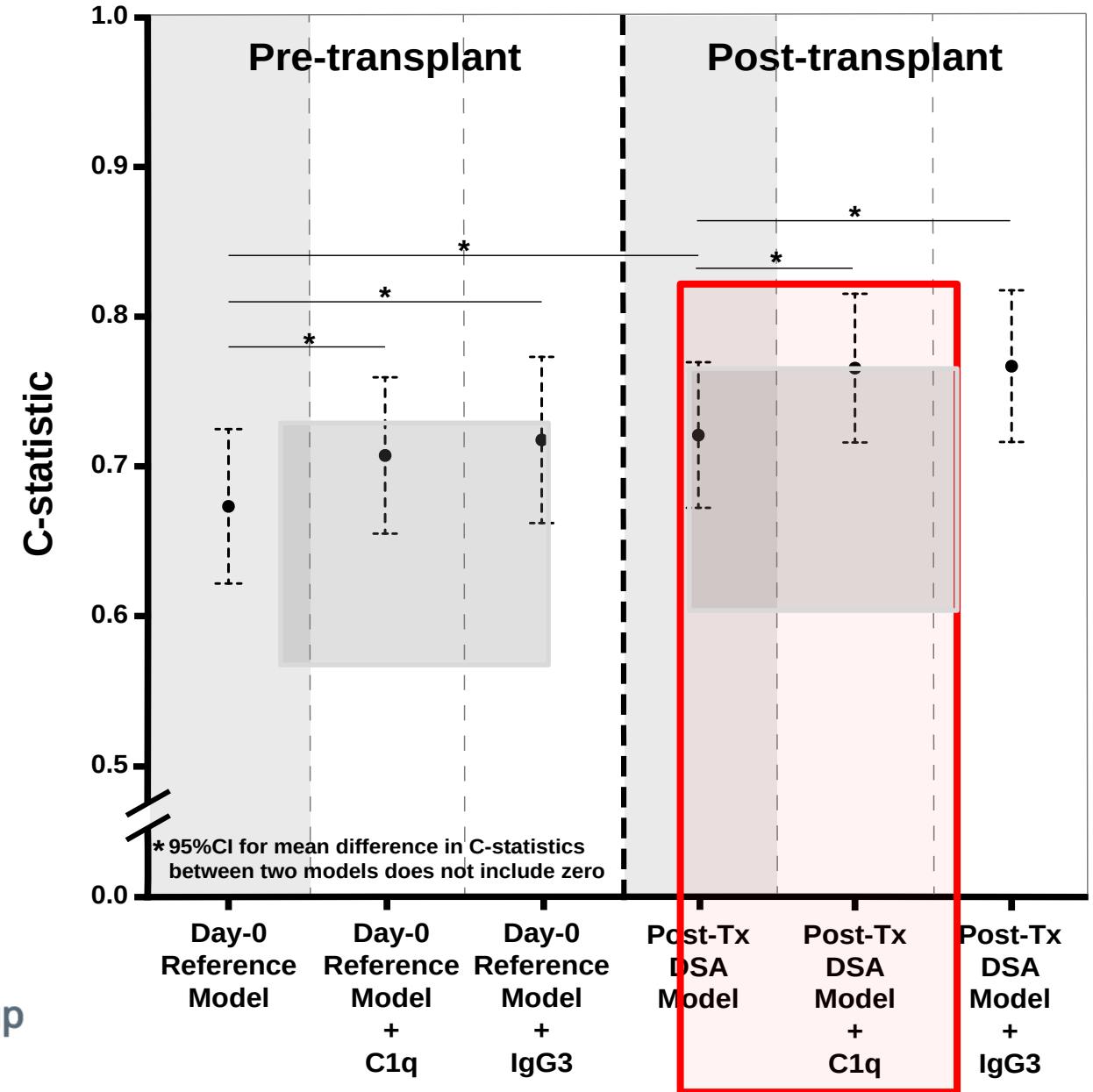
Viglietti D, JASN, 2016

PREDICTIVE VALUE OF DSA MONITORING AND CHARACTERIZATION

Overall population
N=851



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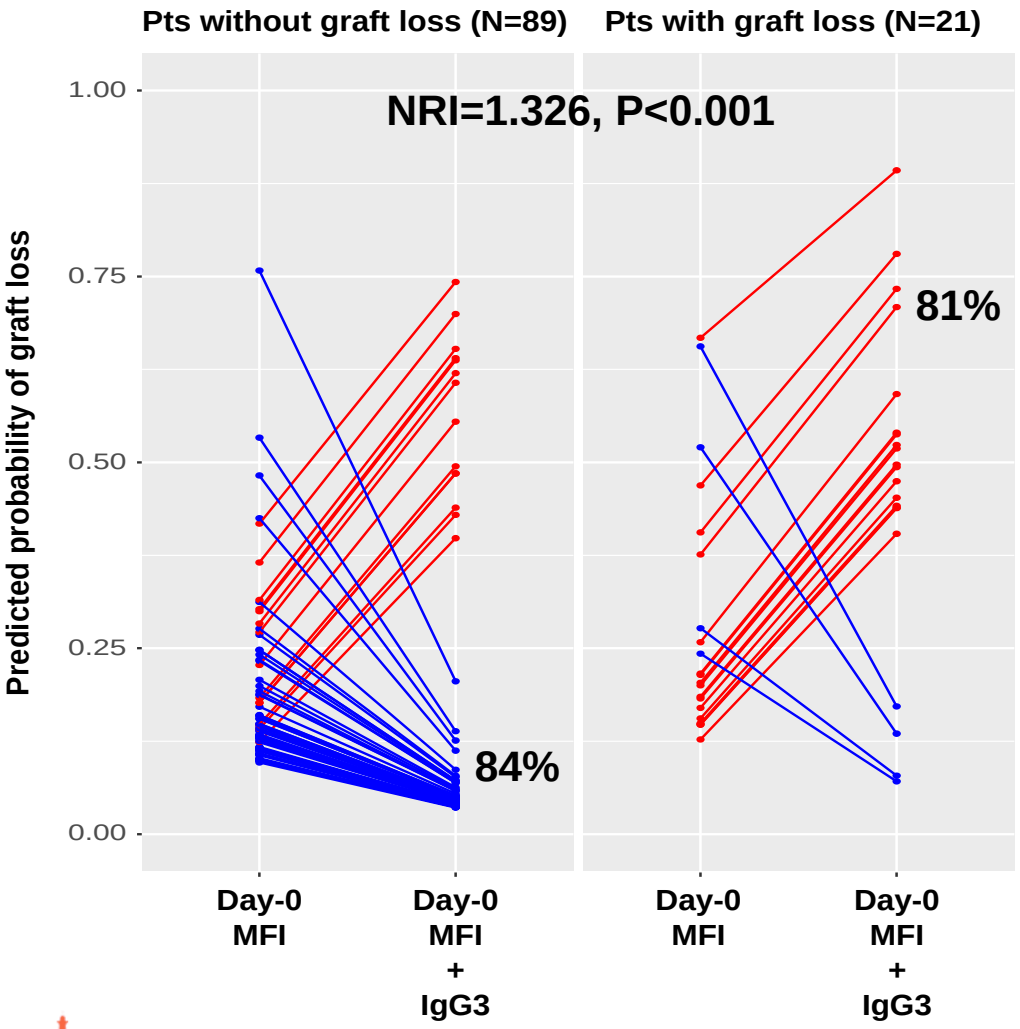
RISK RECLASSIFICATION BY IgG3 AND C1q BEYOND MFI

