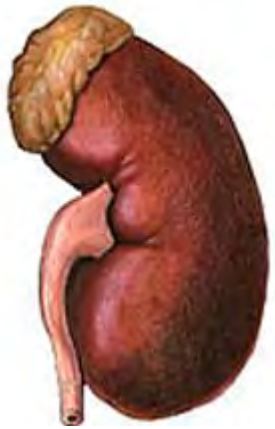
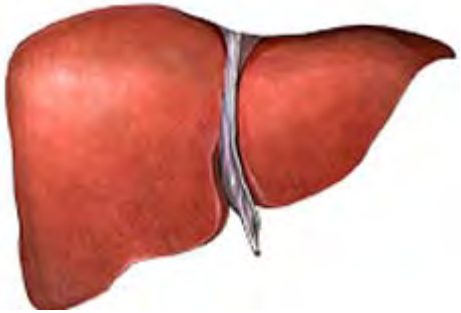


Clinical Implications of HLA Antibodies in Solid Organ Transplantation



Amy Hahn, PhD, D(ABHI)
Albany Medical College
Albany, NY

In the beginning…

Lancet. 1966;2:662-5. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O.

Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells.

- 21 transplants, 2 with hyperacute rejection, both had transfusions and pregnancies
- both had extremely strong IgG leucocyte agglutinins with titers above 1/512

"…*humoral antibodies* active against antigens in the graft may have a decisive effect on the fate of the graft."

N Engl J Med. 1969;280:735-9. Patel R, Terasaki PI.

Significance of the positive crossmatch test in kidney transplantation.

- 225 transplants
- eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch ($p<0.001$)
- Immediate failure occurred in significantly higher numbers among patients with a higher risk of having antibodies, such as multiparous females and patients receiving secondary transplants.

"The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation."

Transplantation. 1989;48:953-8. Taylor CJ, Chapman JR, Ting A, Morris PJ.

Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and regraft outcome.

- 123 renal transplants performed in the presence of a positive cytotoxic crossmatch
  - either peak +/current + or peak +/current –
  - IgG vs IgM by DTT treatment
- IgG HLA class I Ab
  - poor survival
- IgM HLA class I Ab: peak +/current –
  - acceptable survival
- positive B cell XM due to IgM or IgG HLA antibodies
  - good primary survival but poor regraft survival
- IgM non-HLA Ab: peak +/current +
  - good primary and regraft survival

flow crossmatch
solid phase antibody testing

- much more sensitive
- detecting antibodies that previously went undetected by CDC
  - low titer
  - didn’t activate complement
- are these antibodies clinically relevant?

Detection and clinical relevance of donor specific HLA antibodies: a matter of debate

Table 1. The clinical relevance of DSA varies amongst different studies.

Reference	*N*	DSA+ (*n*)	Type	Donor	Selection	AMR (C4d+)	Graft survival
Gibney *et al.* [11] 2006	136	20	AHG-XM	DD/LD	No		↓
Patel *et al.* [12]	60	20	FCXM	LD	No	↑	↔
Gupta *et al.* [13]	121	16	CDCXM	DD	No		↓
Berg Loonen *et al.* [14]	34	13	CDCXM	DD	AM		↔
Billen *et al.* [15]	165	32	CDCXM	DD/LD	No		↓ I ↔ II
Eng *et al.* [16]	471	27	CDCXM	DD	BXM+	↑	↓
Vlad *et al.* [17]	325	27	CDCXM	DD	No	↑	↔
Amico *et al.* [18]	334	67	CDCXM	DD/LD	No	↑	↓
Wahrmann *et al.* [19]	338	39	CDCXM	DD/LD	No	↑	↓
Aubert *et al.* [20]	114	11	CDCXM	??	No	↔	↔
Phelan *et al.* [21]	64	12	CDCXM (AHG)	LD	No	↔	↔
Morris *et al.* [22]	149	15	CDC/FCXM	LD	No		↔
Lefaucheur *et al.* [23]	402	76	CDCXM	DD	No	↑	↓
Riethmüller *et al.* [24]	37	20	CDCXM	LD	Sens	↑ I ↔ II	↓
Bartel *et al.* [25]	68	51	CDCXM	DD	IA	↔	↔
Couzi *et al.* [26]	45	34	CDC/FCXM	DD	FCXM+	↑	↓
Couzi *et al.* [26]	45	11	CDC/FCXM	DD	FCXM−	↔	↔
Willicombe *et al.* [27]	480	45	CDC/FCXM	DD/LD	AL	↑	↓
Caro-Oleas *et al.* [28] 2012	892	50	CDC	DD	No	↑	↓

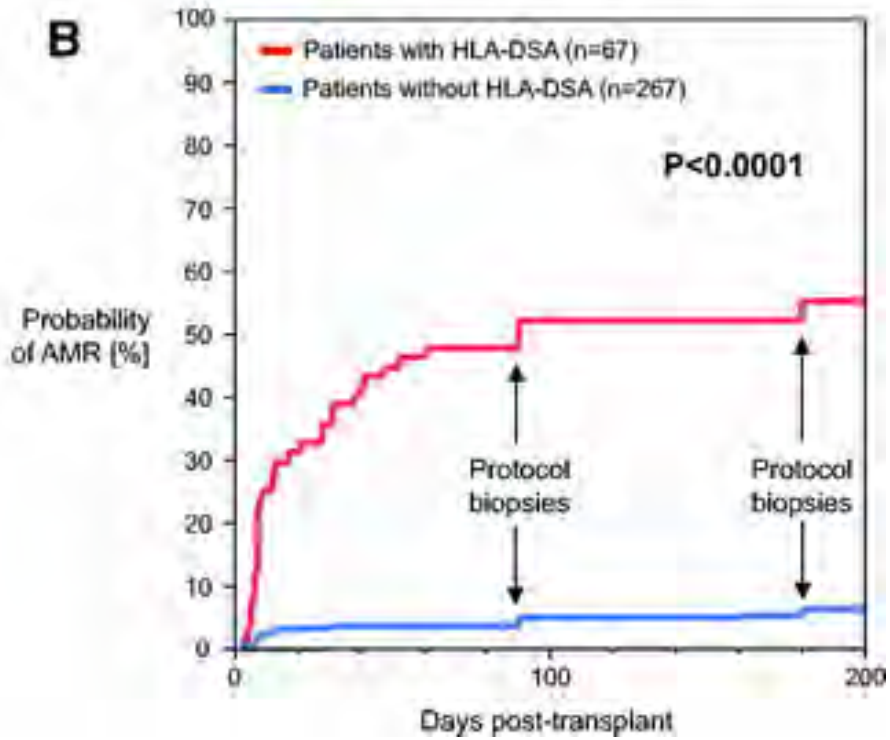
AL, Induction with alemtuzumab; AHG-XM, complement dependent cytotoxicity crossmatch in the presence of anti-human-immunoglobulin; AMR antibody-mediated rejection; BXM, B cell crossmatch; CDC, complement-dependent cytotoxicity; CDCXM, crossmatch in complement dependent cytotoxicity; DD, deceased donor; DSA, donor specific HLA antibodies; FCXM, flow cytometric crossmatch; IA, immunoadsorption; LD, living donor.
↔ No difference.
↑ or ↓ Increased or decreased.

