

**2017 BANFF-SCT  
Joint Scientific Meeting**

**BARCELONA  
27-31 March 2017**

# **Impact of Donor –Specific HLA Antibodies on Lung Allograft**

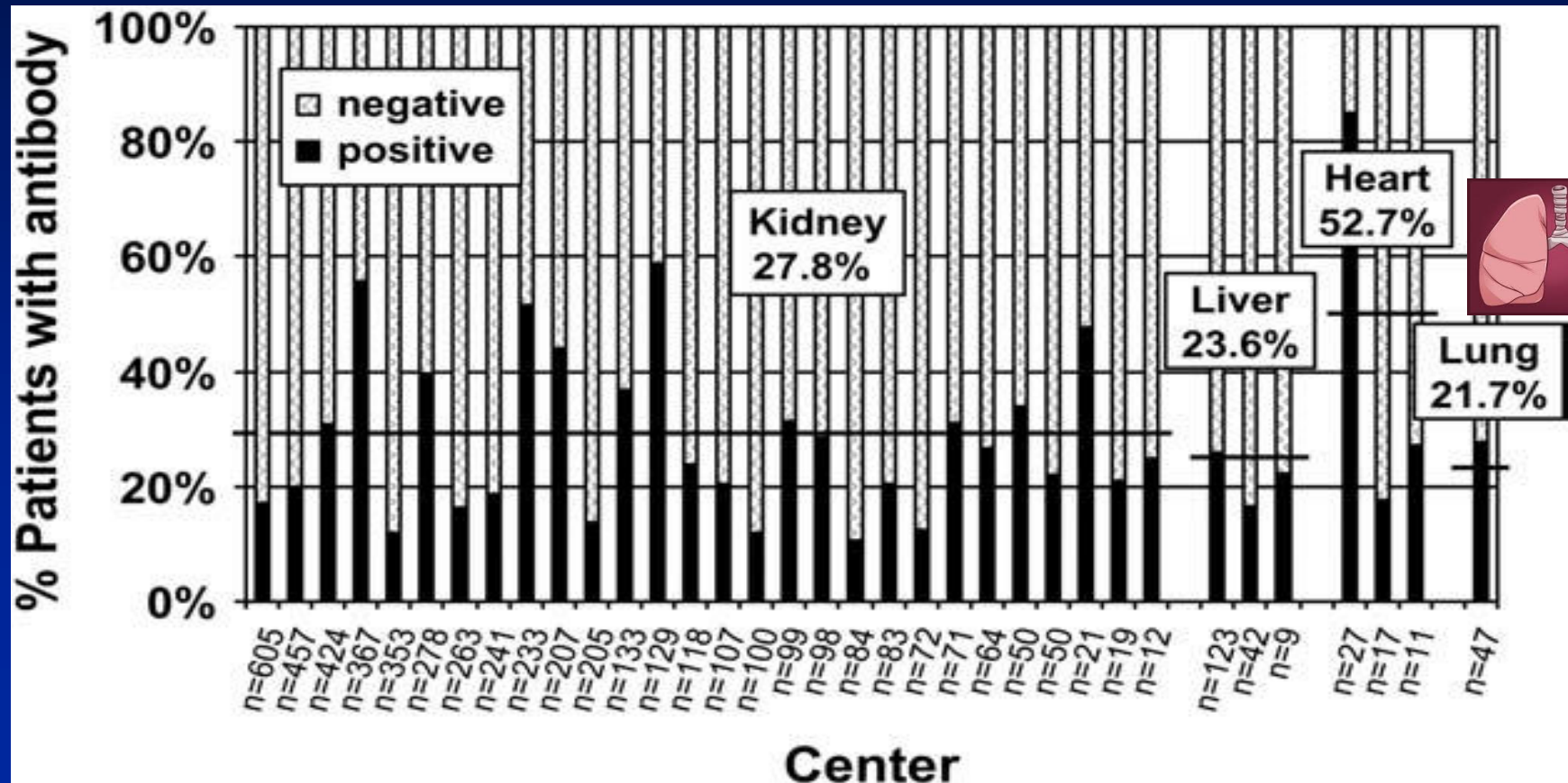
**Adriana Zeevi PhD (D) ABHI  
Professor of Pathology, Surgery and Immunology  
Director of Histocompatibility Laboratory  
University of Pittsburgh Medical Center**