In patients with Day-0 DSA



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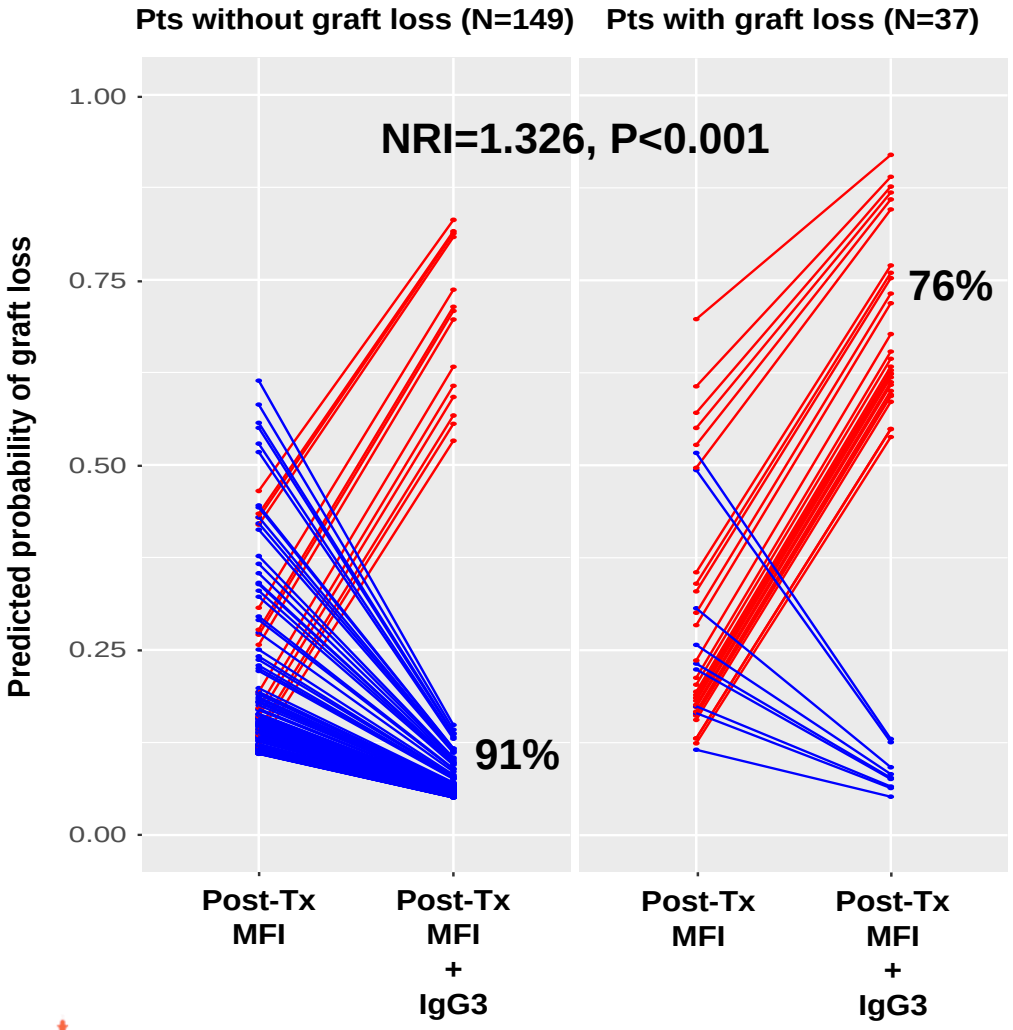
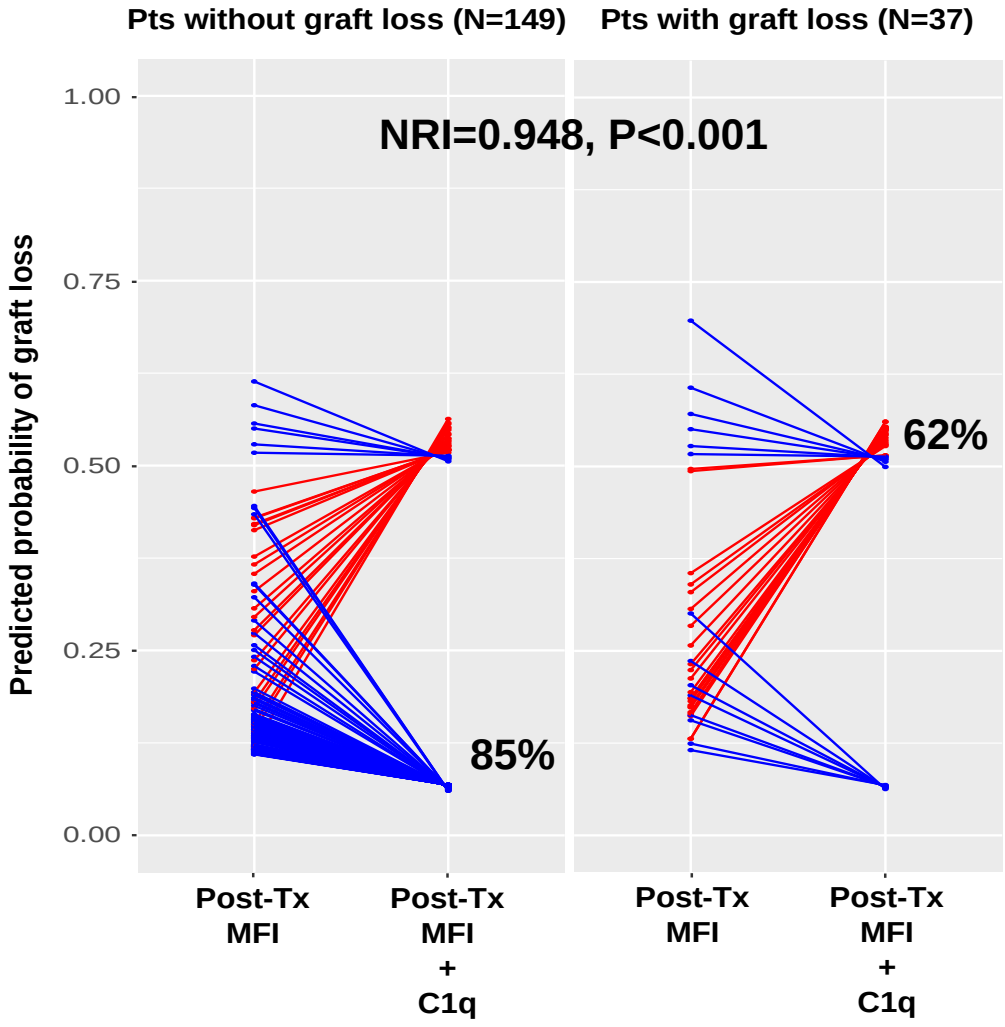
RISK RECLASSIFICATION BY IgG3 AND C1q BEYOND MFI

In patients with Post-Tx DSA





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CLINICAL RESEARCH www.jasn.org

Value of Donor–Specific Anti–HLA Antibody Monitoring and Characterization for Risk Stratification of Kidney Allograft Loss

Denis Viglietti,*† Alexandre Loupy,†‡ Dewi Vernerey,§ Carol Bentlejewski,‖ Clément Gosset,¶ Olivier Aubert,† Jean-Paul Duong van Huyen,** Xavier Jouven,† Christophe Legendre,†‡ Denis Glotz,*† Adriana Zeevi,‖ and Carmen Lefaucheur*†