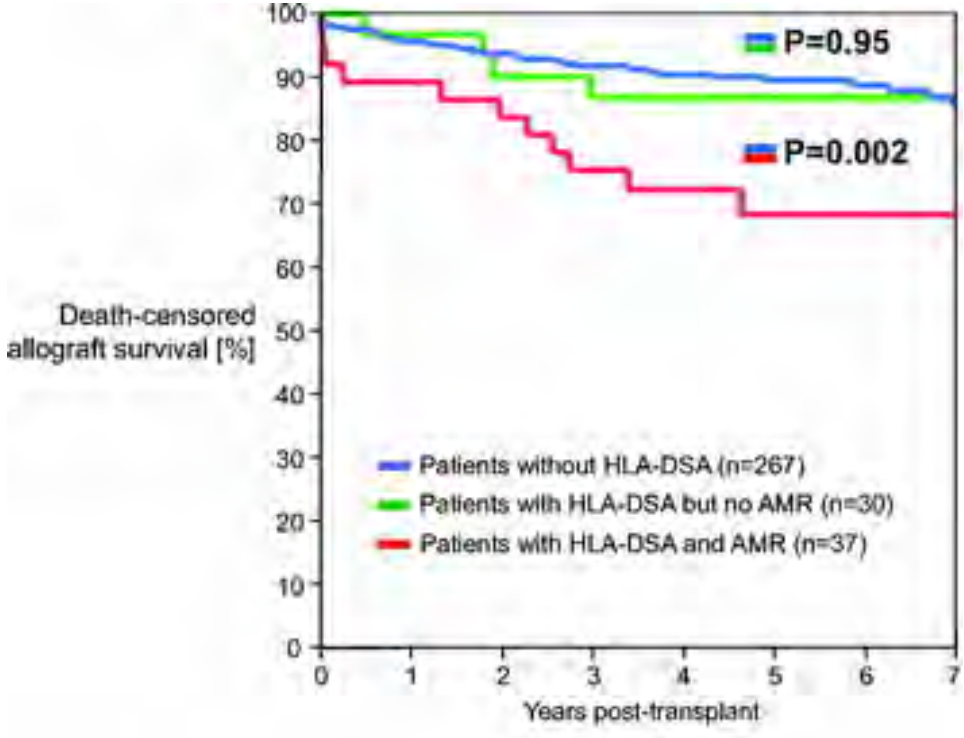
Roelen et al. Transp Int. 2012;25:604

PRE-TRANSPLANT DSA

Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads

Retrospective study of 334 kidney transplants 1999-2004
current CDC XM– (2 had remote B CDC XM+) no prospective FXM
test day of txp serum for DSA by Luminex (pos>500 MFI): 67 DSA+



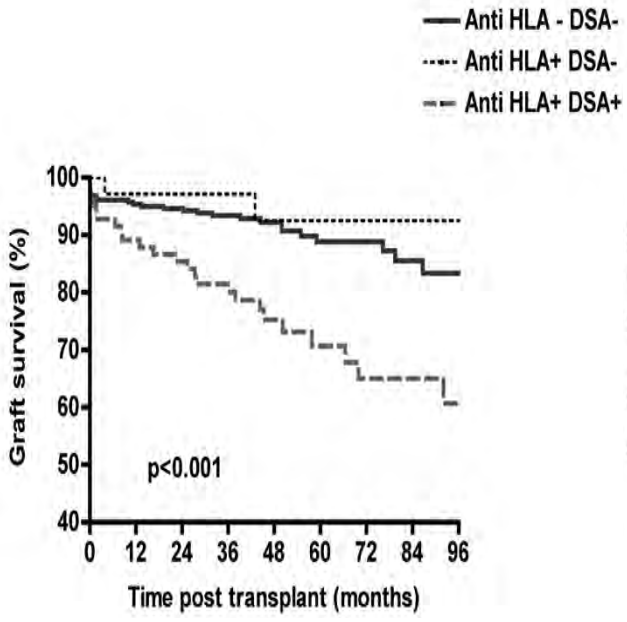


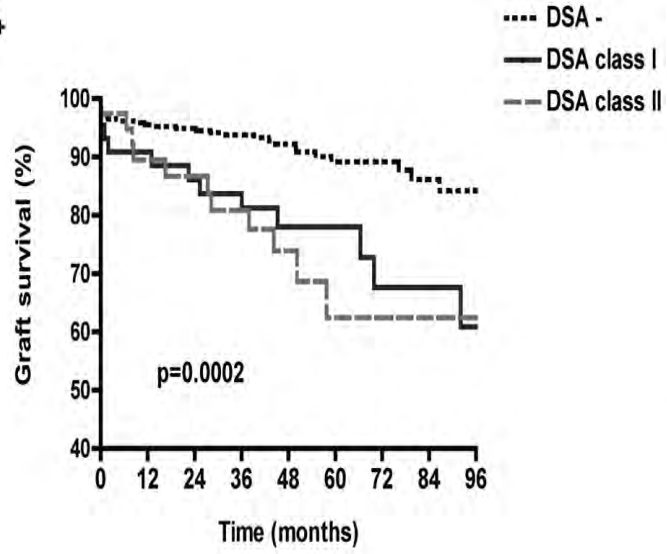
Amico, et al. Transplantation. 2009;87:1681-8

Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation

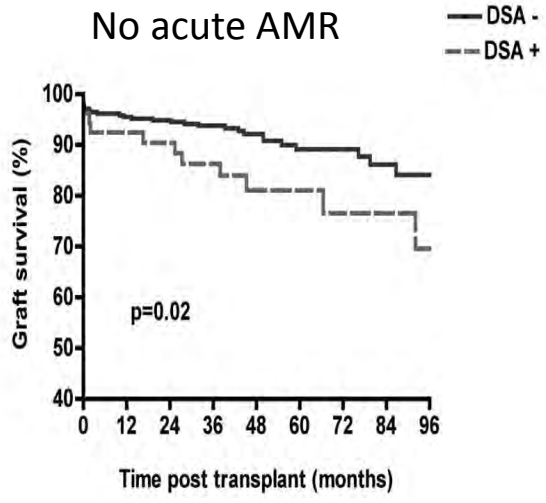
observational study of 402 deceased donor kidney recipients 1998-2006
historic: 118 I/II Ab positive, 46 DSA by ELISA, 83 DSA by SAB, 24 remote CDC XM+
current: 76 DSA by SAB, CDC XM – (DSA pos >300 MFI)

historic (peak) DSA, class I or II, is associated with decreased graft survival
even in absence of AMR





No acute AMR



Lefaucheur, et al. J Am Soc Nephrol. 2010;21:1398-406.

