AST AMERICAN SOCIETY OF TRANSPLANTATION

HLA Antibody Complement Based Assays

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Atlanta, GA

CEOT CUTTING EDGE of TRANSPLANTATION

TRANSPLANT SUMMIT 2019

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

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

Disclosure

Neither presenter has any financial relationships related to this presentation

AND

The presentation *does NOT* include discussion of "off-label" or "investigational" use.

Learning Objectives

1) To understand the rationale behind tests to assess the complement fixing abilities of HLA antibodies.

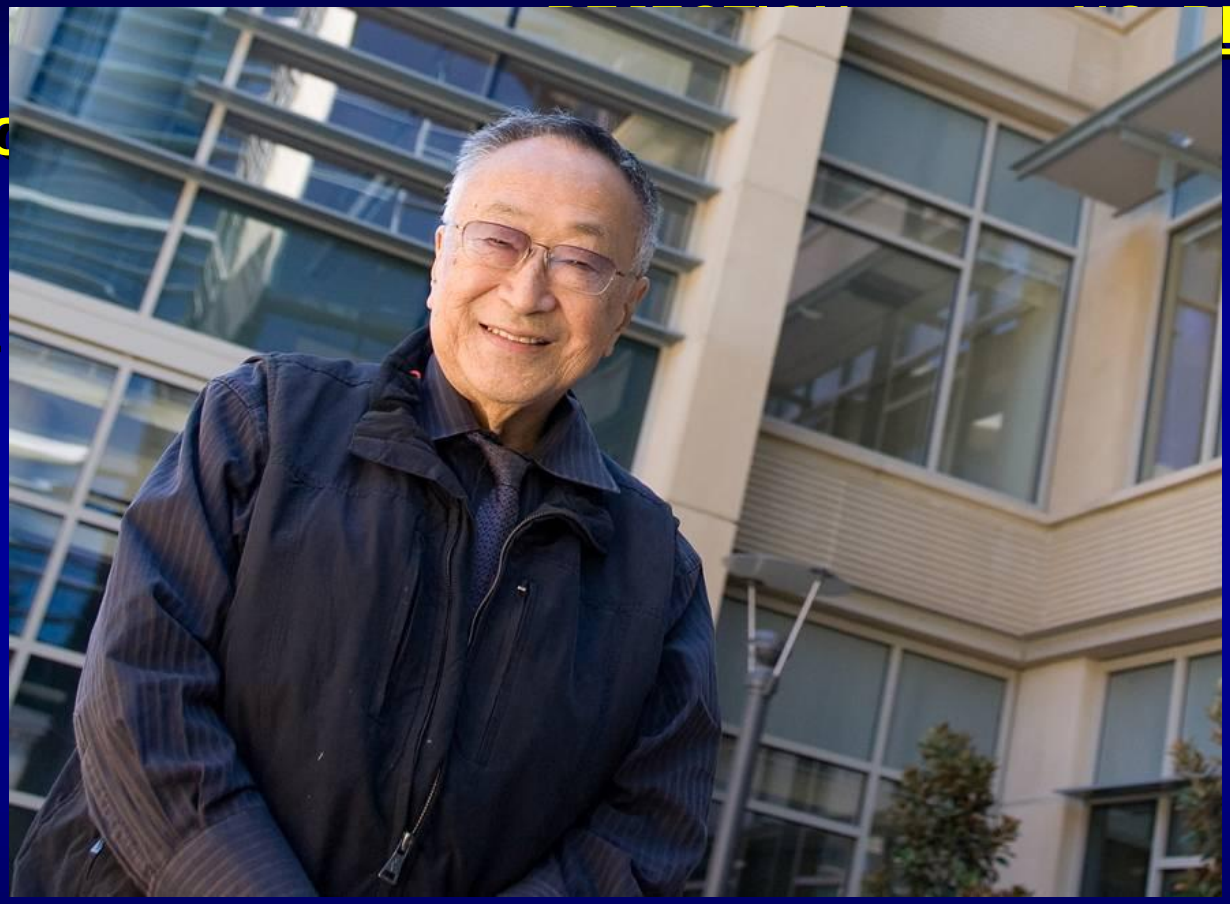
2) To discuss the clinical impact of complement fixing HLA antibodies in solid organ transplant recipients.

3) To recognize the limitations of complement fixation assays.

The Beginning…

PATEL AND TERASAKI, NEJM 280:735,1969

	EJECTION
POSITIVE C	6
NEGATIVE	187

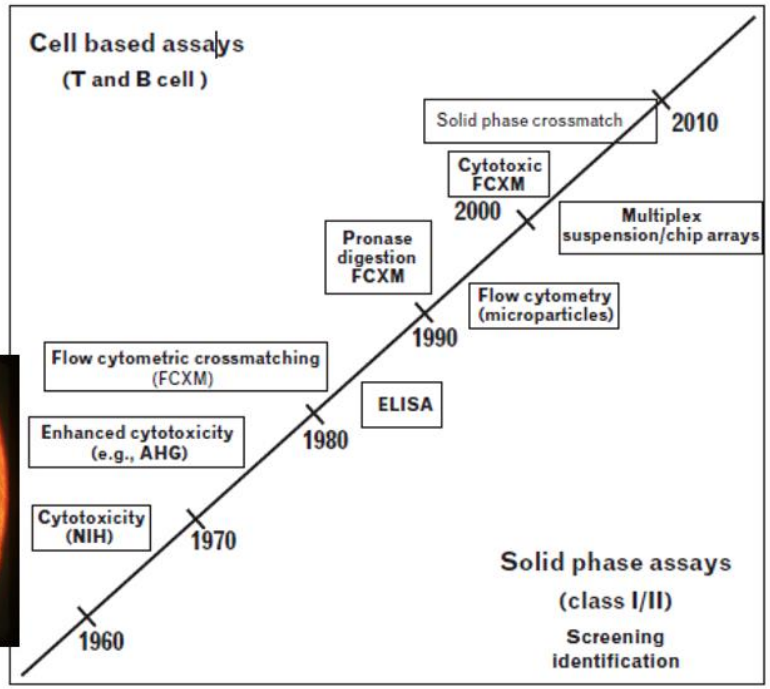


The evolution and clinical impact of Human Leukocyte Antigen technology

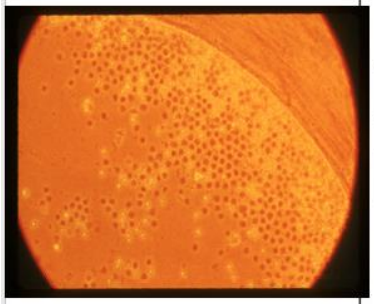
Howard M. Gebel and Robert A. Bray

Current Opinion in Nephrology and Hypertension 2010, 19:598–602

Figure 1 Evolution of human leukocyte antigen antibody testing

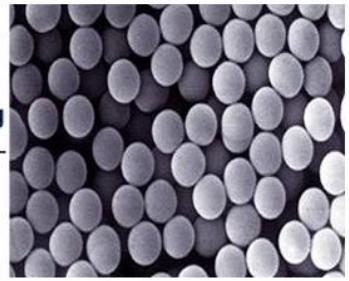


- Cumbersome cell-based assays for XM, anibody ID and HLA typing.



Cytotoxicity

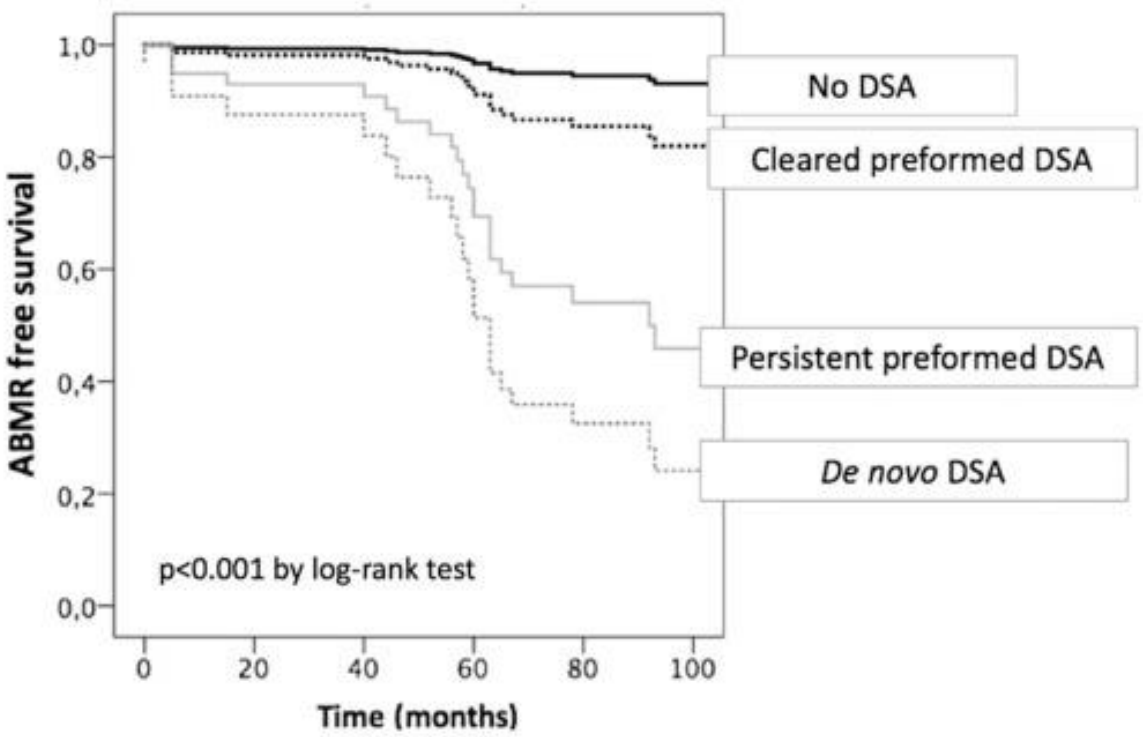
Solid Phase Assays



- Highly sensitive and specific bead-based assays for antibody ID and Molecular-based HLA typing.

Impact of persistent and cleared preformed HLA DSA on kidney transplant outcomes

Dolores Redondo-Pachón[a,b], María José Pérez-Sáez[a,b], Marisa Mir[a,b], Javier Gimeno[b,c], Laura Llinás[a,b], Carmen García[d], Juan José Hernández[d], Jose Yélamos[b,e], Julio Pascual[a,b,*,1], Marta Crespo[a,b,*,1]



Human Immunology 79 (2018) 424–431

C4d and Early Graft Loss

(H.E. Feucht et al, Kidney Int 43: 1333-8, 1993)

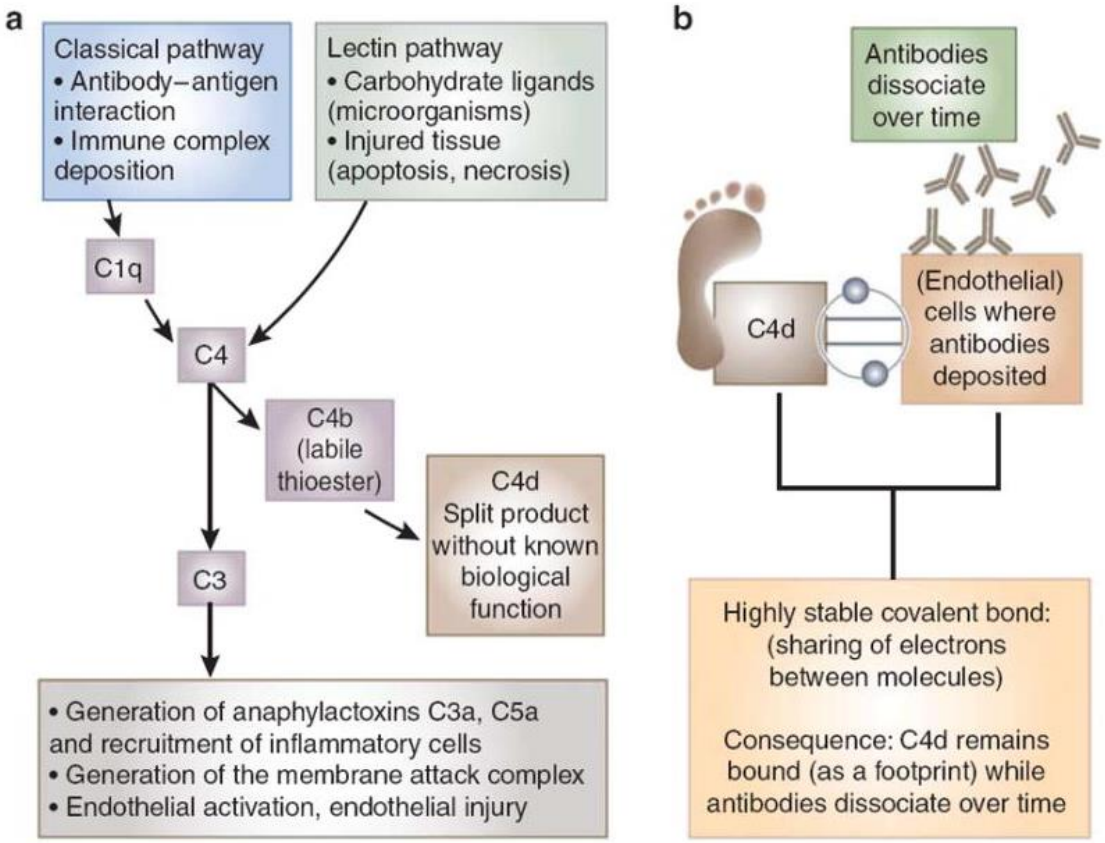
- 93 renal allografts biopsied for early dysfunction (mean 11 days post-transplant)
- 43 biopsies – diffuse PTC C4d
  - 18 graft losses in 1st year (58% graft survival)
- 8 biopsies – focal PTC C4d
  - 3 graft losses in 1st year (63% graft survival)
- 42 biopsies – C4d negative in PTC
  - 4 graft losses in 1st year (90% graft survival)
- 3/4 cases of graft loss in C4d- group were C4d+ on a later biopsy
- C4d+ associated with re-transplant, elevated PRA

"Because of its association with preformed antibodies to HLA in recipients, vascular presence of complement fragment C4d has finally been assumed to represent an otherwise undetectable humoral immune reaction against graft endothelial cells."

http://www.kidney-international.org

Pros and cons for C4d as a biomarker

Danielle Cohen[1], Robert B. Colvin[2], Mohamed R. Daha[3], Cinthia B. Drachenberg[4], Mark Haas[5], Volker Nickeleit[6], Jane E. Salmon[7], Banu Sis[8], Ming-Hui Zhao[9], Jan A. Bruijn[1] and Ingeborg M. Bajema[1]



Kidney International (2012) 81, 628–639

review http://www.kidney-international.org

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Problems with C4d as THE marker of AMR

- **Difficulties of interpreting focal staining patterns (especially in lungs)**
- **Relatively low sensitivity (i.e. C4d negative AMR)**
- **Accommodation (e.g., in ABO incompatible recipients)**
- **AND-AMR has already begun….**

Antibody-mediated rejection: New approaches in prevention and management

R. A. Montgomery[1] | A. Loupy[2] | D. L. Segev[3]

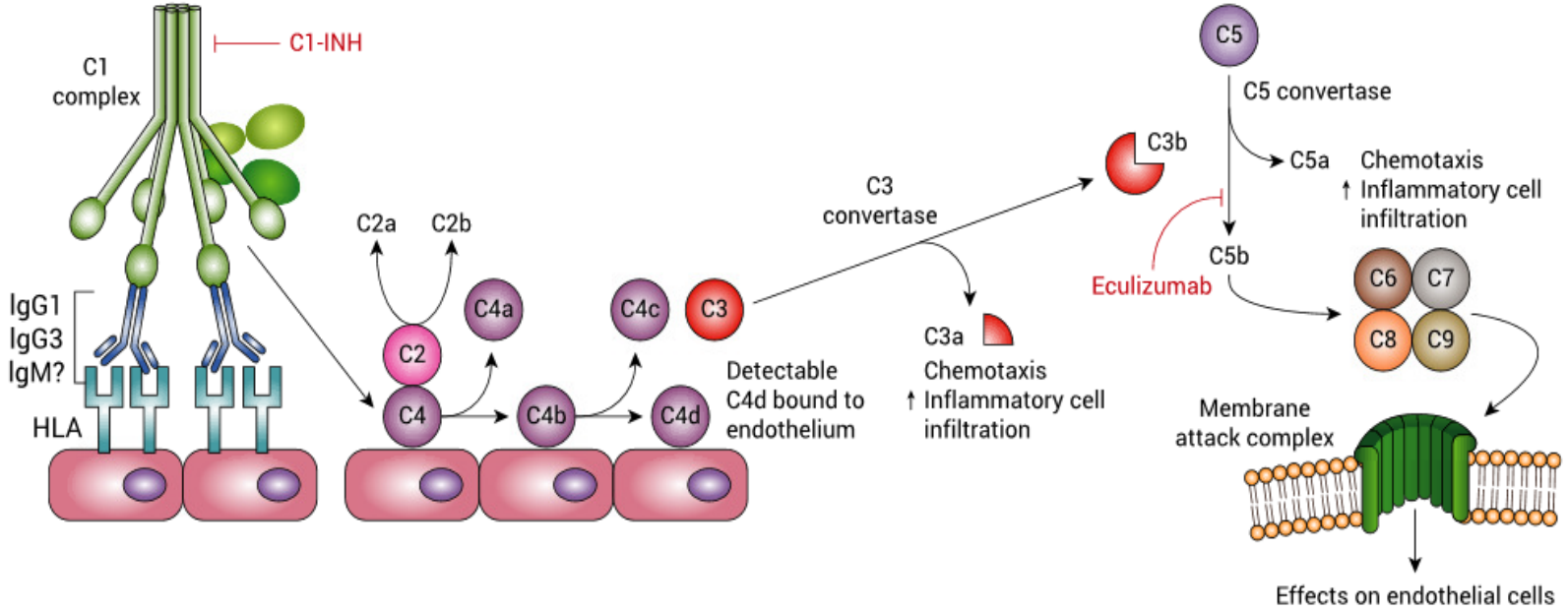
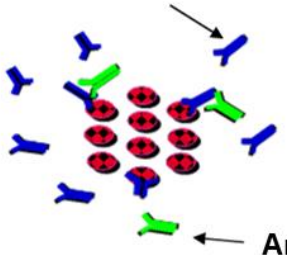


FIGURE 1 The role of the classical complement pathway in acute AMR in sensitized renal transplant recipients. Following binding of DSA to the allograft vascular endothelium, the C1 complex activates the serine esterases C1s and C1r, resulting in the cleavage of C4, deposition of C4d, and the assembly of the classical pathway C3 convertase. C3 convertase cleaves C3 into C3a, a potent pro-inflammatory mediator, and C3b, which propagates the complement cascade and leads to the formation of the pro-inflammatory mediator C5a and the lytic membrane attack complex (C5b-C9). Investigational therapies are shown in red. Adapted from Stegall et al.[12] AMR, antibody-mediated rejection; C1-INH, C1 esterase inhibitor; DSA, donor-specific antibody; HLA, human leukocyte antigen

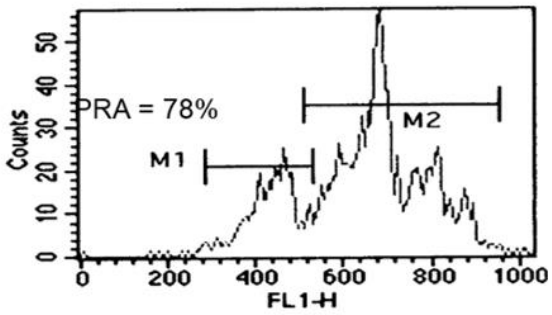
Solid Phase HLA antibody detection

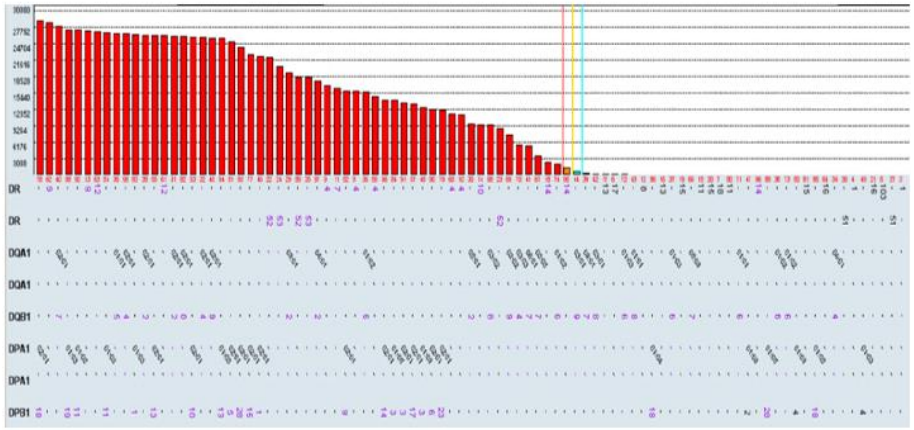
HLA alloantibody



Anti-IgG-FITC (or PE)

Suspension Arrays





Adapted from Gebel and Bray. Transplantation Reviews 20: 189-194, 2006

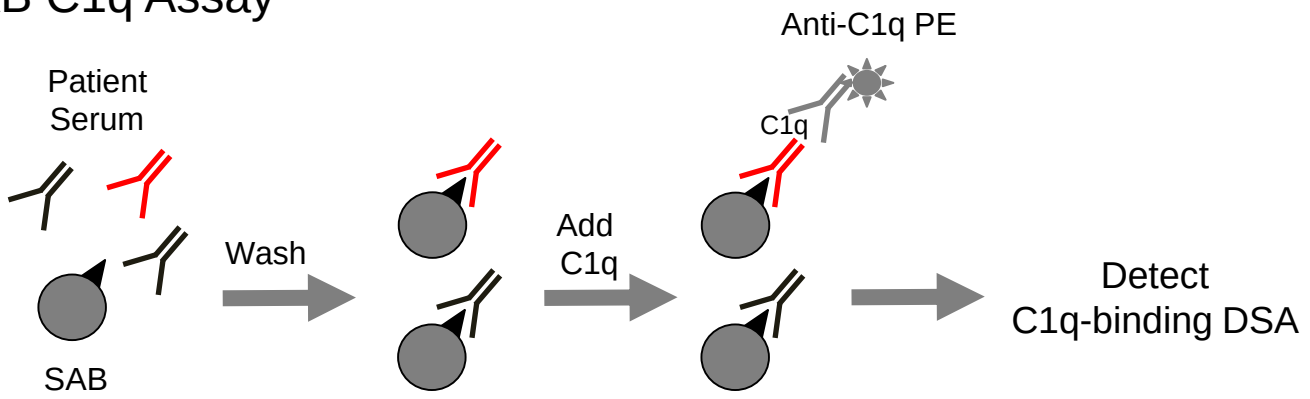
C1q-Fixing Human Leukocyte Antigen Antibodies Are Specific for Predicting Transplant Glomerulopathy and Late Graft Failure After Kidney Transplantation

Julie M. Yabu,[1,5] John P. Higgins,[2] Ge Chen,[2,3] Flavia Sequeira,[2,3] Stephan Busque,[4] and Dolly B. Tyan[2,3]

342 | www.transplantjournal.com

Transplantation • Volume 91, Number 3, February 15, 2011

SAB C1q Assay



Clinical relevance of preformed C4d-fixing and non-C4d-fixing HLA single antigen reactivity in renal allograft recipients

Markus Wahrmann,[1] Gregor Bartel,[1] Markus Exner,[2] Heinz Regele,[3] Günther F. Körmöczi,[4] Gottfried F. Fischer[4] and Georg A. Böhmig[1]

Transplant International 22 (2009) 982–989

American Journal of Transplantation 2007; 7: 2809–2815
Blackwell Munksgaard

Brief Communication

doi: 10.1111/j.1600-6143.2007.01991.x

C4d Fixing, Luminex Binding Antibodies—A New Tool for Prediction of Graft Failure After Heart Transplantation

J. D. Smith[a], I. M. Hamour[b], N. R. Banner[b] and M. L. Rose[a,*]

Detection of C3d-Binding Donor-Specific Anti-HLA Antibodies at Diagnosis of Humoral Rejection Predicts Renal Graft Loss

Antoine Sicard,*†‡ Stéphanie Ducreux,§ Maud Rabeyrin,‖ Lionel Couzi,¶** Brigitte McGregor,‖ Lionel Badet,‡†† Jean Yves Scoazec,‡‖ Thomas Bachelet,¶** Sébastien Lepreux,‡‡ Jonathan Visentin,§§ Pierre Merville,¶** Véronique Fremeaux-Bacchi,‖‖¶¶ Emmanuel Morelon,*†‡ Jean-Luc Taupin,§§ Valérie Dubois,§ and Olivier Thaunat*†‡

J Am Soc Nephrol 26: 457–467, 2015.

RESEARCH ARTICLE

Complement-activating donor-specific anti-HLA antibodies and solid organ transplant survival: A systematic review and meta-analysis

Antoine Bouquegneau[1,2☯], Charlotte Loheac[1☯], Olivier Aubert[1,3☯], Yassine Bouatou[1,4], Denis Viglietti[1,5], Jean–Philippe Empana[1], Camilo Ulloa[6], Mohammad Hassan Murad[7], Christophe Legendre[1,3], Denis Glotz[1,5], Annette M. Jackson[8], Adriana Zeevi[9], Stephan Schaub[10], Jean–Luc Taupin[11], Elaine F. Reed[12], John J. Friedewald[13], Dolly B. Tyan[14], Caner Süsal[15], Ron Shapiro[16], E. Steve Woodle[17], Luis G. Hidalgo[18], Jacqueline O'Leary[19], Robert A. Montgomery[20], Jon Kobashigawa[21], Xavier Jouven[1,22], Patricia Jabre[1,23,24,25☯], Carmen Lefaucheur[1,5☯*], Alexandre Loupy[1,3☯*]

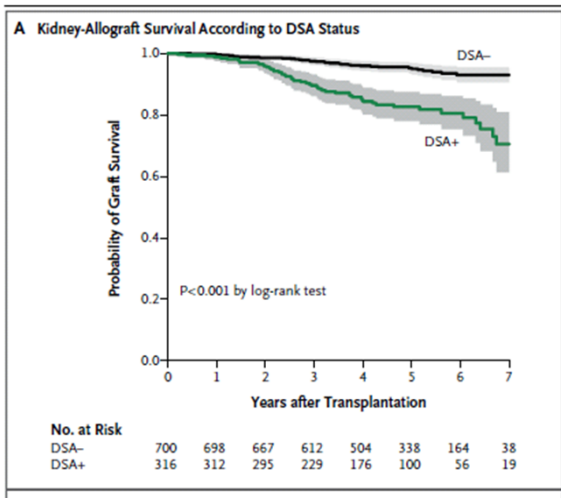
PLOS Medicine | https://doi.org/10.1371/journal.pmed.1002572 May 25, 2018

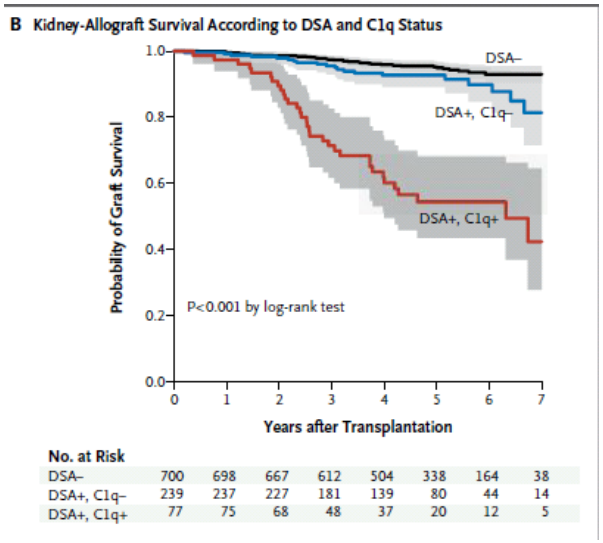
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival

Alexandre Loupy, M.D., Ph.D., Carmen Lefaucheur, M.D., Ph.D., Dewi Vernerey, M.P.H., Christof Prugger, M.D., Jean-Paul Duong van Huyen, M.D., Ph.D., Nuala Mooney, Ph.D., Caroline Suberbielle, M.D., Ph.D., Véronique Frémeaux-Bacchi, M.D., Ph.D., Arnaud Méjean, M.D., François Desgrandchamps, M.D., Dany Anglicheau, M.D., Ph.D., Dominique Nochy, M.D., Dominique Charron, M.D., Ph.D., Jean-Philippe Empana, M.D., Ph.D., Michel Delahousse, M.D., Christophe Legendre, M.D., Denis Glotz, M.D., Ph.D. Gary S. Hill, M.D.,* Adriana Zeevi, Ph.D., and Xavier Jouven, M.D., Ph.D.