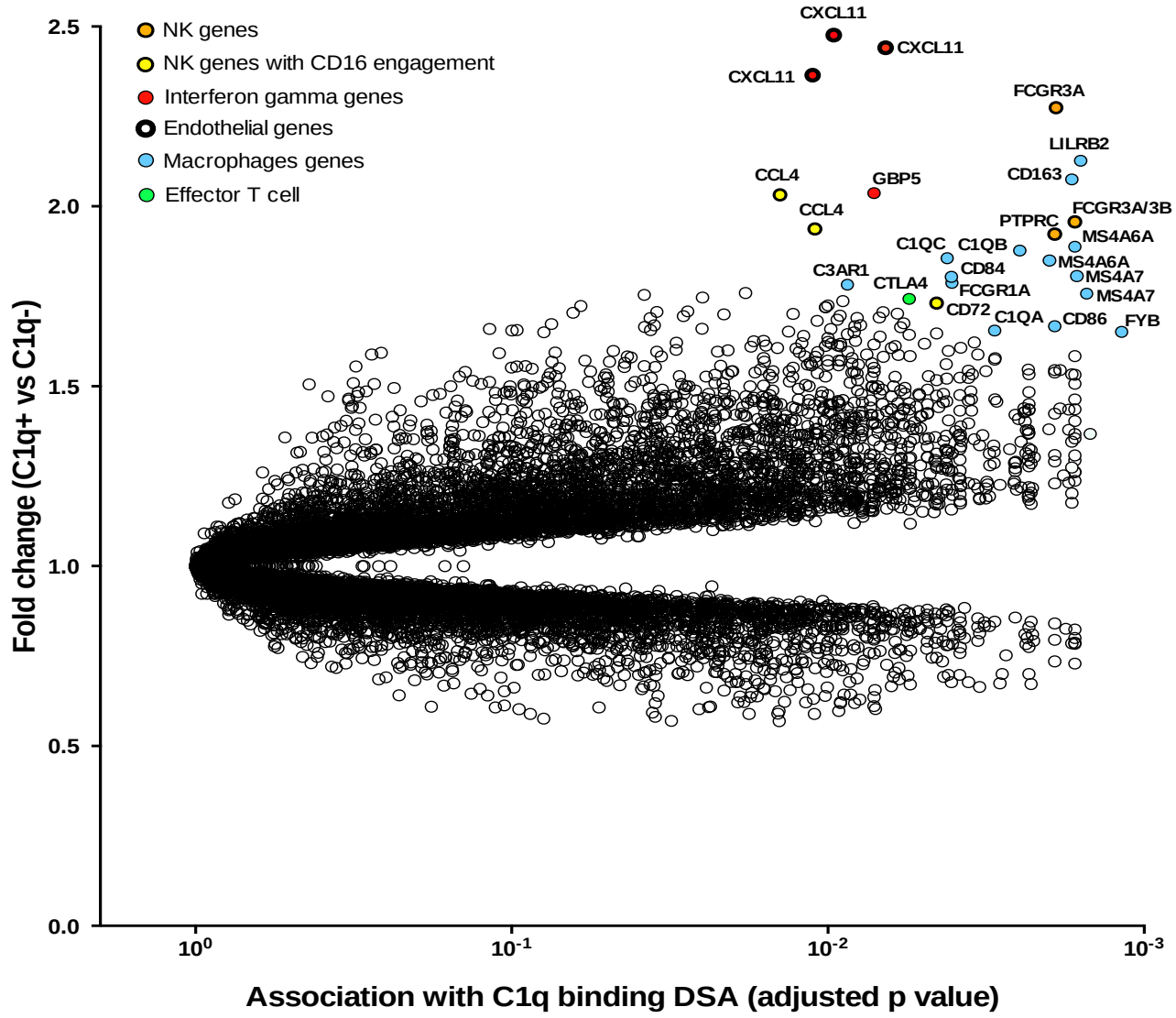
- Prospective, systematic monitoring of DSA improved risk stratification for allograft loss beyond traditional determinants
- IgG3 positivity and C1q-binding capacity were the most informative DSA characteristics for classifying patients according to their risk of allograft loss
- IgG3 positivity or C1q-binding capacity improved risk stratification at the population level and also in patients with DSA beyond MFI level
- Further studies are needed to determine the most cost efficient DSA screening policies

Anti-HLA DSA characteristics induce distinct injuries in kidney allografts

- **Complement binding capacity**
- **IgG subclass composition**

INTRAGRAFT GENE EXPRESSION ACCORDING TO DSA C1q STATUS

9954 IQR-filtered genes



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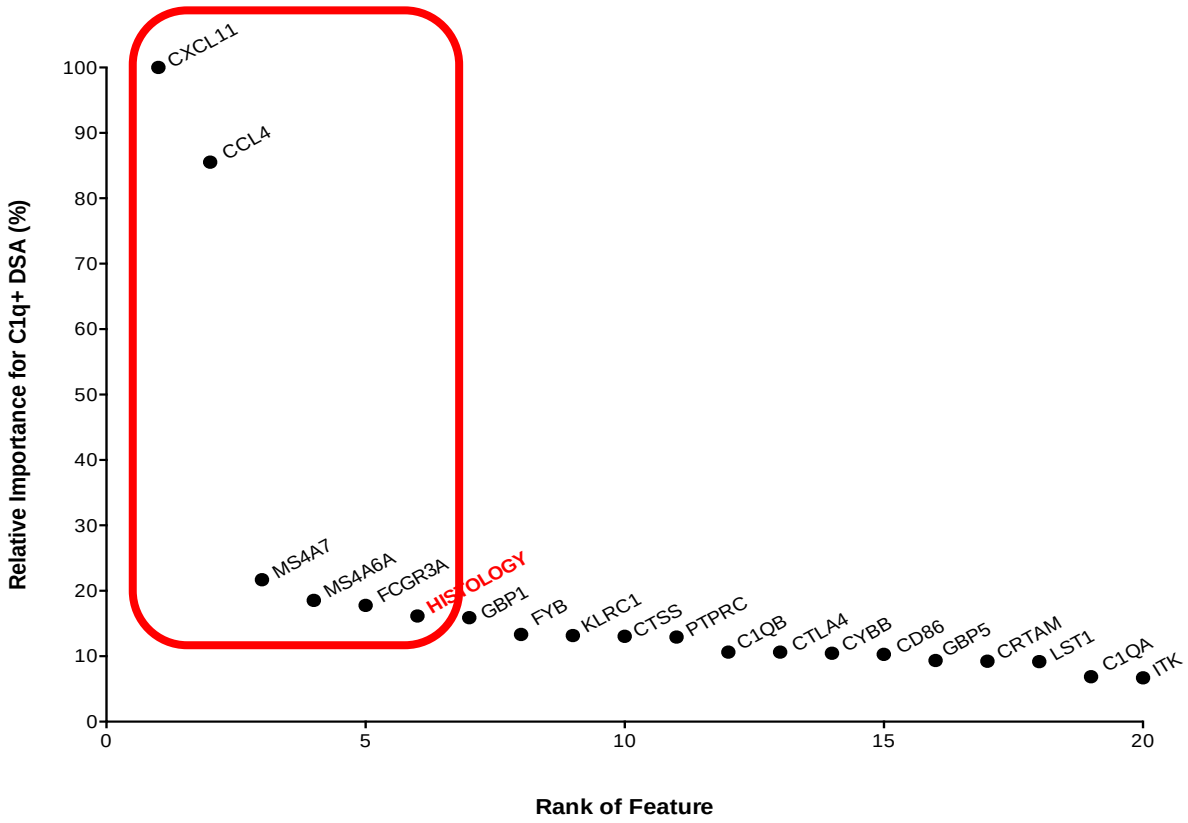
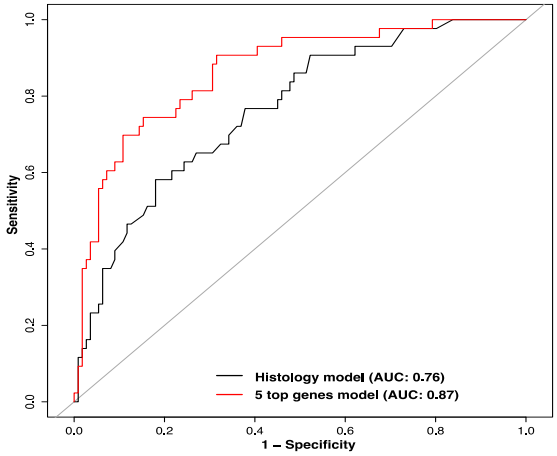
RELATIVE IMPORTANCE OF C1q SELECTIVE GENES AND HISTOLOGY IN DETERMINING DSA C1q STATUS