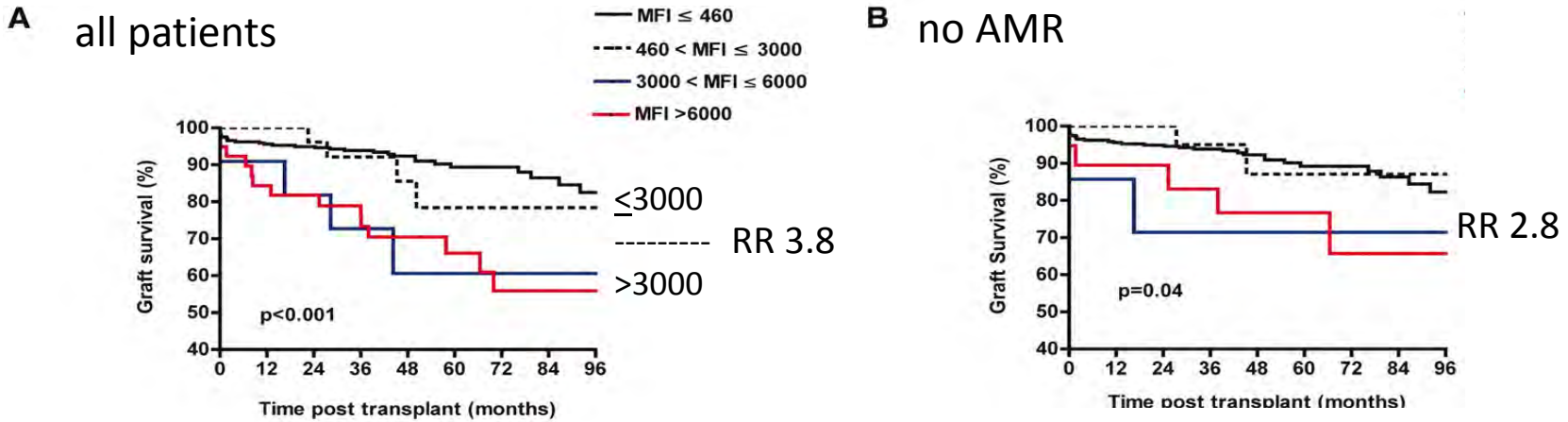
Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation

Risk of AMR is higher in patients with preexisting DSA >465 MFI (by ROC analysis)

Table 2. RR for acute AMR according to the MFI of highest pregraft ranked DSA detected by Luminex (logistic regression)

DSA MFI_{max} class	RR (95% CI)	*P*
≤465	1.0	
465 to 1500	24.8 (4.6 to 134.8)	<0.001
1500 to 3000	23.9 (3.5 to 160.8)	0.001
3000 to 6000	61.3 (11.5 to 327)	<0.001
>6000	113.0 (30.8 to 414)	<0.001

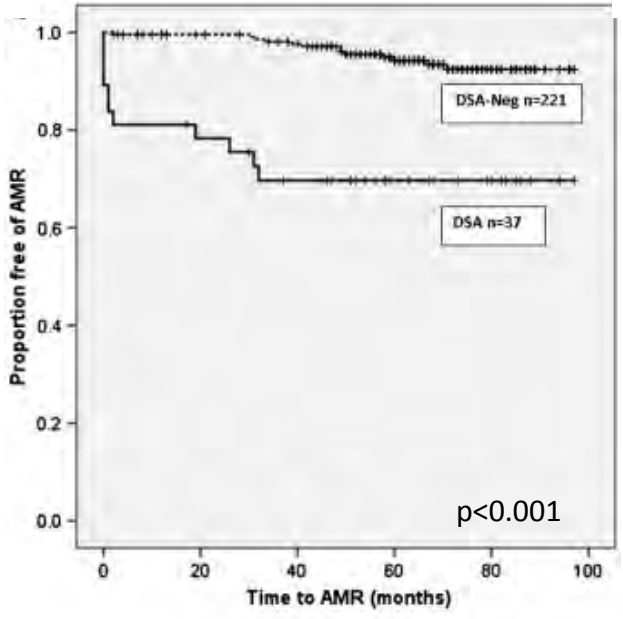
graft survival is lower in patients with preexisting DSA >3000 MFI

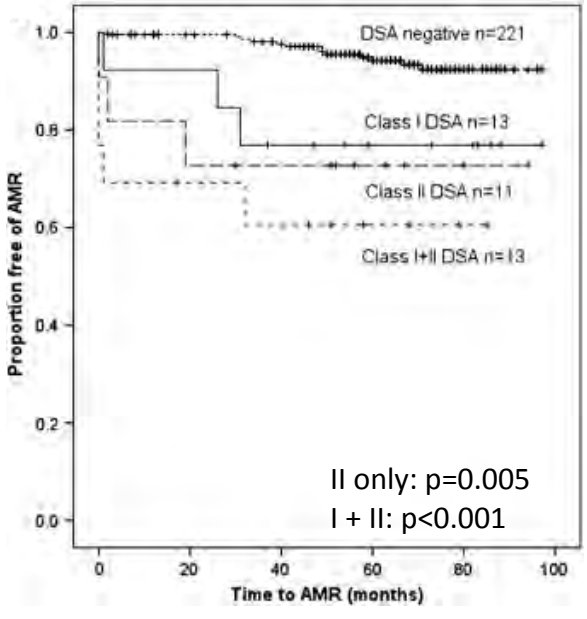


Lefaucheur, et al. J Am Soc Nephrol. 2010;21:1398-406.

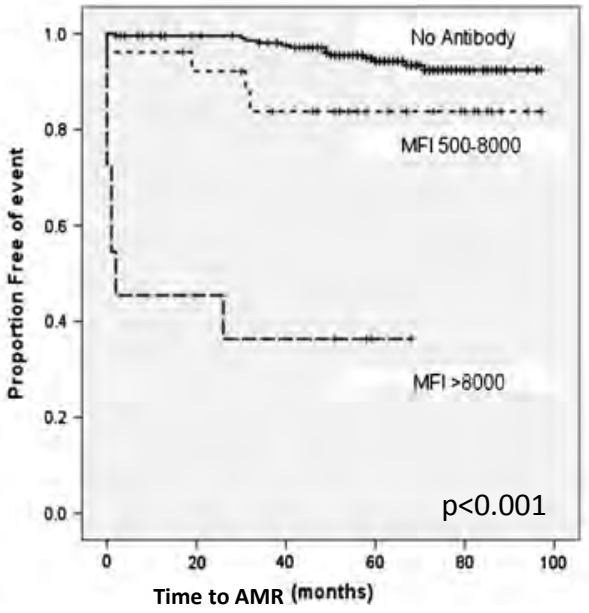
Pre-transplant donor specific anti-HLA antibody is associated with antibody-mediated rejection, progressive graft dysfunction and patient death

retrospective study of 258 renal transplants 2003-2007 median 5.6 yrs follow-up
all CDC T XM–, 4 BXM+ DSA+, 30 BXM+ DSA–, 1 DSA+ no BXM
day of txp serum screened by luminex mixed then SAB if pos (DSA pos>500 MFI)