Acquisition of C3d-Binding Activity by *De Novo* Donor-Specific HLA Antibodies Correlates With Graft Loss in Nonsensitized Pediatric Kidney Recipients

P. Comoli[1,†], M. Cioni[2,†], A. Tagliamacco[3], G. Quartuccio[1], A. Innocente[4], I. Fontana[5], A. Trivelli[2], A. Magnasco[2], A. Nocco[4], C. Klersy[6], L. Rubert[1], M. Ramondetta[4], M. Zecca[1], G. Garibotto[3], G. M. Ghiggeri[2], M. Cardillo[4], A. Nocera[3,†] and F. Ginevri[2,*,†]

American Journal of Transplantation 2016; 16: 2106–2116

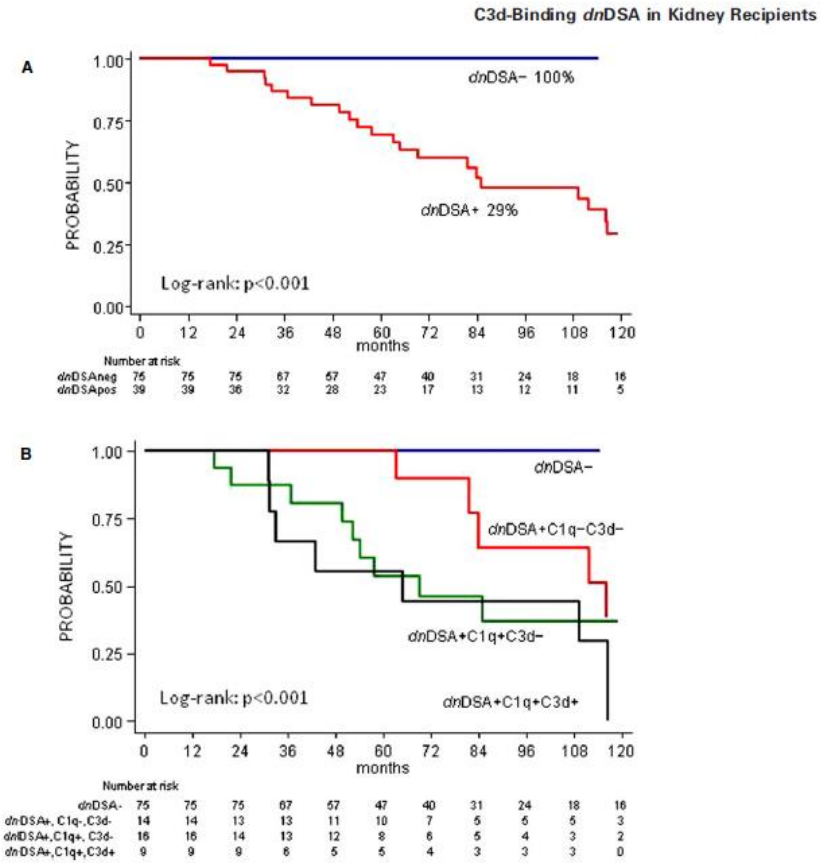
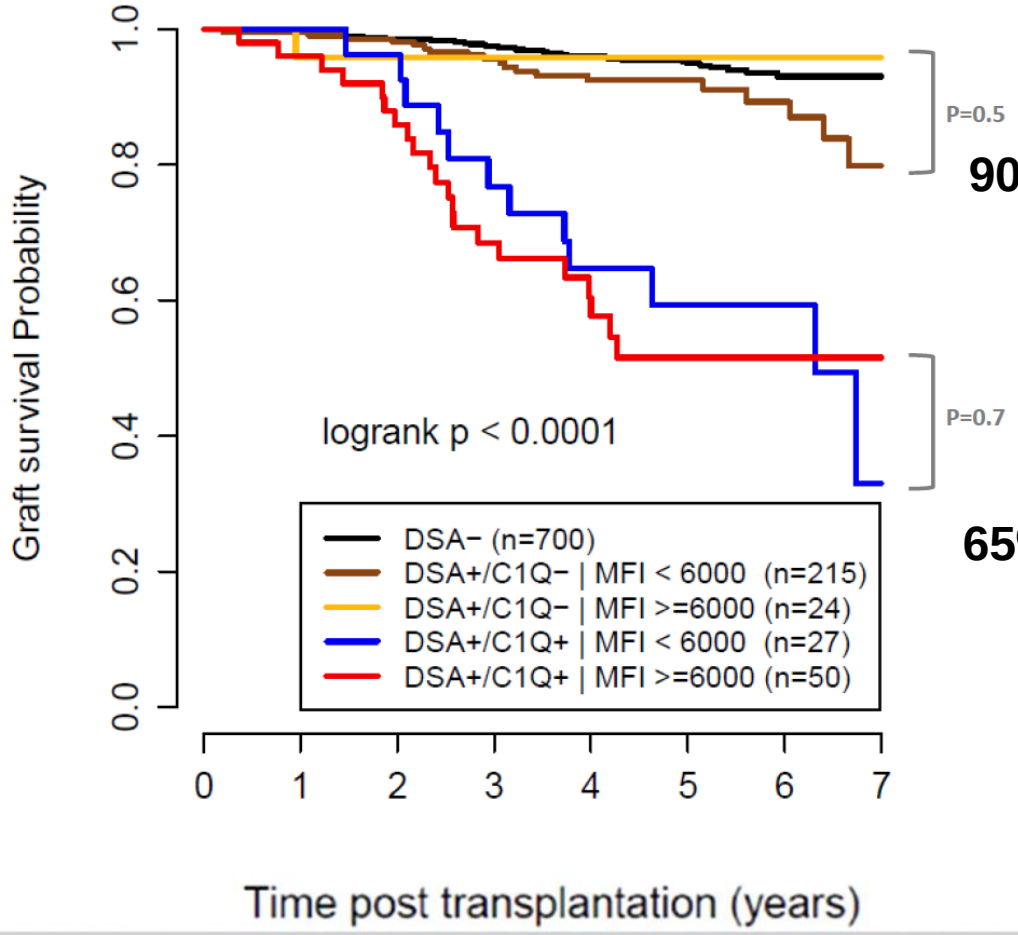


Figure 3: *De novo* donor-specific antibodies (*dn*DSAs) and risk of developing antibody-mediated rejection (AMR) in the analyzed cohort. (A) AMR-free allograft survival stratified by presence or absence of *dn*DSAs. (B) AMR-free allograft survival in kidney graft recipients stratified by development of non-complement-binding or complement-binding antibodies at *dn*DSA emergence. The statistical difference between Kaplan–Meier survival curves was evaluated by log-rank test, and differences with p-values <0.05 were considered statistically significant.

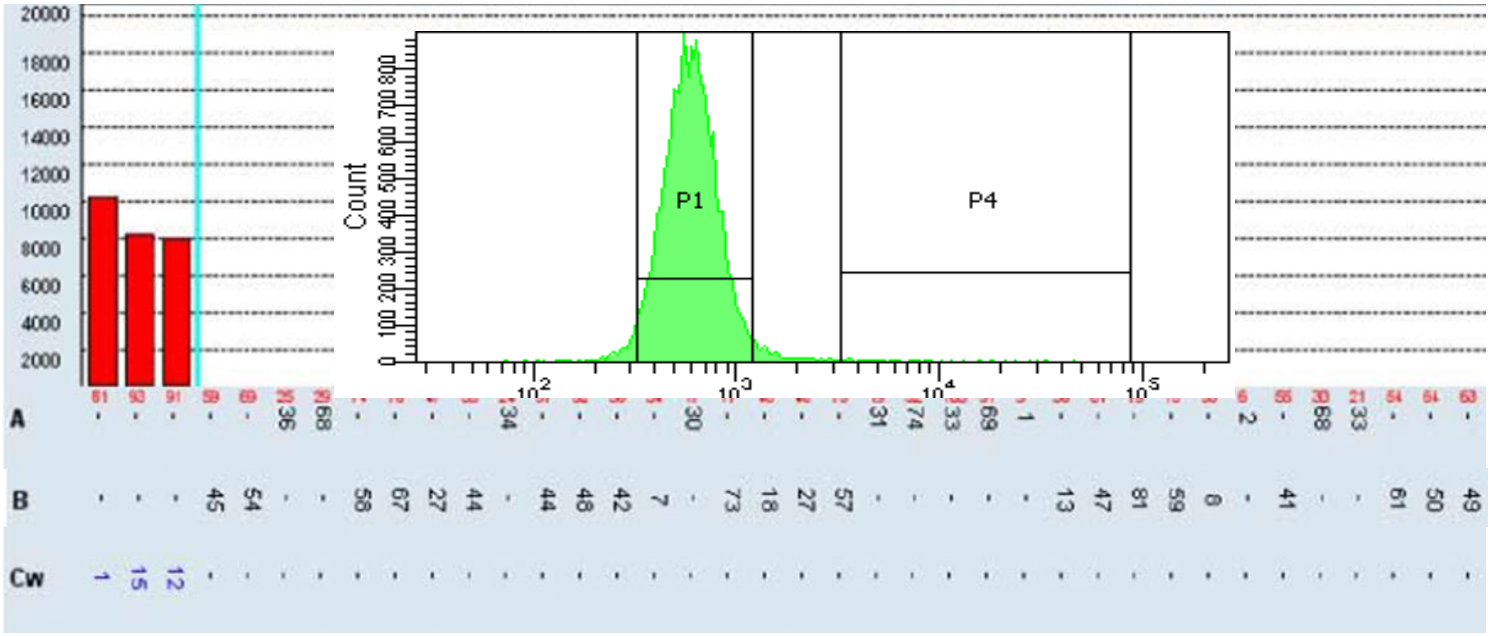
Kaplan Meier Analysis of graft outcome according to post-transplant DSA-MFI and complement-binding status



90% of C1q- pts <6000 MFI

65% of C1q+ pts >6000 MFI

Supplement to: Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013;369:1215-26. DOI: 10.1056/NEJMoa1302506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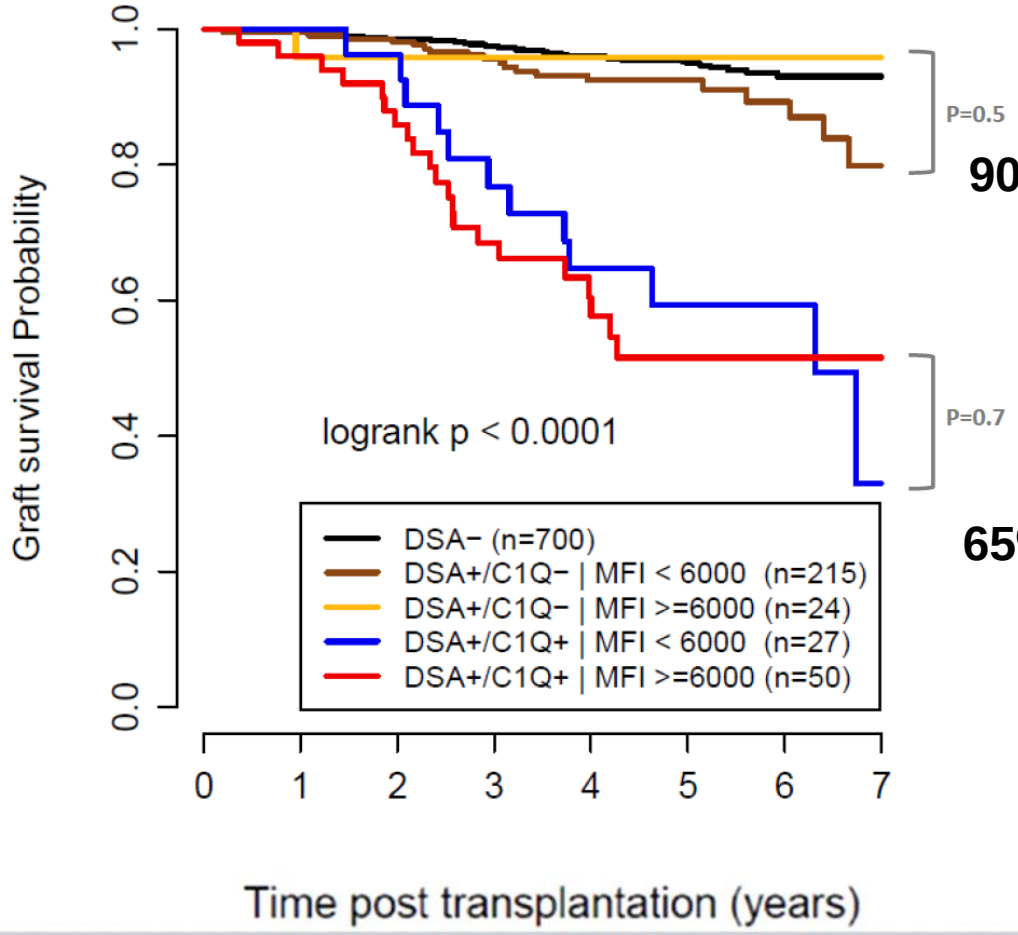
Kaplan Meier Analysis of graft outcome according to post-transplant DSA-MFI and complement-binding status



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Supplement to: Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013;369:1215-26. DOI: 10.1056/NEJMoa1302506

The Prozone Effect

INTERNATIONAL JOURNAL OF
IMMUNOGENETICS

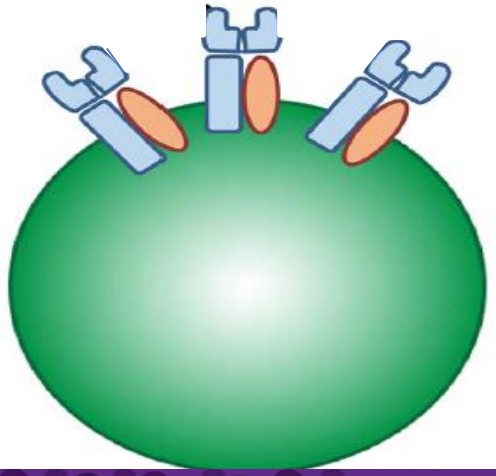
Review

doi: 10.1111/j.1744-313X.2012.01147.x

The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera

C. Weinstock* & M. Schnaidt†

International Journal of Immunogenetics, 2013, **40**, 171–177



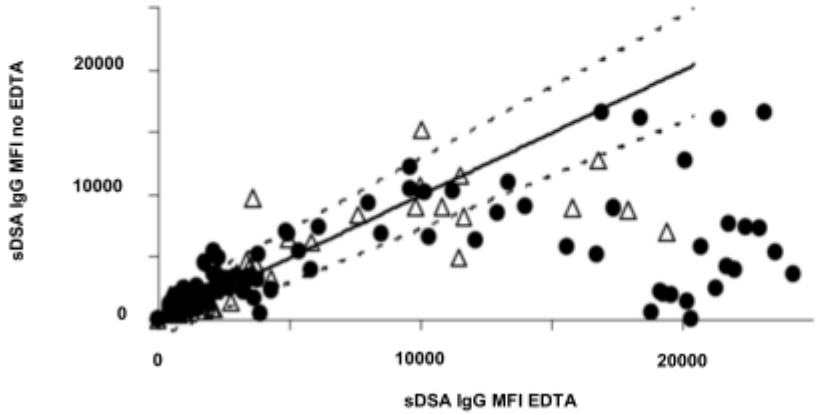
The disappointing contribution of anti-human leukocyte antigen donor-specific antibodies characteristics for predicting allograft loss

Nephrol Dial Transplant (2018) 33: 1853–1863

Maxime Courant[1,*], Jonathan Visentin[2,3,*], Gabriel Linares[2], Valérie Dubois[4], Sébastien Lepreux[5,6], Gwendaline Guidicelli[4], Olivier Thaunat[7,8], Pierre Merville[1,3], Lionel Couzi[1,3,*] and Jean-Luc Taupin[2,3,9,*]

f

serum.



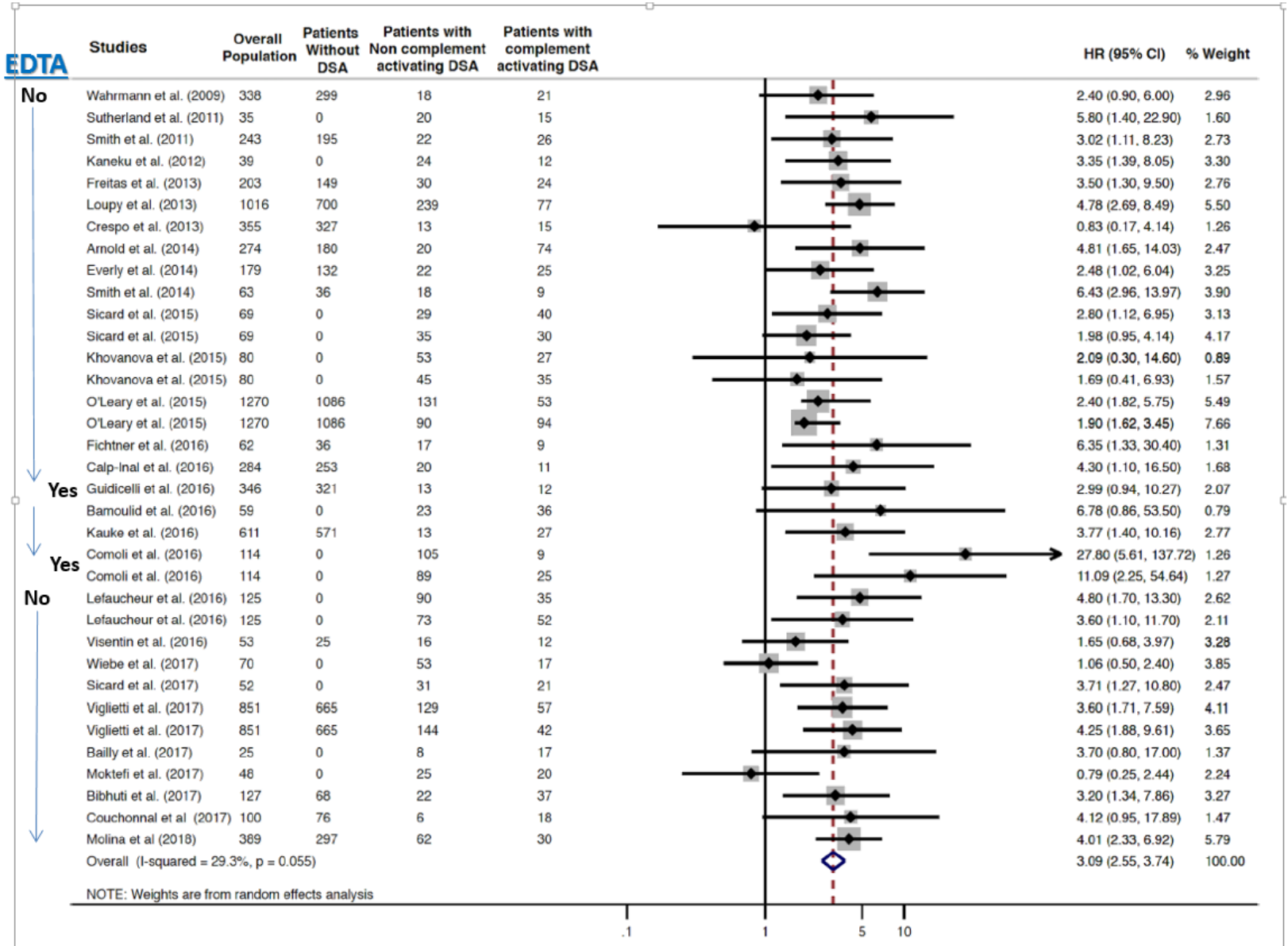


Fig 3. Association between circulating complement-activating anti-HLA DSAs and the risk of allograft loss. Fig 3 shows the forest plot of the association between complement-activating anti-HLA DSAs and the risk of allograft loss for each study and overall (n = 29). Studies are listed by date of publication. Number of patients are

Acquisition of C3d-Binding Activity by *De Novo* Donor-Specific HLA Antibodies Correlates With Graft Loss in Nonsensitized Pediatric Kidney Recipients

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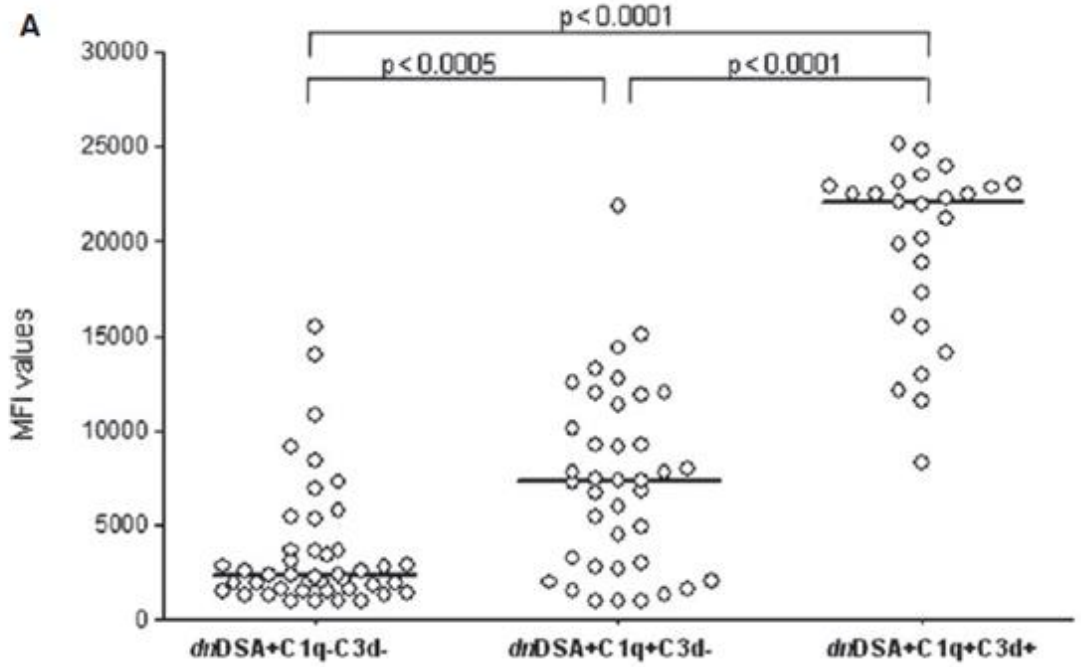
American Journal of Transplantation 2016; 16: 2106–2116



All samples pre-treated with EDTA

Figure 1: Relation between MFI and complement-binding activity of de novo DSA.

C1q Binding Activity of De Novo Donor-specific HLA Antibodies in Renal Transplant Recipients With and Without Antibody-mediated Rejection

Maggie Yell, MD,[1] Brenda L. Muth, RN, MS,[2] Dixon B. Kaufman, MD, PhD,[3] Arjang Djamali, MD,[2] and Thomas M. Ellis, PhD[1]

TABLE 5.

Effects of normalization of C1q + DSA MFI values to levels comparable C1q – on Luminex-C1q activity

	N	MFI	Luminex-C1q
C1q + DSA	12	18,233 + 4268	+
C1q + DSA-diluted	12	6784 + 3386	–
C1q – DSA	22	5864 + 2686	–

TABLE 6.

Effects of serum concentration on C1q-binding activity of C1q – DSA

	Neat		Concentrated	
Sample	MFI	Luminex-C1q	MFI	Luminex-C1q
1	5489	Neg	12,243	Pos
2	4924	Neg	10,125	Pos
3	6985	Neg	13,112	Pos
4	5573	Neg	11,832	Pos
5	6323	Neg	7125	Neg
6	3794	Neg	5793	Neg

Transplantation 2015;99: 1151–1155

Acquisition of C3d-Binding Activity by *De Novo* Donor-Specific HLA Antibodies Correlates With Graft Loss in Nonsensitized Pediatric Kidney Recipients

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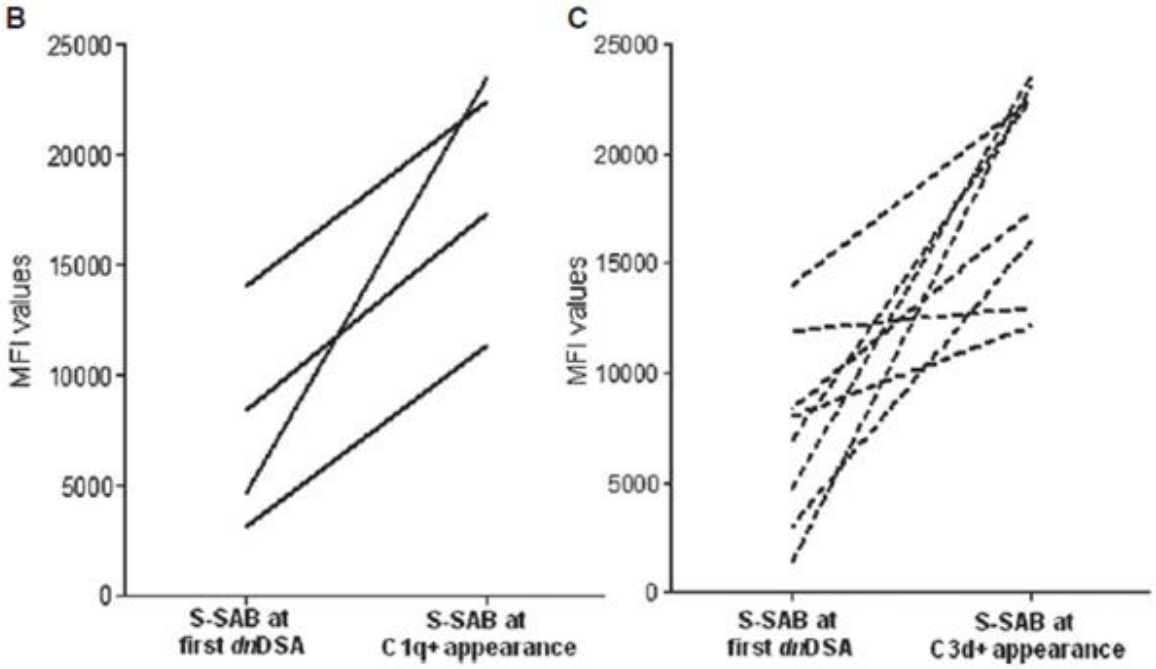
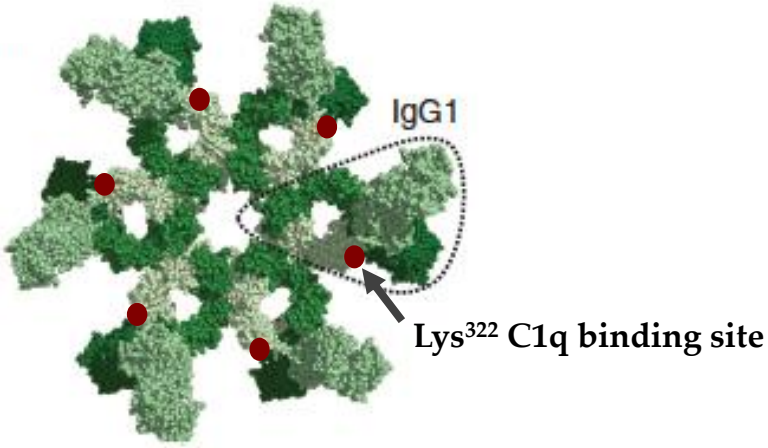
American Journal of Transplantation 2016; 16: 2106–2116

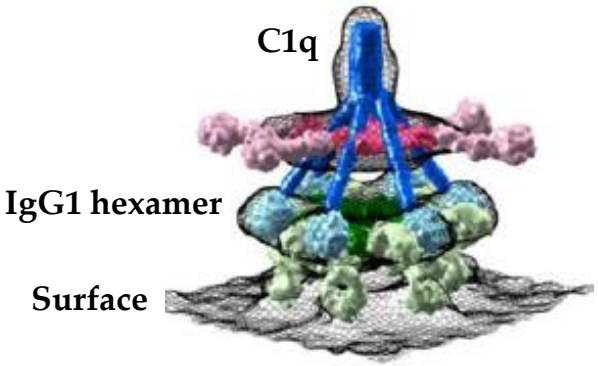


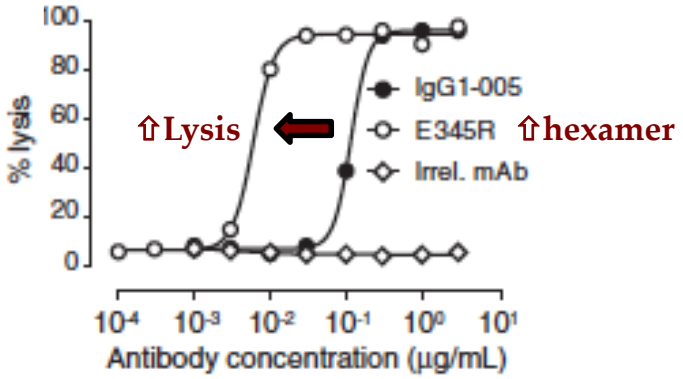
All samples pre-treated with EDTA

Figure 1: Relation between MFI and complement-binding activity of de novo DSA.

Complement is Activated by IgG Hexamers Assembled at the Cell Surface



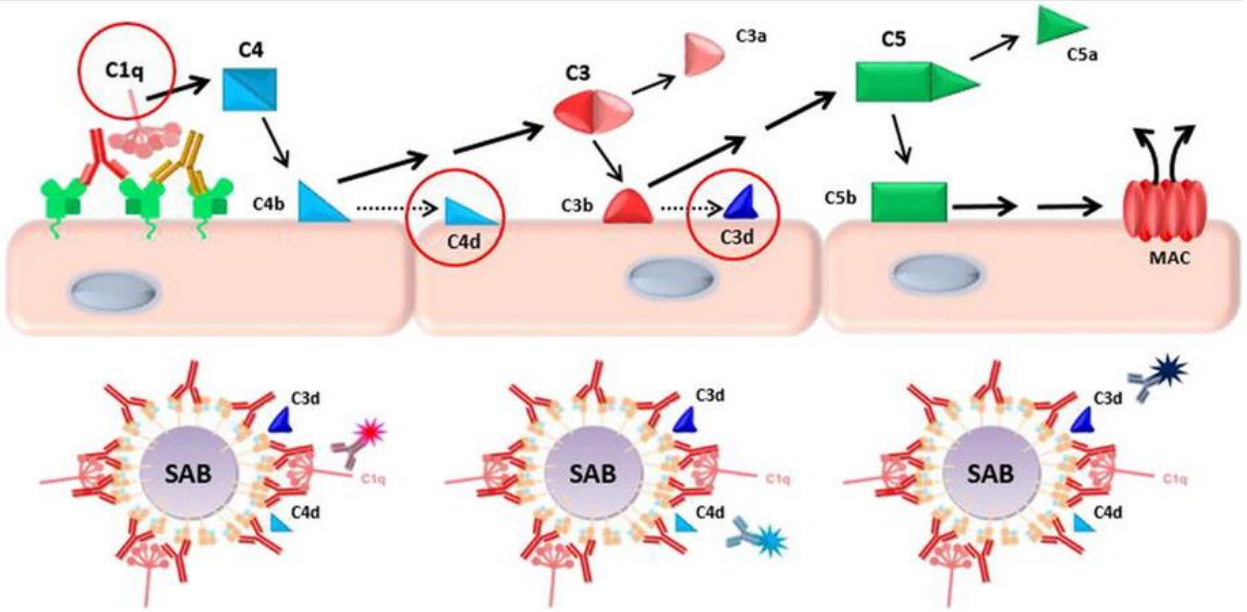




Science (2014) 343:1260-1263

Clinical Utility of Complement Dependent Assays in Kidney Transplantation

James H. Lan, MD, FRCP(C), D(ABHI)[1] and Kathryn Tinckam, MD, MMSc, FRCPC[2]



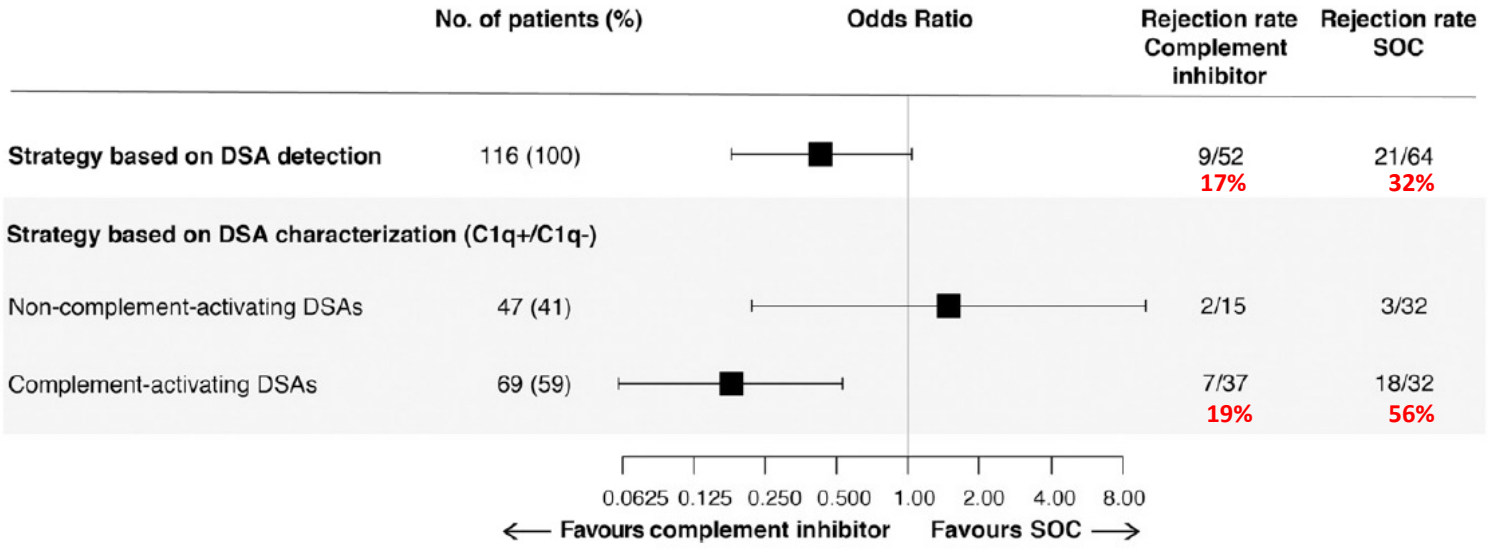
	C1q Assay	C4d Assay	C3d Assay
Complement source	Purified exogenous C1q	Normal human serum	Normal human serum
Reagent	Anti-C1q antibody	Anti-C4d antibody	Anti-C3d antibody

FIGURE 1. Schematic of the classical complement pathway. Different components of the pathway (C1q, C4d, C3d) are targeted in modified solid phase tests to evaluate the complement-activating potential of HLA antibodies.