Hierarchical ranking (random forest)





Histology: g+ptc+v+i+t+C4d Banff scores

Top genes	Biological association
CXCL11	ENDOTHELIAL IFNG RESPONSE
CCL4	NK CELL CD16-ENGAGEMENT/MACROPHAGE IFNG RESPONSE
MS4A6A	MONOCYTE/MACROPHAGE
MS4A7	MONOCYTE/MACROPHAGE
FCGR3A	NK CELL

BIOLOGICAL RELEVANCE OF C1q DSA TRANSCRIPTS

Human rejection effector cells culture

CCL4

CXCL11

FCGR3A

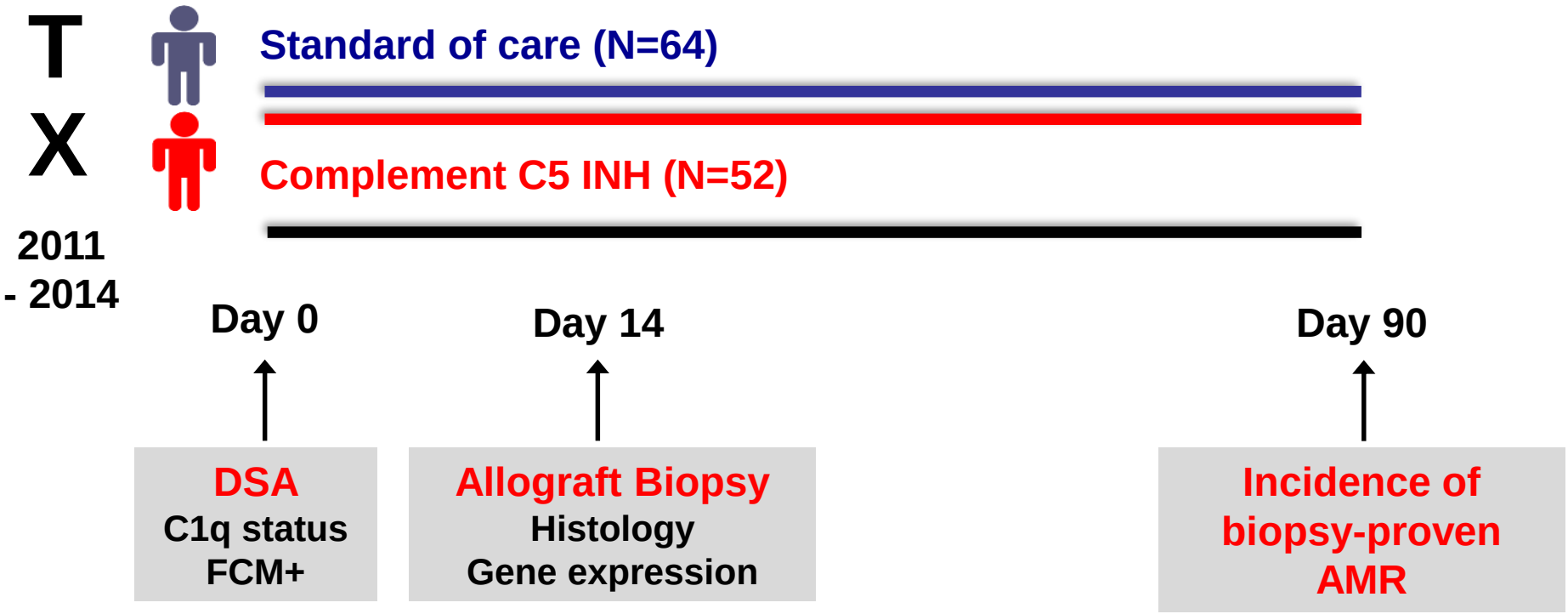
MS4A6A
MS4A7

Gene Symbol	Control Kidney	CD4	CD8	NK Unstimulated	NK CD16-Stimulated	B cell	Monocyte	Macrophage unstim	Macrophage + IFNG	HUVEC	HUVEC + IFNG	Avg signal
AIM2	-0.66	0.06	-0.03	-0.55	-0.53	2.00	0.43	-0.33	2.23	-0.67	-0.60	162
C1QA	0.13	-0.44	-0.46	-0.44	-0.42	-0.44	0.01	0.35	3.13	-0.46	-0.43	208
C1QB	-0.17	-0.33	-0.31	-0.32	-0.31	-0.33	-0.25	0.04	3.31	-0.35	-0.33	318
C1QC	-0.18	-0.33	-0.32	-0.33	-0.33	-0.33	-0.32	0.22	3.29	-0.34	-0.34	394
C3AR1	-0.57	-0.34	-0.07	0.02	-0.51	-0.69	1.56	1.53	1.98	-0.71	-0.71	458
CCL4	-0.55	-0.50	0.03	0.60	2.95	-0.56	-0.53	0.13	0.71	-0.57	-0.57	1258
CD163	-0.45	-0.48	-0.48	-0.48	-0.48	-0.48	0.72	2.71	1.36	-0.48	-0.48	641
CD72	-0.40	-0.35	-0.30	-0.33	3.09	0.97	-0.40	-0.32	-0.34	-0.40	-0.41	137
CD84	-0.57	-0.24	-0.23	-0.55	-0.50	-0.21	0.33	2.66	1.58	-0.56	-0.57	706
CD86	-0.54	-0.55	-0.42	-0.57	-0.52	-0.08	2.36	1.19	1.50	-0.58	-0.55	103
CRTAM	-0.29	-0.29	-0.27	-0.27	3.33	-0.29	-0.27	-0.24	-0.22	-0.29	-0.29	85
CTLA4	-0.40	2.85	1.48	-0.38	-0.38	-0.39	-0.40	-0.39	-0.39	-0.40	-0.40	80
CTSS	-0.72	-0.58	-0.61	-0.50	-0.55	-0.16	1.53	1.87	1.75	-0.72	-0.07	3789
CXCL11	-0.34	-0.34	-0.34	-0.35	-0.33	-0.35	-0.34	-0.34	-0.13	-0.34	3.28	708
CYBB	-0.53	-0.54	-0.54	-0.53	-0.53	-0.33	2.28	1.43	1.44	-0.54	-0.54	1260
EMR2	-0.46	-0.45	-0.45	-0.44	-0.45	-0.46	2.93	1.08	0.49	-0.45	-0.46	136
EVI2A	-1.06	0.96	0.48	0.31	1.49	-0.35	1.49	0.63	0.33	-1.05	-1.06	974
FCGR1A///FCGR1B///FCGR1C	-0.34	-0.34	-0.34	-0.34	-0.33	-0.34	0.05	0.05	3.29	-0.34	-0.34	553