# 14<sup>th</sup> International HLA and Immunogenetics Workshop: Report on the prospective chronic rejection project (Ozawa et al Tissue Antigens 2007)

**Frequency of human leukocyte antigen antibodies post-transplant by organ, n = 5219.**



# Impact of Preformed HLA-Ab

Panel Reactive Antibody (PRA) detected by cell based methods (pre 2010)

- Influence of PRA on post-transplant outcomes in LTx recipients [Lau et al Ann Thorac Surg 2000 \(n=200 LTx, method CDC\)](#)
  - Sensitized LTx experienced more acute and chronic complications after LTx (BOS 56% vs. 23 %).
- Pretransplant PRA in LTx is associated with significantly worse post-transplant survival in a multicenter study [Hadjiliadis et al JHLT 2005 \(n=656 LTx, method CDC\)](#)
  - Patients with PRA >25% had decreased median survival at 1 month, 1 and 5 years (at 5 years 31% vs. 50%).
- Pretransplant PRA in human LTx: an analysis of over 10,000 patients. [Shah et al Ann Thorac Surg 2008 \(method CDC\)](#)
  - UNOS database from 1995-2008, PRA >25 % associated with increased mortality, this effect was not seen in more recent era.

**Based on PRA using PBMC: only class I HLA-Abs were considered and no information on donor-specific HLA antibodies**

# Impact of Preformed HLA-Ab

PRA and DSA detected by solid-phase methods  
(after 2010)

- Lung transplantation in patients with pre-transplantation donor-specific antibodies (DSA) detected by luminex assay **Brugiere et al Transplantation 2013 (n=56)**
  - Freedom from BOS and survival was lower in patients with pre-formed HLA class II DSA vs. patients with HLA class I DSA or without DSA.
- Impact of pre-transplant anti-HLA antibodies on outcomes in LTx candidates. **Kim et al Am J of Resp and Crit. Care 2014 (n=224)**
  - The presence of HLA-Ab at >3000 MFI was associated with lower transplant rate and higher AMR rate as compared with patients with lower threshold HLA-Abs.
- Pre-transplant donor HLA-specific antibodies: characteristics causing detrimental effects on survival after lung transplantation. **Smith et al JHLT 2014 (n=425)**
  - Complement fixing pre-formed DSA and high MFI were associated with poor survival within the first year post LTx.

**determination by single antigen bead assay  
correlated with poor outcome**



# 2016 ISLT Consensus

The Journal of  
Heart and Lung  
Transplantation

<http://www.jhltonline.org>

## ISHLT CONSENSUS REPORT

### Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation



Deborah J. Levine, MD,<sup>a</sup> Allan R. Glanville, MBBS, MD,<sup>b</sup> Christina Aboyoun, BA, MBA,<sup>b</sup> John Belperio, MD,<sup>c</sup> Christian Benden, MD, FCCP,<sup>d</sup> Gerald J. Berry, MD,<sup>e</sup> Ramsey Hachem, MD,<sup>f</sup> Don Hayes Jr., MD, MS,<sup>g</sup> Desley Neil, MBBS, PhD,<sup>h</sup> Nancy L. Reinsmoen, PhD, D(ABHI),<sup>i</sup> Laurie D. Snyder, MD,<sup>j</sup> Stuart Sweet, MD, PhD,<sup>f</sup> Dolly Tyan, PhD,<sup>e</sup> Geert Verleden, MD, PhD,<sup>k</sup> Glen Westall, MBBS, PhD,<sup>l</sup> Roger D. Yusen, MD, MPH,<sup>f</sup> Martin Zamora, MD,<sup>m</sup> and Adriana Zeevi, PhD<sup>n</sup>