(*Transplantation* 2018;102: S14–S22)

Complement-Activating Anti-HLA Antibodies in Kidney Transplantation: Allograft Gene Expression Profiling and Response to Treatment

Carmen Lefaucheur,[1,2] Denis Viglietti,[1,2] Luis G. Hidalgo,[3] Lloyd E. Ratner,[4] Serena M. Bagnasco,[5] Ibrahim Batal,[6] Olivier Aubert,[1] Babak J. Orandi,[7] Federico Oppenheimer,[8] Oriol Bestard,[9] Paolo Rigotti,[10] Anna V. Reisaeter,[11] Nassim Kamar,[12] Yvon Lebranchu,[13] Jean-Paul Duong Van Huyen,[1,14] Patrick Bruneval,[1,15] Denis Glotz,[1,2] Christophe Legendre,[1,16] Jean-Philippe Empana,[1] Xavier Jouven,[1] Dorry L. Segev,[17] Robert A. Montgomery,[18] Adriana Zeevi,[19] Philip F. Halloran,[3] and Alexandre Loupy[1,16]

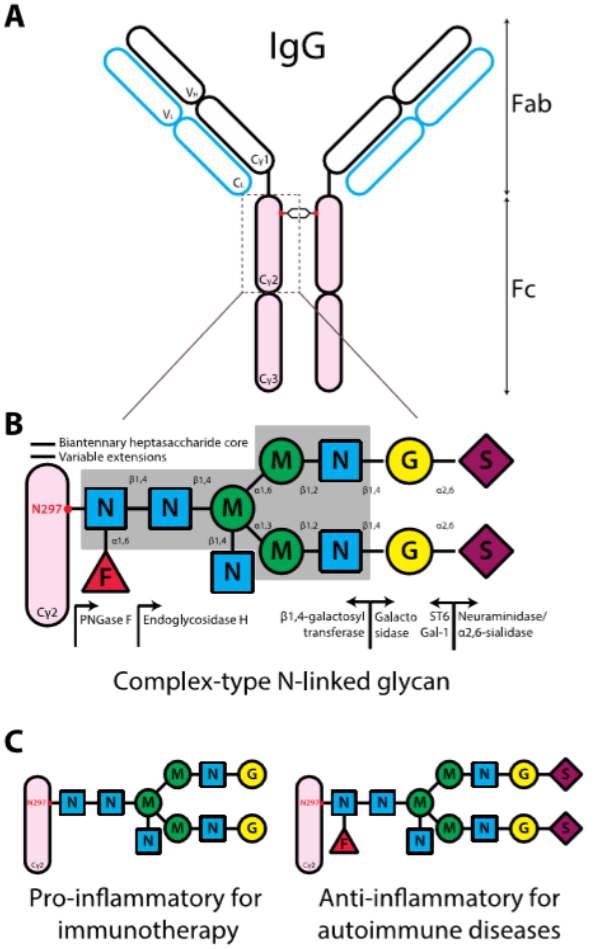


J Am Soc Nephrol 29: 620–635, 2018

Review

Antibody Glycosylation and Inflammation

Kai-Ting C. Shade and Robert M. Anthony * *Antibodies* **2013**, *2*, 392-414

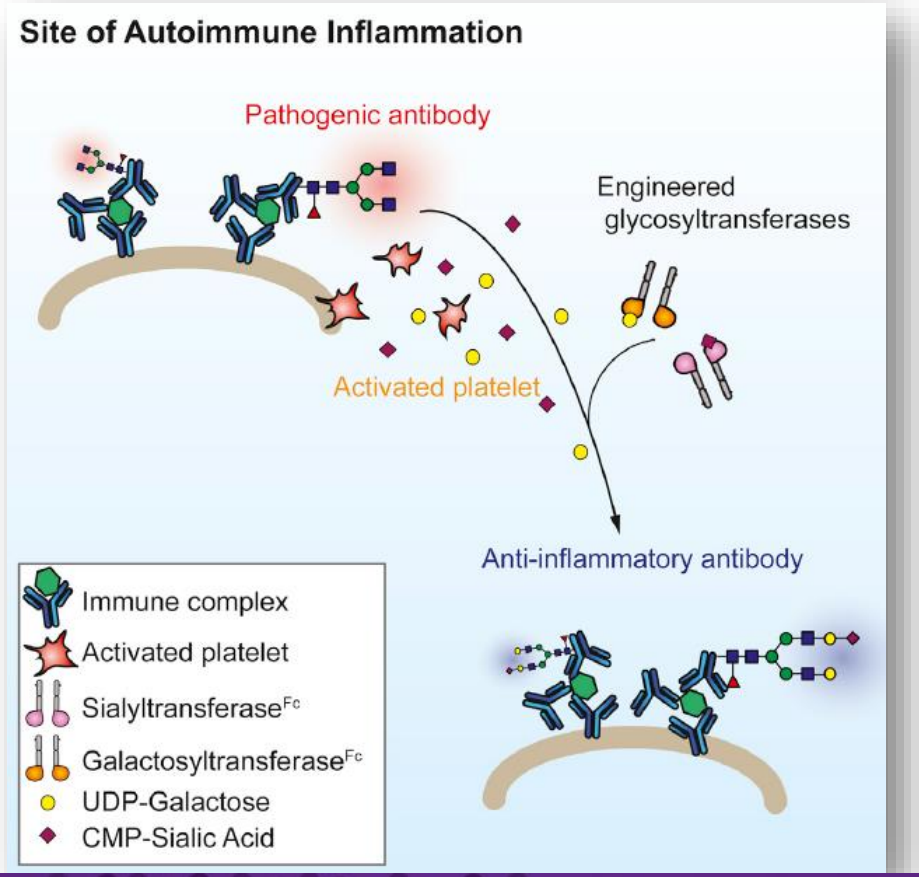


Cell

January 25, 2018

Engineered Sialylation of Pathogenic Antibodies *In Vivo* Attenuates Autoimmune Disease

Jose D. Pagan,[1,2] Maya Kitaoka,[1,2] and Robert M. Anthony[1,3,*]

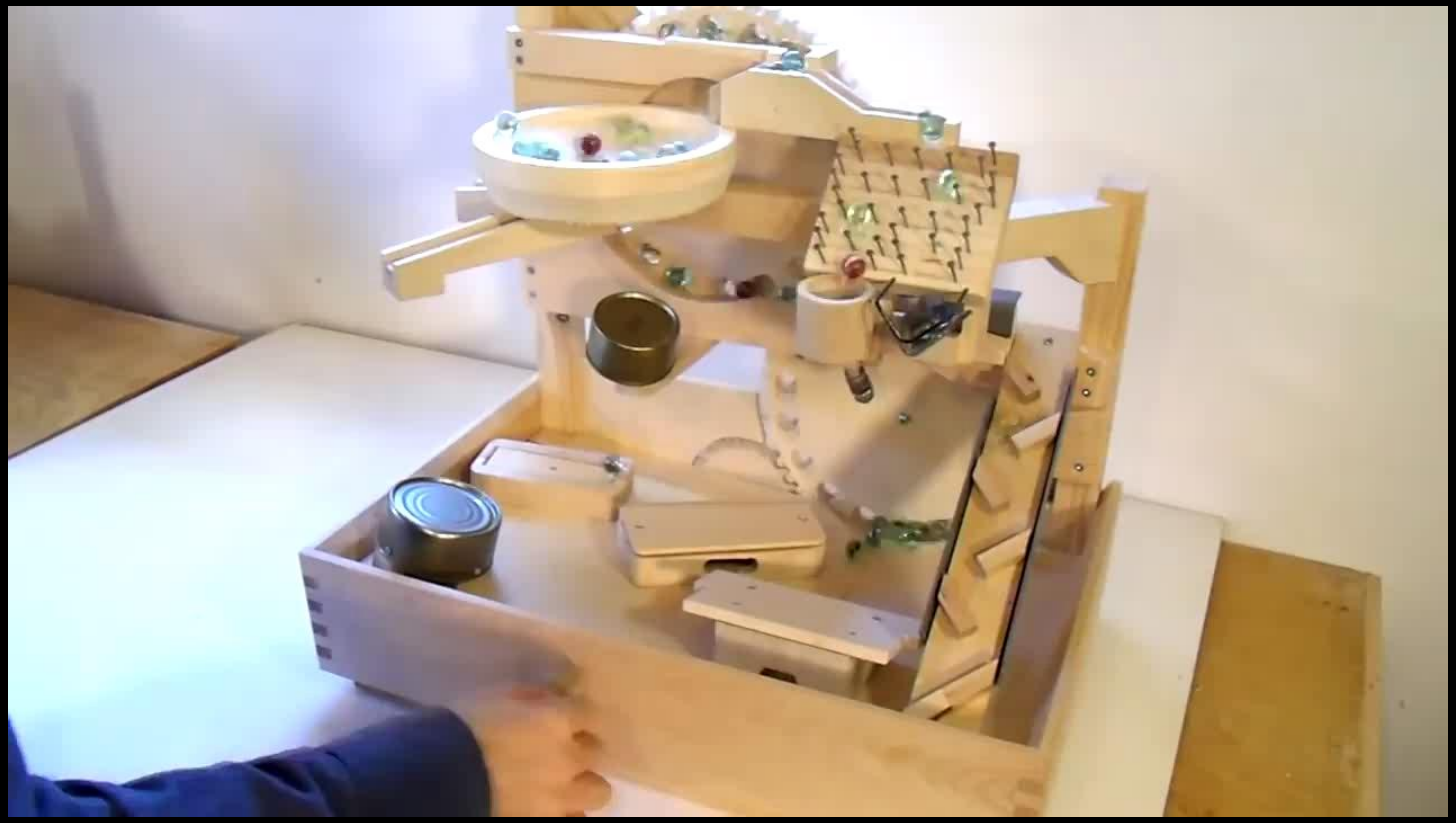


CONCLUSIONS

- **Strong association between DSAs that bind complement (C1q, C3d, C4d) and graft loss/graft dysfunction.**
- **MFI levels (*EDTA*) typically correspond with their ability to fix complement**
- ***In-vitro* complement binding is not synonymous with *in-vivo* complement binding.**
  - **Concentration**
  - **Conformation**
  - **Continuum**
- **DSAs can mediate graft damage via complement-independent mechanisms.**

CONCLUSIONS

- Published evidence supports the following recommendations.
  - Pre-transplant:
    - Routine - Not indicated
    - Selected Indications – Yes
  - Post-transplant:
    - – Routine monitoring – Not indicated
    - - Selected indications - Maybe
- Rather than merely identifying whether antibodies fix complement, a better strategy would be to focus on strategies to mitigate their pathogenesis.



Banff survey on antibody-mediated rejection clinical practices in kidney transplantation: Diagnostic misinterpretation has potential therapeutic implications

Carrie A. Schinstock[1] | Ruth Sapir-Pichhadze[2] | Maarten Naesens[3,4] | Ibrahim Batal[5] | Serena Bagnasco[6] | Laurine Bow[7] | Patricia Campbell[8] | Marian C. Clahsen-van Groningen[9] | Matthew Cooper[10] | Emanuele Cozzi[11] | Darshana Dadhania[12] | Fritz Diekmann[13] | Klemens Budde[14] | Fritz Lower[15] | Babak J. Orandi[16] | Ajda T. Rowshani[17] | Lynn Cornell[18] | Edward Kraus[19]

TABLE 1 Interpretation of Banff ABMR classification in clinical practice

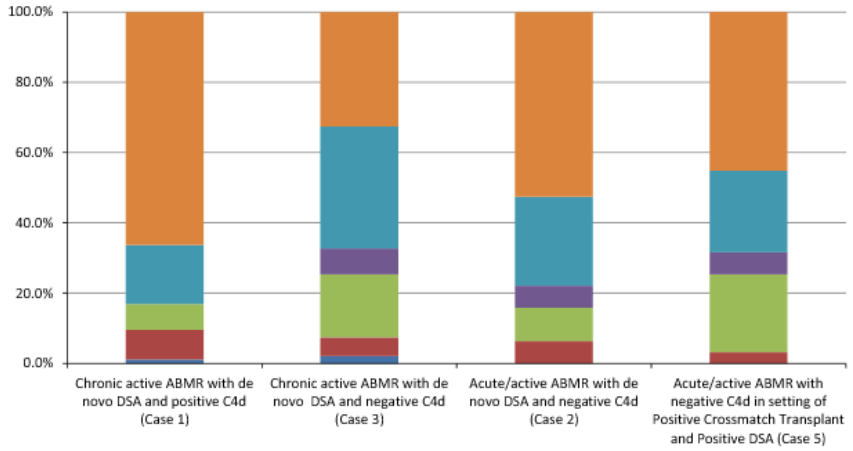
Scenario		% (n/N) of cases the respondents' assigned diagnosis differed from the reference standard (intended Banff diagnosis)		
		Pathologists	Clinicians	*P* value
Case 1	Chronic active ABMR with de novo DSA and positive C4d	27.8% (20/72)	46.3% (44/95)	.02
Case 2	Acute/active ABMR with de novo DSA and negative C4d	22.9% (16/70)	34.7% (33/95)	.14
Case 3	Chronic active ABMR with de novo DSA and negative C4d	27.5% (19/69)	24.2% (23/95)	.76
Case 4	Histologic features of ABMR without detectable anti-HLA antibody	20.6% (14/68)	49.4% (43/87)	.0004
Case 5	Acute/active ABMR with negative C4d in setting of positive crossmatch transplant and positive DSA	33.8% (23/68)	44.2% (42/95)	.24
Case 6	Mixed T cell–mediated rejection and C4d negative ABMR	14.7% (10/68)	10.6% (10/94)	.59
Mean (SD) number of cases/respondent		26.1% (SD 28.1%)	34.5% (SD 23.3%)	.04

ABMR, antibody-mediated rejection; DSA, donor-specific antibody.

Am J Transplant. 2019;19:123–131

Banff survey on antibody-mediated rejection clinical practices in kidney transplantation: Diagnostic misinterpretation has potential therapeutic implications

Carrie A. Schinstock[1] | Ruth Sapir-Pichhadze[2] | Maarten Naesens[3,4] | Ibrahim Batal[5] | Serena Bagnasco[6] | Laurine Bow[7] | Patricia Campbell[8] | Marian C. Clahsen-van Groningen[9] | Matthew Cooper[10] | Emanuele Cozzi[11] | Darshana Dadhania[12] | Fritz Diekmann[13] | Klemens Budde[14] | Fritz Lower[15] | Babak J. Orandi[16] | Ajda T. Rowshani[17] | Lynn Cornell[18] | Edward Kraus[19]

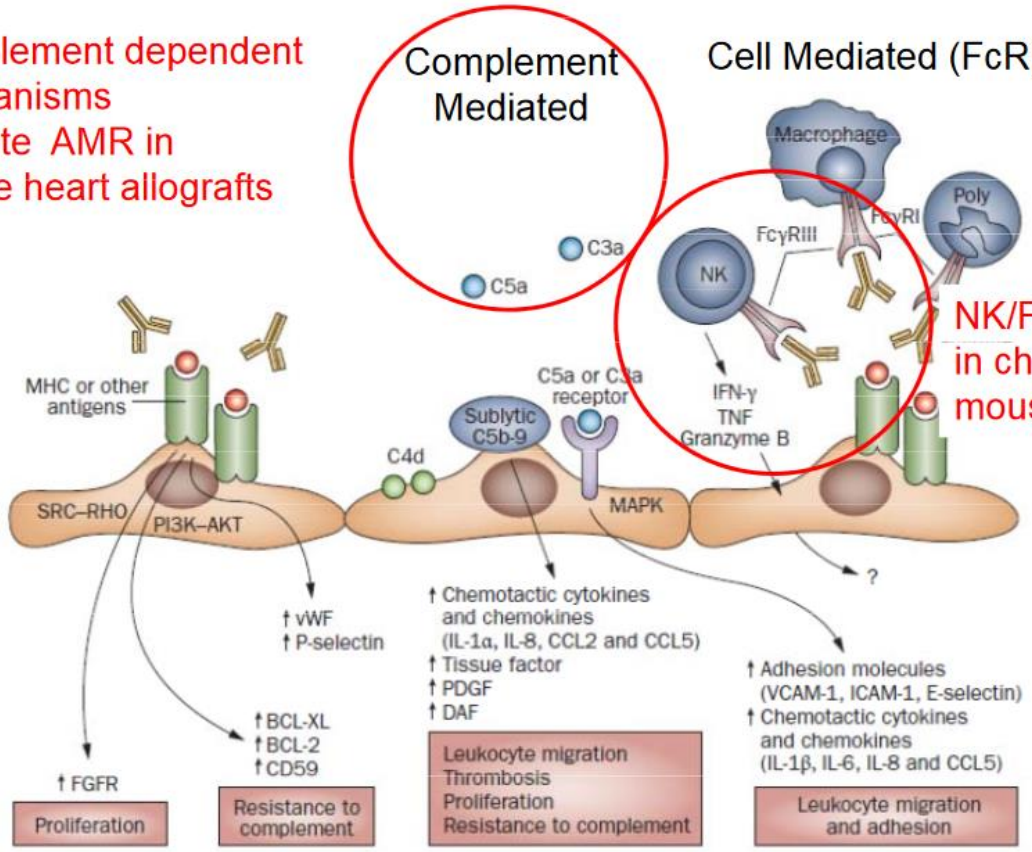


Plasmapheresis, IVIG, +/- rituximab and/or bortezomib	66.3% (63)	32.6% (31)	52.6% (50)	45.3% (43)
IVIG +/- steroids	16.8% (16)	34.7% (33)	25.3%(24)	23.2% (22)
Steroids only	0.0% (0)	7.4% (7)	6.3% (6)	6.3% (6)
Conservative treatment only	7.4% (7)	17.9% (17)	9.5% (9)	22.1% (21)
Other	8.4% (8)	5.3% (5)	6.3% (6)	3.2% (3)
Unanswered	1.1% (1)	2.1(2)	0.0% (0)	0.0% (0)

Am J Transplant. 2019;19:123–131

Mechanism Theory

Complement dependent mechanisms in acute AMR in mouse heart allografts



NK/Fc mechanisms in chronic AMR in mouse heart allografts

Farkash and Colvin, Nat Rev Nephrol, 2012

STAR2: Antibody Attributes Report

Howard Gebel, Kathryn Tinckam, Michael Mengel, Elaine Reed

February 18, 2019

1. Complement fixation assays (Humans; in vivo and in vitro studies)

Known:

Current assays focus on whether a DSA fixes complement (C1q,C3d, C4d) and have correlated that attribute with a greater likelihood that recipients will develop AMR and have a higher incidence of graft loss. The studies tend to be retrospective and population based. Most of the studies reveal a strong association between complement fixation and MFI levels/ab titers of the antibodies with high MFIs/high antibody titers being predictive of C' fixation. There appears to be a distinct (specific?) molecular signature for AMR that occurs in the context of complement fixing antibodies and therapeutic strategies designed to avert such AMR are warranted.

Needed:

a. Intervention/outcome studies-who/how frequently to monitor/what types of intervention/endpoints (in vivo).

b. Biochemical analysis of antibody attributes associated with function (e.g., sialyation status of DSAs). Baseline studies of Class I vs class II antibodies with high vs low MFIs; at times of detection/at times of AMR; impact of sialyation (and de-siaylation) in vitro assays; translation to clinical trials.

2. HLA Antibody Subclass

Known:

Different routes of allosensitization trigger distinct patterns of IgG subclasses directed against HLA. Cell based and solid phase assays testing for HLA antibodies, traditionally identify antibodies of the IgG isotype. The CDC assay identifies high titered HLA antibodies that initiate the complement cascade culminating in the MAC complex and potential cell death. Single antigen and flow crossmatch assays are more sensitive and detect binding of HLA antibodies to donor cells. These assays do not distinguish between complement activating IgG1 and IgG3 subclasses from the weaker complement activating IgG2 and IgG4 subclasses of IgG. Preliminary studies of HLA antibody subclasses suggests that IgG2 and IgG4 do not constitute a large proportion of HLA DSA; HLA IgG1 and IgG3 are the predominant subclasses associated with graft rejection/ graft loss. However, the hypothesis has been put forth that donor specific IgG2and IgG4, (later class switch subclasses) represent a chronic humoral response with active T cell help. The current single antigen class I and class II IgG subclasss antibody identification assays suffer from lack of specificity The concentrations of the different IgG subclasses cannot be directly compared to relative abundance of each subclass. The sensitivities of the IgG subclass single antigen assay are different. Most patients make a mixture of HLA DSA subclasses making it difficult to determine significant differences in incidence of HLA subclass and transplant outcome.

Needed:

a. Laboratories must develop robust assays that provide information on the different strengths/titers of IgG antibody subclass.

b. The mechanisms of graft injury by different subclasses are unknown and need to be confirmed in experimental transplant models and in situ in allografts.

c. Studies are needed to assess the treatment of strategies to prevent or manipulate IgG subclass diversity to prevent graft injury and rejection.

d. Data are needed to understand the details of IgG subclass specification by cytokines and other signals.

3. HLA Antibody and Outside In Signaling

Known:

Crosslinking of HLA is a universal function of HLA I and II antibodies irrespective of subclass and triggers outside in signal transduction and endothelial cell survival, proliferation and migration. Outside in signaling also mobilizes Weibel-Palade bodies, externalizing P-selectin to the endothelial cell surface and supports monocyte tethering through PSGL-1. Complement fixing subclasses of DSA can concurrently engage FcgR to amplify P-selectin-mediated adhesion of monocytes. P-selectin-PSGL-1 and antibody-FcgR interactions promote firm adhesion to ICAM-1 by activating monocyte MAC-1 integrin in the monocyte. HLA recruits co-receptors such as integrin $\beta 4$ for HLA class I to form protein:protein complexes to transduce intracellular signals via Src/FAK/mTOR and mediate endothelial cell proliferation, migration and protein synthesis. HLA outside in signaling in endothelial cells causes actin cytoskeleton remodeling via Src/FAK/Rho to mediate ICAM-1 clustering enabling firm adhesion of monocytes. The capacity for HLA antibodies to induce outside in signaling is dependent upon HLA antigen expression on graft cells and HLA antibody titer and affinity/avidity. The mTOR signaling axis is activated by HLA class I and class II antibodies and represents a viable diagnostic criteria for AMR and therapeutic target to reduce endothelial cell activation during antibody-mediated rejection.

Needed:

a. Studies testing different IgG subclasses, titer and specificities of HLA class I and class II antibodies to better understand which qualitative aspects of the DSA are most relevant for the outcome (ie. leukocyte recruitment, cell survival, cell proliferation & migration, complement activation) and the pathology caused.

b. Clinical trials to assess the effect of signal transduction inhibitors such as mTOR on HLA class I and class II –mediated outside in signaling pathways in acute and chronic AMR.

c. *In vivo* studies assessing the role of signal transduction inhibitors on leukocyte recruitment including NK cells and monocyte/macrophages.

d. Assessing the role of P-selectin-PSGL-1 and antibody-FcgR interactions in monocyte recruitment in *in vivo* experimental and human models of AMR.

e. Further corroboration that signaling pathways elicited by outside in HLA signaling (i.e., p70S6K and pS6RP) are potential biomarkers and drug targets for ABMR.

4. Leukocyte recruitment and recipient FC receptor genotype.

Known:

A hallmark of AMR is the presence of intracapillary mononuclear cells in the allograft. Interactions between endothelial-bound IgG and myeloid and NK cell FCgRs facilitate tethering and adhesion of leukocytes in autoimmune inflammation and in response to HLA antibodies. Intragraft macrophages have a proinflammatory phenotype during acute rejection and a repair/profibrotic phenotype during chronic rejection suggesting that macrophage effector functions differentially contribute to acute and chronic rejection. Endothelial cells exposed to HLA antibodies produce cytokines and growth factors that can signal in an autocrine and paracrine manner and promote inflammation. Data from in vitro experimental assays indicate that FCGR2A polymorphisms (H131 vs R131) on monocytes govern interactions with distinct HLA IgG subclasses. Retrospective human studies suggest that FCGR2A polymorphisms constitute a risk factor for graft loss following kidney transplantation when anti-HLA antibodies are present. Similarly, the FcgRIIIA V158 high-affinity allele (CD16a) expressed on NK cells could enhance the ability of anti-HLA DSA to trigger inflammation in the microcirculation, resulting in adverse long-term allograft outcomes.

Needed:

a. Studies to understand the mechanistic role of graft infiltrating leukocytes in AMR.
b. Studies are needed to understand interplay between HLA DSA titer, subclass, activated complement split products, Fc receptor polymorhphism and mechanisms of graft injury by infiltrating cells.
c. There are currently no therapies aimed at preventing leukocyte recruitment or function of leukocytes infiltrating the allograft.
d. Larger prospective studies are needed to define the impact of FCgR polymorphisms on long-term graft outcomes. If confirmed, risk assessment for AMR may include determining recipient FcgR polymorphisms and the IgG subclass of DSA.

CONCLUSIONS

1. Strong association between DSAs that bind complement (C1q, C3d, C4d) and graft loss/graft dysfunction.

2. MFI levels of DSA typically correspond with their ability to fix complement.

3. In vitro complement binding does not equal in vivo complement binding.

4. DSAs can mediate graft damage by complement independent mechanisms

5. Insufficient evidence to warrant routine (and costly) testing to determine whether HLA antibodies fix complement.

6. Rather than merely identifying whether antibodies fix complement, a better strategy would be to focus on strategies to mitigate their pathogenesis.

HLA Antibody Complement Based Assays

Howard M. Gebel, PhD, D(ABHI)
Robert A. Bray, PhD, D(ABHI)
Emory University Hospital
Atlanta, GA

OBJECTIVES

1) To understand the rationale behind tests to assess the complement fixing abilities of HLA antibodies.

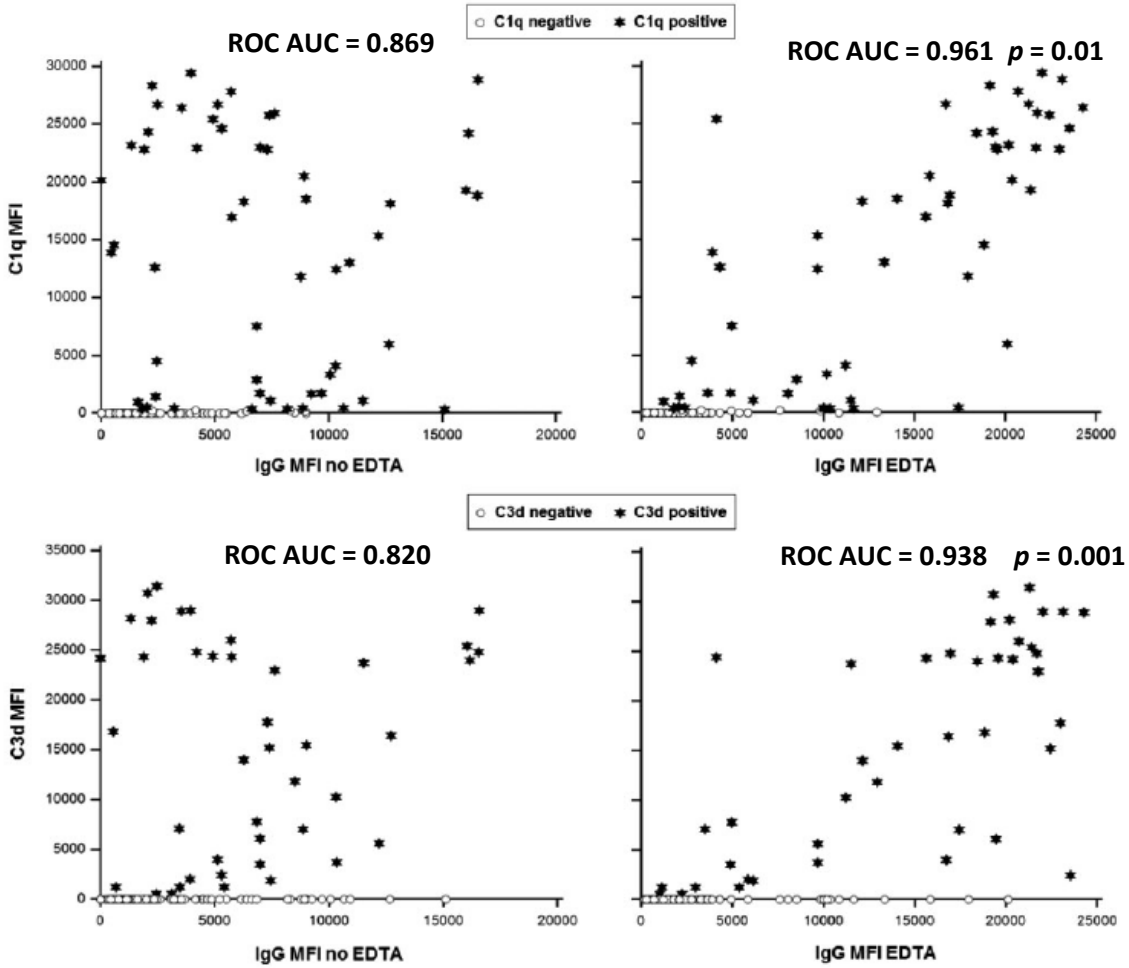
2) To discuss the clinical impact of complement fixing HLA antibodies in solid organ transplant recipients.