MFI of highest DSA bead

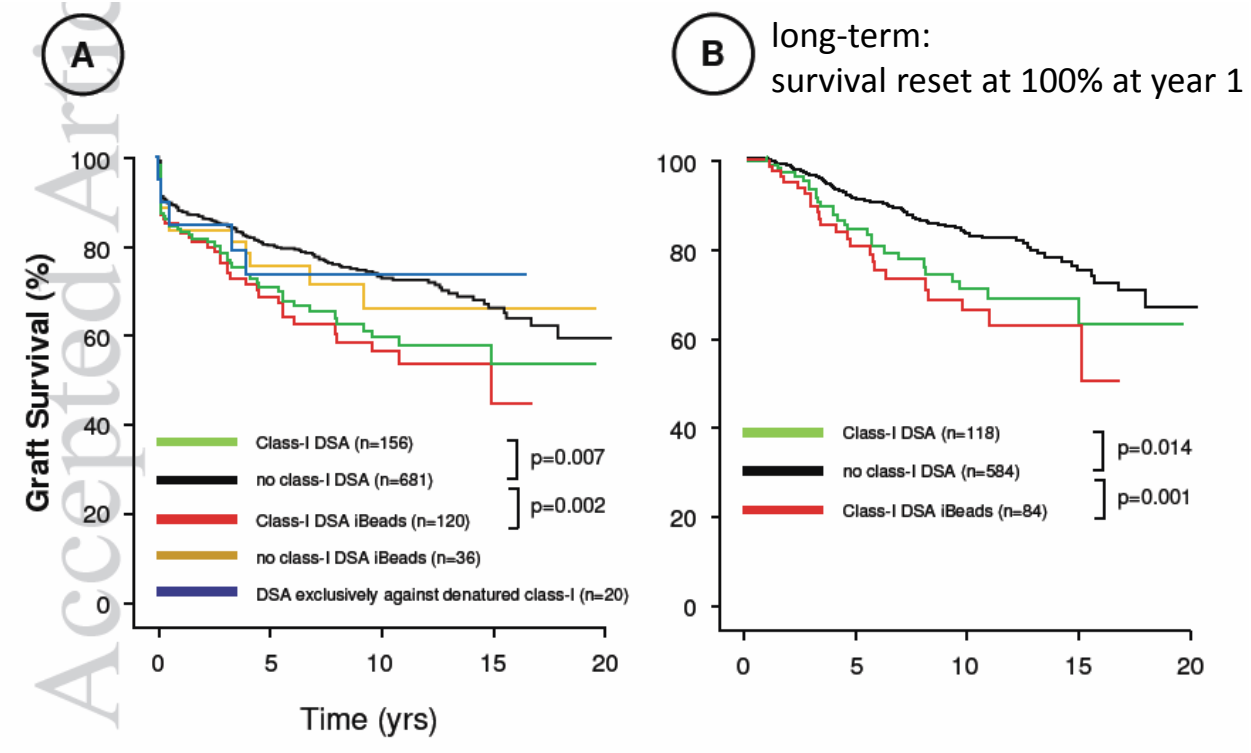


DSA >8000 MFI, especially class II or class I +II leads to increased rates of acute AMR

Fidler, et al. Transp Immun. 2013;28:148

The Significance of Pretransplant Donor-Specific Antibodies Reactive with Intact or Denatured HLA in Kidney Transplantation

pretransplant sera with DSA from 156 kidney transplant recipients
tested on class I regular SAB, iBeads and SAB with denatured antigen



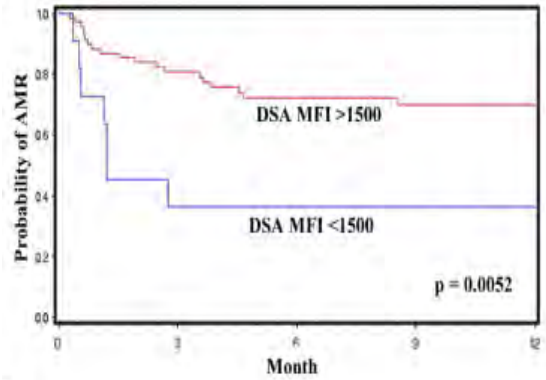
reactivity to exclusively denatured HLA was not associated with decreased graft survival

Otten et al, Clin Exp Imm. 2013

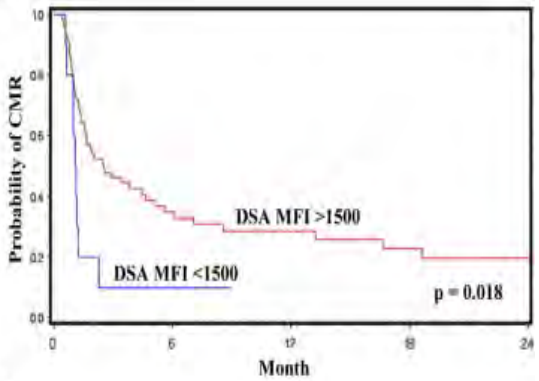
Effect of Pretransplant Human Leukocyte Antigen Antibodies Detected by Solid-Phase Assay on Heart Transplant Outcomes

retrospective study of 85 heart transplants T CDC XM-
tested pre-txp sera with flow XM(retrospective) and luminex SAB (DSA pos >1500 MFI)

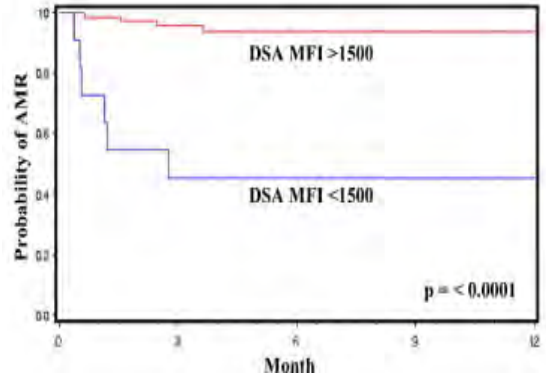
A AMR with C4d or CD68 staining



C ISHLT CMR grade ≥1R/1A



B ISHLT AMR grade ≥1



D ISHLT CMR grade ≥2R/3A

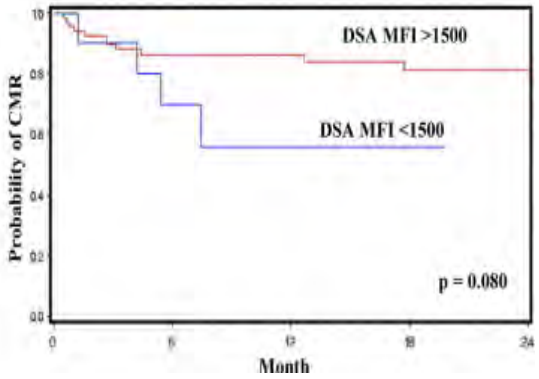


Table 1 Histomorphologic Criteria for AMR and ACR Grading as per ISHLT 2005 Guidelines