*From the <sup>a</sup>Pulmonary Disease and Critical Care Medicine, University of Texas Health Science Center San Antonio, San Antonio, Texas, USA; <sup>b</sup>The Lung Transplant Unit, St. Vincent's Hospital, Sydney, New South Wales, Australia; <sup>c</sup>Pulmonary Disease and Critical Care Medicine, University of California, Los Angeles, California, USA; <sup>d</sup>Division of Pulmonary Medicine, University Hospital Zurich, Zurich, Switzerland; <sup>e</sup>Division of Pathology, Stanford University Medical Center, Palo Alto, California, USA; <sup>f</sup>Division of Pulmonology, Washington University, St. Louis, Missouri, USA; <sup>g</sup>Department of Pulmonology, The Ohio State University, Columbus, Ohio, USA; <sup>h</sup>Department of Pathology, Queen Elizabeth Hospital, Birmingham, UK; <sup>i</sup>Department of Immunology, Cedars-Sinai Hospital, Los Angeles, California, USA; <sup>j</sup>Department of Pulmonology, Duke University, Durham, North Carolina, USA; <sup>k</sup>Department of Pulmonology, University Hospitals Leuven, Leuven, Belgium; <sup>l</sup>Department of Pulmonology, Alfred Hospital, Melbourne, Victoria, Australia; <sup>m</sup>Department of Pulmonology, University of Colorado, Denver, Colorado, USA; and the <sup>n</sup>Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.*

## Key criteria:

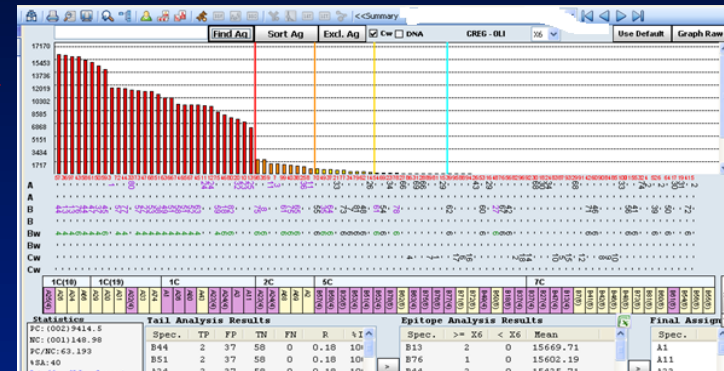
## Presence of DSA

## Lung Histology



# HLA-Ab Testing

## Single Antigen Bead



Specificity  
DSA

Risk Assessment for AMR  
*Persistent DSA- Monitoring*  
*Increase Titer -Ab Burden*  
*Complement Binding- IgG Subtype*

# MFI $\neq$ TITER

## Determining Antibody Strength

**Table 2:** Comparison between neat MFI values of several HLA-DQ antibody specificities with the corresponding MFI values as the serum was diluted, in a patient presenting with AMR and in need for antibody removal therapy

Bead ID	Neat MFI					
	Neat	1:4	<u>1:16</u>	1:64	1:256	<u>1:1024</u>
1	15 785	14 736	11 846	6688	2276	644
2	14 718	12 654	10 533	6119	2100	577
3	14 619	17 645	16 283	9755	3473	1072
4	13 993	12 512	10 202	5818	1693	355
5	12 392	11 235	8875	4637	1445	377
6	11 184	17 457	16 734	9371	3342	1007
7	10 995	17 359	15 605	8692	2886	896
8	10 606	5514	2162	448	21	0
9	8942	3953	1066	117	0	0
10	8227	3417	1018	74	0	0
1	DQA1*03:01/DQB1*02:01			6	DQA1*03:02/DQB1*03:03	
2	DQA1*03:01/DQB1*03:01			7	DQA1*03:02/DQB1*03:02	
3	DQA1*03:03/DQB1*04:01			8	DQA1*01:03/DQB1*06:01	
4	DQA1*03:01/DQB1*03:03			9	DQA1*01:03/DQB1*06:03	
5	DQA1*03:01/DQB1*03:02			10	DQA1*01:02/DQB1*06:09	

AMR, antibody-mediated rejection; MFI, mean fluorescence intensity.

