The new Banff vision of the role of HLA antibodies in organ transplantation:

Improving diagnostic system and design of clinical trials

Carmen Lefaucheur

1	**Banff 2015: Integration of HLA-Ab for improving diagnosis**
2	Banff 2017: HLA-Ab for surrogate endpoint in clinical trials

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BANFF 2015: KIDNEY

Criterion 3 for diagnosis of ABMR in the kidney allograft: requirement of serologic evidence of DSAs against HLA or other antigens

Can DSA be waived for the diagnosis of ABMR in biopsies showing both morphologic evidence of acute or chronic tissue injury and C4d staining?

Opinion of the majority of experts at Banff 2015: *« Biopsies meeting histologic criteria of ABMR and showing diffuse or focal linear peritubular capillary C4d staining on frozen on paraffin sections are associated with a high probability of ABMR and should prompt expedited DSA testing »*

Potential role of DSAs currently not tested for in many centers (HLA DP, non-HLA antigens)

BANFF 2015: HEART

« The vital role and importance of serologic data in the overall assessment of the patient is heavily underscored »

« DSA testing shows outstanding sensitivity and negative predictive value for biopsy-diagnosed AMR »

« Quantitative DSA should be an essential component in the surveillance for AMR »

« Investigators have raised the issue of reintroducing HLA DSA testing information for use in the diagnosis of AMR and for risk assessment of persistent AMR and chronic allograft vasculopathy (CAV) »

BANFF/ASHI HLA panel experts

Implementing HLA-Ab detection into the AMR classification: Question identified and recommendations

Question	Recommendations	Definintions
What is the optimum timing of DSA testing post-transplantation?	Stratify the patients based on risk for AMR and monitor: • High and intermediate risk with each biopsy early post-transplant, 3, 6, 9, 12 months first year and yearly if no clinical indication. • Low risk minimum 3,6,12 months, yearly after and anytime clinically indicated	• High Risk: presence of DSA at the time of transplant • Intermediate Risk: presence of DSA in historical samples

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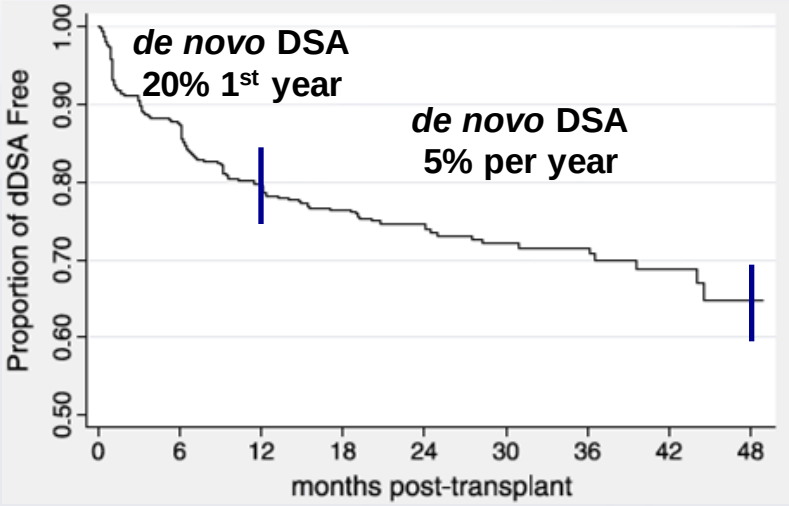
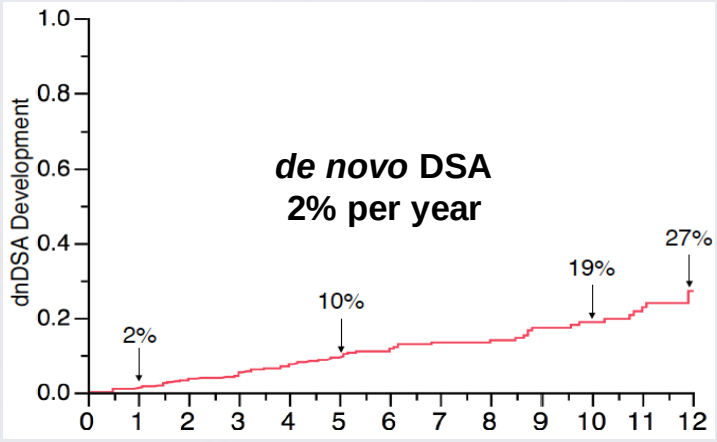
Importance of post-transplant DSA monitoring



DeVos et al., Transplantation (2014)



Wiebe et al., AJT (2015)

Reference	*De novo* DSA		
	1st Month	1st Year	>1st Year
Cooper	15.6%	27.0%	0% yr 2
DeVos	8.0%	20.0%	5.0%/yr
Heilman	8.2%	17.6%	n.a.
Everly	3.0%	11.0%	2.3%/yr
Wiebe	0.0%	2.0%	2.0%/yr

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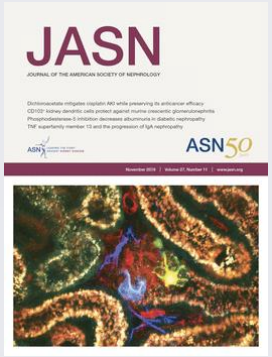
Post-Tx DSA monitoring improves risk stratification for allograft loss

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

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



Donor-specific HLA alloantibodies: Impact on cardiac allograft vasculopathy, rejection, and survival after pediatric heart transplantation

Andrew Tran, MD,[a] David Fixler, MD,[a] Rong Huang, MS,[b] Tiffany Meza, MBA, MHSM,[c] Chantale Lacelle, PhD,[d] and Bibhuti B. Das, MD[a]