	Histomorphologic criteria
ACR grade	
Grade 0R	No ACR
Grade 1R	Mild, low-grade, ACR: interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage
Grade 2R	Moderate, intermediate-grade, acute cellular rejection: 2 or more foci of infiltrate with associated myocyte damage
Grade 3R	Severe, high-grade, ACR: diffuse infiltrate with multifocal myocyte damage ± edema ± hemorrhage ± vasculitis
AMR grade	
AMR 0	No AMR
AMR 1	A. Evidence of cardiac dysfunction B. If the immunofluorescence or IHC staining supports the histologic features of AMR C. Histologic features include: • Capillary endothelial changes (swelling or denudation with congestion) • Macrophages in capillaries (morphologic or by CD68 IHC) • Neutrophils in capillaries • Interstitial edema and/or hemorrhage and fibrin in vessels

ACR, acute cellular rejection; AMR, antibody-mediated rejection; IHC, immunohistochemistry.

patients with DSA >1500 and positive flow XM have higher rates of AMR and CMR

Gandhi et al. Transp Proc. 2011;43:3840

Correlation of circulating donor-specific anti-HLA antibodies and presence of C4d in endomyocardial biopsy with heart allograft outcomes: a single-center, retrospective study

112 cardiac allografts, 109 patients, 170 biopsies with concurrent C4d IF and DSA by luminex mixed, phenotype and SAB (pos >1,000 MFI) pre- and post-transplant DSA measurement

Table 4 (A) Endomyocardial Biopsy During Allograft Dysfunction With Corresponding C4d IF Staining[a]

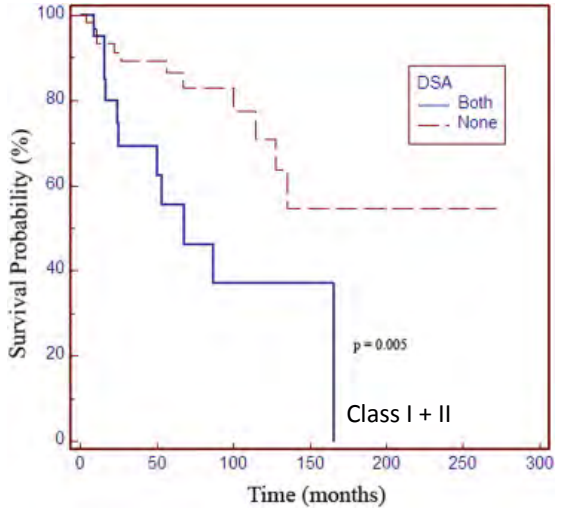
DSA ($n = 170$ biopsies)	No C4d	Weak C4d[b]	C4d >50%
HLA Class I ($n = 10$)	5 (50%)	3 (30%)	2 (20%)
HLA Class II ($n = 61$)	28 (46%)	24 (39%)	9 (15%)
HLA Class I and II ($n = 39$)	15 (39%)	9 (23%)	15 (38%)
Any DSA ($n = 110$)	48 (44%)	36 (32%)	26 (24%)
No DSA ($n = 60$)	43 (72%)	14 (23%)	3 (5%)

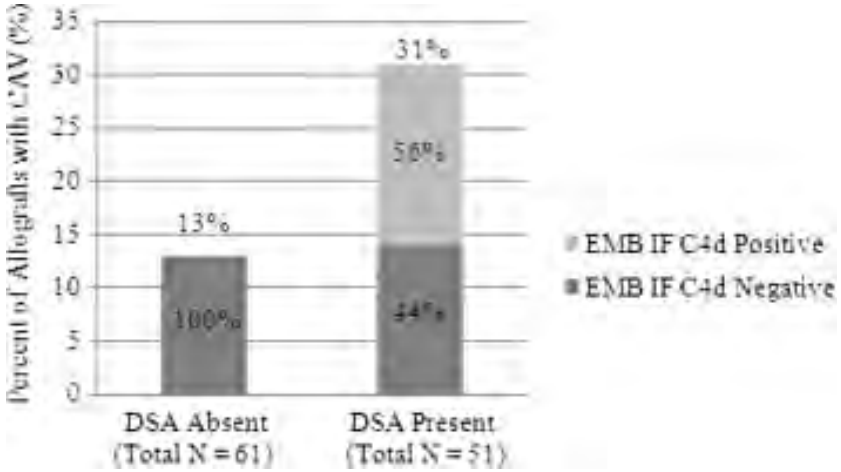
(B) Biostatistically Significant p-Values for Comparison of C4d IF Staining in Patients With DSA to Class I and/or Class II vs Without DSA

DSA	Class I and II	No DSA
Class I	$p = 0.01$	$p = 0.0004$
Class II	$p = 0.004$	$p = 0.003$

[a]C4d IF staining scores in EMBs during episodes of graft dysfunction in patients with positive DSA.
[b]Weak C4d: $\leq 1^+$ interstitial capillary staining in any percentage of myocardial parenchyma or any intensity of staining in <50% of interstitial capillaries.

48 DSA+ post/13 DSA+ pre
61 DSA- post/2 DSA+ pre



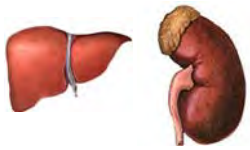


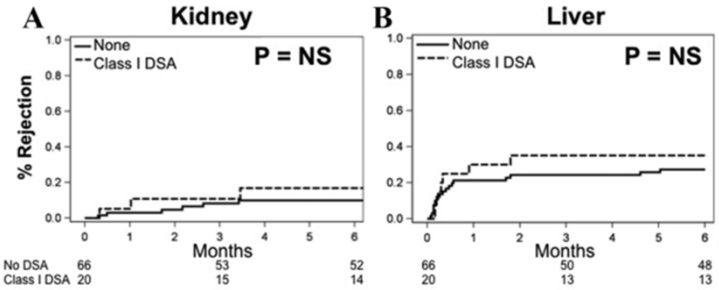
DSA to class I or class I+II correlates with C4d deposition and graft failure

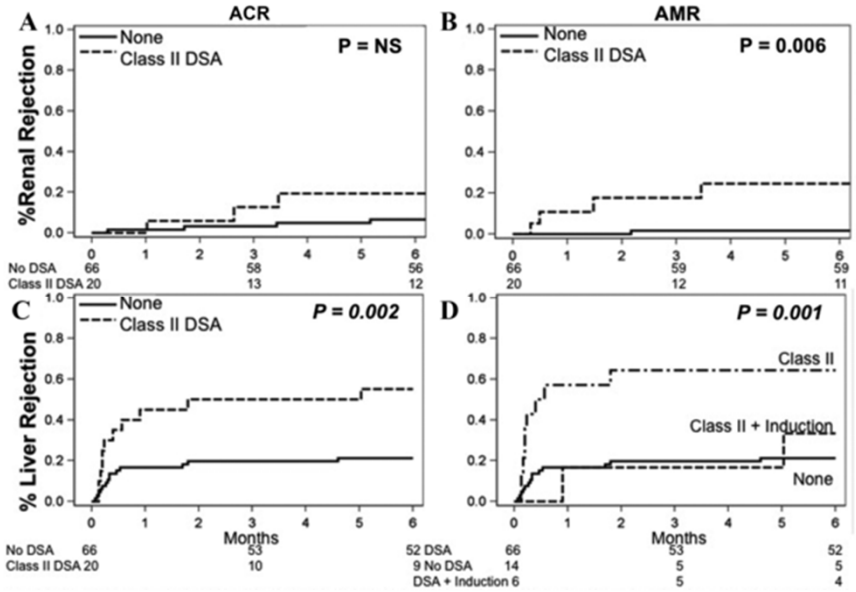
Frank et al. J Heart Lung Transp. 2013;32:410