DSA bead 6 and 8 show the same neat MFI- during AMR very different titers and required treatment- the kinetics of response to removal therapy different

# C1q Screen



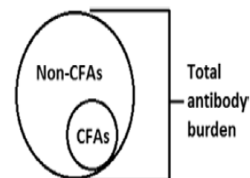
## New approaches for detecting complement-fixing antibodies

Dolly B. Tyan

## Histocompatibility

## KEY POINTS

- Complement-fixing capability of HLA antibodies can be determined using C1q and C4d solid phase assays.
- The C1q assay has high sensitivity and specificity.
- The IgG mean fluorescence intensity (MFI) cannot be used to predict which antibodies can fix complement.
- C1q+ donor-specific antibody correlates with antibody mediated rejection and graft loss in kidney and heart transplant recipients.
- The C1q assay can be used to predict and monitor resolution of antibody mediated rejection (AMR) in heart transplant patients.
- The C1q assay can be used to predict and monitor desensitization by intravenous immunoglobulin (IVIg)



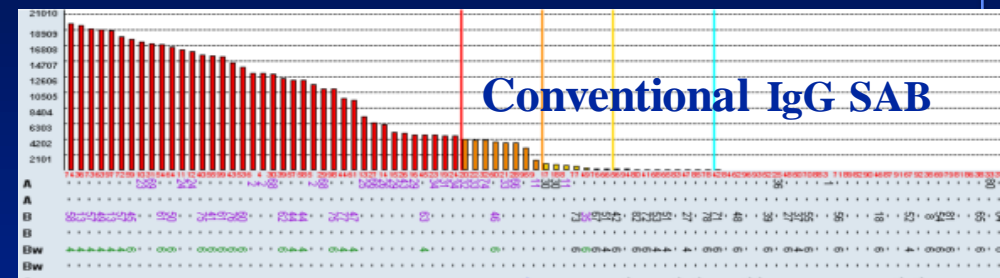
**Figure.** The total antibody burden present in a sensitized patient is composed of non-complement fixing antibodies (Non-CFAs) and complement fixing antibodies (CFAs).

## Challenges and Opportunities in Pediatric Heart Failure and Transplantation

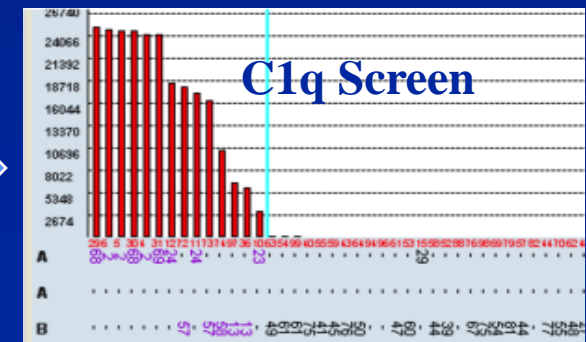
## Transplantation in the Highly Sensitized Pediatric Patient

Chesney Castleberry, MD; Thomas D. Ryan, MD, PhD; Clifford Chin, MD

## Total Antibody Burden



## Complement Fixing IgG





# From Humoral Theory to Performant Risk Stratification in Kidney Transplantation

C. Lefaucheur,<sup>1,2</sup> D. Viglietti,<sup>1,2</sup> M. Mangiola,<sup>3</sup> A. Loupy,<sup>1,4</sup> and A. Zeevi<sup>3</sup>