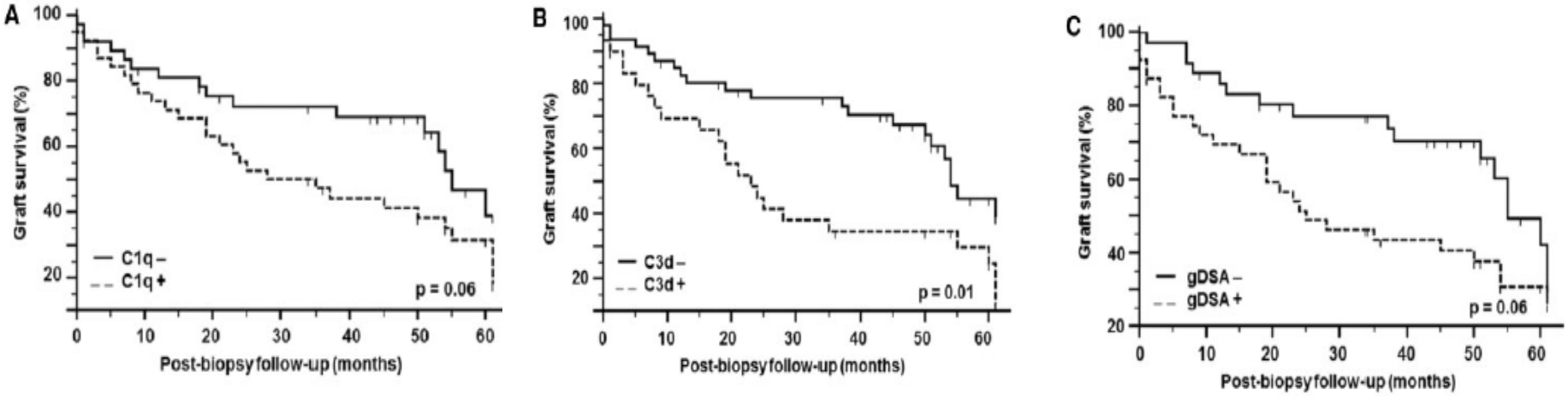
3) To recognize the limitations of complement fixation assays.

The disappointing contribution of anti-human leukocyte antigen donor-specific antibodies characteristics for predicting allograft loss

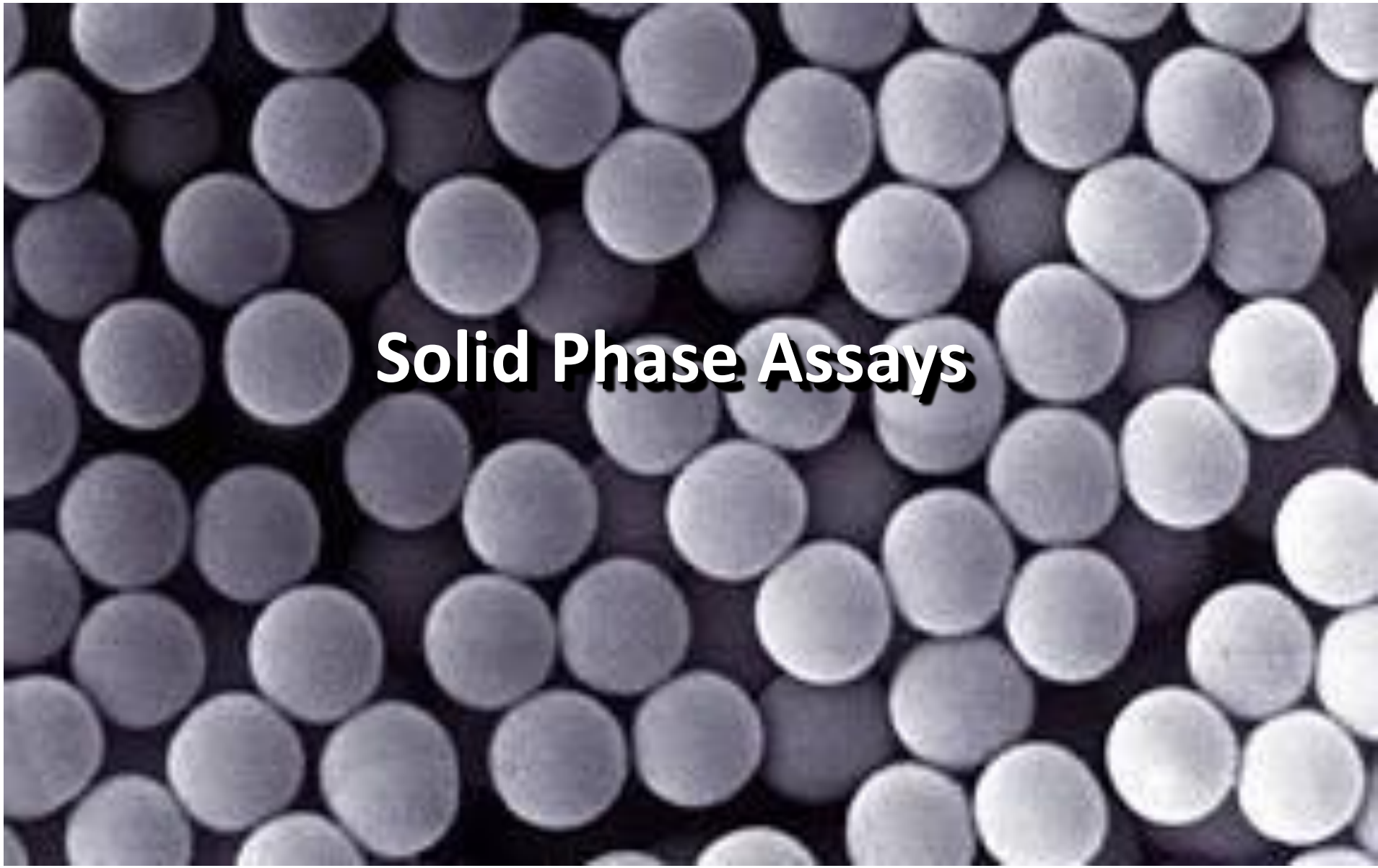
Nephrol Dial Transplant (2018) 33: 1853–1863

Maxime Courant[1,*], Jonathan Visentin[2,3,*], Gabriel Linares[2], Valérie Dubois[4], Sébastien Lepreux[5,6], Gwendaline Guidicelli[4], Olivier Thaunat[7,8], Pierre Merville[1,3], Lionel Couzi[1,3,*] and Jean-Luc Taupin[2,3,9,*]





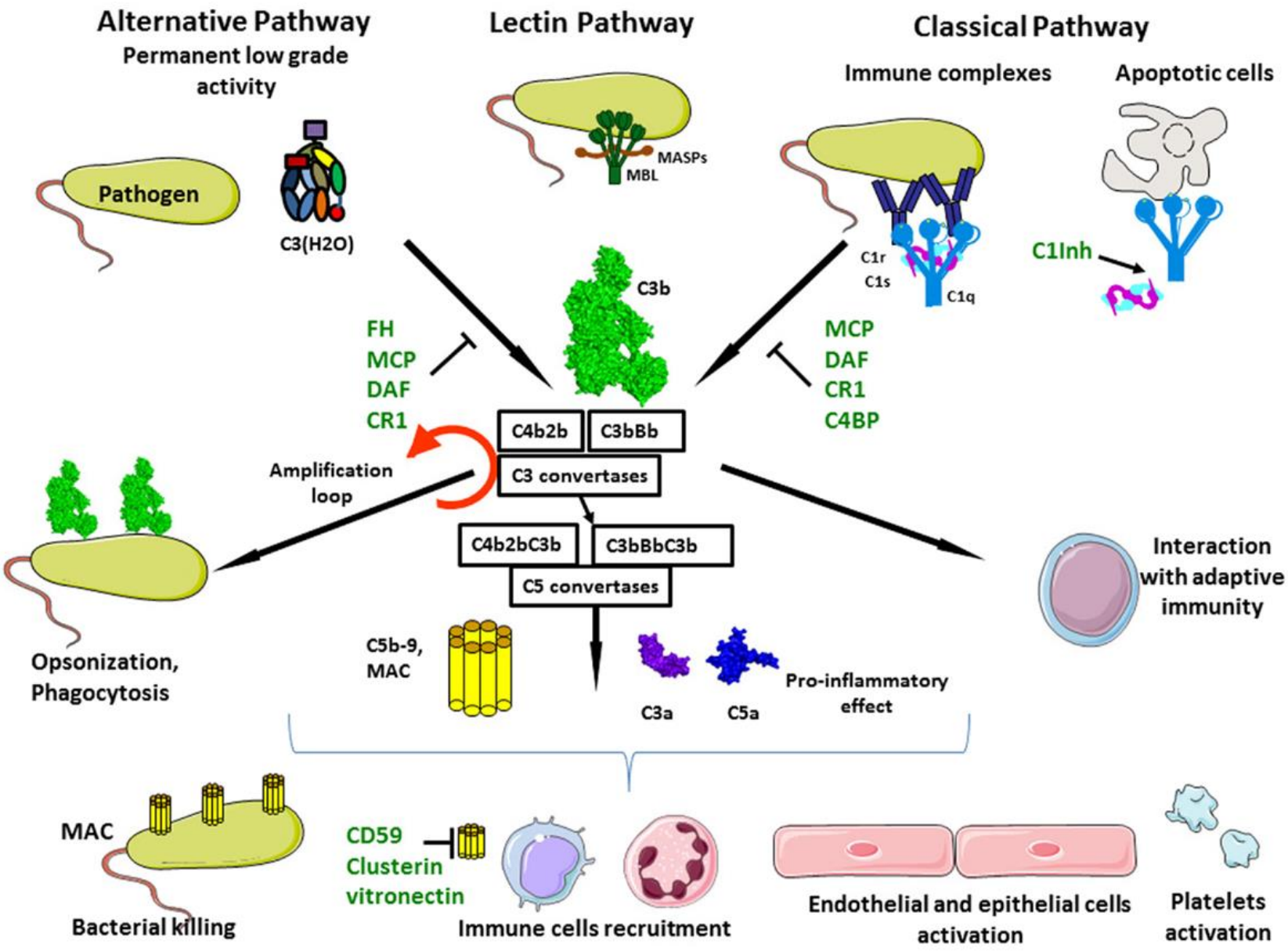
In conclusion, at the time of a for-cause biopsy, our findings do not plead for systematically implementing any of the C1q, C3d or gDSA assays. Indeed, none of them independently predicted ABMR nor was associated with graft loss, while eGFR and histopathologic criteria of chronic ABMR remained the best prognostic factors for graft loss.

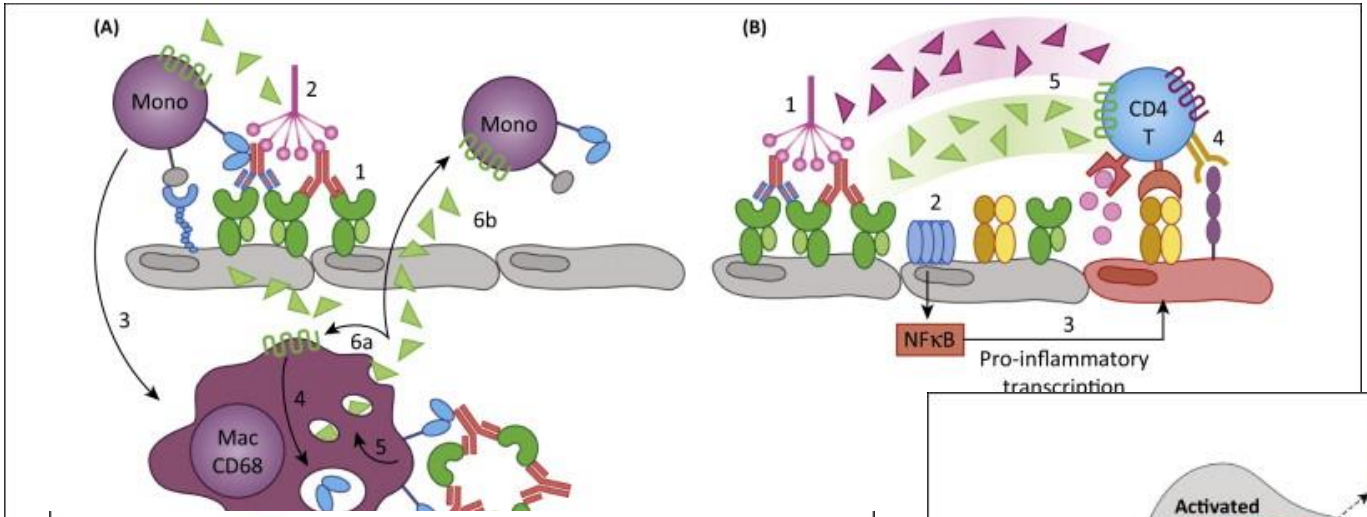

Solid Phase Assays

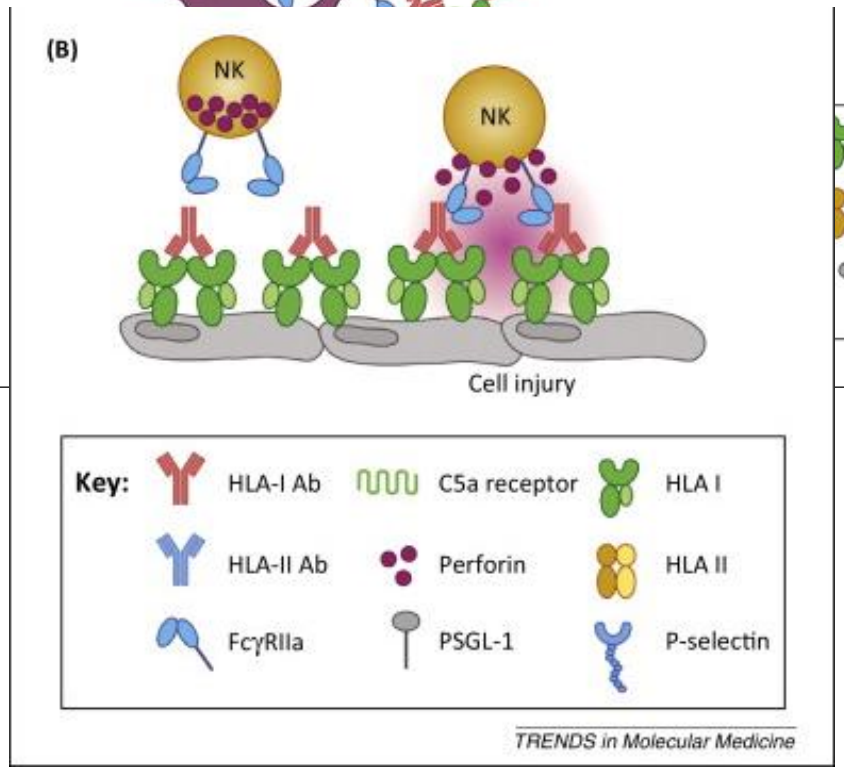
Differences

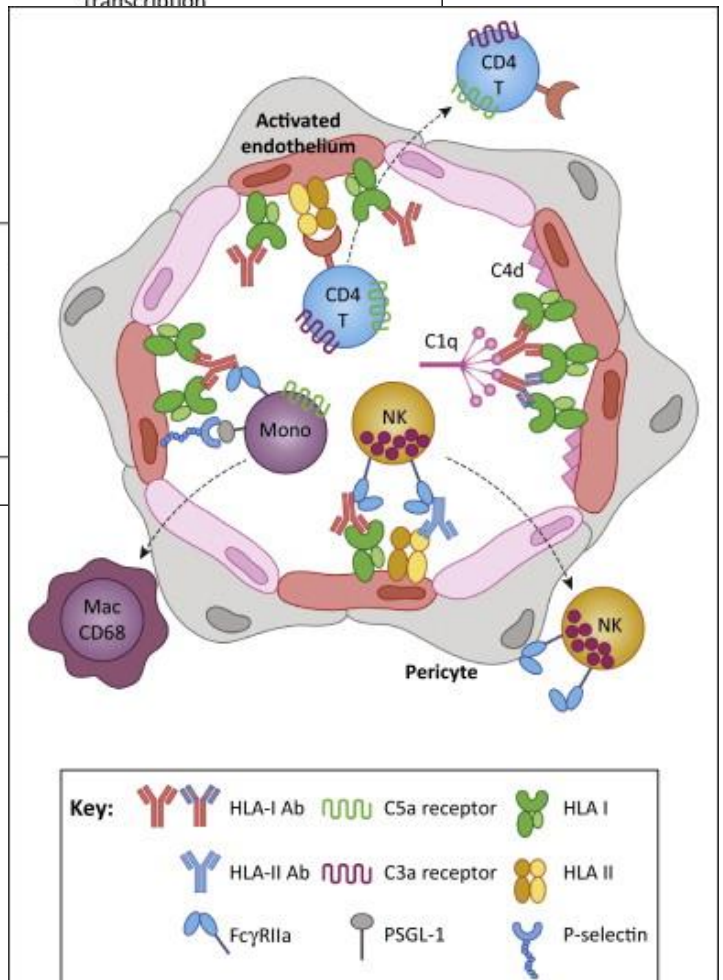
Acute AMR	Chronic AMR
Usually presensitized	Usually de novo DSA Association with TCMR
Rapid loss of function (days)	Insidious loss of function (months-years) Most cases not associated with acute AMR
Anti donor HLA class I or II	Anti-class II DSA common
Widespread C4d deposition common	Minimal C4d common
Capillaritis/glomerulitis neutrophils/mononuclears	Capillaritis/glomerulitis macrophages/NK

***from* Acute and Chronic AMR: A continuum or distinct diseases?**
FDA Workshop
Antibody Mediated Rejection in Kidney Transplantation April 12, 2017 Silver Spring MD


Alternative Pathway
Permanent low grade activity
Lectin Pathway
Classical Pathway
Immune complexes
Apoptotic cells
Pathogen
C3(H2O)
MASPs
MBL
C1r
C1s
C1q
C1Inh
C3b
FH
MCP
DAF
CR1
MCP
DAF
CR1
C4BP
C4b2b
C3bBb
C3 convertases
Amplification loop
C4b2bC3b
C3bBbC3b
C5 convertases
Opsonization, Phagocytosis
C5b-9, MAC
C3a
C5a
Pro-inflammatory effect
Interaction with adaptive immunity
MAC
Bacterial killing
CD59
Clusterin
vitronectin
Immune cells recruitment
Endothelial and epithelial cells activation
Platelets activation


(A)
Mono
2
1
Mono
6b
3
6a
4
Mac
CD68
5
(B)
1
5
CD4
T
4
2
3
NFκB
Pro-inflammatory
transcription


(B)
NK
NK
Cell injury
Key:
HLA-I Ab
C5a receptor
HLA I
HLA-II Ab
Perforin
HLA II
FcγRIIa
PSGL-1
P-selectin
TRENDS in Molecular Medicine


CD4
T
Activated
endothelium
C4d
CD4
T
C1q
Mono
NK
Mac
CD68
NK
Pericyte
Key:
HLA-I Ab
C5a receptor
HLA I
HLA-II Ab
C3a receptor
HLA II
FcγRIIa
PSGL-1
P-selectin

Zhang CJASN 2018;13:182-192

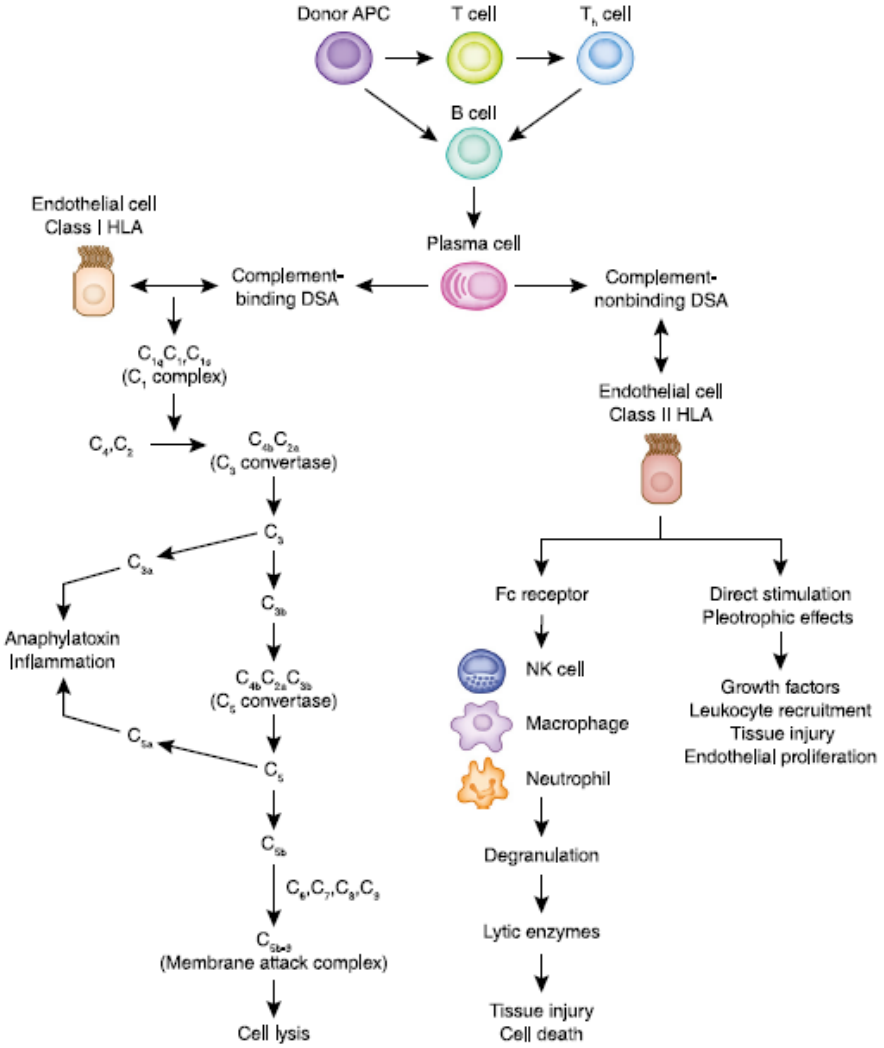


Figure 1. | The three proposed pathogeneses of donor-specific antibodies (DSAs) in antibody-mediated rejection. Donor antigen-presenting cells include macrophages, dendritic cells, and B cells. Complement binding DSAs target the class 1 HLA on endothelial cells, activate the classic complement cascade, and deliver complement-dependent cytotoxicity in acute antibody-mediated rejection. Complement nonbinding DSAs recruit innate immune cells (NK cells, macrophages, and neutrophils) through Fc receptors and lead to antibody-dependent cellular toxicity. In addition, complement nonbinding DSAs have direct stimulation and pleotrophic effects that cause tissue injury, cellular recruitment, and endothelial proliferation. The latter two mechanisms play an important role in acute antibody-mediated rejection with negative C4d deposit in peritubular capillaries as well as chronic antibody-mediated rejection, transplant glomerulopathy, and vasculopathy (4,8,11,21–24). APC, antigen-presenting cells; NK, natural killer cells.

NATURE REVIEWS | NEPHROLOGY VOLUME 9 | DECEMBER 2013 | 713

TRANSPLANTATION

Complementing donor-specific antibody testing

Kathryn J. Tinckam and Peter S. Heeger

"C1q binding might simply be an indirect measure of the strength of the antibody."

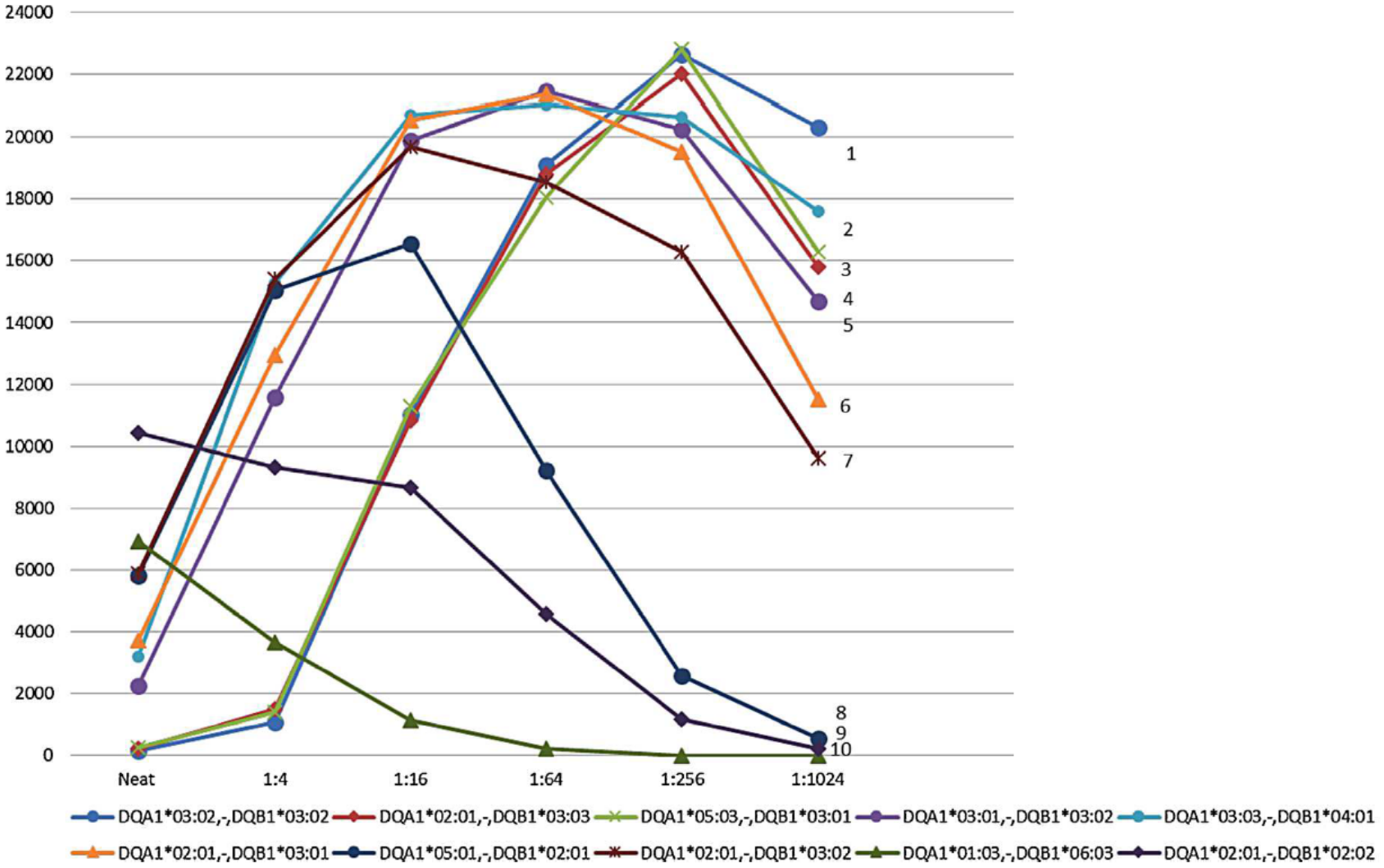
"When deciding to implement C1q DSA testing, programs need to consider the burden of extra testing as opposed to using MFI data already available."

Burden = $$$s

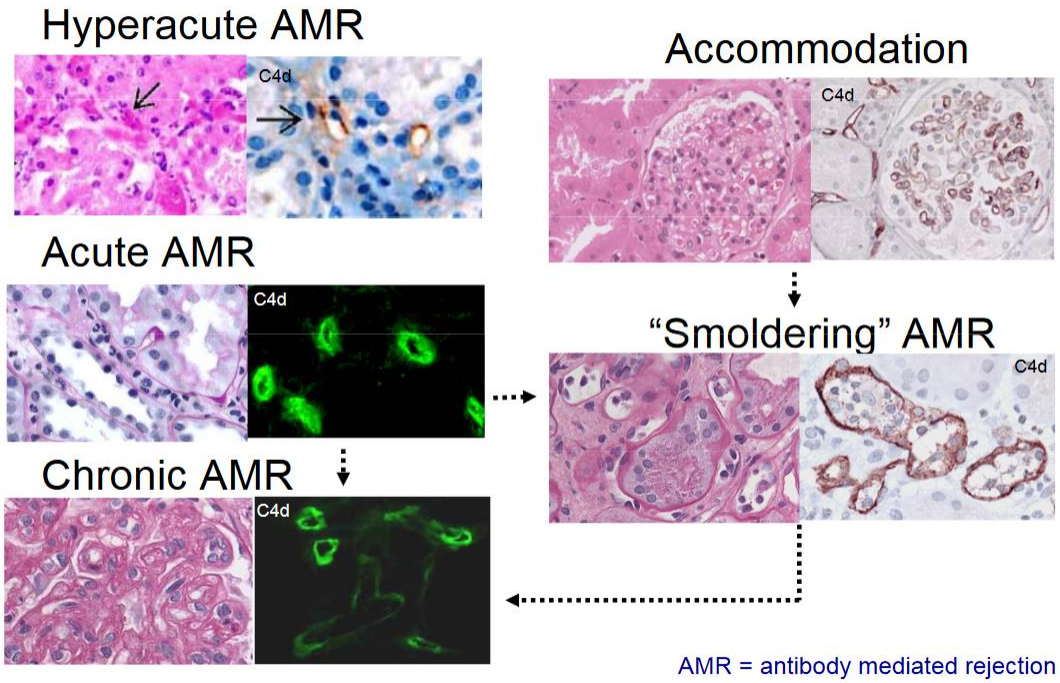
Assessing Antibody Strength: Comparison of MFI, C1q, and Titer Information

A. R. Tambur[1,5,*], N. D. Herrera[1,5], K. M. K. Haarberg[1,5], M. F. Cusick[1,5], R. A. Gordon[2,5], J. R. Leventhal[3,5], J. J. Friedewald[4,5] and D. Glotz[6]

American Journal of Transplantation 2015; 15: 2421–2430
Wiley Periodicals Inc.