FCGR2A	-0.49	-0.56	-0.56	-0.47	-0.50	-0.48	1.66	1.66	1.92	-0.57	-0.57	389
FCGR3A///FCGR3B	-0.49	-0.50	-0.49	2.32	2.12	-0.49	0.06	-0.31	-0.24	-0.50	-0.50	642
FKBP5	-0.39	-0.48	-0.69	-0.64	-0.26	-0.81	0.09	0.26	-0.12	-0.71	-0.46	1494
FYB	-0.85	0.95	1.35	0.26	-0.59	-0.87	1.93	0.41	0.79	-0.84	-0.78	208
GBP1	-0.65	-0.36	-0.56	-0.45	-0.30	-0.70	-0.41	-0.30	1.96	-0.66	2.08	1884
GBP5	-0.78	0.01	-0.26	0.54	0.13	-0.74	-0.63	-0.72	2.72	-0.78	0.88	1037
IL7R	-0.64	2.72	0.33	-0.19	-0.03	-0.63	-0.64	1.29	0.24	-0.60	-0.54	560
ISG20	-1.16	0.59	1.43	-0.18	0.25	1.75	-0.96	-0.65	1.14	-1.08	0.11	832
ITK	-0.63	1.38	1.08	1.24	1.96	-0.63	-0.63	-0.63	-0.63	-0.63	-0.63	455
KLRC1///KLRC2	-0.40	-0.40	-0.24	1.34	2.92	-0.40	-0.40	-0.40	-0.40	-0.40	-0.40	309
LILRB2	-0.52	-0.53	-0.53	-0.52	-0.53	-0.52	1.79	1.60	1.86	-0.53	-0.53	354
LST1	-0.49	-0.25	-0.21	-0.45	-0.46	-0.43	3.02	0.48	0.83	-0.51	-0.50	582
MEGF11	-0.27	-0.29	-0.30	-0.31	-0.31	-0.31	-0.31	-0.30	-0.32	-0.30	-0.31	19
MS4A4A	-0.36	-0.41	-0.41	-0.41	-0.41	-0.41	0.23	3.14	0.67	-0.41	-0.41	137
MS4A6A	-0.39	-0.47	-0.47	-0.45	-0.45	-0.47	2.92	0.84	0.83	-0.46	-0.47	502
MS4A7	-0.44	-0.51	-0.51	-0.51	-0.51	-0.29	2.70	1.29	0.82	-0.51	-0.51	632
PRKCB	-0.66	-0.03	0.40	0.33	-0.32	2.59	1.45	-0.55	-0.53	-0.67	-0.67	178
PTPRC	-1.03	0.44	0.14	1.51	1.63	0.44	1.05	0.07	-0.02	-1.06	-1.06	1473
SOD2	-0.43	-0.97	-1.03	-0.95	-0.76	-0.40	1.54	-0.04	0.41	-0.95	0.62	287

Anti-HLA DSA characteristics & gene expression to identify responders to Eculizumab therapy

THERAPEUTIC STUDY: EFFECT OF COMPLEMENT INHIBITION

Multi-center, international study in HLA incompatible kidney recipients
11 centers in the US and Europe: NCT01567085 & NCT01399593

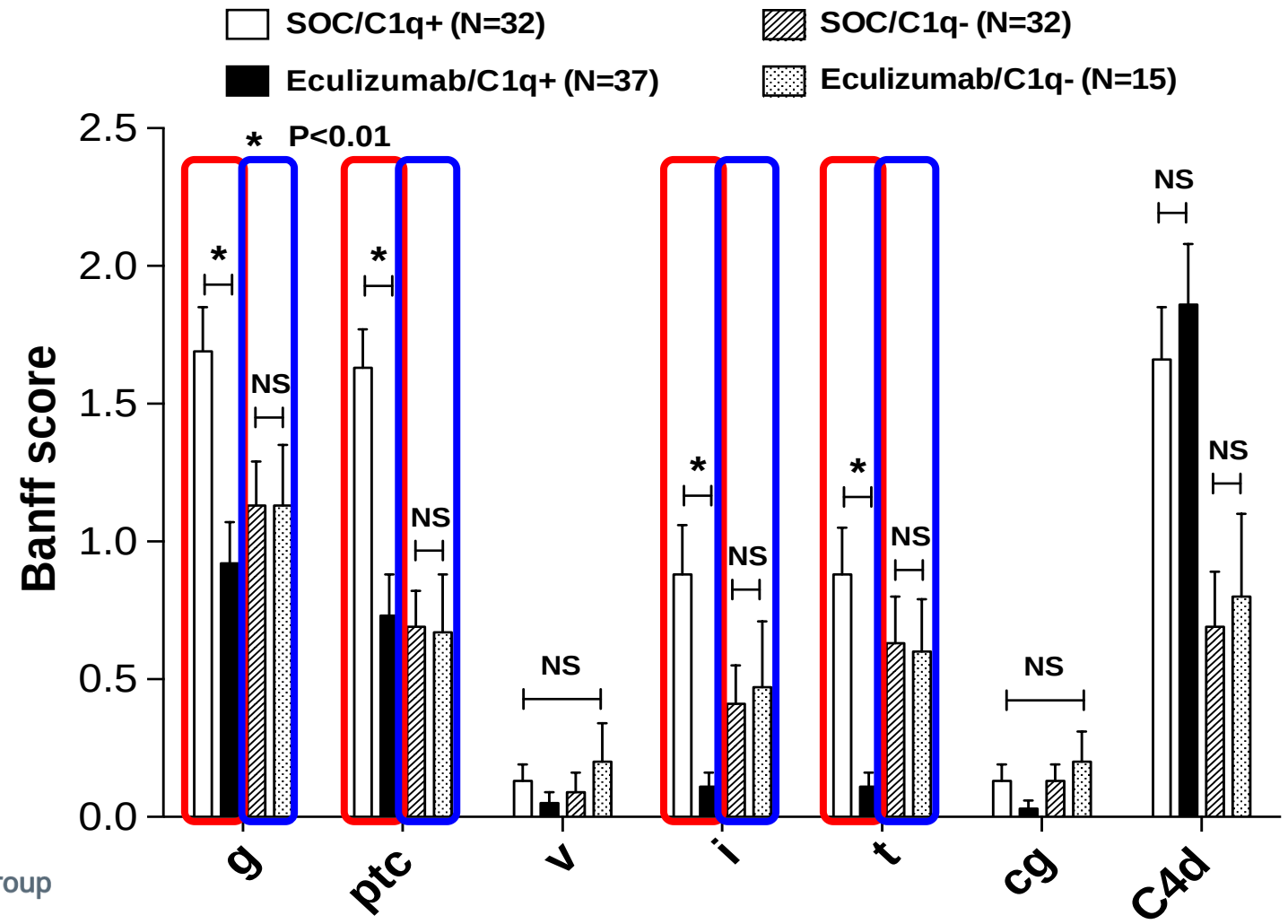


Standard of care: PE and IVIG according to local centers' protocol
C5 INH: Eculizumab 1200 mg at Tx, 900 mg/week x4 and 1200 mg at week 5, 7, 9