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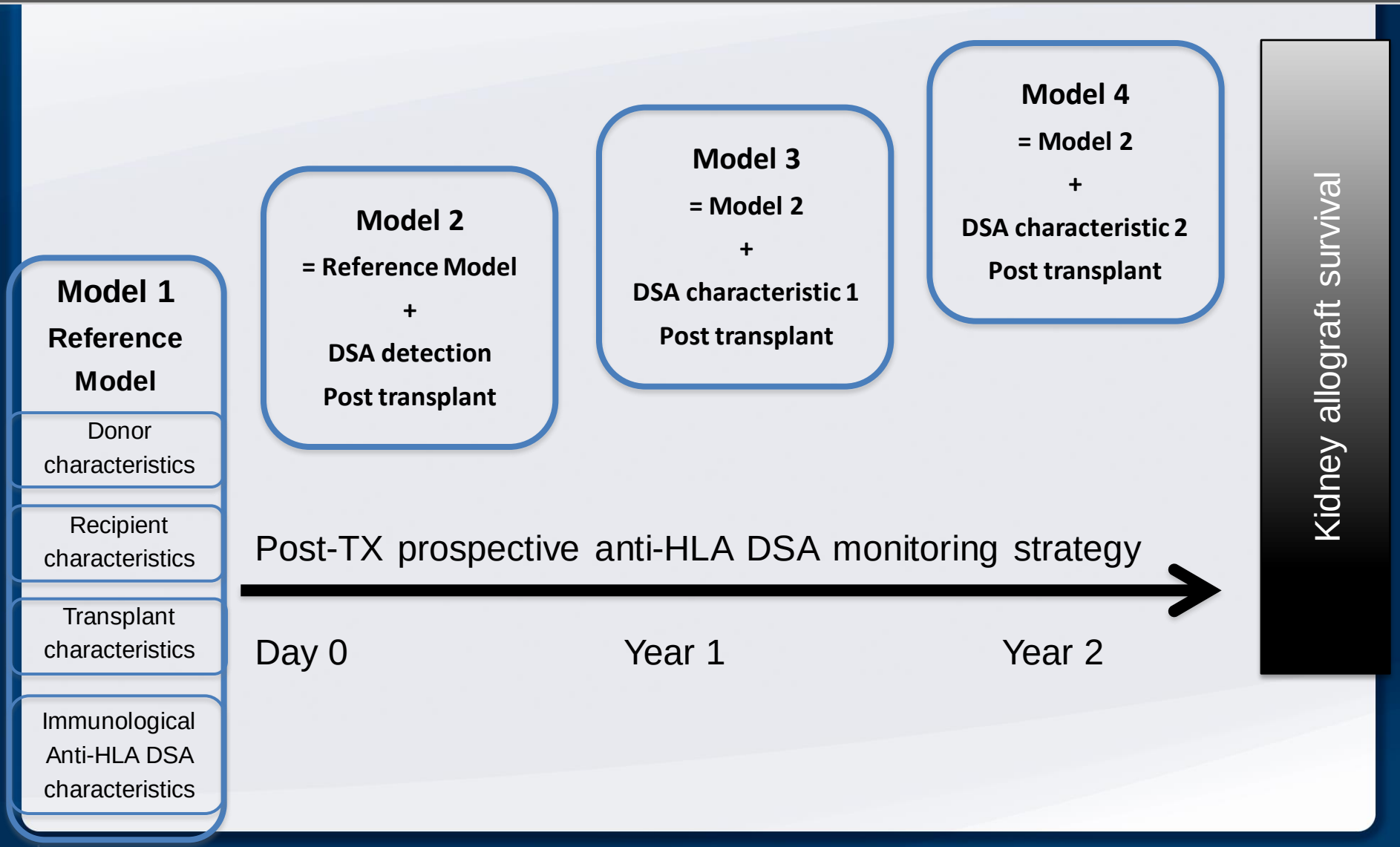
DYNAMIC MODELING TO ASSESS IMPROVEMENT IN RISK PREDICTION ACCORDING TO DSA MONITORING AND CHARACTERIZATION



BANFF/ASHI HLA panel experts

Implementing HLA-Ab detection into the AMR classification: Question identified and recommendations

Question	Recommendations	Definintions
When DSA should be treated?	• Increased level (Titer and MFI) of persistent DSA should be biopsied to rule out subclinical rejection • Strong correlation of persistent DSA with graft dysfunction	• Level DSA levels assessed by MFI strength and/or titration of sera • Persistent DSA: presence of DSA in serial samples • Transient DSA: presence of DSA only in one sample

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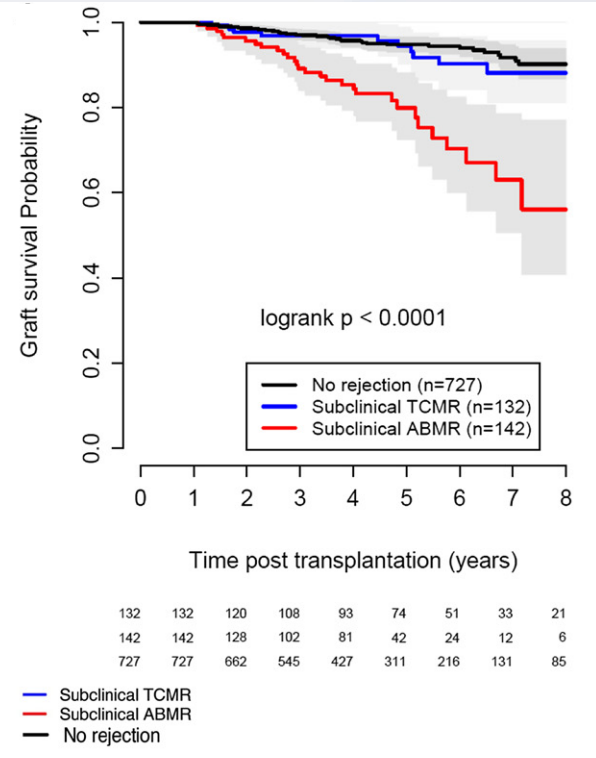
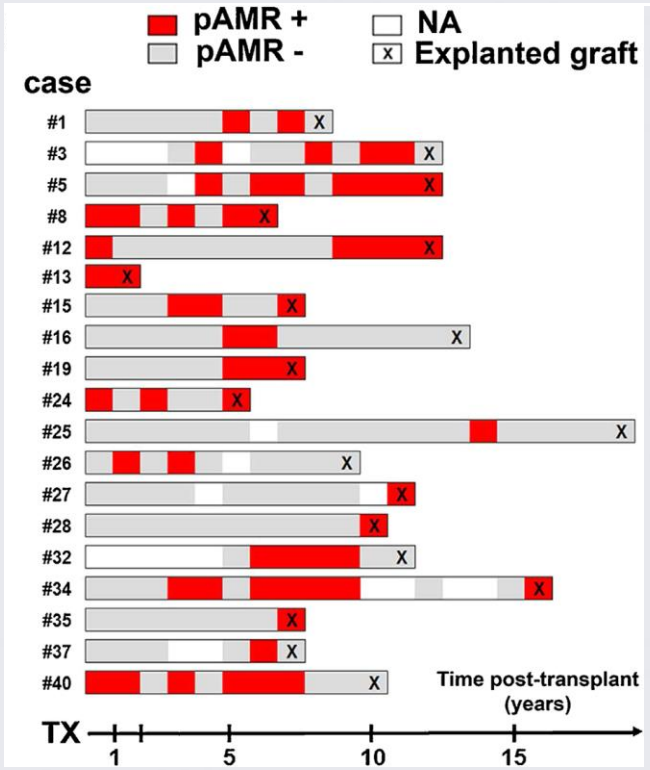
Major impact of subclinical AMR on allograft outcomes