Multiple Effects of Antibody



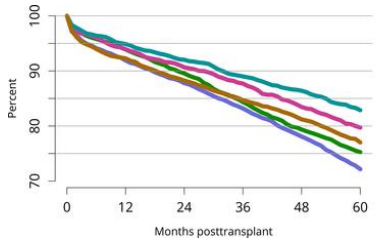
from **Acute and Chronic AMR: A continuum or distinct diseases?**
FDA Workshop
Antibody Mediated Rejection in Kidney Transplantation April 12, 2017 Silver Spring MD

Long-term solid organ allograft survival rates

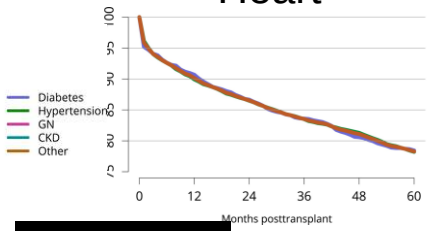
- Late allograft loss is an increasing focus area for improving outcomes

- Mechanisms of chronic rejection are an increasing focus to improve long-term graft survival

Renal

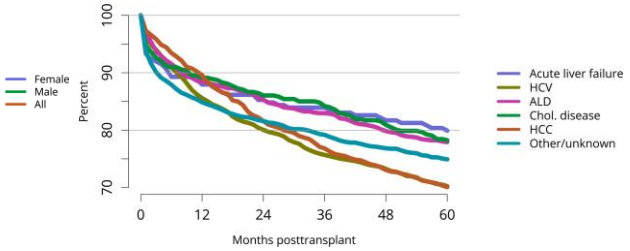


Heart



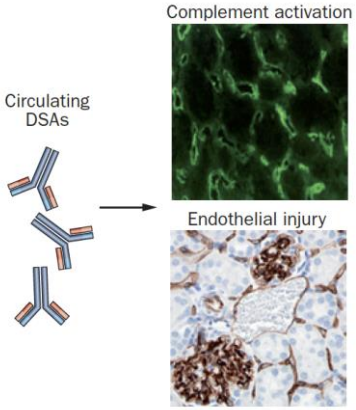
???
Keep or discard?

Liver



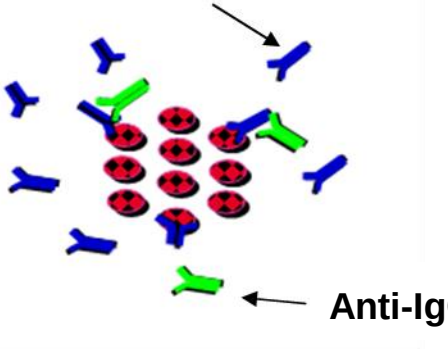
OPTN/SRTR 2016 Annual Report

- Antibody-mediated rejection (ABMR) is recognized as an immunologic driver of chronic rejection

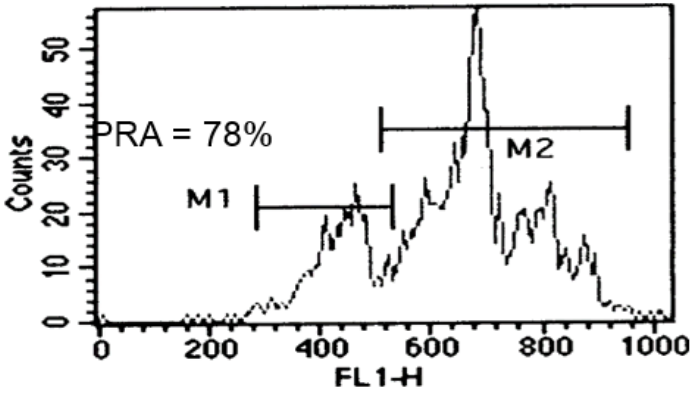


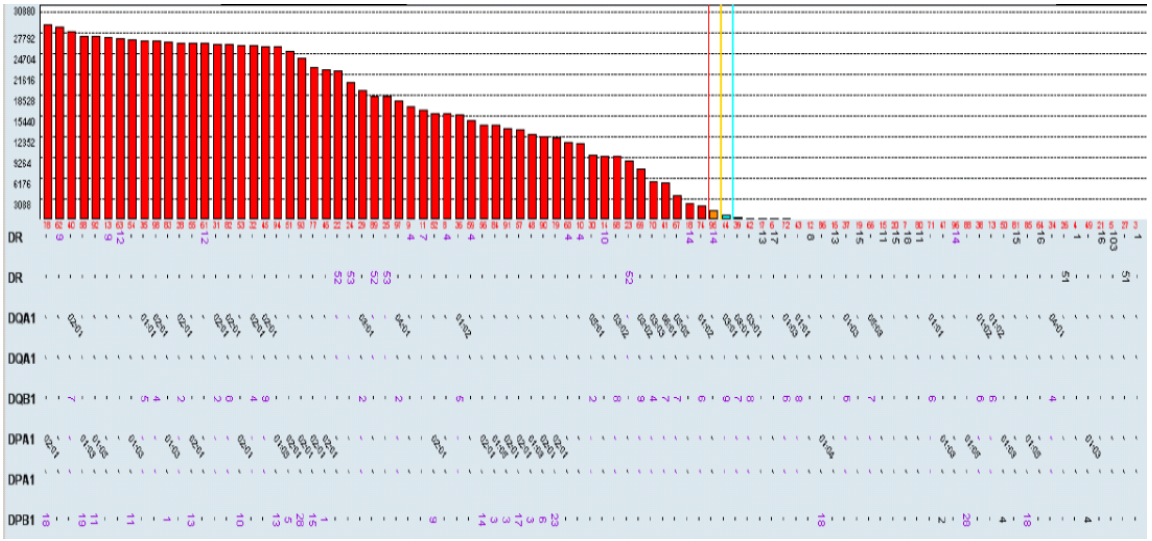
Solid Phase HLA antibody detection

HLA alloantibody



Suspension Arrays





Adapted from Gebel and Bray. Transplantation Reviews 20: 189-194, 2006

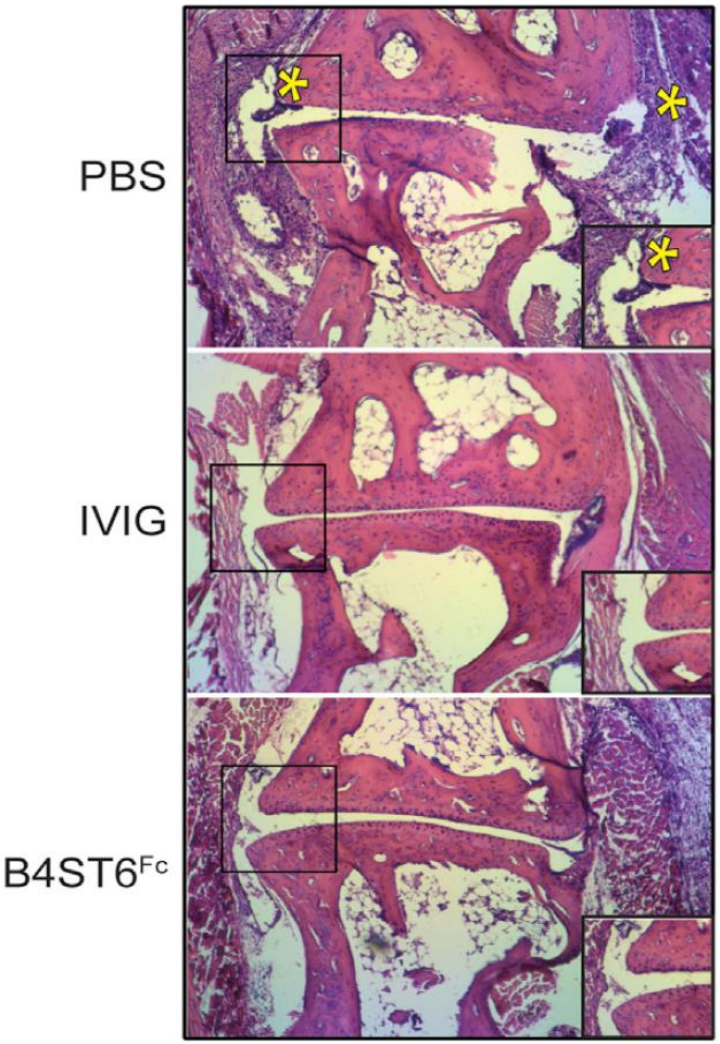
Issues with Flow Cytometric Crossmatch

Relies on adequate numbers of viable cells

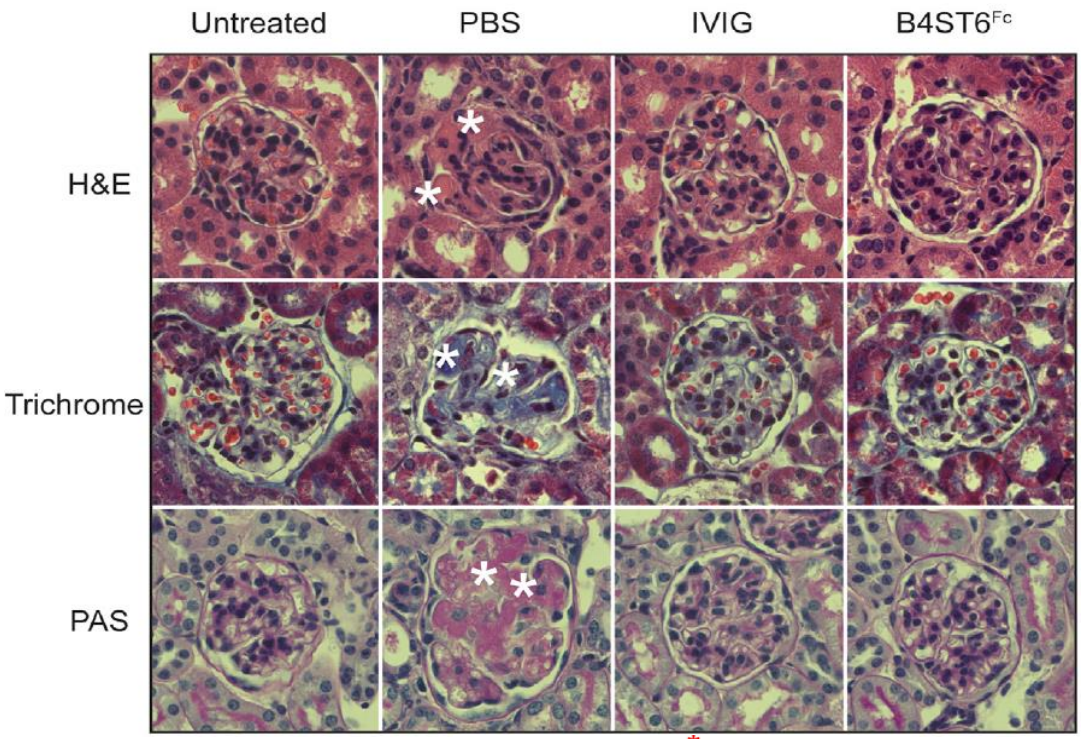
Antigen masking/density

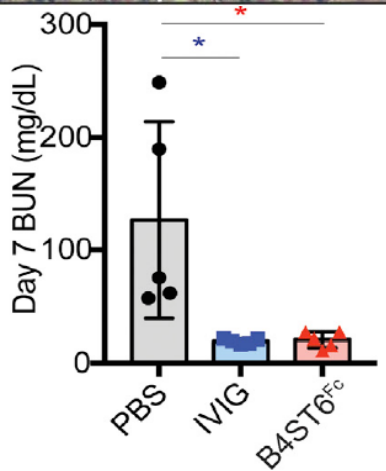
Low specificity (false positives)

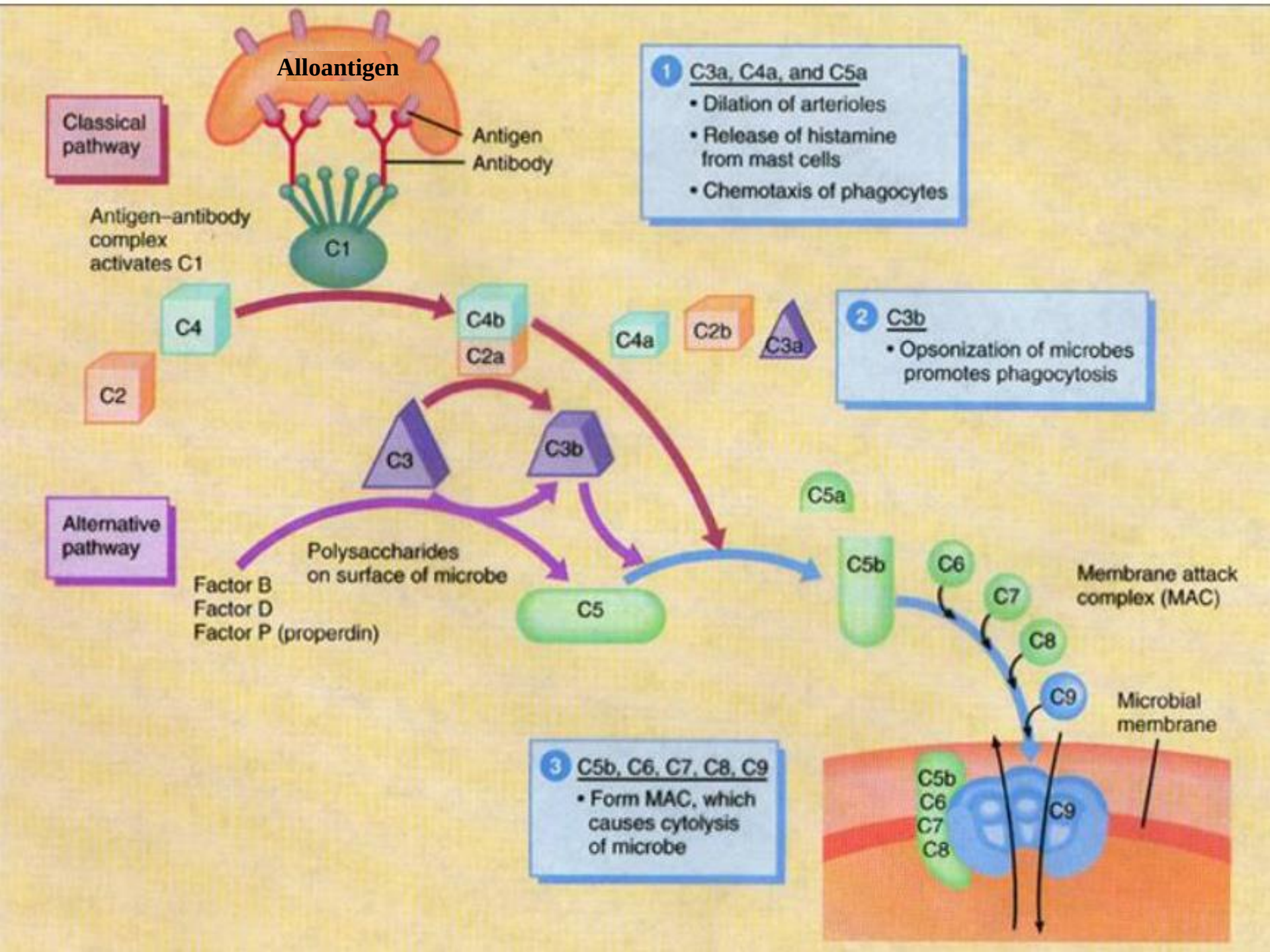
Arthritogenic Murine Model



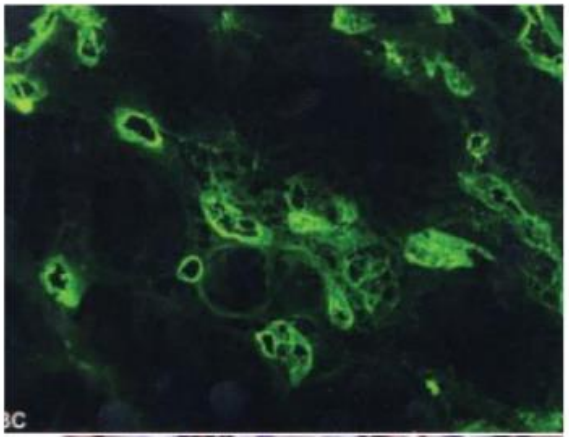
Nerphotoxic Nephritis in Murine Goodpasture Disease Model



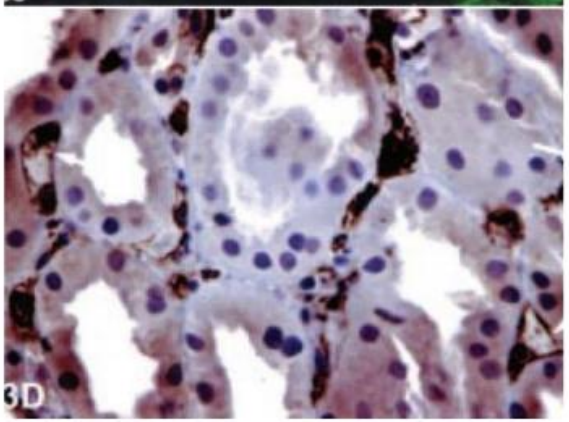



Alloantigen
Classical pathway
Antigen
Antibody
Antigen–antibody complex activates C1
C1
C4
C2
C4b
C2a
C4a
C2b
C3a
1 C3a, C4a, and C5a
• Dilation of arterioles
• Release of histamine from mast cells
• Chemotaxis of phagocytes
2 C3b
• Opsonization of microbes promotes phagocytosis
C3
C3b
Alternative pathway
Polysaccharides on surface of microbe
Factor B
Factor D
Factor P (properdin)
C5
C5a
C5b
C6
C7
C8
C9
Membrane attack complex (MAC)
Microbial membrane
3 C5b, C6, C7, C8, C9
• Form MAC, which causes cytolysis of microbe

???
Keep or
discard?



Peritubular capillary
Immunofluorescent
Staining
for C4d



Peritubular capillary
Immunohistochemistry
staining
for C4d.

Nickeleit, Neph Dial and Transplant 18: 2232-2239, 2003

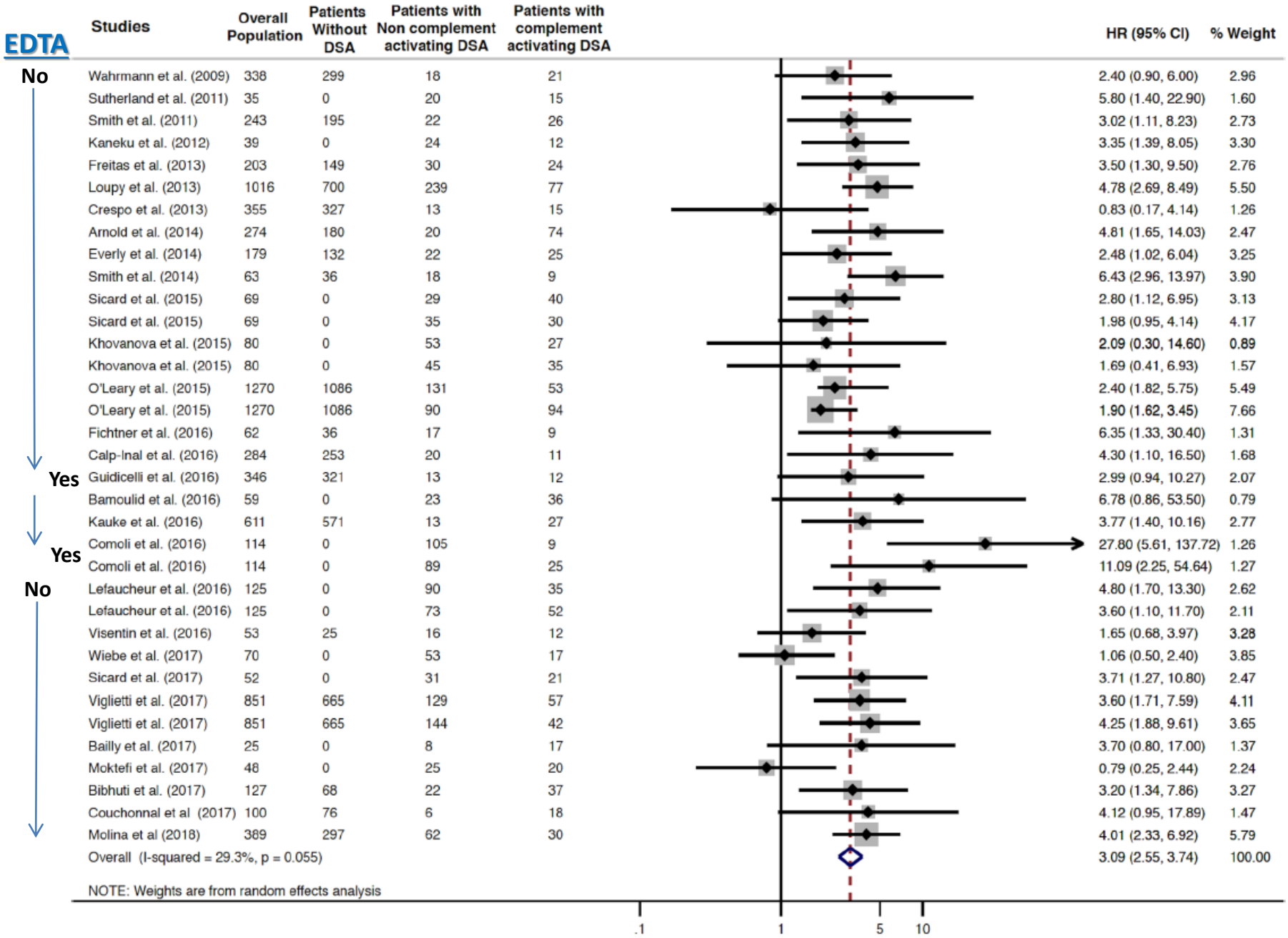
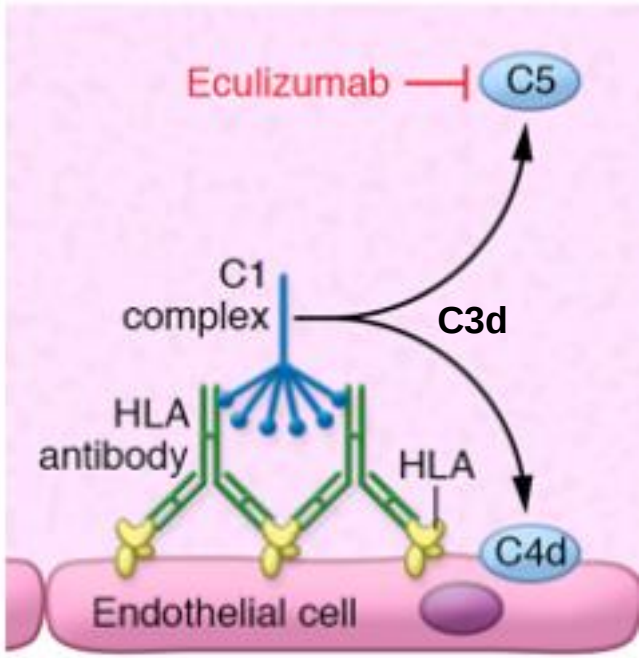


Fig 3. Association between circulating complement-activating anti-HLA DSAs and the risk of allograft loss. Fig 3 shows the forest plot of the association between complement-activating anti-HLA DSAs and the risk of allograft loss for each study and overall ($n = 29$). Studies are listed by date of publication. Number of patients are



Complement-mediated injury

C4d
C3d

In severe cases

Loss of endothelial cell integrity, as in hyperacute rejection

Nicole M. Valenzuela, Elaine F. Reed *J Clin Invest.* 2017;127(7):2492-2504.

The Prozone Effect

INTERNATIONAL JOURNAL OF
IMMUNOGENETICS

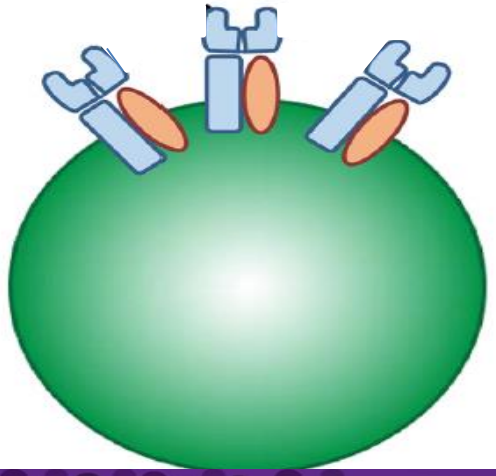
Review

doi: 10.1111/j.1744-313X.2012.01147.x

The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera

C. Weinstock* & M. Schnaidt†

International Journal of Immunogenetics, 2013, **40**, 171–177



The Prozone Effect

INTERNATIONAL JOURNAL OF
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Review

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The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera

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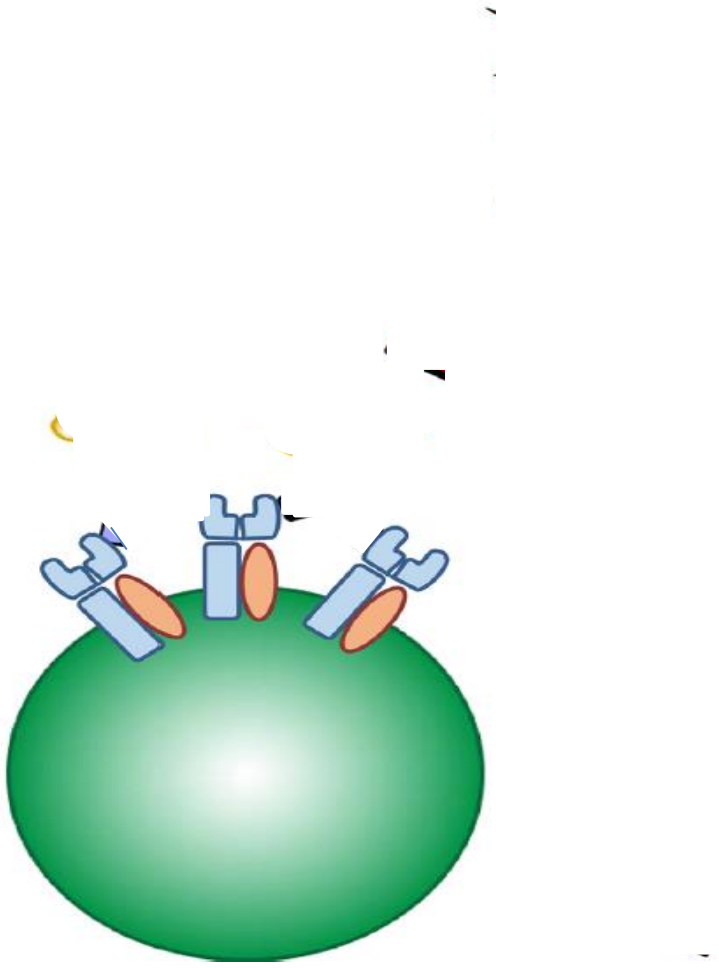
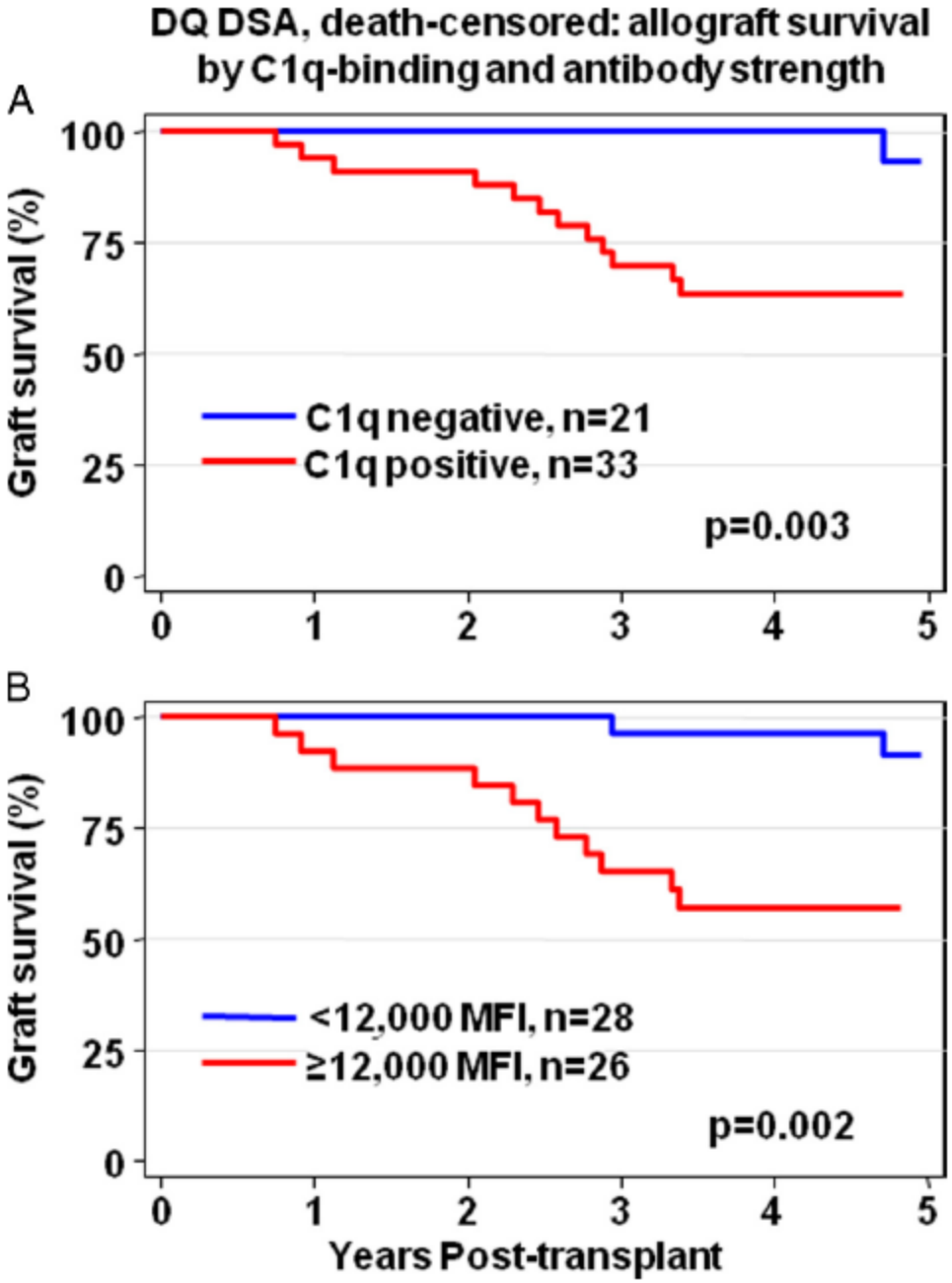