EFFECT OF COMPLEMENT INHIBITION ON HISTOLOGY

Eculizumab specifically decreased acute injury in C1q+DSA patients (Day 14 allograft biopsy)

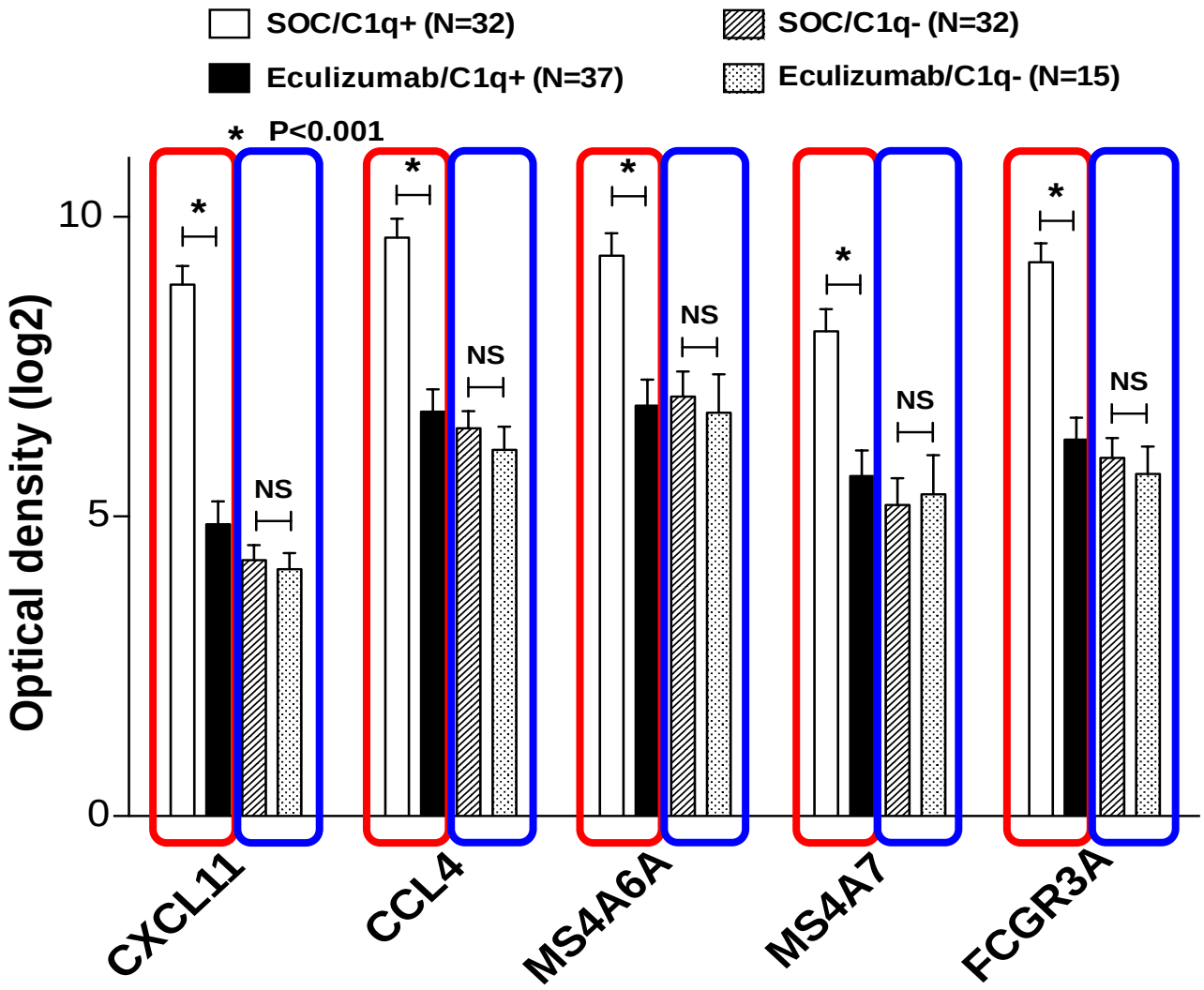
C1q+ DSA



EFFECT OF COMPLEMENT INHIBITION ON THE C1q DSA GENE SET

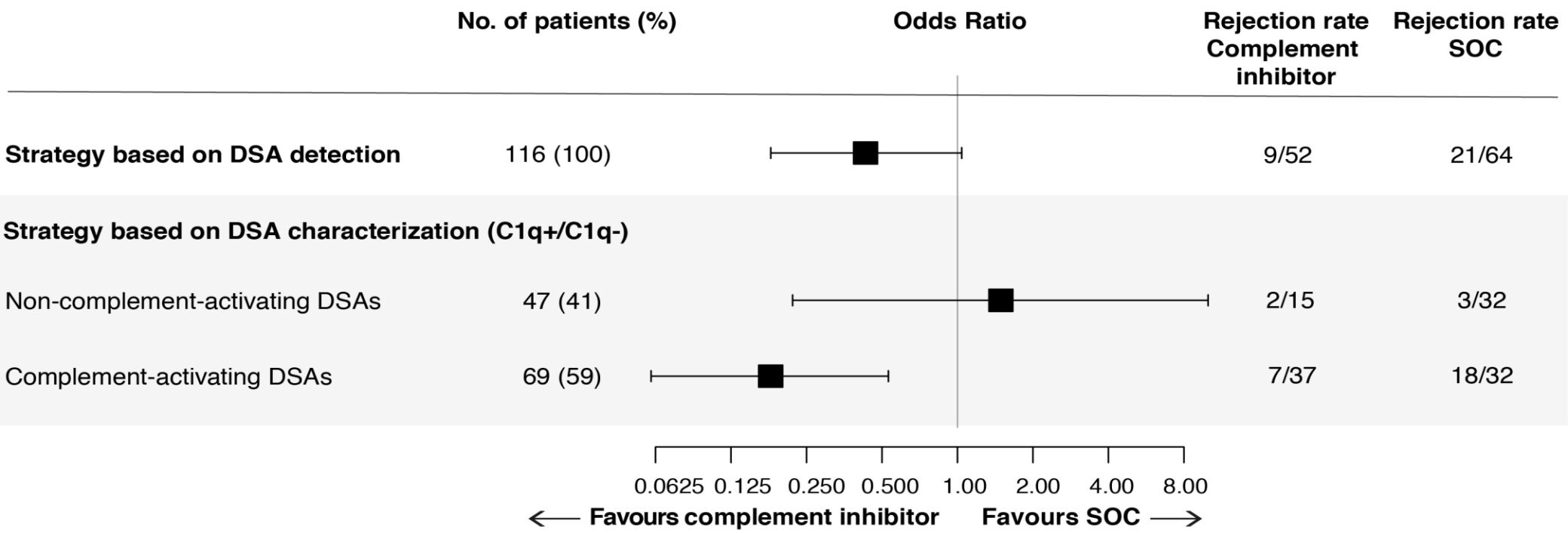
Eculizumab specifically decreased the C1q gene set expression