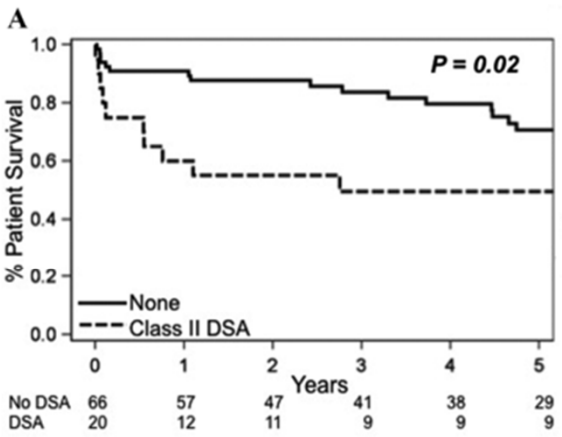
Class II Alloantibody and Mortality in Simultaneous Liver-Kidney Transplantation

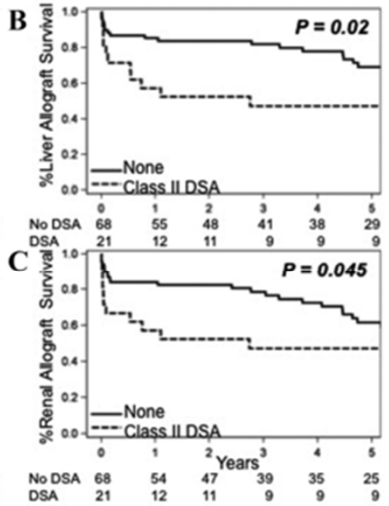
86 SLKT patients with pretransplant serum sample (76% had posttransplant serum sample)
luminex SAB pos ≥2000 MFI: 30 with preformed DSA, 9 with *de novo* DSA







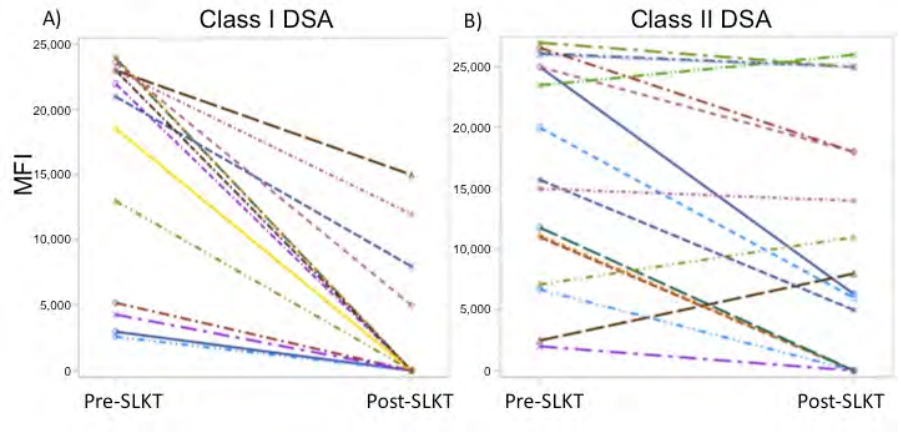


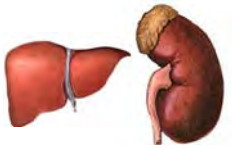


preformed class II DSA increased risk of renal AMR, liver AMR and ACR, and graft loss

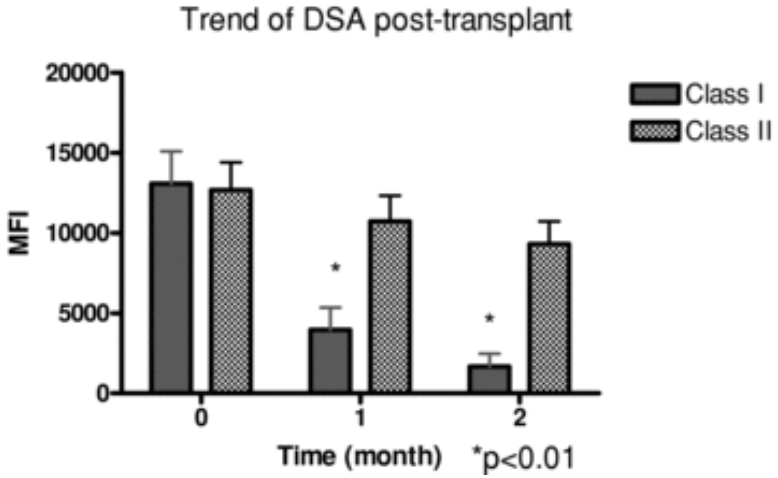
O'Leary et al. Am J Transp. 2013;13:954

Class II Alloantibody and Mortality in Simultaneous Liver-Kidney Transplantation





Donor-Directed MHC Class I Antibody Is Preferentially Cleared from Sensitized Recipients of Combined Liver/Kidney Transplants



liver allografts may not fully protect renal allografts in the presence of class II DSA

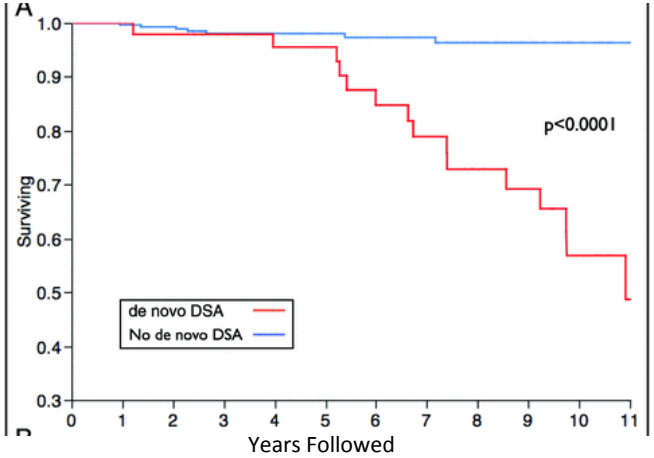
Dar, et al. Am J Transp. 2011;11:841

POST-TRANSPLANT *DE NOVO* DSA

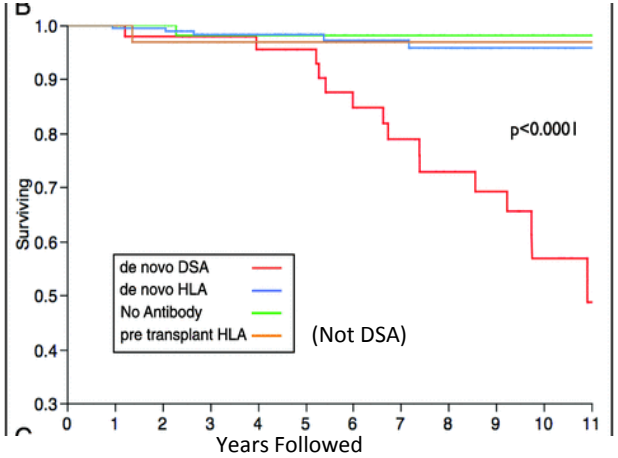
Evolution and Clinical Pathologic Correlations of *De Novo* Donor-Specific HLA Antibody Post Kidney Transplant