Loupy et al., JASN (2015)



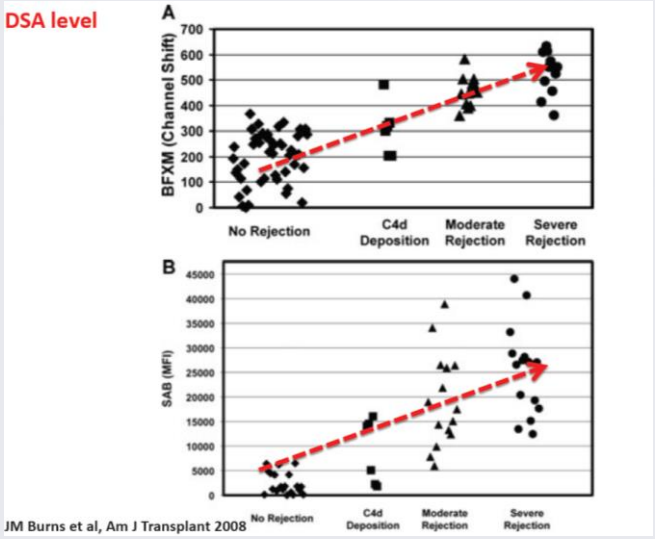
Loupy et al., AJT (2016)

BANFF/ASHI HLA panel experts

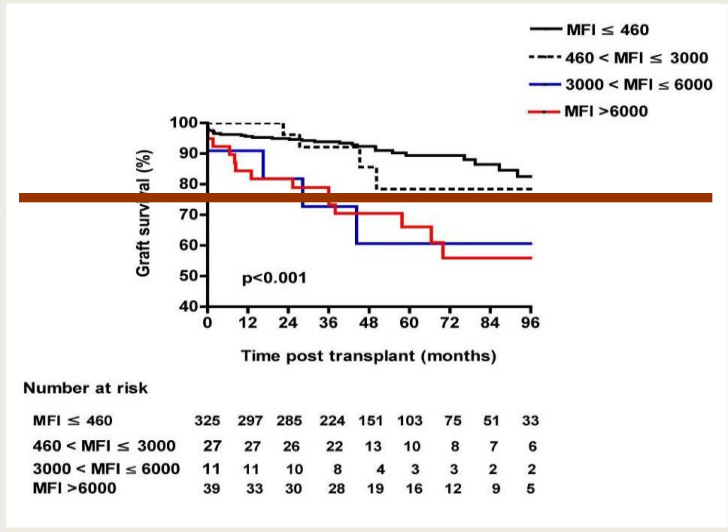
Implementing HLA-Ab detection into the AMR classification: Question identified and recommendations

Question	Recommendations	Definintions
Should DSA testing be performed with diagnosis of pAMR?	Testing for DSA presence and level (HLA and non HLA) should be performed to: a) correlate with severity of pAMR b) assess efficacy of treatment	

Post-Tx DSA level correlate with the severity of allograft injury and the risk of allograft loss

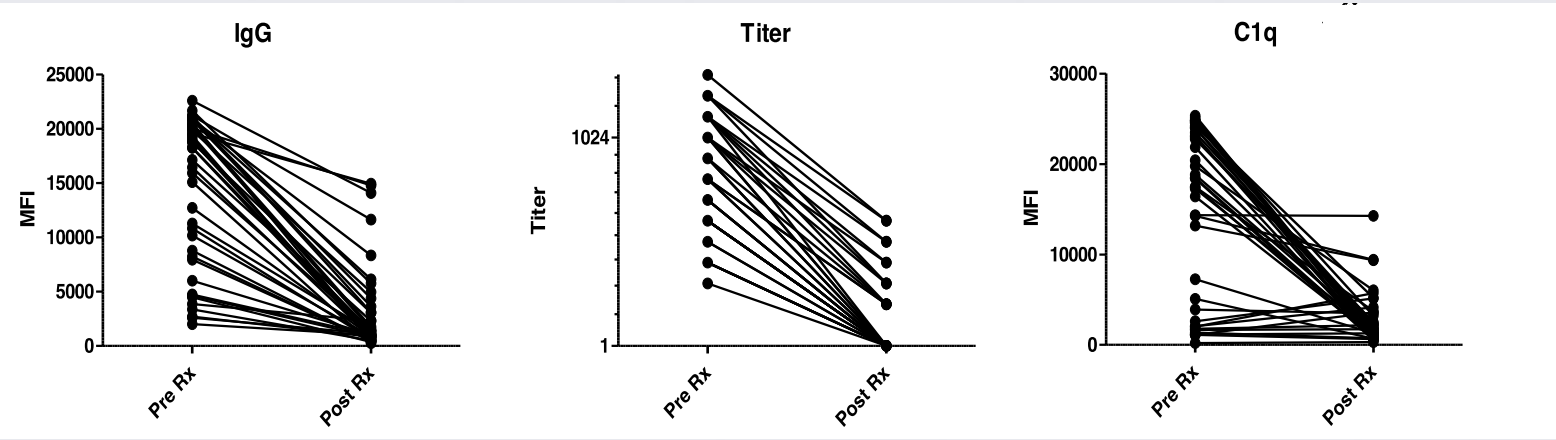


JM Burns et al, Am J Transplant 2008



Lefaucheur et al., JASN (2010)

SAB assays to assess antibody removal by PP/IVIG/Rituximab



Tambur et al, Hum Immunol (2016)

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HLA-Ab detection: limitations and potential solutions

Problem	Interpretation	Resolution
HLA-Ab to denatured antigens	False positive results: HLA-Ab to cryptic epitopes, clinically irrelevant	Repeat testing after acid treatment of SAB; surrogate crossmatch
Intrinsic and extrinsic factors inhibiting the SAB assay	False low MFI or negative results: due to inhibition of SAB assay	Dilution of sera pre-testing, adsorption, inhibition of C1q, addition of EDTA, heat treatment
Low MFI on SAB resulting in higher reactivity using cellular targets	False low MFI: DSA to a shared target present on multiple beads	Adequate analysis of specific DSA epitope
Using MFI to evaluate level and strength of DSA for risk stratification	Low or high MFI level of DSA may not correlate with risk of AMR, or response to treatment following antibody removal therapies	Modified SAB assay to distinguish between C' and non-C' binding DSA and determining titer of DSA (serial dilutions)