C1q+ DSA



IMPACT OF A THERAPEUTIC STRATEGY BASED ON DSA C1q STATUS *vs.* DSA DETECTION

Response rate to complement inhibition improved when characterizing DSA C1q status at transplantation



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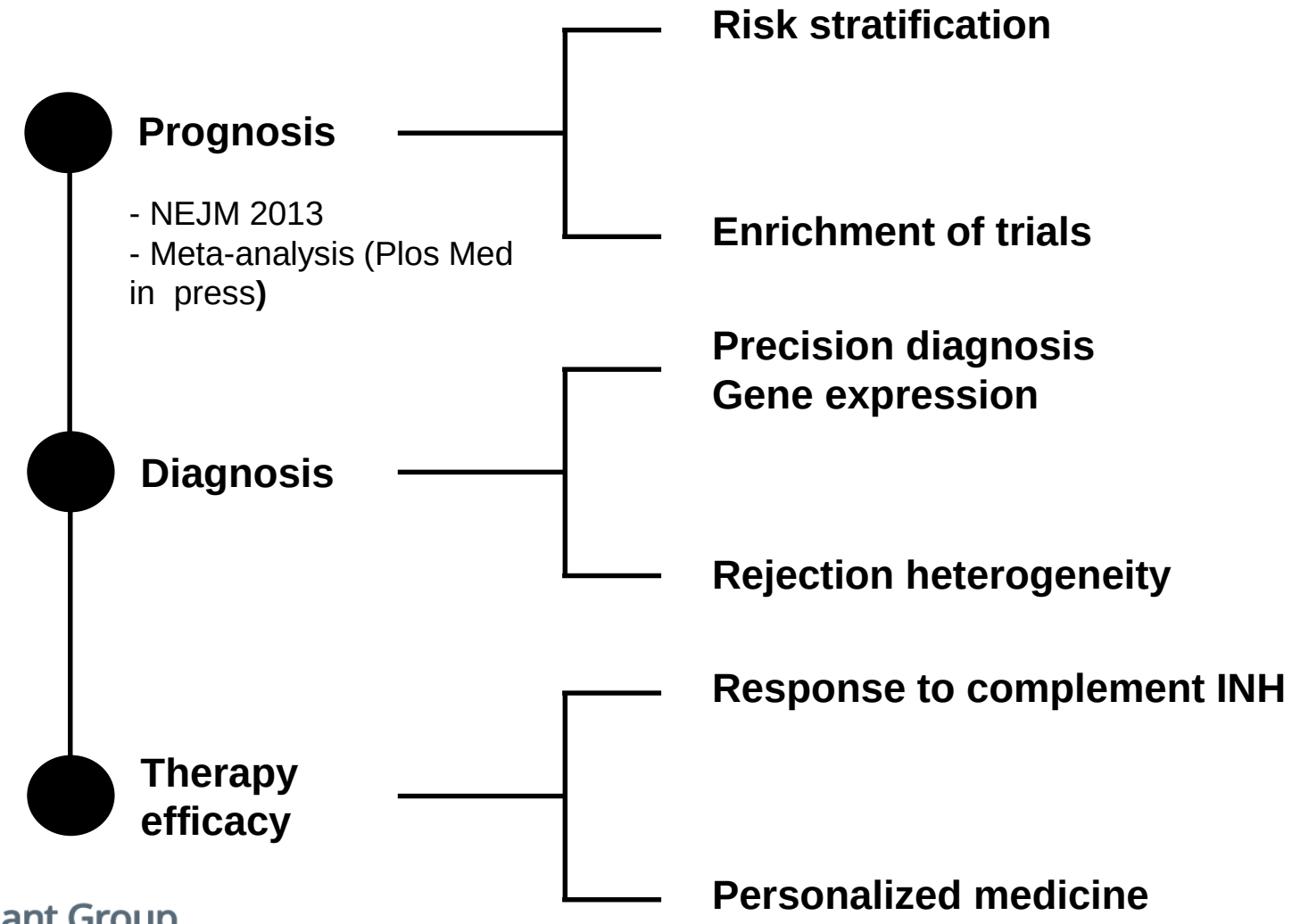
CONCLUSION

C'-binding HLA DSA as a biomarker in the era of new technologies



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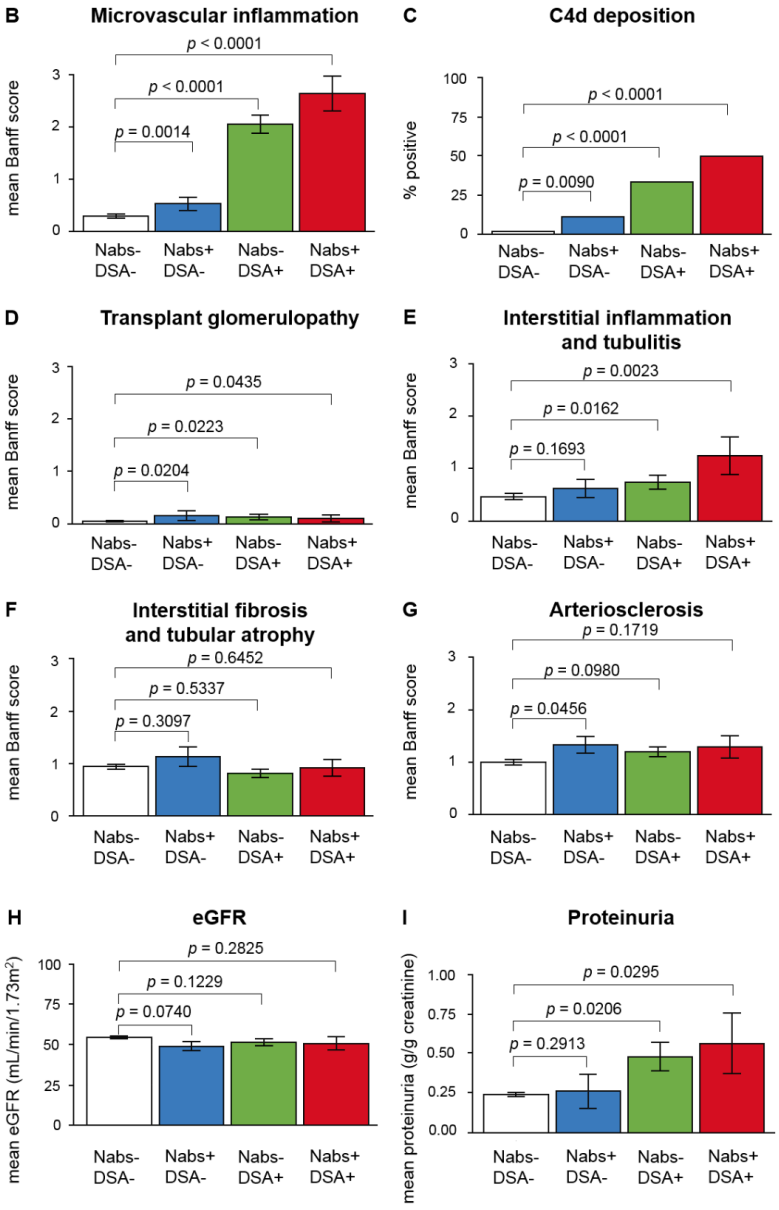
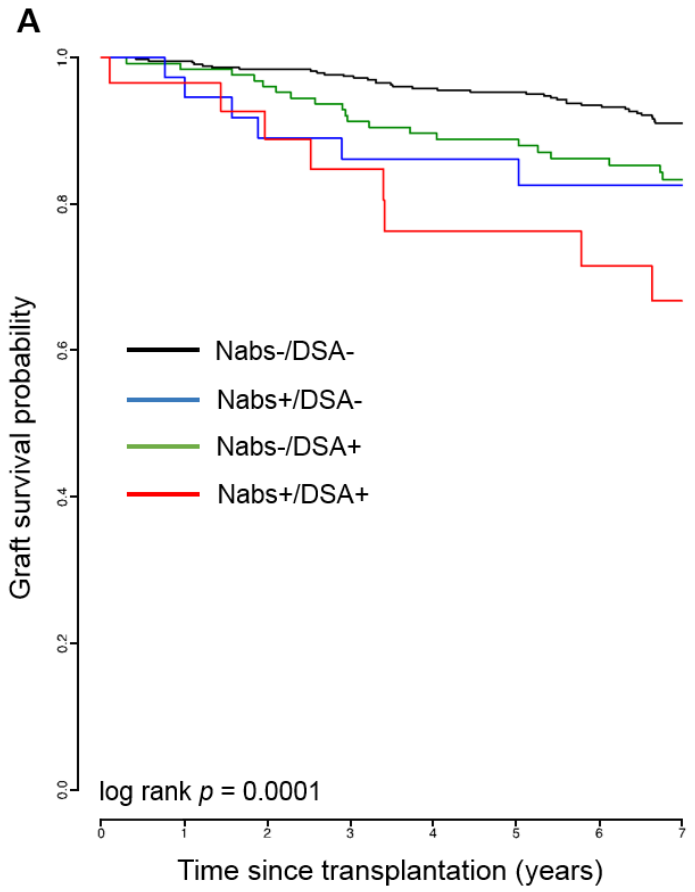
CONCLUSION