Journal of  
Immunol  
Research 2017

HLA-Ag	T IgG	C1q	IgG1	IgG2	IgG3	IgG4	
B53	14522	1247	5280	2023	1022	19999	C1q+
B51	13778	949	4239	2195	1079	20023	
DQ5	16026	20787	14030	5668	26	8066	
DQ6	16639	22113	14577	6045	20	9009	
A32	13967	11	5498	1615	0	0	C1q-
A23	11440	89	4733	1413	40	0	
DR12	11741	30	3864	89	0	5	

## C1q Reactivity was not predicted by total IgG MFI

**Table 1:** Correlation between different approaches currently used to assign antibody strength, for different HLA loci

Correlation	HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ	HLA-DP
C1q vs. Neat	0.395	0.529	0.484	0.788	0.344	0.197
C1q vs. Peak	0.820	0.779	0.750	0.856	0.660	0.689
C1q vs. Titers	0.709	0.830	0.911	0.891	0.870	0.973

Tambur  
AJT  
2015

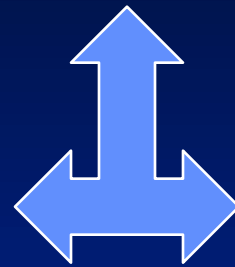
# Complement Binding HLA-Ab Characteristics