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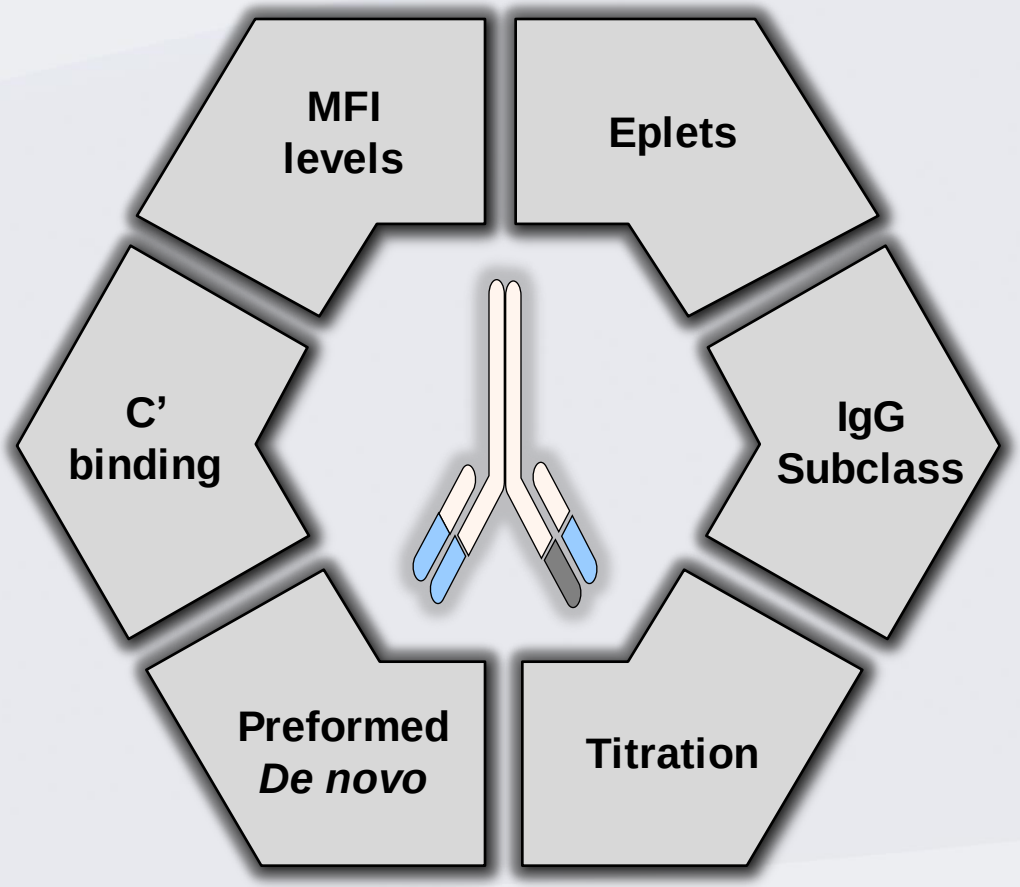
Integrative assessment of DSA



Graft failure
Disease progression
Risk prediction
Response to therapy

- Tambur et al, AJT (2015)
- Wiebe et al, AJT (2016)
- Loupy et al, NEJM (2013)
- Lefaucheur al, JASN (2015)
- Tait et al, Transplantation (2013)

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Allograft injury in relation to DSA properties

« Accumulating evidence supports the concept that not all DSA are equivalent and that DSA properties (ability to bind complement or IgG subclass) beyond simple positivity and mean MFI are associated with distinct outcomes and injury phenotypes »

« These distinct DSA properties and their relationship with distinct allograft injury patterns is also increasingly demonstrated in other solid organ transplants such as liver and heart. »

Biological rationale:
Effects of complement-activating IgG subclasses

	abundance	complement activation	FcγRI	FcγRIIA-H131	FcγRIIA-R131	FcγRIIB	FcγRIIIA-F158	FcγRIIIA-V158	FcγRIIIB NA1	FcγRIIIB NA2
IgG1	++++	+++	+++	+++	+++	+	++	+++	+++	+++
IgG2	++	+	-	++	-	-	--	+	-	-
IgG3	+	++++	+++	+++	+++	++	+++	+++	+++	+++
IgG4	+	-	+++	+	+	+	-	+	-	-
Cell Expression			monocytes, activated neutrophils	monocytes, neutrophils	monocytes, neutrophils	monocytes, neutrophils, B cells, dendritic cells	NK cells	NK cells	neutrophils, some monocytes	neutrophils, some monocytes

Valenzuela et al, Transplantation (2016)

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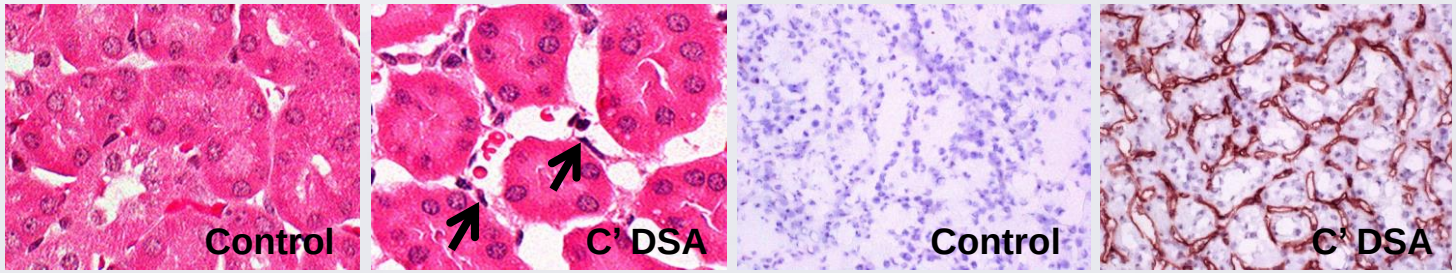
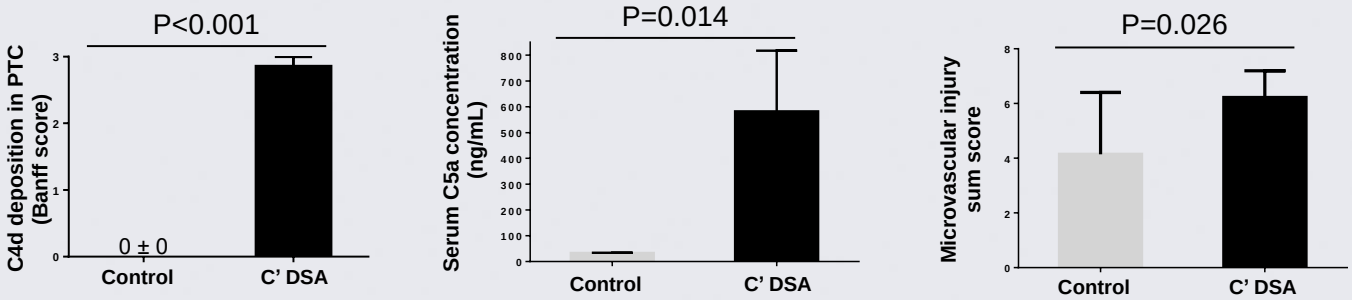
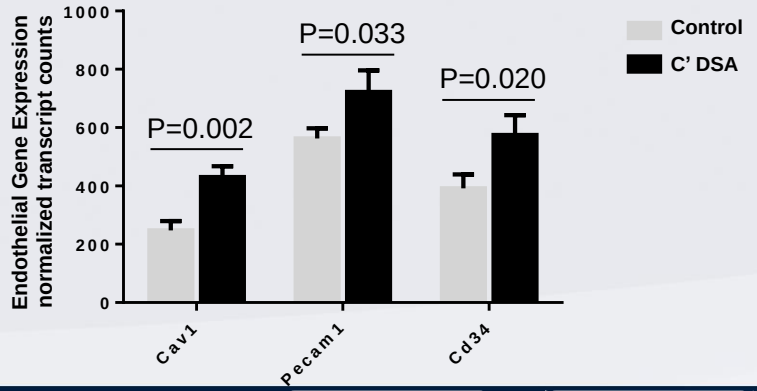
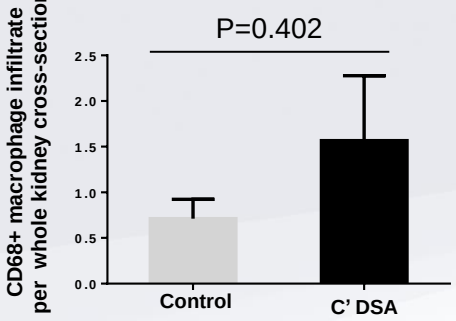
Animal model of ABMR:

C' activation by DSA induces distinct allograft injury phenotype









Sis et al. (submitted)

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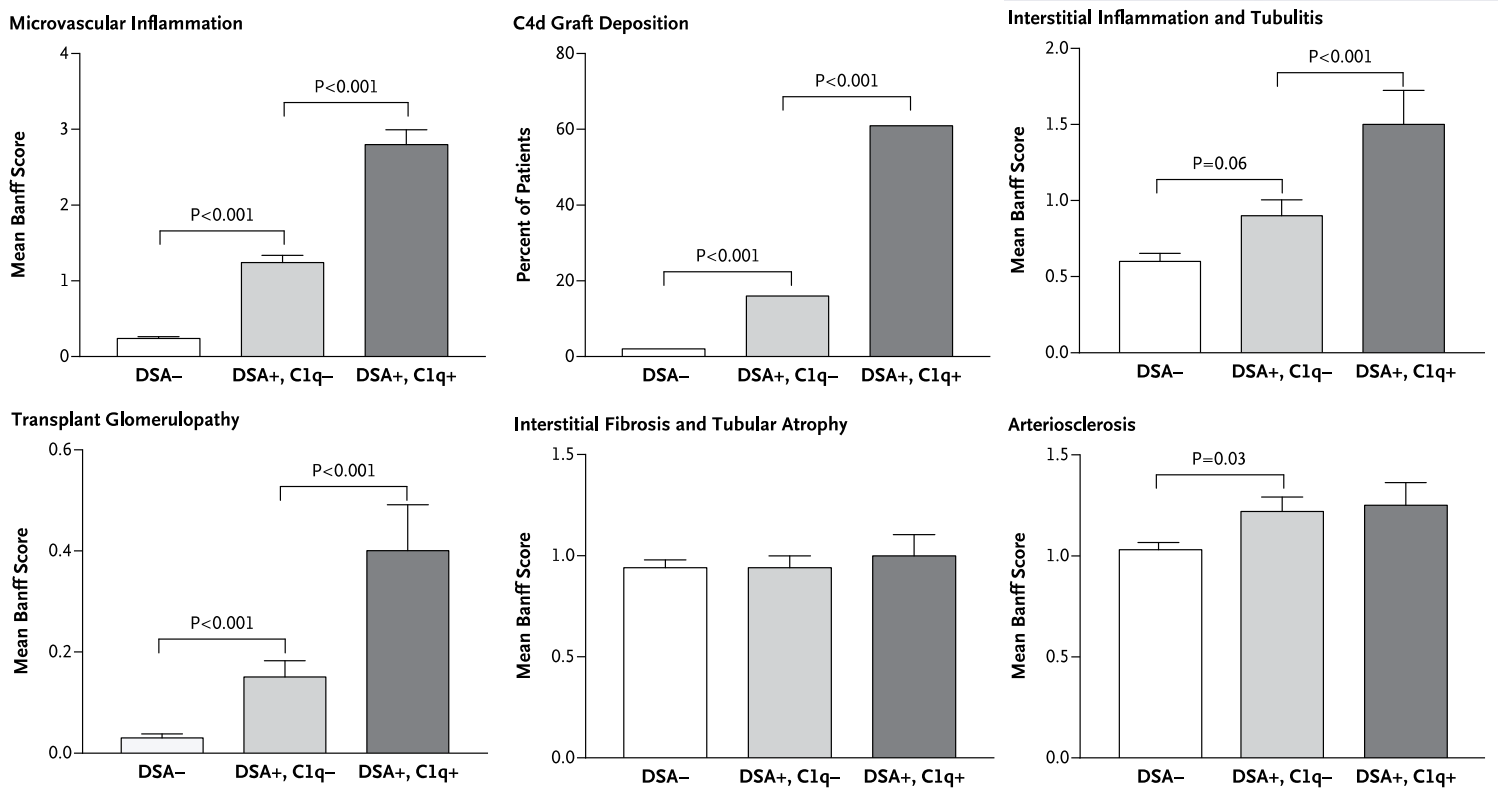
Clinical correlations in kidney transplant patients: HLA-DR and -DQ Eplet mismatches and transplant glomerulopathy

Odds Ratio of Developing TG based upon Total Eplet Threshold		
	Univariate	**Multivariate ****
DR + DQ: ≥36 *vs.* <36	2.01 [1.01-4.01]	3.21 [1.26-7.56]
DQ: ≥18 *vs.* <18	1.50 [0.75-3.00]	2.42 [1.03-5.70]
DR: ≥15 *vs.* <15	2.44 [1.16-5.12]	3.64 [1.42-9.37]