N at risk

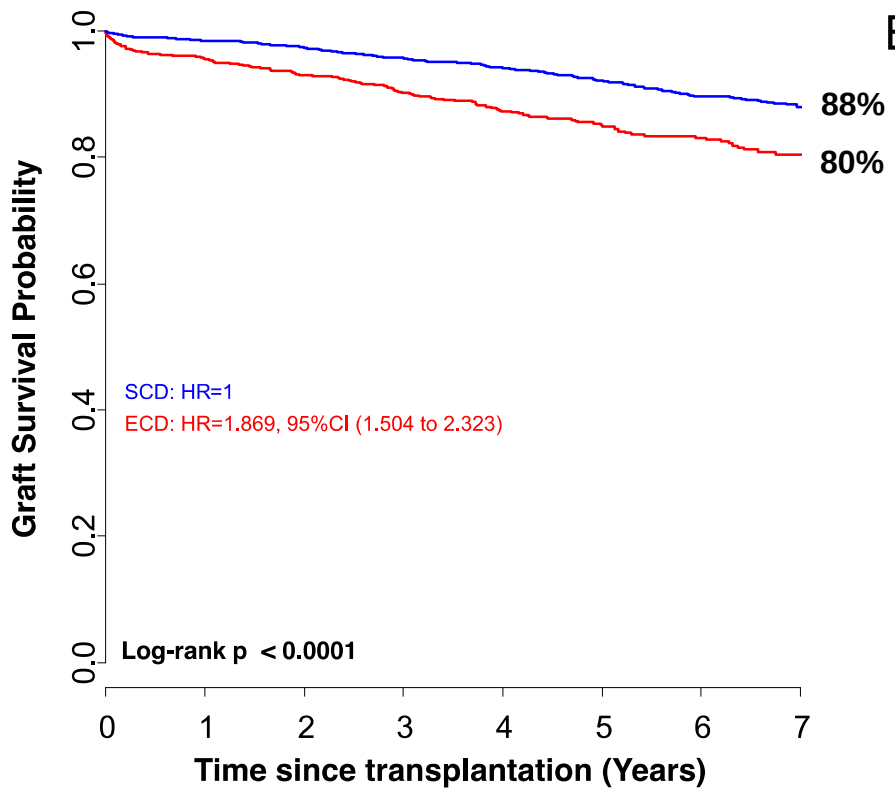
Nabs-/DSA-	441	435	424	414	393	374	354	329
Nabs+/DSA-	37	36	31	30	30	25	20	19
Nabs-/DSA+	128	125	121	115	108	103	92	81
Nabs+/DSA+	29	26	23	20	18	17	15	12

Lee S & Aubert O et al. JASN in press

CONCLUSION

Kaplan-Meier curves of kidney allograft survival by donor type

A

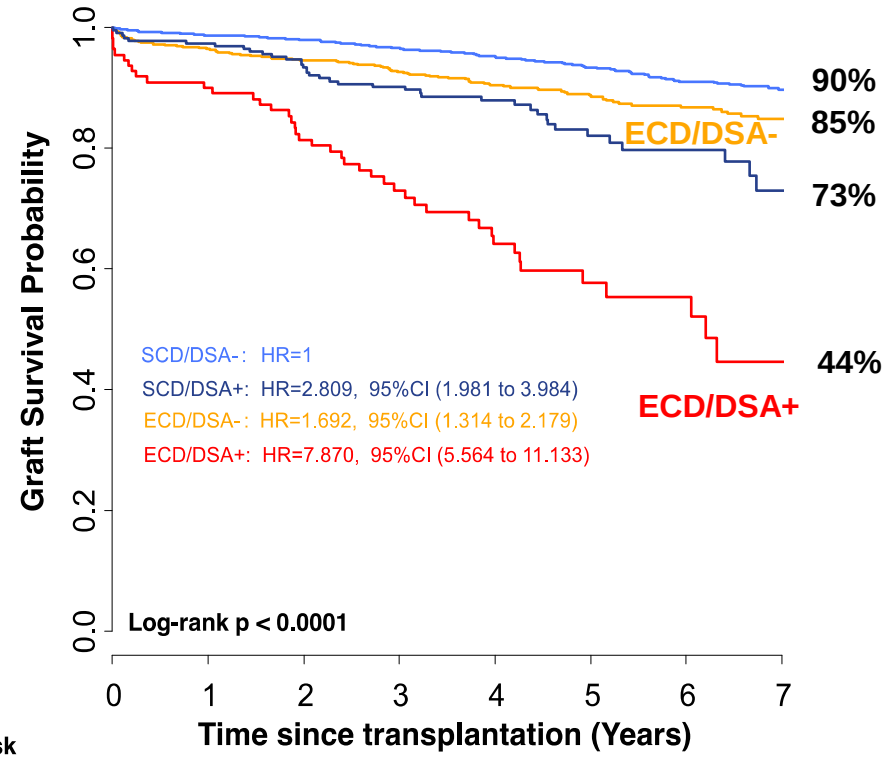


N at risk

SCD	1847	1810	1760	1594	1349	1039	758	526
ECD	916	854	806	704	558	419	292	179

Kaplan-Meier curves of kidney allograft survival by donor type and the presence of DSA

B



N at risk

SCD/DSA-	1622	1591	1552	1419	1216	962	708	504
SCD/DSA+	225	219	208	175	133	77	50	22
ECD/DSA-	806	756	723	641	511	393	275	175
ECD/DSA+	110	98	83	63	47	26	17	4

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Aubert O et al. BMJ 2015

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Acknowledgments