**C1q Screen Positive**

**Relative Ratio of IgG subtype:**

**CF: IgG1 and IgG3**

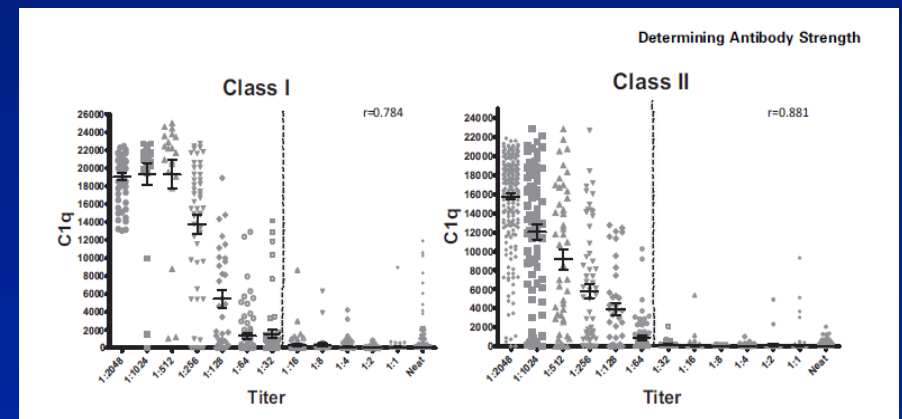
**NCF: IgG2, IgG4**



**Level of IgG:**

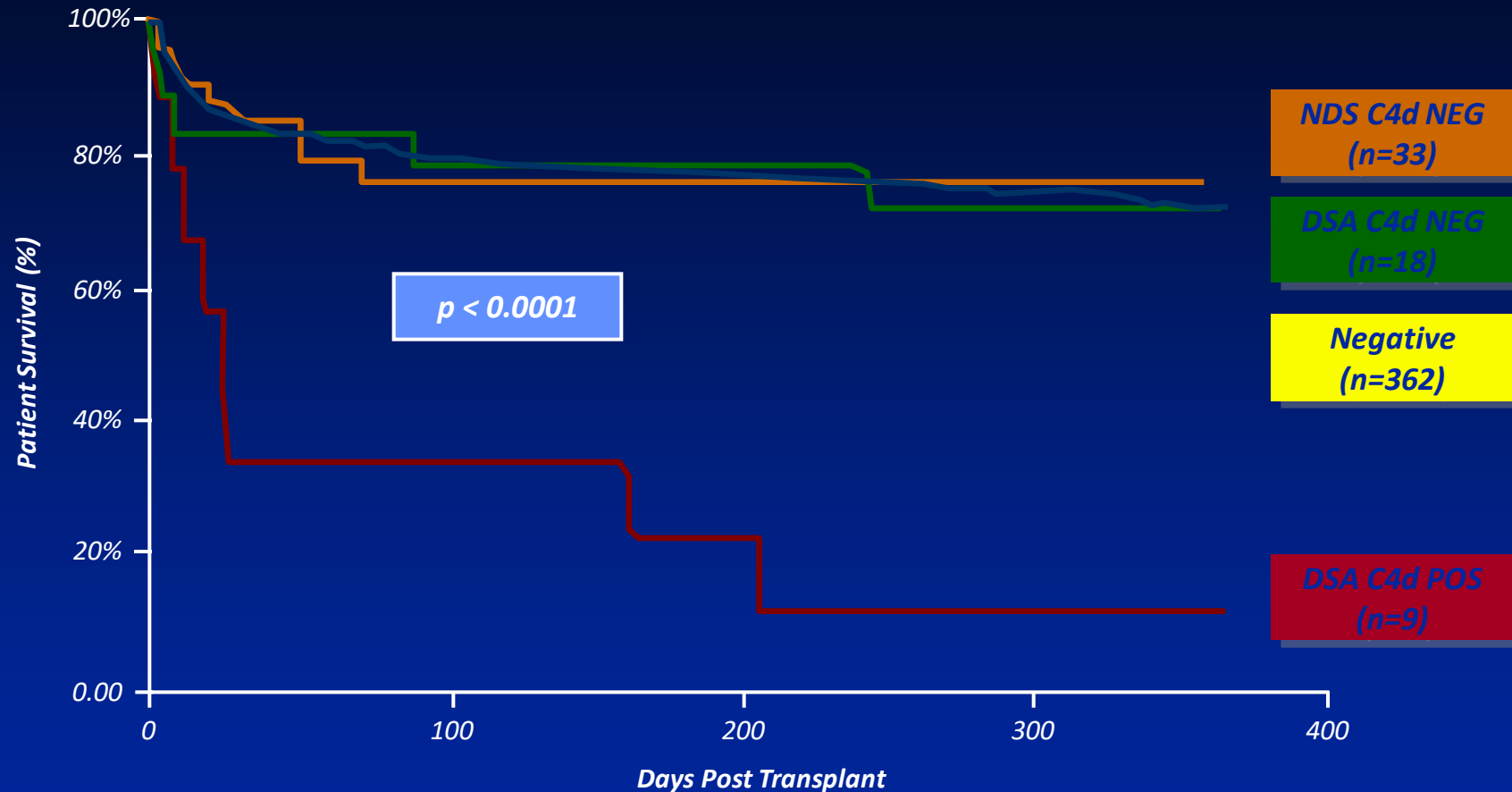
**Titer > 1:16 to 1:32**

COMPLEMENT FIXING ABILITY OF DSA	IgG SUBCLASS OF DSA	MICROVASCULAR INFLAMMATION	INFLAMMATORY CELLS PRESENT	TIME TO INDUCE GRAFT DAMAGE
C1Q NEG	IgG4	+	MONOCYTES	LONG
	IgG2	+	MONOCYTES	LONG
C1Q POS	IgG1	+++	NK CELLS, MONOCYTES	MODERATE
	IgG3	++++	NK CELLS, MONOCYTES	SHORT



# Pretransplant DSA Characteristics Causing Detrimental Effects on Survival after Lung Transplantation:

## Effect of Complement-Fixing DSA on Patient Survival



**Conclusion:** Complement-fixing DSA had significantly lower 1-year survival (11.1%) than DSA that do not fix complement (72.2%).