** Model includes Eplet exposure, recipient age, sex, peak PRA, race, induction and donor type.

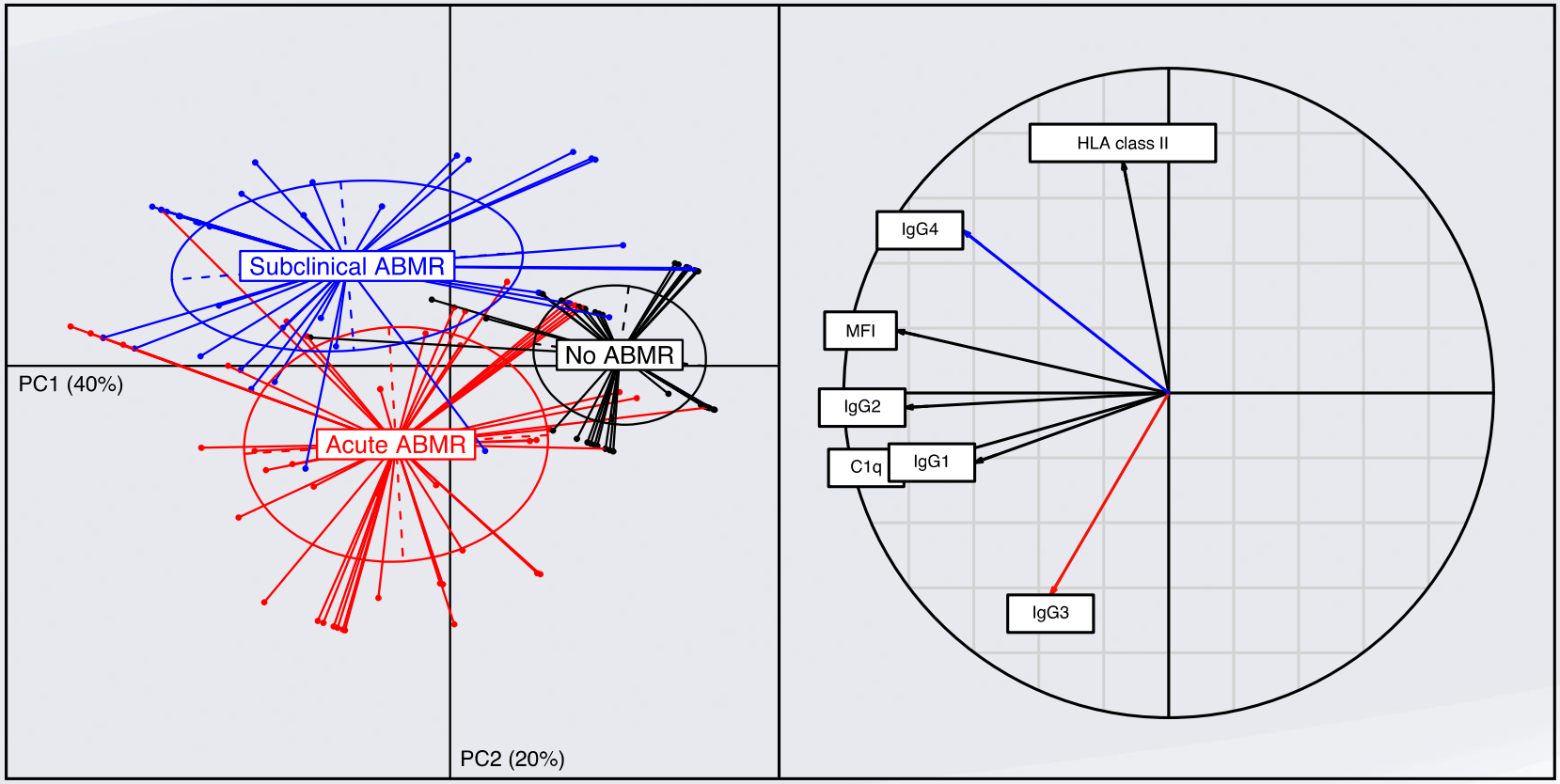
Sapir-Pichhadze et al. AJT (2015)

Clinical correlations in kidney transplant patients: DSA C'-binding capacity and kidney allograft injury phenotype



Loupy et al. NEJM (2013)

Clinical correlations in kidney transplant patients: DSA IgG subclasses and kidney allograft injury phenotype



Lefaucheur et al. JASN (2016)

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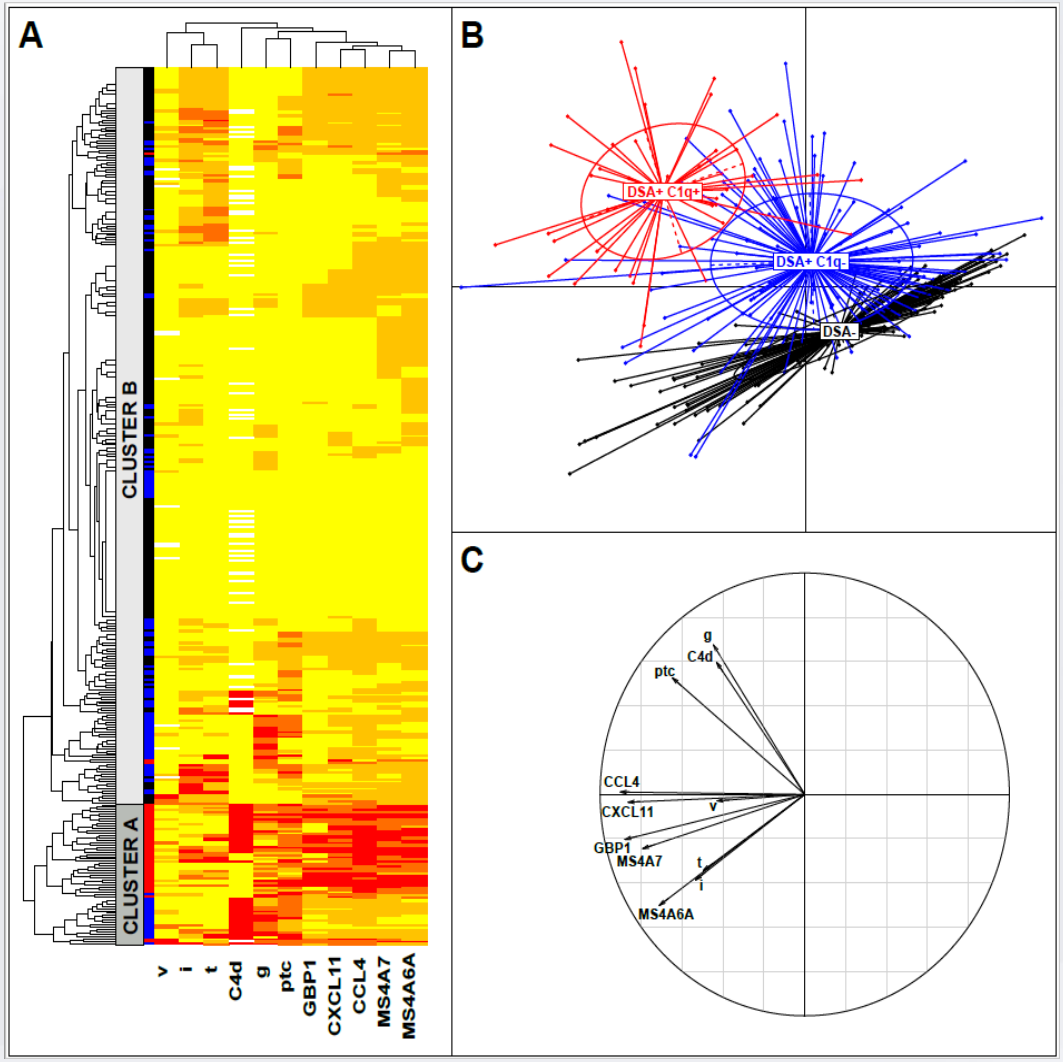
Gene expression profiling to define subtypes of ABMR