DQ DSA, death-censored: allograft survival
by C1q-binding and antibody strength
A
Graft survival (%)
100
75
50
25
0
0
1
2
3
4
5
C1q negative, n=21
C1q positive, n=33
p=0.003
B
Graft survival (%)
100
75
50
25
0
0
1
2
3
4
5
<12,000 MFI, n=28
≥12,000 MFI, n=26
p=0.002
Years Post-transplant

The Role of Immunoglobulin-G Subclasses and C1q in De Novo HLA-DQ Donor-Specific Antibody Kidney Transplantation Outcomes

Maria Cecilia S. Freitas,[1,7] Lorita M. Rebellato,[2] Miyuki Ozawa,[3] Anh Nguyen,[1] Nori Sasaki,[3] Matthew Everly,[1] Kimberly P. Briley,[2] Carl E. Haisch,[4] Paul Bolin,[5] Karen Parker,[5] William T. Kendrick,[6] Scott A. Kendrick,[6] Robert C. Harland,[4] and Paul I. Terasaki[1]

Transplantation • Volume 95, Number 9, May 15, 2013

American Journal of Transplantation 2015; 15: 1003–1013
Wiley Periodicals Inc.

doi: 10.1111/ajt.13153

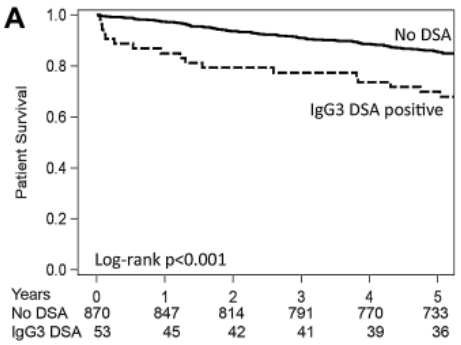
Impact of IgG3 Subclass and C1q-Fixing Donor-Specific HLA Alloantibodies on Rejection and Survival in Liver Transplantation

J. G. O'Leary[1,*], H. Kaneku[2], N. Banuelos[3], L. W. Jennings[1], G. B. Klintmalm[1] and P. I. Terasaki[2,3]

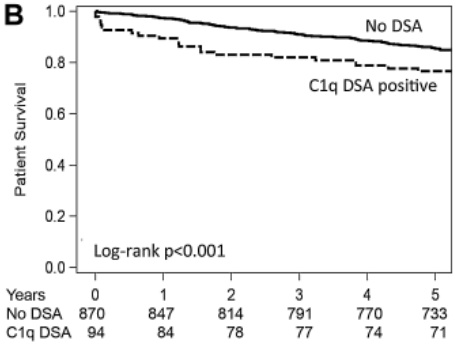
Introduction

Studies in different types
shown that donor-specific

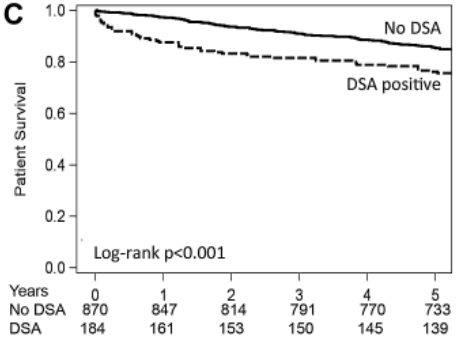
IgG3 and C1q-Fixing DSA in Liver Transplantation



	HR	P-value
Preformed IgG3 DSA	**2.4**	**<0.001**
AA recipient	1.9	<0.001
HCV	1.7	<0.001
Donor age >50	1.4	0.01
CMV	1.2	0.20



	HR	P-value
Preformed C1q DSA	**1.9**	**<0.001**
AA recipient	1.8	0.001
HCV	1.7	<0.001
Donor age >50	1.3	0.02
CMV	1.2	0.30



	HR	P-value
Preformed DSA	**1.6**	**<0.001**
AA recipient	1.8	<0.001
HCV	1.7	<0.001
Donor age >50	1.4	0.006
CMV	1.2	0.20

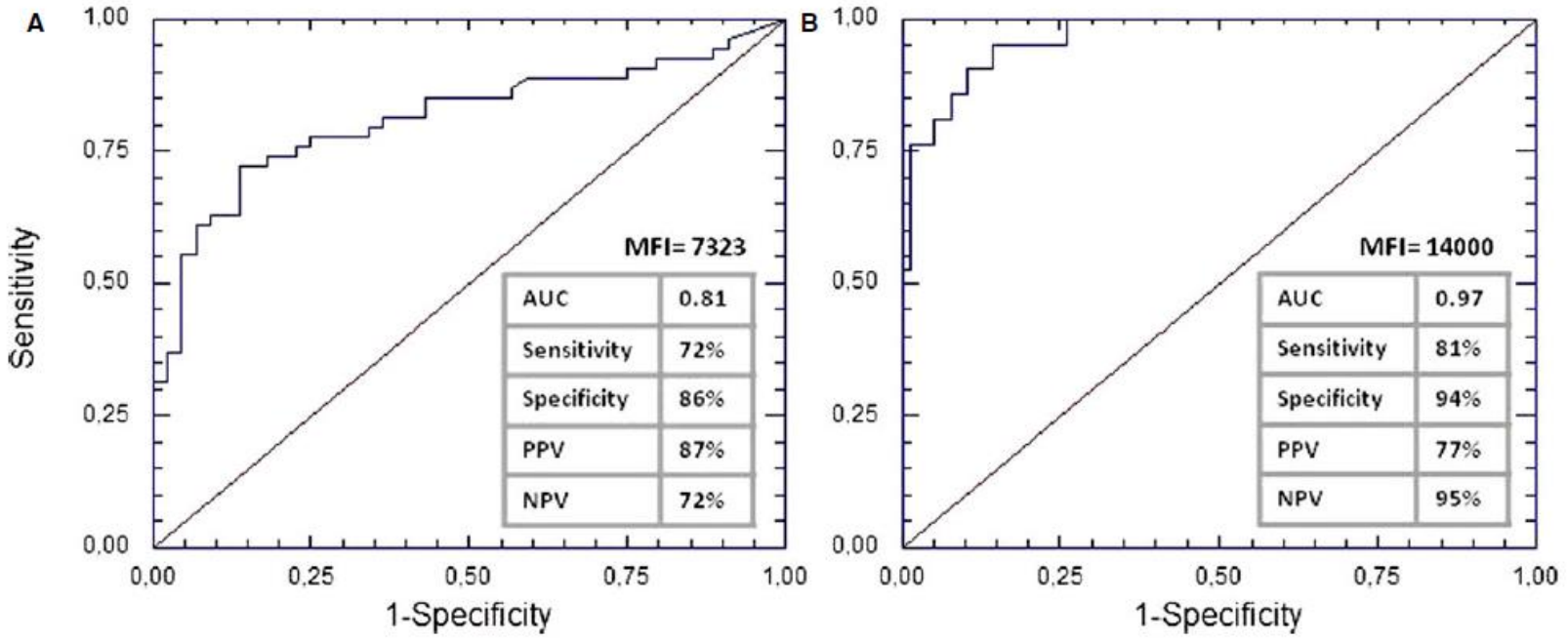


Figure 2: Complement-binding activity prediction by mean fluorescence intensity (MFI) values of *de novo* donor-specific antibodies (*dn*DSAs). Predictive values of *dn*DSA MFIs for C1q binding (A) and C3d binding (B) by receiver operating characteristic (ROC) analysis. AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

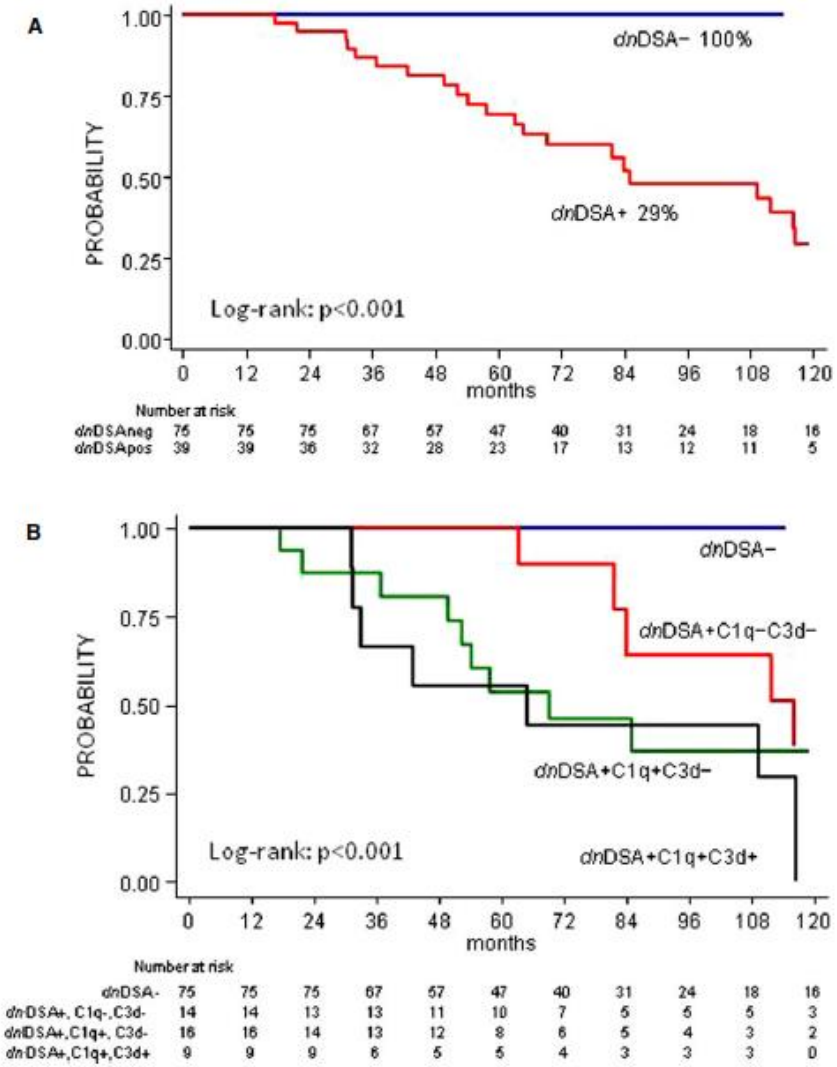


Figure 3: *De novo* donor-specific antibodies (*dn*DSAs) and risk of developing antibody-mediated rejection (AMR) in the analyzed cohort. (A) AMR-free allograft survival stratified by presence or absence of *dn*DSAs. (B) AMR-free allograft survival in kidney graft recipients stratified by development of non-complement-binding or complement-binding antibodies at *dn*DSA emergence. The statistical difference between Kaplan–Meier survival curves was evaluated by log-rank test, and differences with p-values <0.05 were considered statistically significant.

The prevalence and clinical significance of C1q-binding donor-specific anti-HLA antibodies early and late after kidney transplantation

Sumeyye Calp-Inal[1], Maria Ajaimy[1,2], Michal L. Melamed[1], Christina Savchik[2,3], Peter Masiakos[2,3], Adriana Colovai[2,3,4] and Enver Akalin[1,2,4]

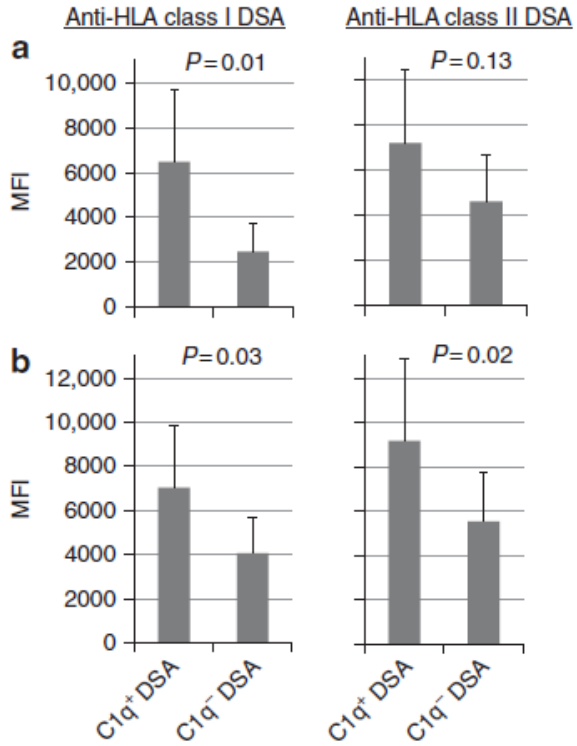


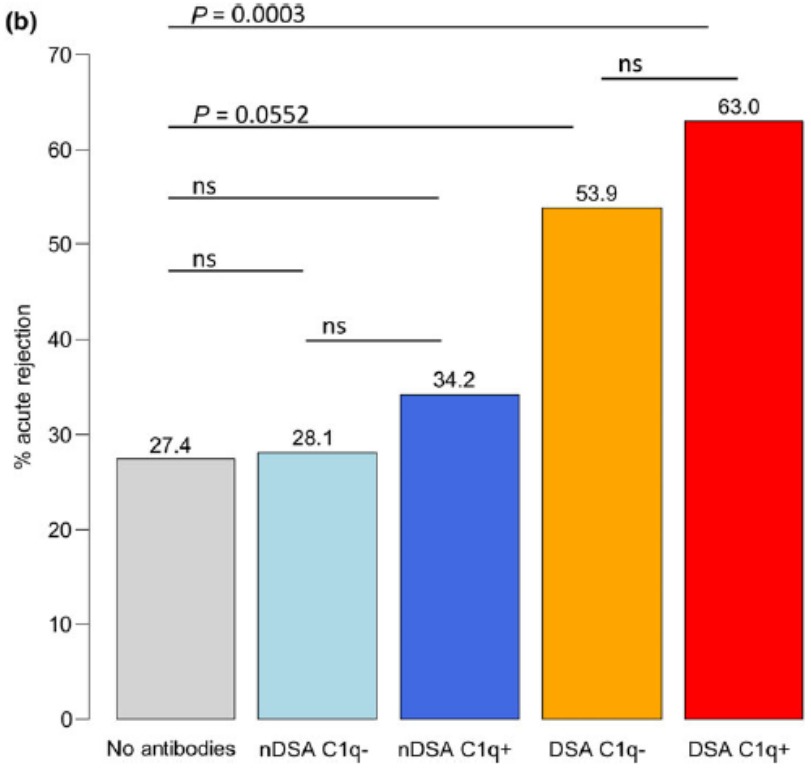
Figure 2 | Mean fluorescence intensity (MFI) values of C1q$^+$ and C1q$^-$ DSAs in Group 1 and Group 2 patients. (**a**) Group 1 and (**b**) Group 2 patients.

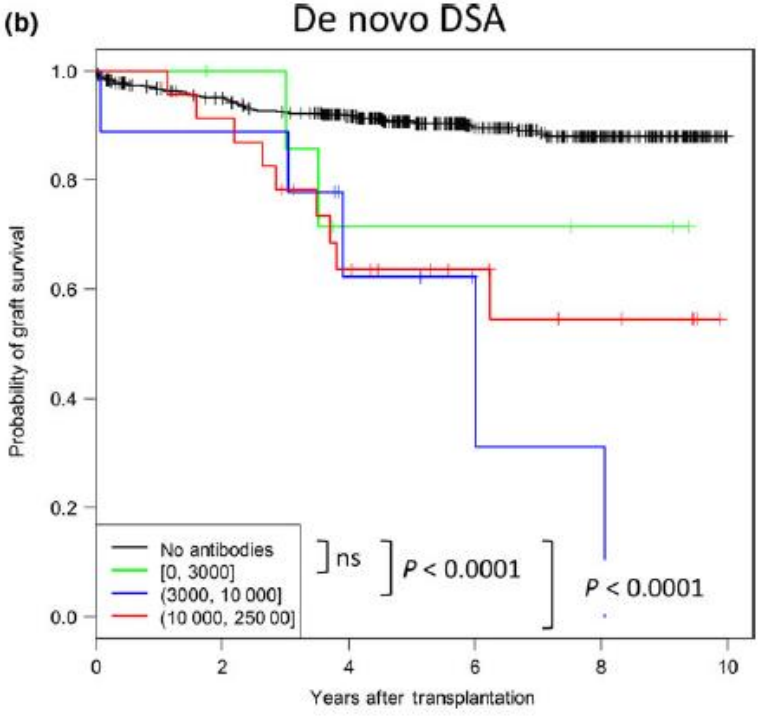
ORIGINAL ARTICLE

De novo donor-specific anti-HLA antibodies after kidney transplantation are associated with impaired graft outcome independently of their C1q-binding ability

Teresa Kauke[1,2], Cornelia Oberhauser[3], Viviane Lin[1,4], Michaela Coenen[3], Michael Fischereder[5], Andrea Dick[1], Ulf Schoenermarck[5], Markus Guba[2], Joachim Andrassy[2], Jens Werner[2], Bruno Meiser[4], Martin Angele[2], Manfred Stangl[2] & Antje Habicht[4]

Transplant International 2017; 30: 360–370





Non-Complement–Binding *De Novo* Donor-Specific Anti-HLA Antibodies and Kidney Allograft Survival

Gwendaline Guidicelli,*†‡ Florent Guerville,†‡§ Sébastien Lepreux,†‖ Chris Wiebe,¶ Olivier Thaunat,**††‡‡ Valérie Dubois,§§ Jonathan Visentin,*†‡ Thomas Bachelet,†‡§ Emmanuel Morelon,**††‡‡ Peter Nickerson,¶ Pierre Merville,†‡§ Jean-Luc Taupin,*†‡ and Lionel Couzi†‡§

J Am Soc Nephrol 27: 615–625, 2016.

to the serum HLA antibody.[23] Chelating bivalent cations with EDTA to block complement activation restored the detection of anti-HLA antibodies.[24,25] Therefore, in the absence of EDTA, the SAFB assay underestimated the MFI strength of these C1q+ *dn*DSAs in these four patients. This finding raises the hypothesis that the EDTA-treated SAFB assay could be better correlated with the C1q assay than the classic IgG SAFB assay. However, our study is insufficiently powered to answer this question. If this hypothesis

Table 2. Effect of serum preincubation with EDTA on MFI of C1q-binding *dn*DSA: evidence for a prozone phenomenon in low MFI DSAs found to be C1q+ DSAs

Patient No.	Target Antigen	SAFB (MFI)	SAFB-EDTA (MFI)	C1q-Binding assay (MFI)
1	DR53	2049	10,739	565
2	DQ7	2964	8414	177
3	DQ7	3435	6464	1650
4	DQ α chain	3878	6748	2182

Complement-Activating Anti-HLA Antibodies in Kidney Transplantation: Allograft Gene Expression Profiling and Response to Treatment

Carmen Lefaucheur,[1,2] Denis Viglietti,[1,2] Luis G. Hidalgo,[3] Lloyd E. Ratner,[4] Serena M. Bagnasco,[5] Ibrahim Batal,[6] Olivier Aubert,[1] Babak J. Orandi,[7] Federico Oppenheimer,[8] Oriol Bestard,[9] Paolo Rigotti,[10] Anna V. Reisaeter,[11] Nassim Kamar,[12] Yvon Lebranchu,[13] Jean-Paul Duong Van Huyen,[1,14] Patrick Bruneval,[1,15] Denis Glotz,[1,2] Christophe Legendre,[1,16] Jean-Philippe Empana,[1] Xavier Jouven,[1] Dorry L. Segev,[17] Robert A. Montgomery,[18] Adriana Zeevi,[19] Philip F. Halloran,[3] and Alexandre Loupy[1,16]

J Am Soc Nephrol 29: 620–635, 2018.

Table 1. Characteristics of patients with post-transplant donor-specific anti-HLA antibodies according to complement-activating capacity in the prospective cohort study

Characteristics	All Patients, *n*=931	C1q-Negative Anti-HLA DSAs, *n*=113	C1q-Positive Anti-HLA DSAs, *n*=44	*P* Value
MFI, mean (SEM)	4801 (371)	2979 (278)	9483 (748)	<0.001
IgG subclasses, no. (%)				
IgG1	112 (71)	70 (62)	42 (95)	<0.001
IgG2	61 (39)	43 (38)	18 (41)	0.74
IgG3	44 (28)	19 (17)	25 (57)	<0.001
IgG4	33 (21)	22 (19)	11 (25)	0.45

Complement-binding anti-HLA antibodies are independent predictors of response to treatment in kidney recipients with antibody-mediated rejection

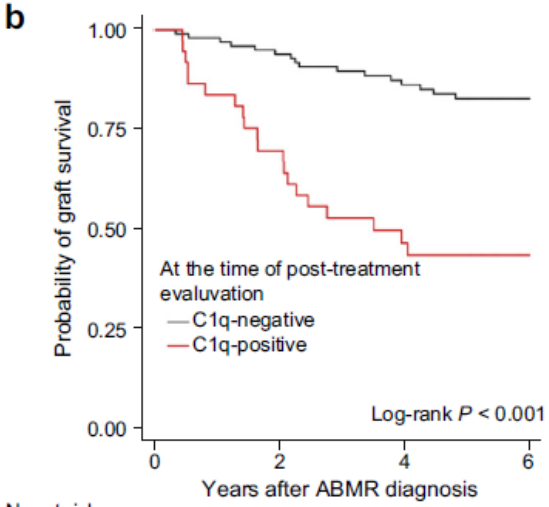
Kidney International (2018) **94,** 773–787

Denis Viglietti[1,2,8], Yassine Bouatou[1,3,4,8], Vissal David Kheav[5], Olivier Aubert[1,6], Caroline Suberbielle-Boissel[5], Denis Glotz[1,2], Christophe Legendre[1,6], Jean-Luc Taupin[5], Adriana Zeevi[7], Alexandre Loupy[1,6,9] and Carmen Lefaucheur[1,2,9]

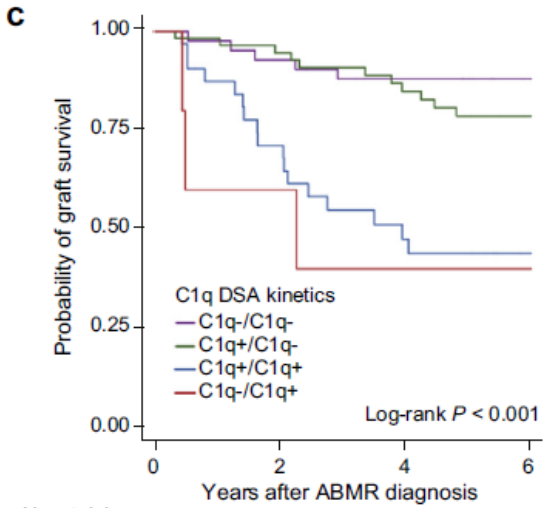
Table 2 | Clinical, histological, and immunological characteristics at the time of antibody-mediated rejection diagnosis according to contemporaneous donor-specific anti-HLA antibody C1q status

	Overall $N = 139$	C1q-negative $N = 48$	C1q-positive $N = 91$	*P* value
MFI, mean (SD, range)	8660 (5610, 1311–23421)	2821 (1370, 1311–9127)	11741 (4414, 4204–23421)	<0.001

DSA, donor-specific antibody; GFR, glomerular filtration rate; HLA, human leukocyte antigen; MFI, mean fluorescence intensity.



No. at risk				
C1q-negative	101	91	75	58
C1q-positive	38	25	15	8



No. at risk				
C1q-/C1q-	43	39	34	27
C1q+/C1q-	58	52	41	31
C1q+/C1q+	33	22	13	7
C1q-/C1q+	5	3	2	1

Role of C1q-binding anti-HLA antibodies as a predictor of lung allograft outcome

Olivier Brugière[1], Antoine Roux[2,8], Jerome Le Pavec[3,8], Deborah Sroussi[1], François Parquin[2], Pauline Pradère[3], Clairelyne Dupin[1], Vincent Bunel[1], Gisele Mourin[1], Gilles Jebrak[1], Gaëlle Dauriat[1], Yves Castier[4], Pierre Mordant[4], Brice Lortat-Jacob[5], Sylvain Jean-Baptiste[5], Herve Mal[1], Caroline Suberbielle[6], Chantal Gautreau[6], Sophie Caillat-Zucman[6], Aurélie Cazes[7], Gabriel Thabut[1,9] and Jean-Luc Taupin[6,9]

Eur Respir J 2018; 52: 1701898

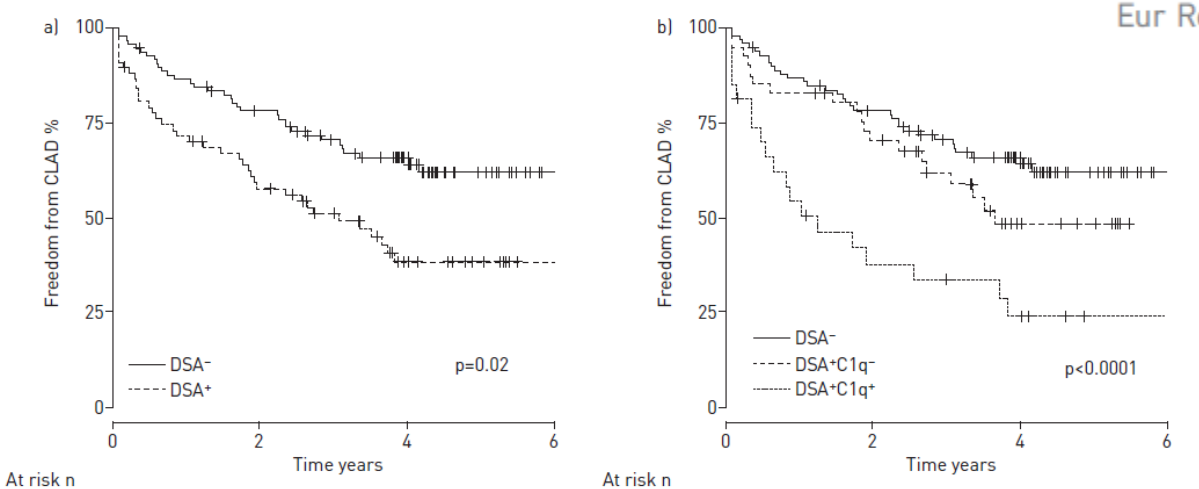


FIGURE 2 Kaplan–Meier curves for freedom from chronic allograft dysfunction (CLAD) according to donor-specific anti-human leukocyte antigen antibody (DSA) status after transplantation. Probability of freedom from CLAD a) by presence or absence of DSA detected by classical historical Luminex single-antigen flow bead assay; b) by presence or absence of DSAs and their C1q-binding capacity. Survival curves are marked by a vertical tick at each censoring time

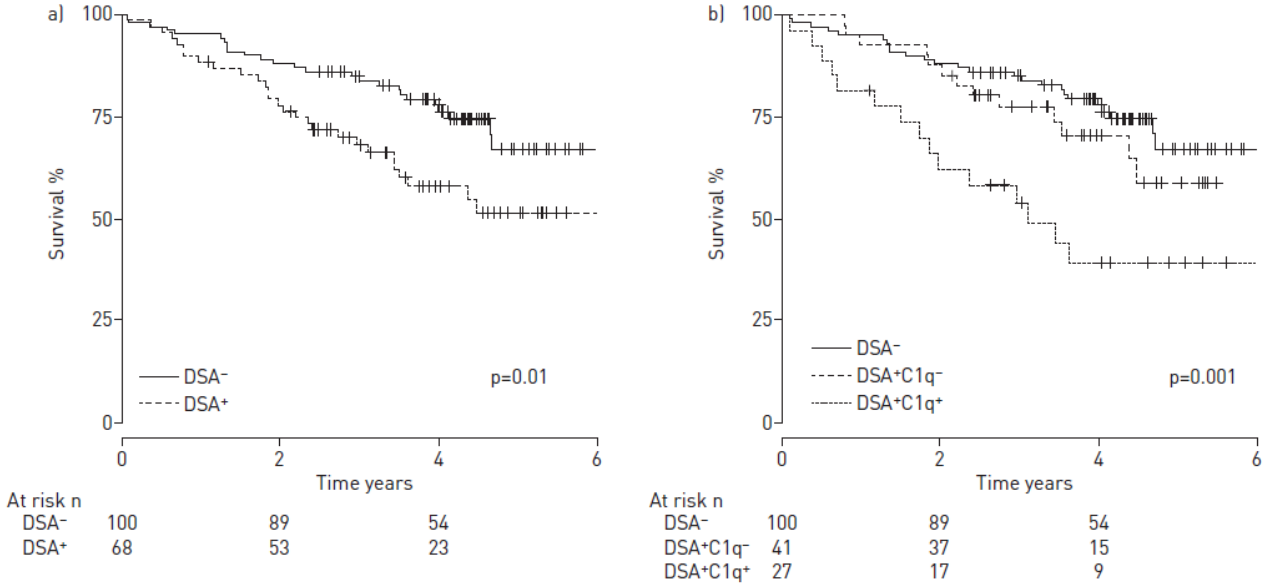
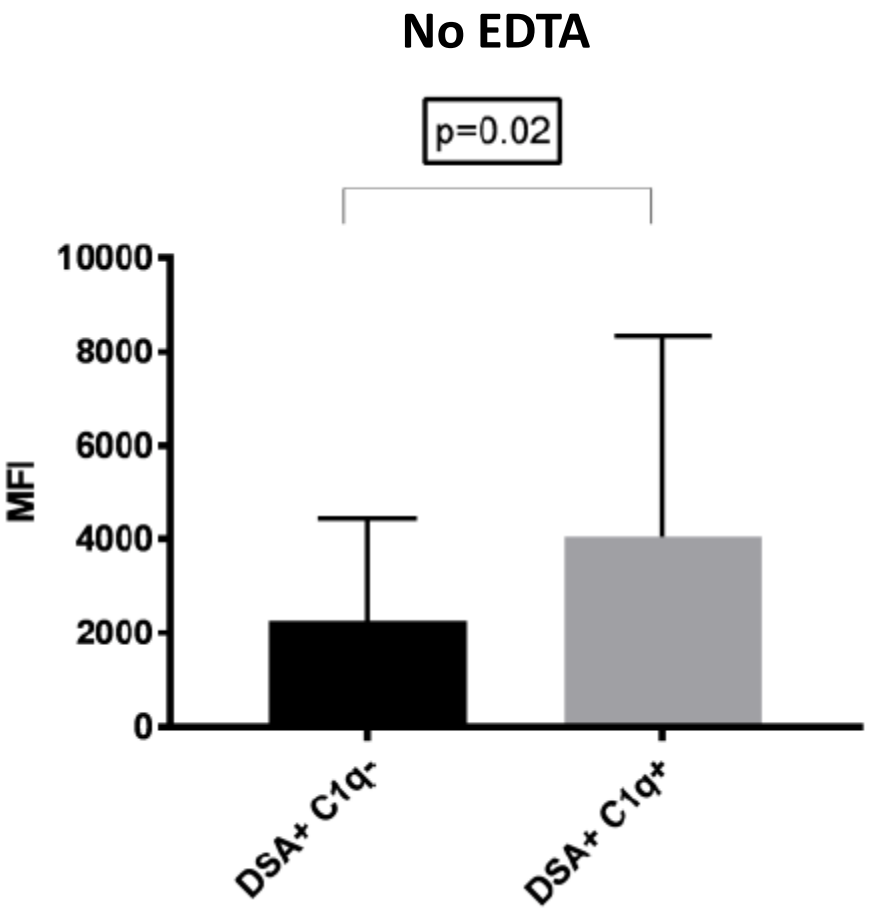
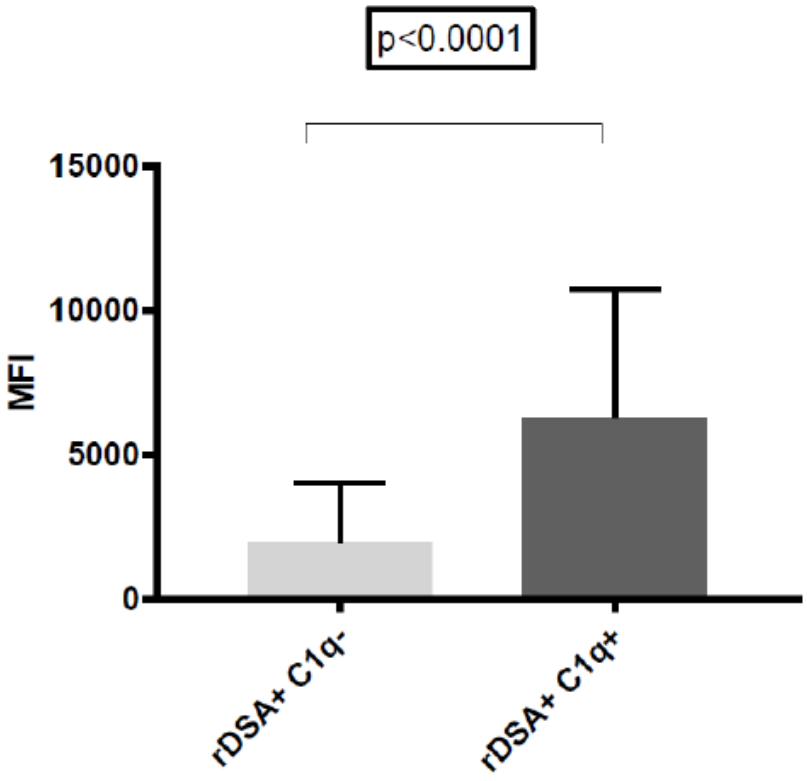