# *The Histopathology of Lung Allograft Dysfunction Associated with the Development of DSA*

*Yousem and Zeevi, American Journal of Surgical Pathology. 2012; 36:987*

**Post LTx de-novo DSA is 72% DQ-specific  
(DQB, DQA and DQB/DQA pairs)**

Lung Phenotype	ACR	DSA	Complement Fixing (C1q)
ACR/AMR	Refractory/ Persistent post IST*	De-novo Rising Titer	Positive Persistent
ACR	Response to IST*	Transient	Negative/ Transient

*\* IST- Immunosuppression Treatment*

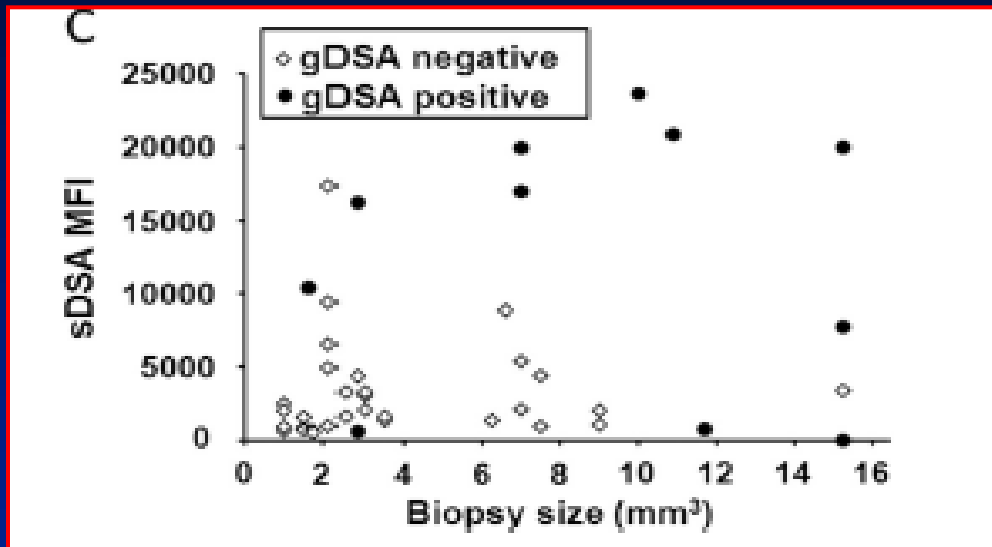
**Donor-Specific Class II HLA-DQ Complement Binding Antibody  
Are Associated with Severe Rejection in Lung Transplantation**

Lobo et al JHLT 2013: DSA are associated with AMR, ACR, BOS after LTx

# Lung intragraft donor-specific antibodies as a risk factor for graft loss



Jonathan Visentin, PharmD, PhD,<sup>a,b</sup> Albane Chartier, MD,<sup>c</sup> Layal Massara,<sup>b</sup> Gabriel Linares,<sup>a</sup> Gwendaline Guidicelli, PharmD, PhD,<sup>a</sup> Elodie Blanchard, MD,<sup>c</sup> Marie Parrens, MD, PhD,<sup>d,e</sup> Hugues Begueret, MD,<sup>d</sup> Claire Dromer, MD,<sup>c</sup> and Jean-Luc Taupin, PharmD, PhD<sup>a,b</sup>



Link between serum MFI,  
biopsy fragment size and gDSA

7/11 >10,000 MFI

8/11 > size 6 mm<sup>3</sup>

28 LTx sDSA (50 DSAs)  
28% Class I (6A, 2B, 6C)  
72% Class II (8DR, 22DQ, 4DP)

15 sDSA C1q + (1DR, 14 DQ)  
15 gDSA (4 Class I, 1DR, 10 DQ)