Cohort of interest with interrogation of the reference set (n=590)

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1	Banff 2015: Integration of HLA-Ab for improving diagnosis
2	**Banff 2017: HLA-Ab for surrogate endpoint in clinical trials**

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ICH HARMONISED TRIPARTITE GUIDELINE

STATISTICAL PRINCIPLES FOR CLINICAL TRIALS

Criteria for validating surrogate variables

- **Biological plausibility of the relationship**
- **Demonstration of the prognostic value of the surrogate for the clinical outcome**
- **Evidence that treatment effects on the surrogate correspond to effects on the clinical outcome**

ICH Guideline E9 (1998)

DSA: 50 years of biological plausibility

HLA antibodies
Hyperacute rejection

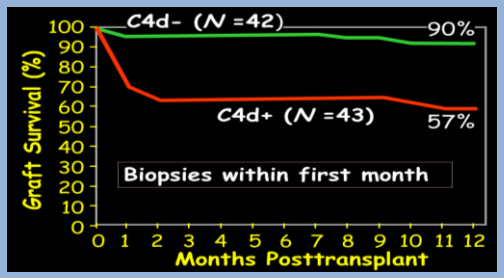
With antibodies, CXM pos

=

24/30 immediate failure

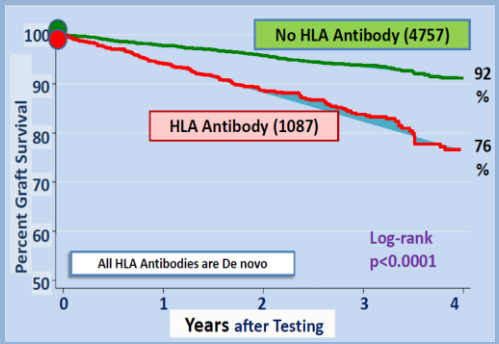
Patel & Terasaki, NEJM(1969)

HLA antibodies
Acute rejection



Feucht et al, KI (1993)

HLA antibodies
Graft survival



Terasaki, 15th Workshop

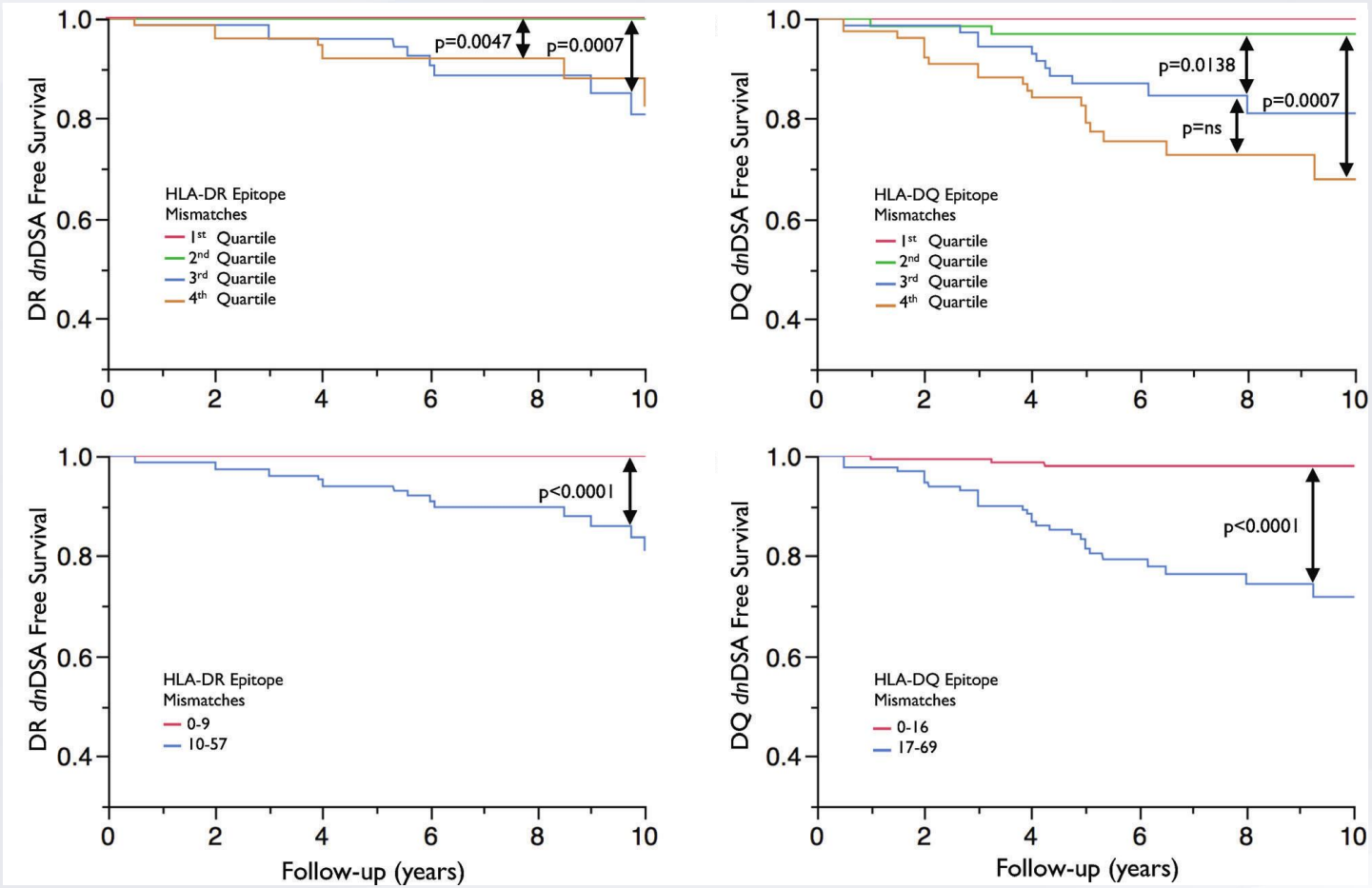
From kidney to other transplants

Heart

Liver

Lung

Class II HLA epitope matching and development of *de novo* DSA



Wiebe et al., AJT (2015)