315 consecutive renal transplants without pre-txp DSA (Flow PRA beads and Flow XM neg)
1999-2008 (mean follow-up 6.2±2.9 years)
serum collected at 0, 1, 2, 3, 6, 12, 18, 24 months and at time of biopsy for graft dysfunction, biopsy when dnDSA was newly detected
protocol biopsy at 6 and 24 months for patients with no dnDSA or graft dysfunction
47 developed dnDSA

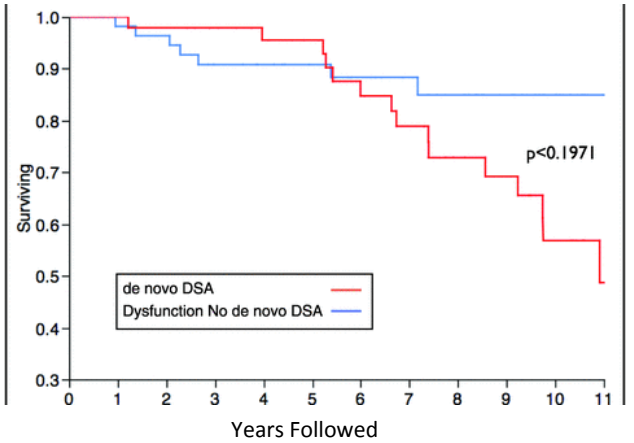
dnDSA vs without



dnDSA vs dnHLA or pretxp HLA



dnDSA vs other dysfunction



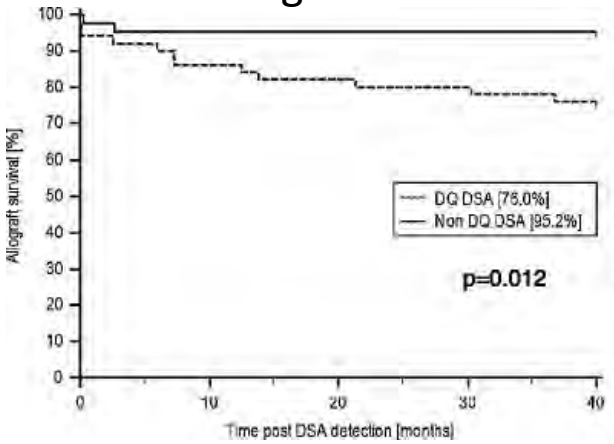
de novo DSA can lead to antibody mediated injury and progress to graft loss

Wiebe et al. Am J Transp. 2012;12:1157

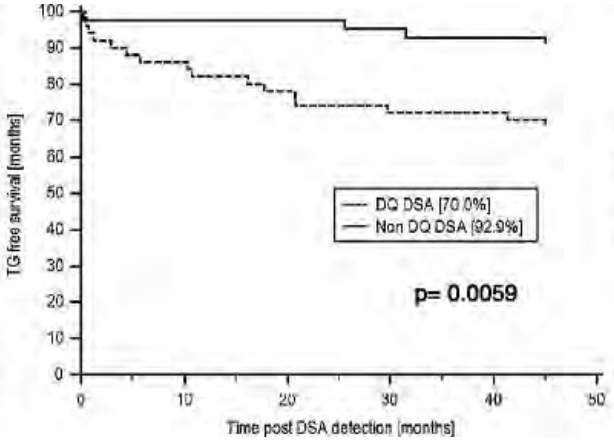
De Novo DQ Donor-Specific Antibodies Are Associated With a Significant Risk of Antibody-Mediated Rejection and Transplant Glomerulopathy

retrospective study of 505 renal txp patients 2005-2010 without pre-txp DSA
CDC B and T XM neg, Flow T XM neg, luminex SAB DSA neg (pos >300 MFI)
92(18.2%) developed DSA (MFI>300)with 50 (54.3% of the 92) having DQ DSA
mixed bead screen first - then positive had SAB

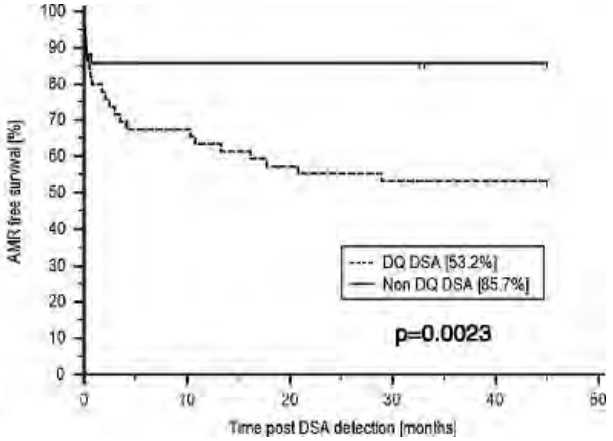
Allograft Survival



TG free survival



AMR free survival



de novo DQ DSA are associated with poor graft outcome

Willicombe, et al. Transplantation 2012;94:172

De Novo Donor HLA-Specific Antibodies after Heart Transplantation Are an Independent Predictor of Poor Patient Survival

retrospective analysis of 243 cardiac txp recipients with no preexisting DSA (CDC XM neg)
measure HLA Ab every year after txp up to 13 years
luminex SAB pos >1000 MFI considering patterns of reactivity and CREGs

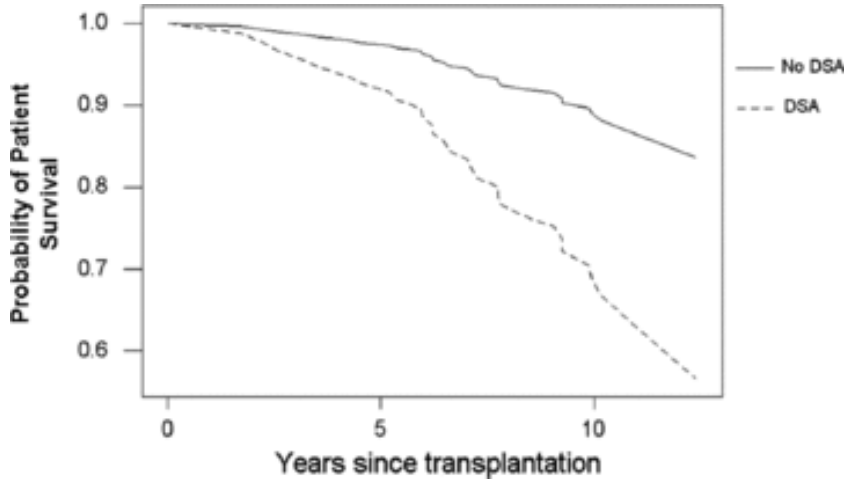


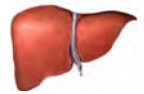
Table 3: Impact of persistent *de novo* DSA on patient survival in a multivariable Cox proportional hazards model