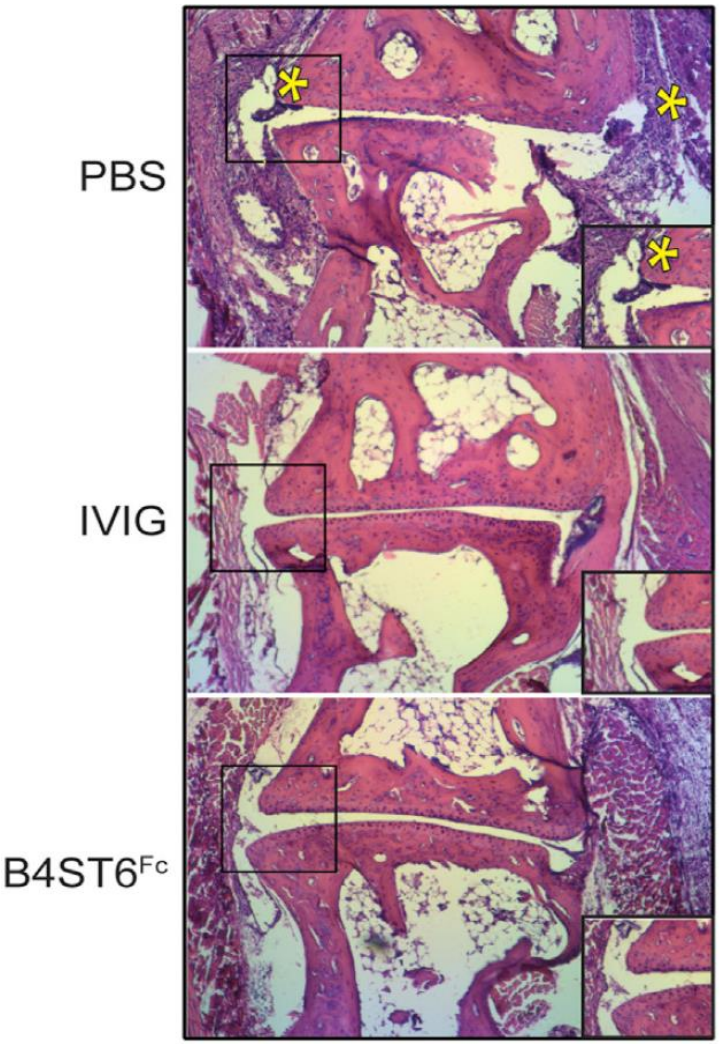
FIGURE 3 Kaplan–Meier curves for graft survival according to donor-specific anti-human leukocyte antigen antibody (DSA) status after transplantation. Probability of graft survival a) by presence or absence of DSA detected using historical Luminex single-antigen flow bead assay; b) by presence or absence of DSAs and their C1q-binding capacity. Survival curves are marked by a vertical tick at each censoring time

Eur Respir J 2018; 52: 1701898

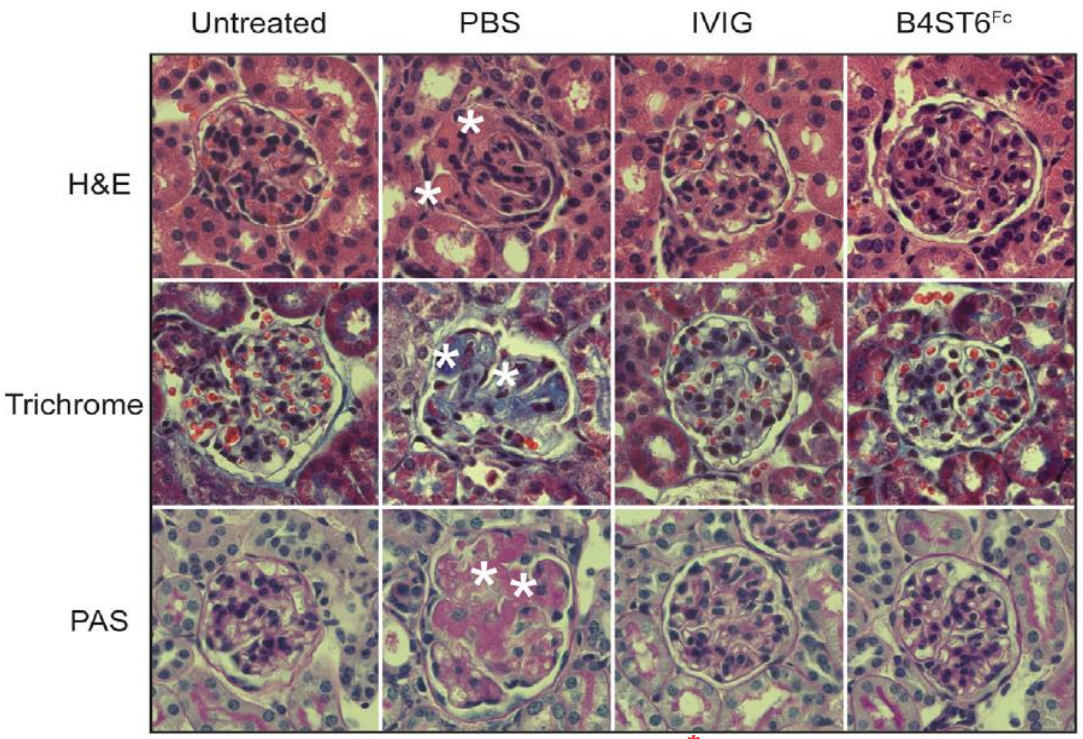


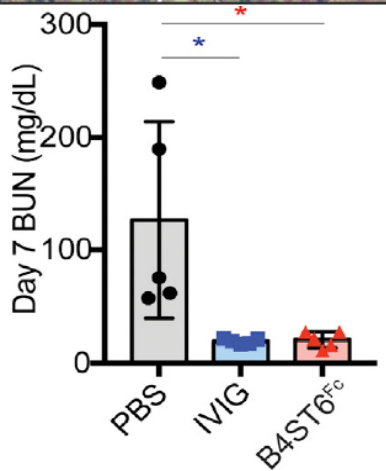


Arthritogenic Murine Model

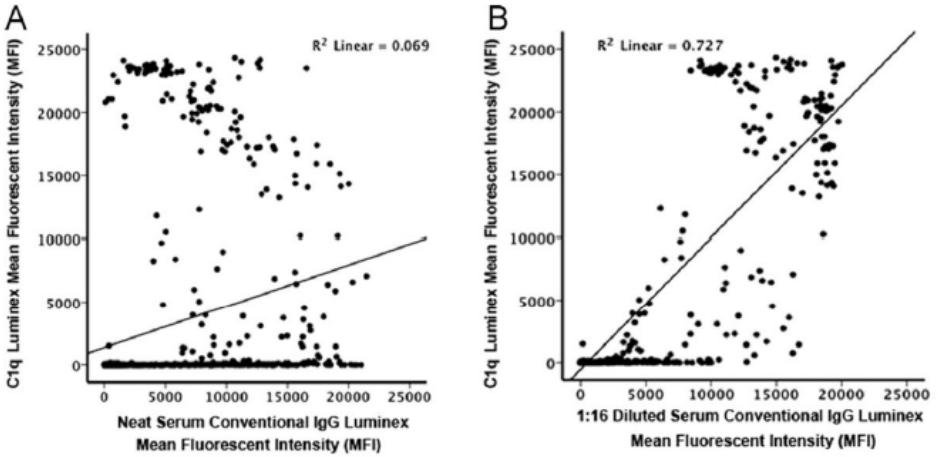


Nerphotoxic Nephritis in Murine Goodpasture Disease Model





https://www.bing.com/videos/search?q=rube+goldberg+honda+commercial&&view=detail&mid=18806D1349E70E48E5BE18806D1349E70E48E5BE&&FORM=VDRVRV



	Conventional IgG Luminex MFI (Neat)	
C1q MFI	≥ 8000	<8000
≥500	91	74
<500	134	574

	Conventional IgG Luminex MFI (1:16 Dilution)	
C1q MFI	≥ 8000	<8000
≥500	140	25
<500	13	695

Figure 1.

Correlation of the HLA Class I antibody single-antigen bead (SAB) mean fluorescent intensity (MFI) results of 873 beads from 9 individual sera assessed by conventional IgG and C1q testing. (A) Comparison of SAB results using undiluted sera vs C1q results. The HLA alleles were segregated using cutoff values of 8,000 MFI in the conventional IgG SAB assay using undiluted sera and 500 MFI for C1q testing. This was used to generate positive and negative predictive values. (B) Comparison of SAB results using sera diluted 1:16 vs C1q results. The HLA alleles were segregated using cutoff values of 8,000 MFI in the conventional IgG SAB assay using 1:16 diluted sera and 500 MFI for the C1q testing for use in generating positive and negative predictive values.

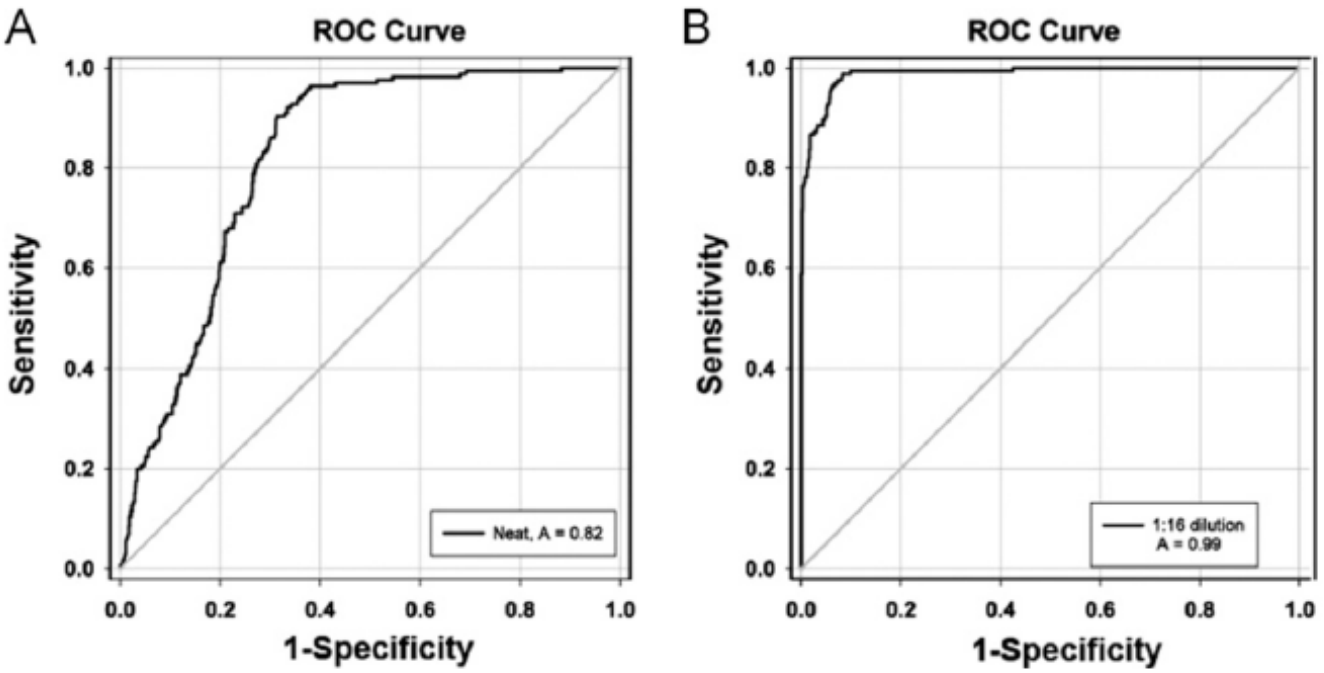
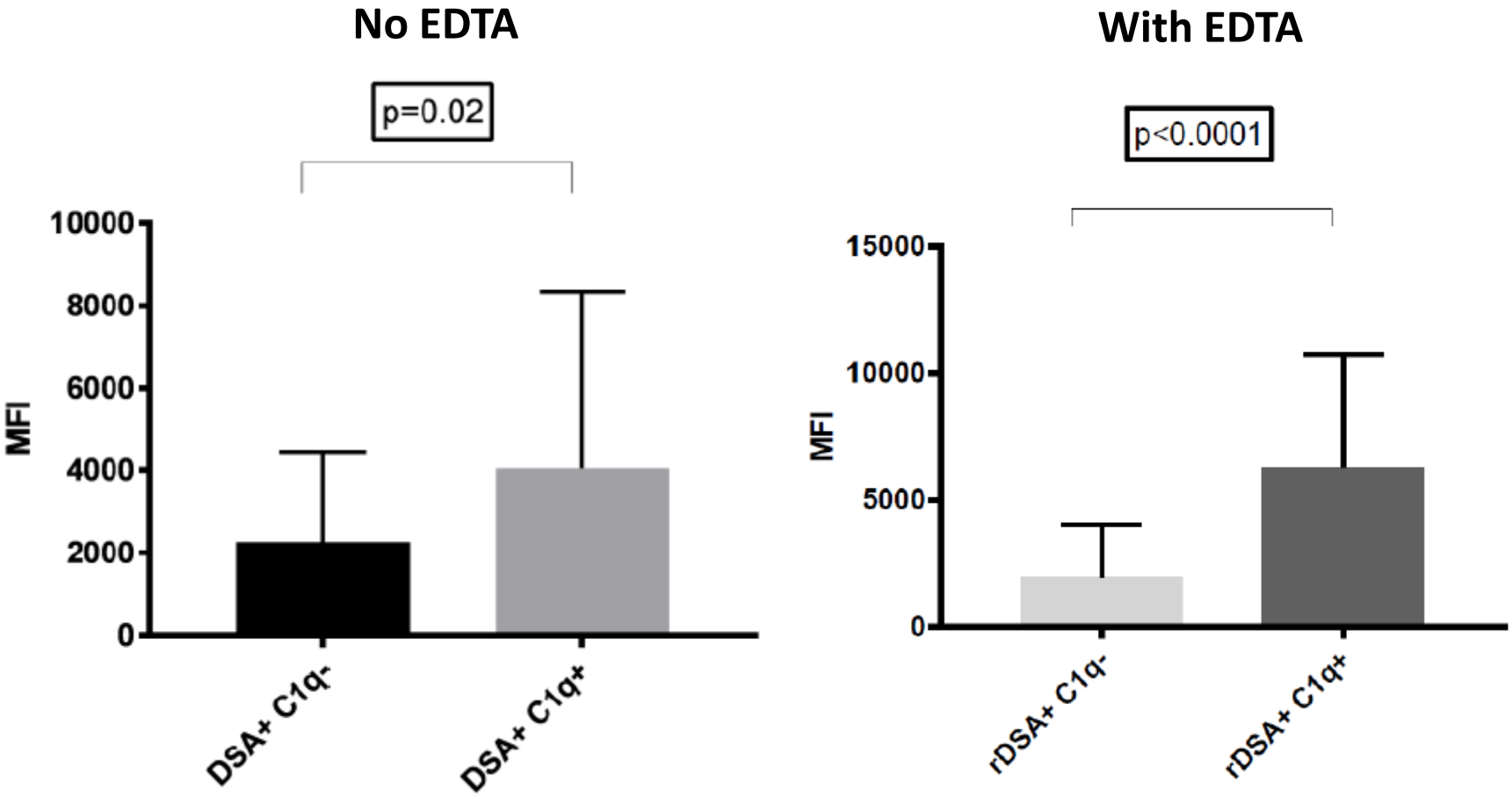
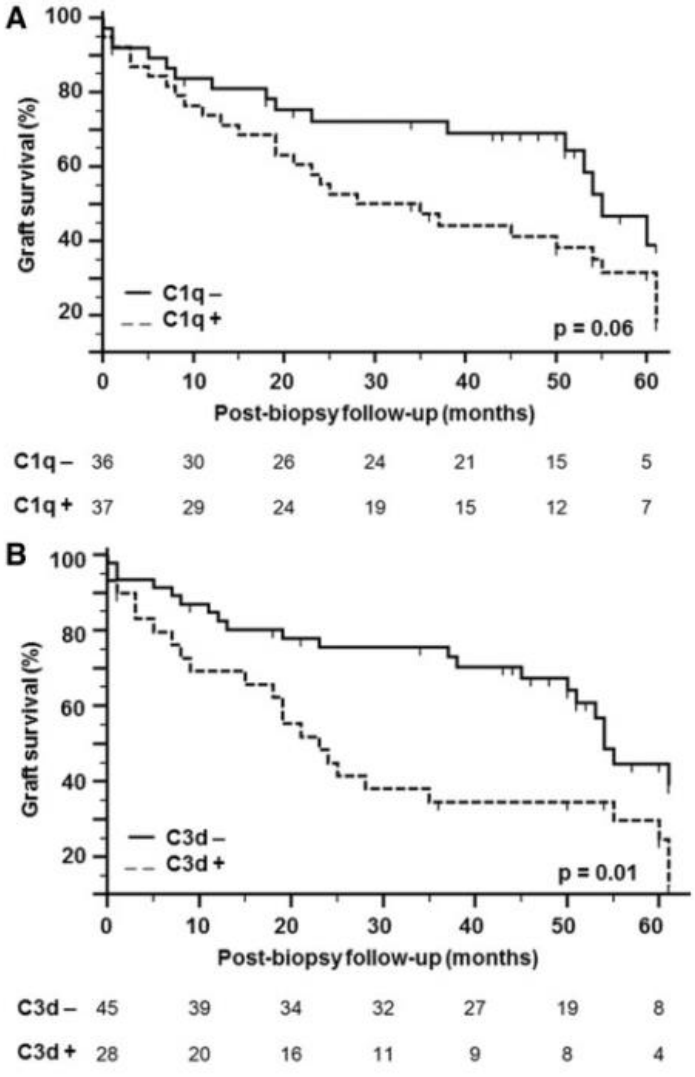


Figure 2.

Receiver–operator characteristic curve analysis examining the ability of a mean fluorescent intensity (MFI) value from conventional single-antigen bead testing using (A) undiluted sera or (B) sera diluted 1:16 to predict a positive C1q test value (MFI >500). A better area under the curve (AUC) was found using the 1:16 diluted sera (AUC = 0.988) compared with the undiluted sera (AUC = 0.821).

Eur Respir J 2018; 52: 1701898





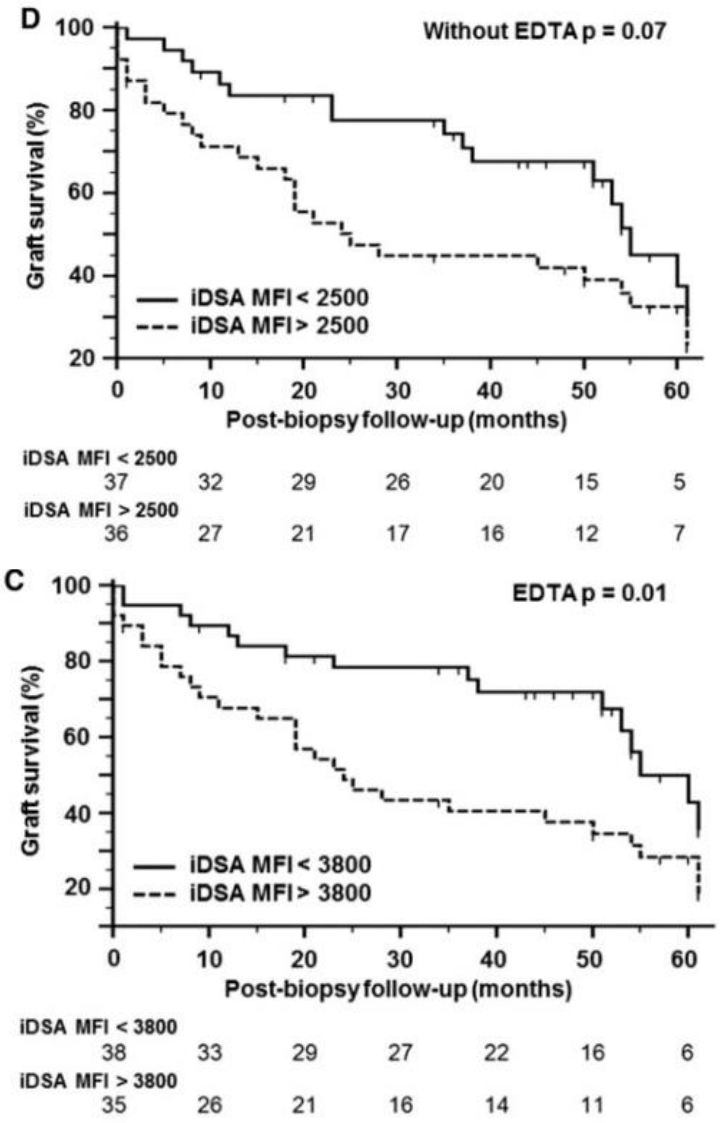


Table 6. Multivariate analyses of factors associated with death-censored graft loss using a Cox proportional hazards regression model

Model	Acute ABMR			Chronic renal injury		
	Variable	HR (95% CI)	P-value	Variable	HR (95% CI)	P-value
C3d	C3d+	1.01 (0.51–1.98)	0.98	C3d+	1.60 (0.83–3.12)	0.16
	eGFR[a]	**0.95 (0.92–0.97)**	**<0.01**	**eGFR[a]**	**0.95 (0.93–0.98)**	**<0.01**
	ptc + g ≥ 2	1.64 (0.83–3.14)	0.15	**cg>0**	**2.40 (1.25–4.60)**	**<0.01**
	C4d staining	**2.13 (1.08–4.23)**	**0.03**	ci + ci[b]	1.20 (0.98–1.46)	0.07
iDSA MFI EDTA	iDSA MFI EDTA > 3800[c]	0.92 (0.43–2.01)	0.84	iDSA MFI EDTA >3800[c]	1.58 (0.85–3.04)	0.15
	eGFR[a]	**0.95 (0.92–0.97)**	**<0.01**	**eGFR[a]**	**0.95 (0.93–0.98)**	**<0.01**
	ptc + g ≥ 2	1.66 (0.85–3.15)	0.14	**cg>0**	**2.65 (1.38–5.13)**	**<0.01**
	C4d staining	**2.22 (1.06–4.84)**	**0.03**	ci + ci[b]	1.12 (0.93–1.35)	0.24

Statistically significant results are depicted in bold parameters.
[a]Each additional mL/min/1.73 m².
[b]Each additional point.
[c]Median iDSA IgG MFI value with EDTA treatment of sera.

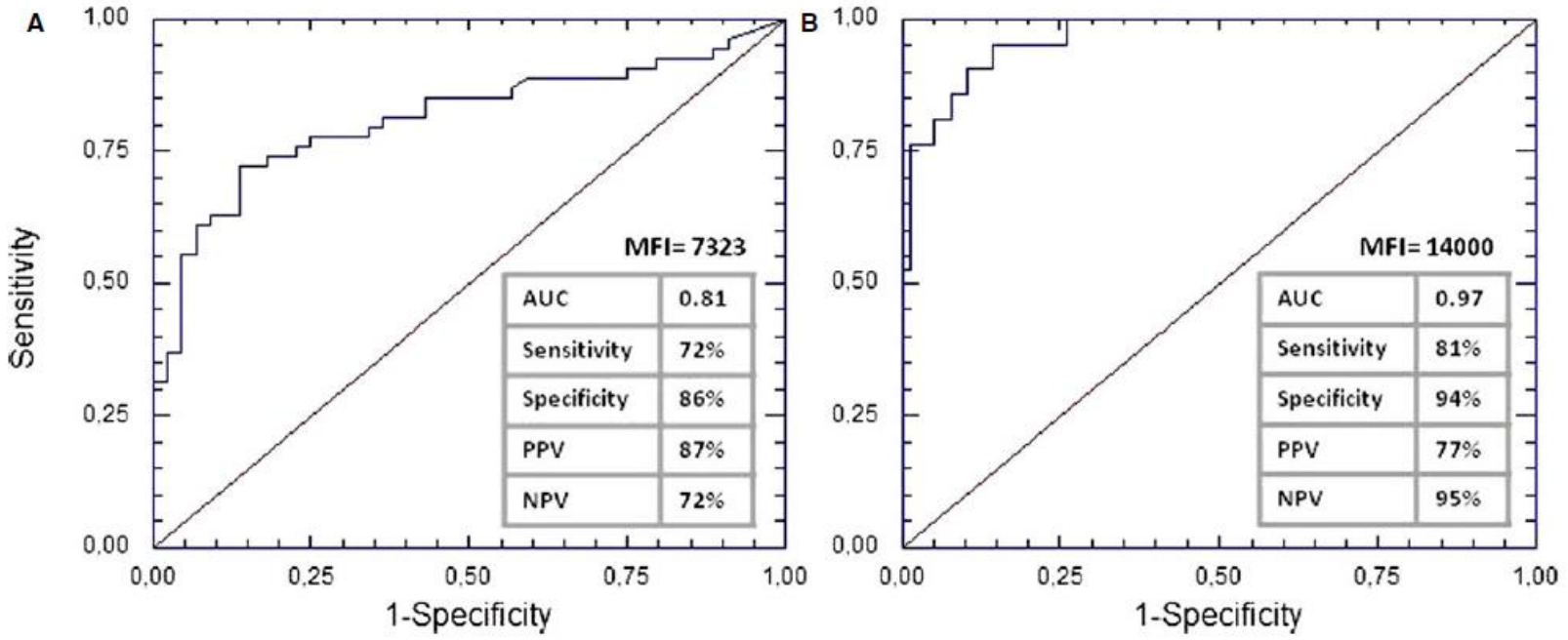


Figure 2: Complement-binding activity prediction by mean fluorescence intensity (MFI) values of *de novo* donor-specific antibodies (*dn*DSAs). Predictive values of *dn*DSA MFIs for C1q binding (A) and C3d binding (B) by receiver operating characteristic (ROC) analysis. AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

REVIEW SERIES: TRANSPLANTATION
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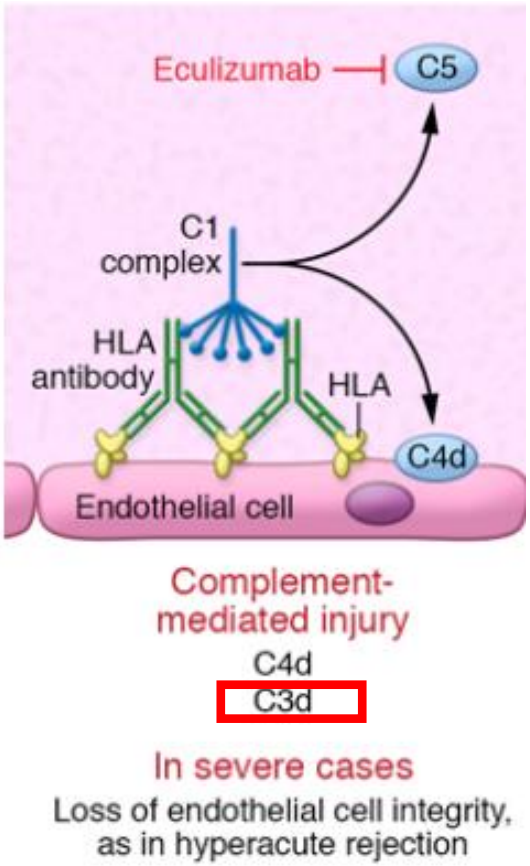
The Journal of Clinical Investigation

Antibody-mediated rejection across solid organ transplants: manifestations, mechanisms, and therapies

Nicole M. Valenzuela and Elaine F. Reed

UCLA Immunogenetics Center, Department of Pathology and Laboratory Medicine, UCLA, Los Angeles, California, USA.



J. Clin Invest 127:2492-2504, 2017

CONCLUSIONS

1. Multiple articles report a strong association between DSAs that bind complement (in solid phase assays detecting C1q, C3d, C4d) and graft loss/graft dysfunction.