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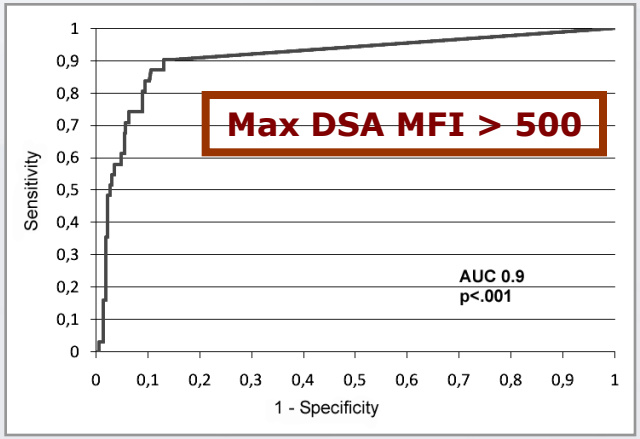
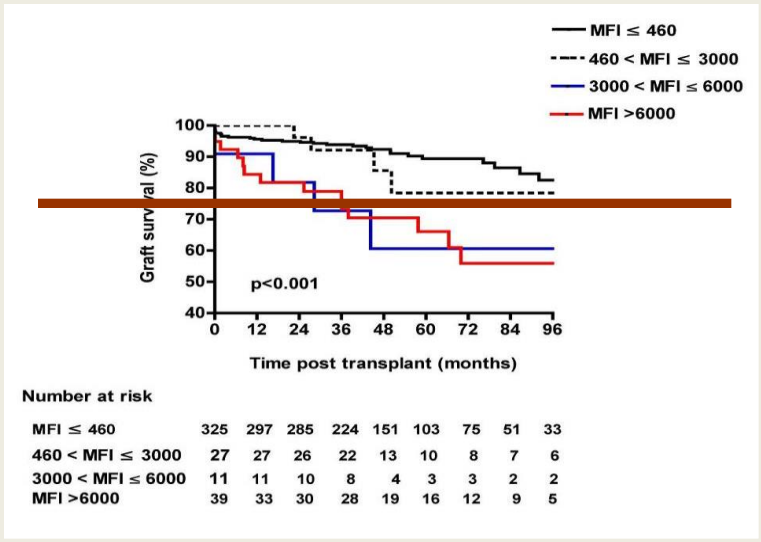
DSA: biological gradient

Risk of ABMR



DSA MFImax	HR	P	[95% CI]
≤ 500	1		
]500 – 1500]	24.8	< 0.001	[4.6 – 134.8]
]1500 – 3000]	23.9	0.001	[3.5 – 160.8]
]3000 – 6000]	61.3	< 0.001	[11.5 – 327]
> 6000	113	< 0.001	[30.8 – 414]

Risk of graft loss



Lefaucheur et al., JASN (2010)

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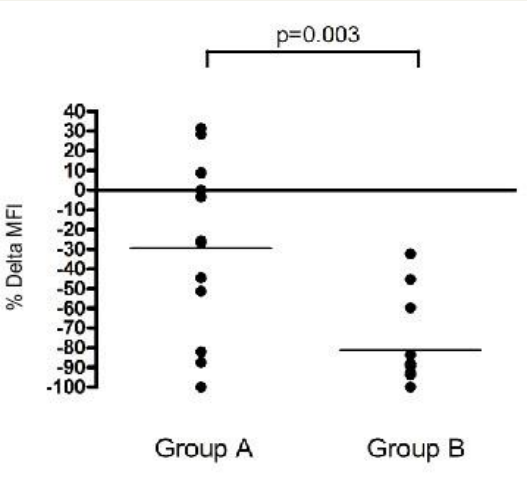
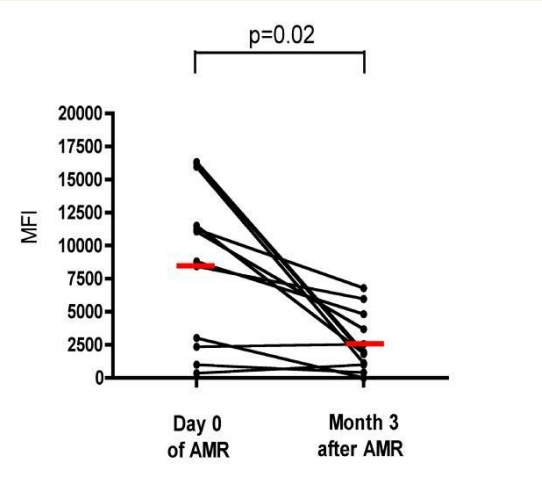
DSA removal: Beneficial effect





Lefaucheur et al, AJT (2009)

	Author	Year	N	Graft survival
OKT3	Feucht	1993	43	57%
IVIg	Lefaucheur	2007	21	70%
PP/IVIg	Rocha	2003	16	81%
PP/Ritux	Faguer	2007	8	75%
PP/IVIg/Ritux	Lefaucheur	2009	12	91.3%
PP/Ritux/Bortezomib	Woodle	2011	107	81%

Post-therapy drop of MFI correlates with improved graft survival independently of graft function and histology

N=278, median FU=3.5 yrs

Multivariate Predictors	HR	95%CI	P
eGFR at ABMR diagnosis	0.93	[0.90-0.95]	<0.001
IF/TA at ABMR diagnosis	2.44	[1.36-4.37]	0.003
Change in eGFR after SOC	0.24	[0.16-0.35]	<0.001
Change in ptc Banff grade after SOC	1.50	[1.16-1.93]	0.002
Change in DSA IgG MFI after SOC	1.30	[1.11-1.52]	0.001

Viglietti et al., ATC 2016

DSA as a surrogate endpoint for interventions in clinical trials

Occurrence of *de novo* DSA

- Efficacy of novel agents for baseline immunosuppression
- Safety of minimization strategies

Change in DSA level/C'-binding capacity

- Therapy efficacy in desenzitization
- Therapy efficacy in ABMR
- Post-Tx prophylaxis protocols in HLA-incompatible patients

Enrichment strategies based on DSA to increase endpoint frequency

Targeted population for graft loss

- High level DSA
- C'-binding DSA

Targeted population for occurrence of *de novo* DSA

- High class II epitope mismatch load

Thank you for your attention

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