	Estimated hazard ratio	95% Confidence interval for hazard ratio	p-Value
De novo and persistent DSA	4.331	1.922–9.7600	<0.0004
HLA-DR mismatch	2.334	1.0782–5.0539	0.0315
Donor age	1.034	1.0048–1.0762	0.0256
Hemodynamic compromise	2.363	1.0003–5.5804	0.0499
Treated rejection in the 1st year	0.417	0.1825–0.9535	0.0382

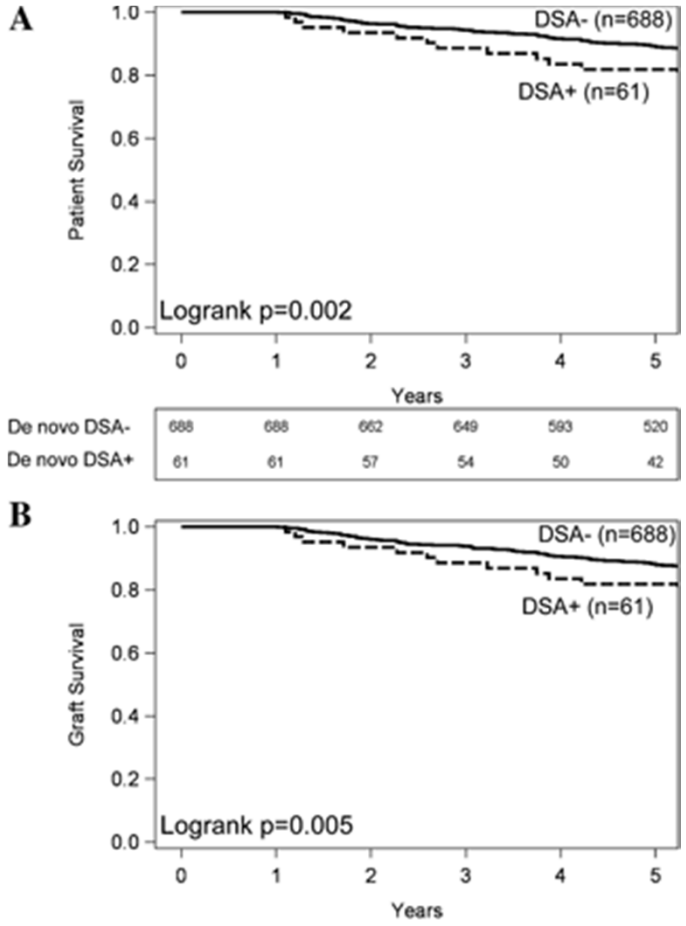
de novo DSA are strongly associated with poor survival

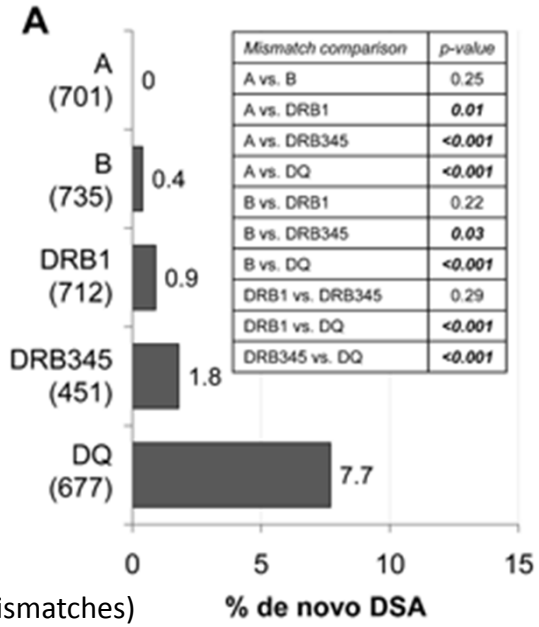
Smith et al. Am J Transp. 2011;11:312

De Novo Donor-Specific HLA Antibodies Decrease Patient and Graft Survival in Liver Transplant Recipients



retrospective study of 749 adult liver txp recipients <u>without</u> pre-txp DSA (pos >5000 MFI) test serum at 1 year posttransplant





Mismatch comparison	p-value
A vs. B	0.25
A vs. DRB1	***0.01***
A vs. DRB345	***<0.001***
A vs. DQ	***<0.001***
B vs. DRB1	0.22
B vs. DRB345	***0.03***
B vs. DQ	***<0.001***
DRB1 vs. DRB345	0.29
DRB1 vs. DQ	***<0.001***
DRB345 vs. DQ	***<0.001***

(# with mismatches)

de novo DSA, mostly against DQ, lead to poor survival

Kaneku, et al. Am J Transp. 2013;13:1541

Things to consider when reading papers

- crossmatching before transplant
  - CDC and/or flow?
  - CDC and flow XM both negative?
  - CDC negative / flow positive?
  - CDC positive / flow negative?
  - what is cutoff for positive flow XM?
  - was pronase used?
  - B cells vs T cells?

Things to consider when reading papers

- antibody analysis
  - what was MFI cutoff for positive?
    - 300-5,000
  - how was MFI cutoff chosen?
    - random, ROC analysis, based on XM positivity? CDC XM or Flow XM?
  - were all loci considered, including Cw and DP?
  - which bead platform – flow or luminex?
  - which vendor's SABs were used?
  - are all donor alleles included in SABs?
  - what resolution of beads?
    - mixed screen, phenotype, single antigen
  - antibody spread across an epitope
    - might look negative
  - IgG vs IgM
  - complement fixing?
    - IgG subclass, C1q, C4d
    - C1q original method or with wash and/or AHG

Things to consider when reading papers

- patient characteristics
  - size of cohort
  - selection criteria
  - prospective or retrospective
  - more immunosuppression or desensitization for patients with DSA?
  - time of serum samples and biopsies
    - protocol or only when graft dysfunction?
  - number of years post-transplant follow-up

Thank
You
Merci
Gracias
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Xièxie
Chokrane
Kiitos
Dziakuju
Grazie
Takk
aDank
ThintKo
Buznyg
Blagodaram
Rahmat
Matondo
Mercé
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Dakujem
Gràcies
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Efharisto
NajisTuke
Nirringrazzjak
Bayarlalaa
Obrigado
Salamat
Saha
Multumesc
CámOn
Gracie
GratiasAgo
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Tenki
Grandmercé
Köszönöm
TapadhLeat
Murakoze
WaadMahadsantahay
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Dhanyavaad
Grazzi
Mauruuru
Barkal
Gratzias
KhobChaiDeu