2. Majority of published literature indicates that MFI levels of DSA correspond with their ability to fix complement.

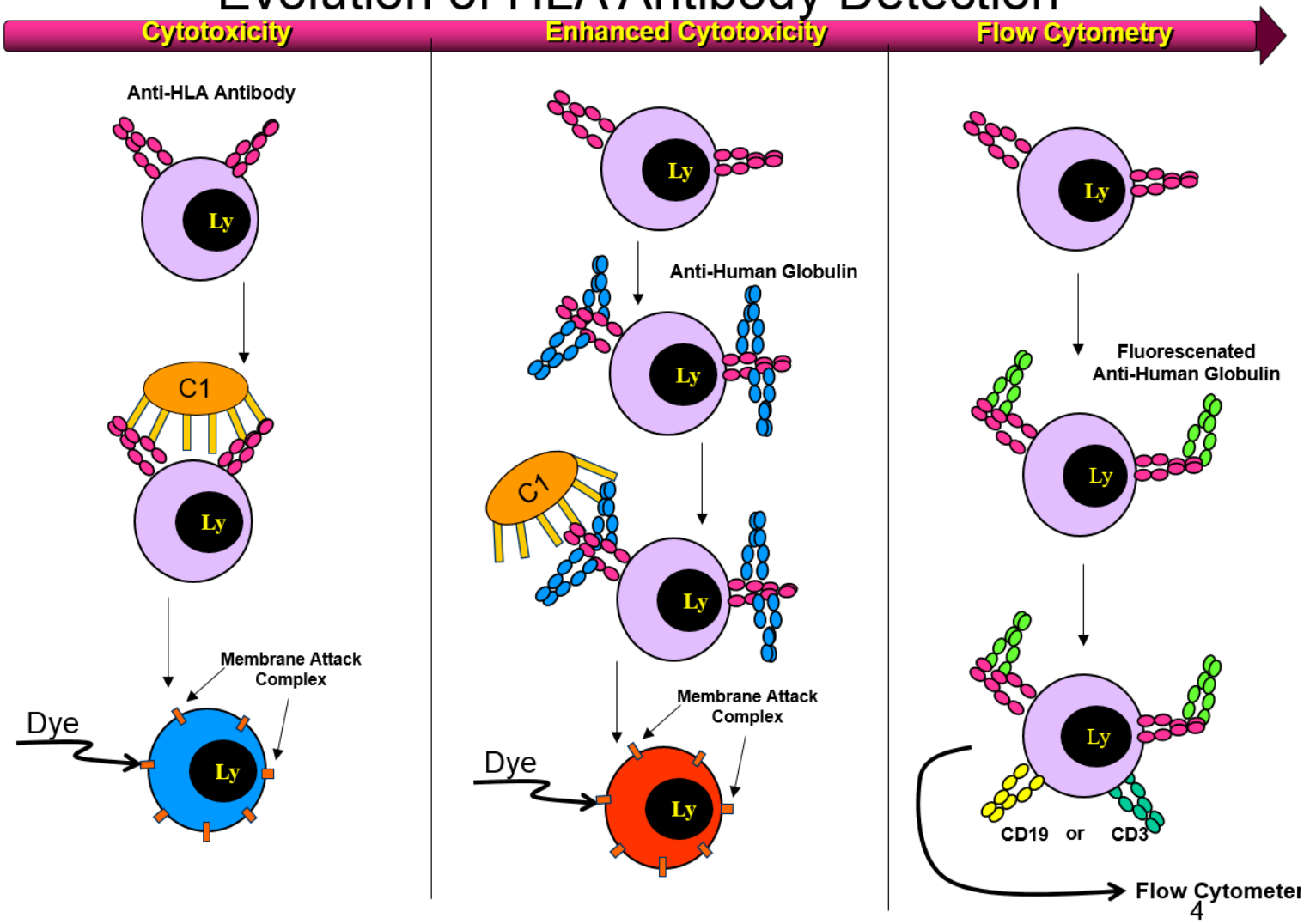
3. In vitro complement binding does not equal in vivo complement binding.

4. DSAs can mediate graft damage by complement independent mechanisms

5. Evidence is insufficient to warrant routine (and costly) testing to determine whether HLA antibodies fix complement.

6. Studies focused on stratgeies…

Evolution of HLA Antibody Detection



Bray et al. Immunol. Res 3:41-53,2004

Persistent strong anti-HLA antibody at high titer is complement binding and associated with increased risk of antibody-mediated rejection in heart transplant recipients

Adriana Zeevi, PhD[a], **John Lunz, PhD**[a], **Brian Feingold, MD, MS**[b], **Michael Shullo, PharmD**[d], **Christian Bermudez, MD**[c], **Jeffery Teuteberg, MD**[c], and **Steven Webber, MBChB**[b]

[a]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

[b]Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

[c]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

[d]University of Pittsburgh School of Pharmacy, Pittsburgh, Pennsylvania

J Heart Lung Transplant. 2013 January ; 32(1): 98–105.

Role of C1q-binding anti-HLA antibodies as a predictor of lung allograft outcome

Olivier Brugière[1], Antoine Roux[2,8], Jerome Le Pavec[3,8], Deborah Sroussi[1], François Parquin[2], Pauline Pradère[3], Clairelyne Dupin[1], Vincent Bunel[1], Gisele Mourin[1], Gilles Jebrak[1], Gaëlle Dauriat[1], Yves Castier[4], Pierre Mordant[4], Brice Lortat-Jacob[5], Sylvain Jean-Baptiste[5], Herve Mal[1], Caroline Suberbielle[6], Chantal Gautreau[6], Sophie Caillat-Zucman[6], Aurélie Cazes[7], Gabriel Thabut[1,9] and Jean-Luc Taupin[6,9]

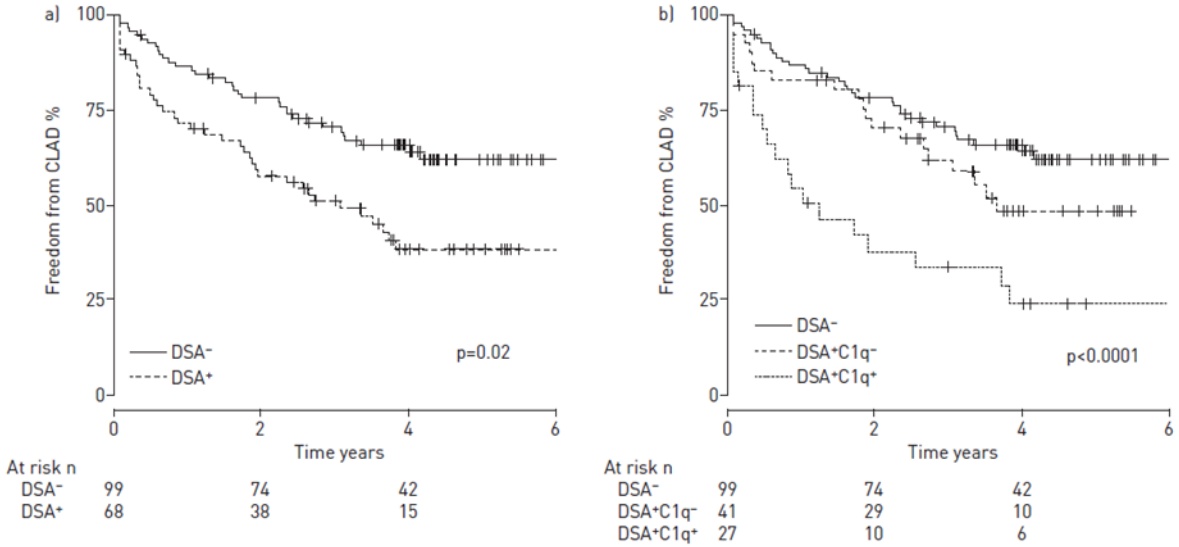


FIGURE 2 Kaplan–Meier curves for freedom from chronic allograft dysfunction (CLAD) according to donor-specific anti-human leukocyte antigen antibody (DSA) status after transplantation. Probability of freedom from CLAD a) by presence or absence of DSA detected by classical historical Luminex single-antigen flow bead assay; b) by presence or absence of DSAs and their C1q-binding capacity. Survival curves are marked by a vertical tick at each censoring time

Eur Respir J 2018; 52: 1701898

Acquisition of C3d-Binding Activity by *De Novo* Donor-Specific HLA Antibodies Correlates With Graft Loss in Nonsensitized Pediatric Kidney Recipients

American Journal of Transplantation 2016; 16: 2106–2116

P. Comoli[1,†], M. Cioni[2,†], A. Tagliamacco[3], G. Quartuccio[1], A. Innocente[4], I. Fontana[5], A. Trivelli[2], A. Magnasco[2], A. Nocco[4], C. Klersy[6], L. Rubert[1], M. Ramondetta[4], M. Zecca[1], G. Garibotto[3], G. M. Ghiggeri[2], M. Cardillo[4], A. Nocera[3,†] and F. Ginevri[2,*,†]

All samples pre-treated with EDTA

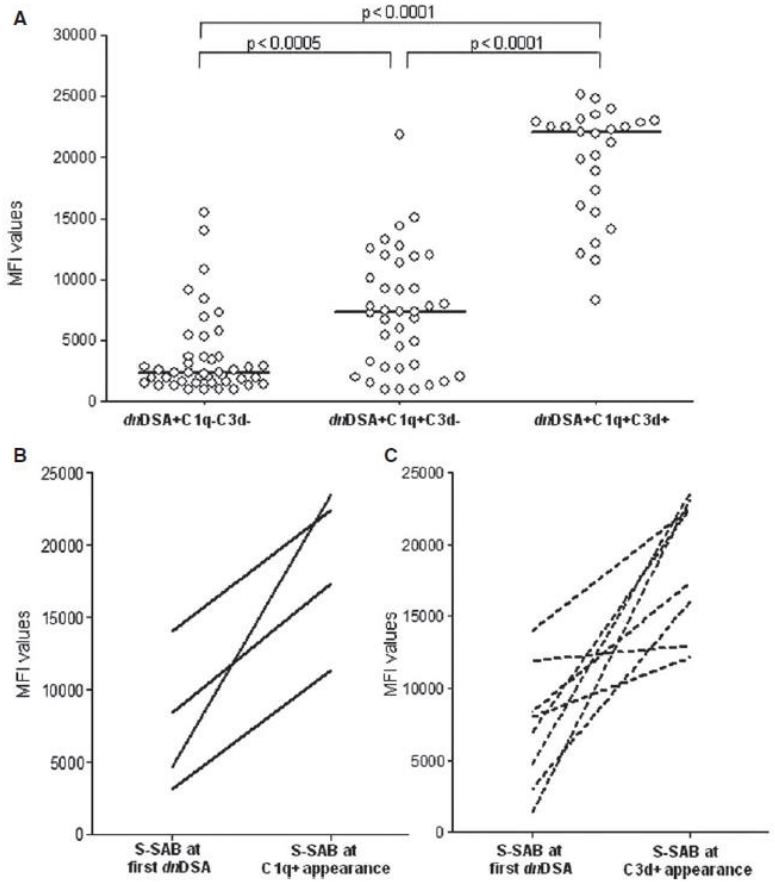


Figure 1: Relation between MFI and complement-binding activity of de novo DSA.

Table 2: Risk of AMR development according to occurrence and characteristics of *dn*DSAs at first appearance (univariable analysis)

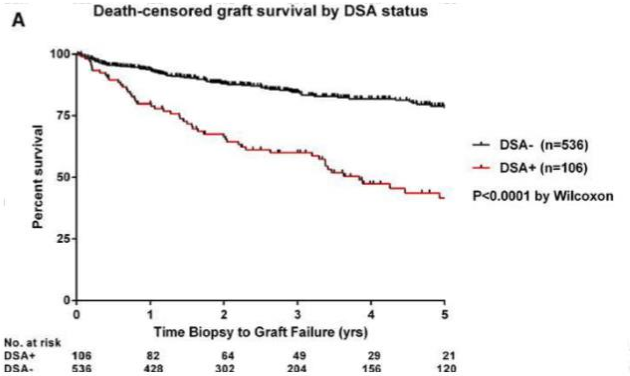
	Total patients (n)	Patients with AMR (n)	HR[1]	95% CI	p-value
*dn*DSAs					
*dn*DSA⁻	75	0			
*dn*DSA⁺	39	21	NE		<0.001
C1q-binding *dn*DSAs					
No	89	5			
Yes	25	16	13.54	4.95–36.99	<0.001
C3d-binding *dn*DSAs					
No	105	14			
Yes	9	7	6.91	2.78–17.18	<0.001
*dn*DSA MFI					
<7323	16	7			
>7323	23	14	11.27	4.51–28.17	<0.001
*dn*DSA MFI					
<14 000	30	15			
>14 000	9	6	7.51	2.86–19.70	<0.005

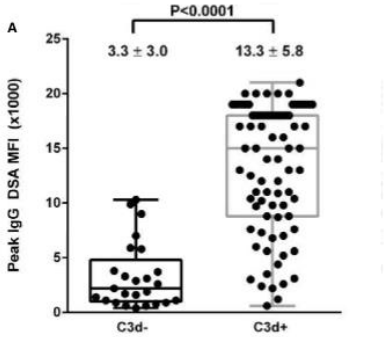
AMR, antibody-mediated rejection; CI, confidence interval; *dn*DSA, *de novo* donor-specific antibody; HR, hazard ratio; MFI, mean fluorescence intensity; NE, not evaluable.
[1]HR and 95% CI, quantified by Cox proportional hazards models, were computed. All variables included are time dependent.

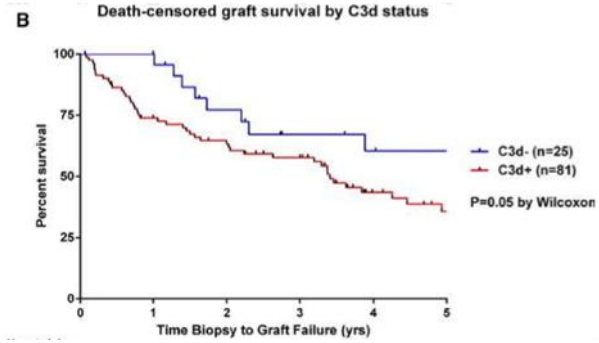
Clinical utility of complement-dependent C3d assay in kidney recipients presenting with late allograft dysfunction

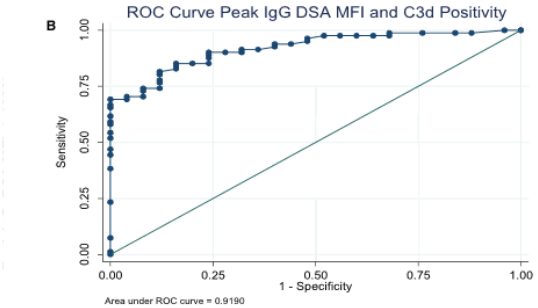
James H. Lan[1] | David Gjertson[2] | Ying Zheng[2] | Stephanie Clark[1] | DeKAF Investigators[†] | Elaine F. Reed[2] | Michael J. Cecka[2,†]

Am J Transplant. 2018;18:2934–2944.



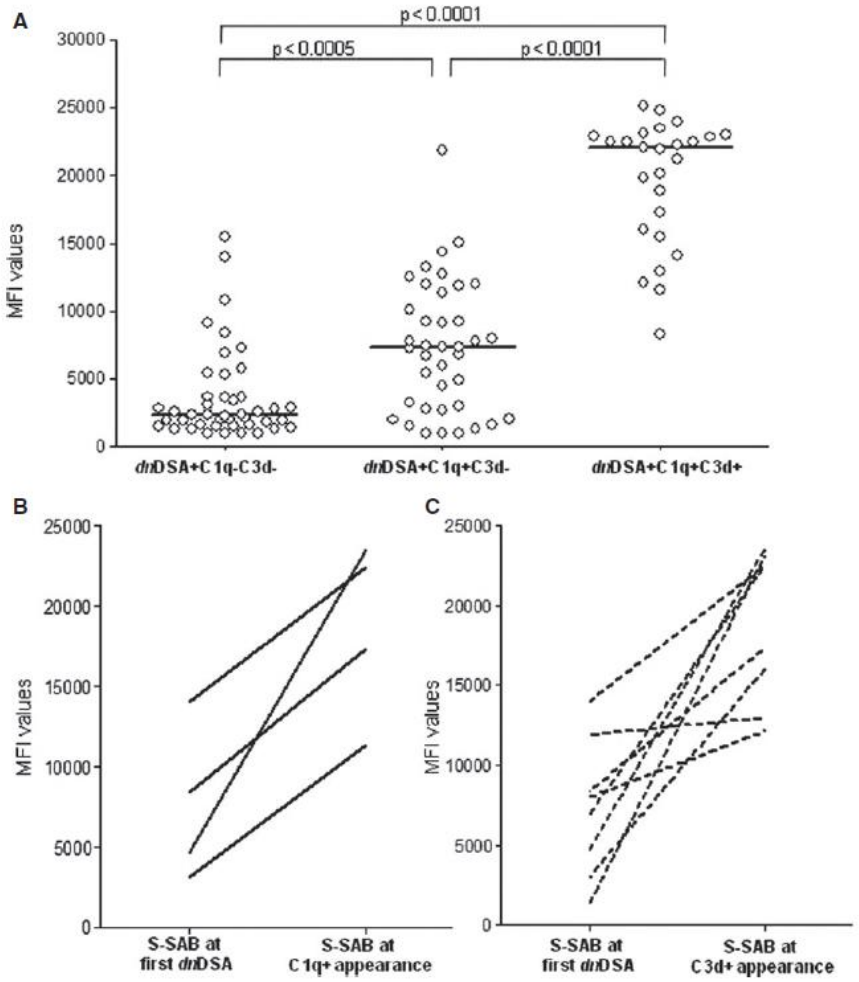






"…these findings suggest that the C3d test provides little discriminatory value, nor is it cost effective, in the risk stratification of strong antibodies where the conventional MFI strength already exceeds the predicted threshold for a positive signal on the assay."

All samples pre-treated with EDTA



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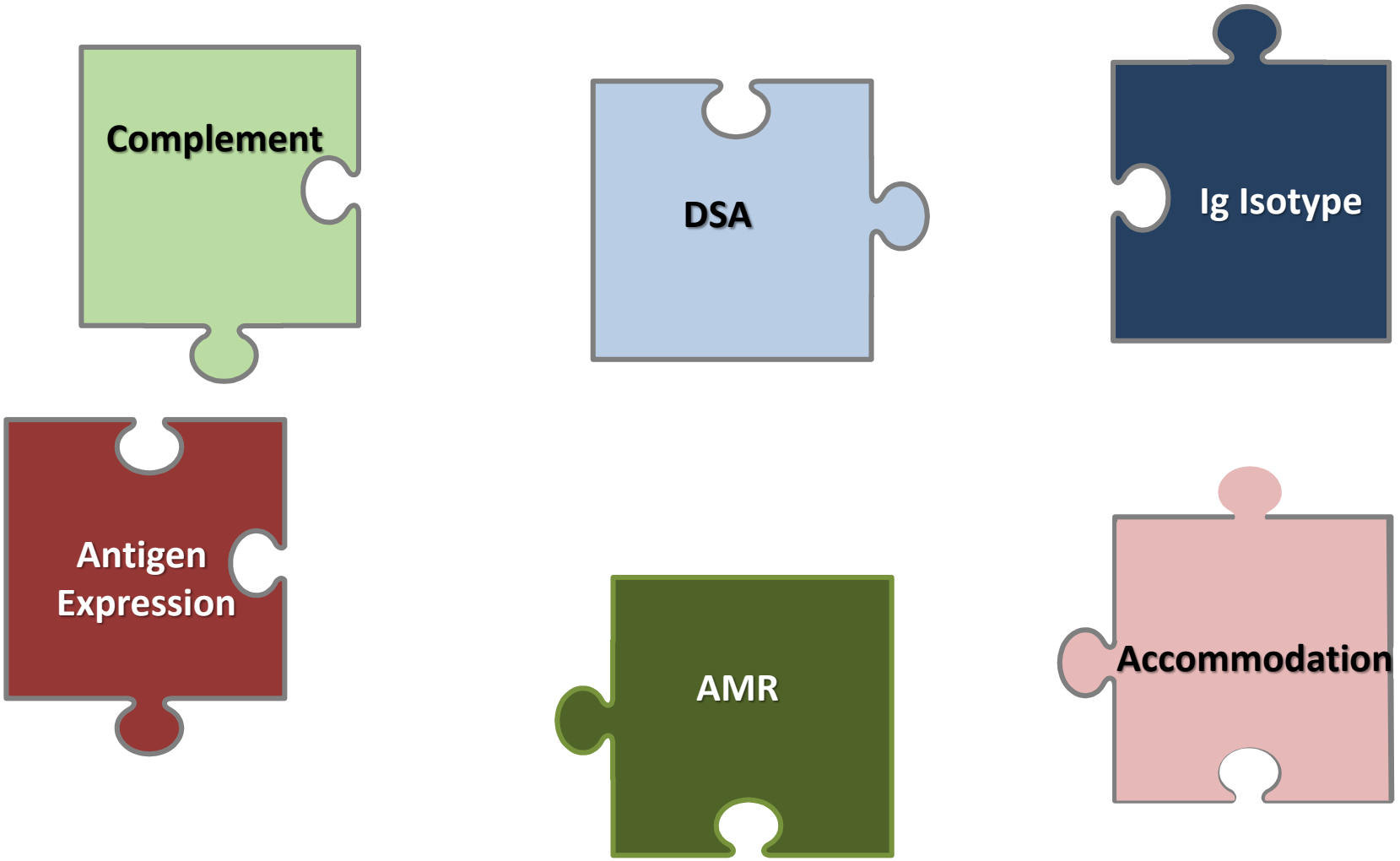
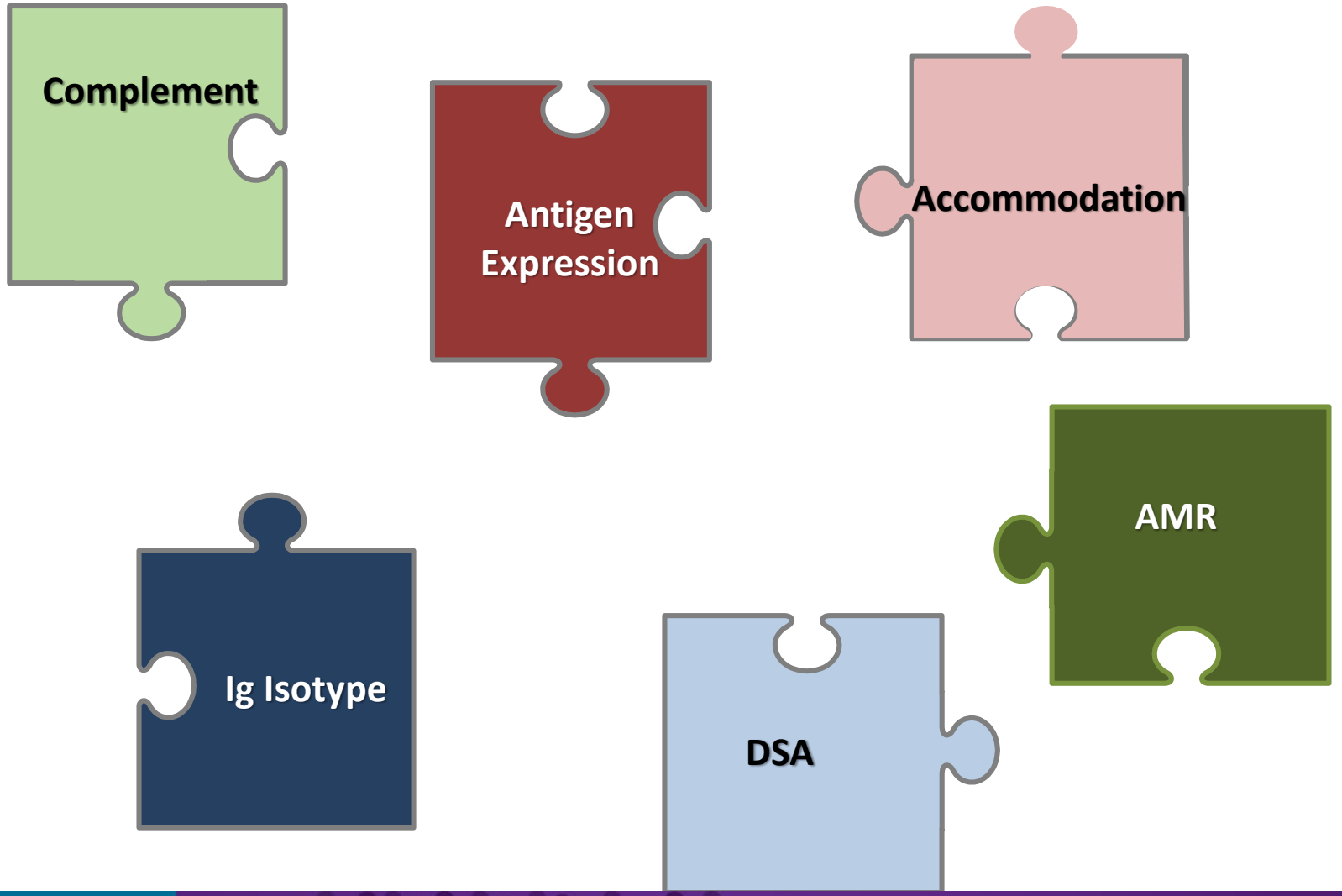
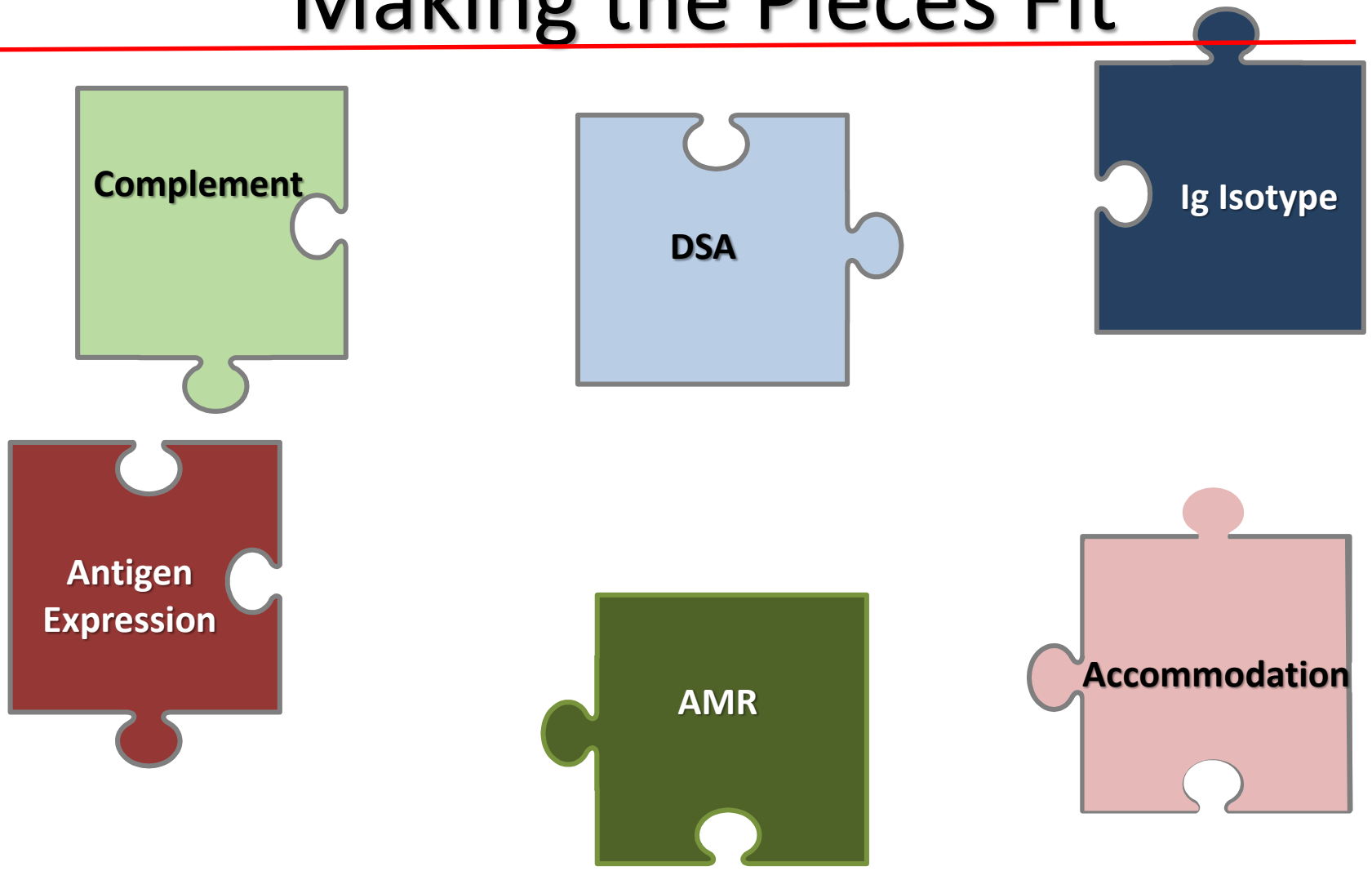
Pieces of the Puzzle



Pieces of the Puzzle



Making the Pieces Fit



RESEARCH ARTICLE

Complement-activating donor-specific anti-HLA antibodies and solid organ transplant survival: A systematic review and meta-analysis

Antoine Bouquegneau[1,2☯], Charlotte Loheac[1☯], Olivier Aubert[1,3☯], Yassine Bouatou[1,4], Denis Viglietti[1,5], Jean–Philippe Empana[1], Camilo Ulloa[6], Mohammad Hassan Murad[7], Christophe Legendre[1,3], Denis Glotz[1,5], Annette M. Jackson[8], Adriana Zeevi[9], Stephan Schaub[10], Jean–Luc Taupin[11], Elaine F. Reed[12], John J. Friedewald[13], Dolly B. Tyan[14], Caner Süsal[15], Ron Shapiro[16], E. Steve Woodle[17], Luis G. Hidalgo[18], Jacqueline O'Leary[19], Robert A. Montgomery[20], Jon Kobashigawa[21], Xavier Jouven[1,22], Patricia Jabre[1,23,24,25☯], Carmen Lefaucheur[1,5☯*], Alexandre Loupy[1,3☯*]

PLOS Medicine | https://doi.org/10.1371/journal.pmed.1002572 May 25, 2018

Conclusions

In this study, we found that circulating complement-activating anti-HLA DSAs had a significant deleterious impact on solid organ transplant survival and risk of rejection. The detection of complement-activating anti-HLA DSAs may add value at an individual patient level for noninvasive biomarker-guided risk stratification.