1/15 gDSA in the biopsy only

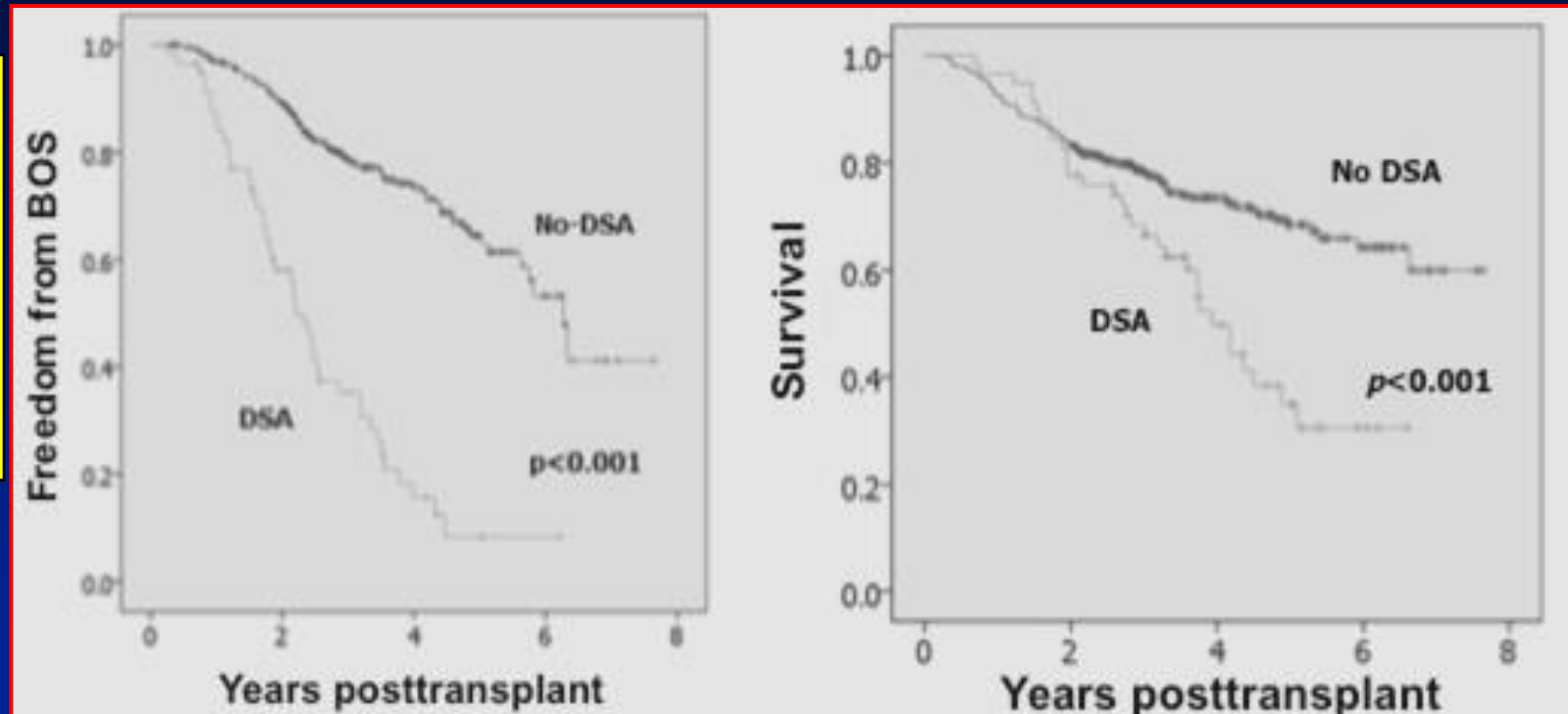
Only 1 LTx had C4d+ biopsy  
45.5% had ACR  
36.4% had BOS (prior biopsy)



# De novo DSA are associated with early and high grade BOS and death after LTx

**Morrell et al JHLT 2014**

- **445 LTx**
- **Follow-up**  
 **$3.3 \pm 1.9y$**
- **14.8% had DSA**
- **41/58 with DSA**  
**Developed BOS**



*Safavi et al JHLT 2014: de novo DSA predict development of BOS after LTx*  
*Ius et al JHLT 2014: DSA in Ltx risk factors and impact on survival*  
*Witt et al JHLT 2013: Acute antibody mediated rejection after LTx*

# De Novo DQ Donor-Specific Antibodies Are Associated with Chronic Lung Allograft Dysfunction after Lung Transplantation

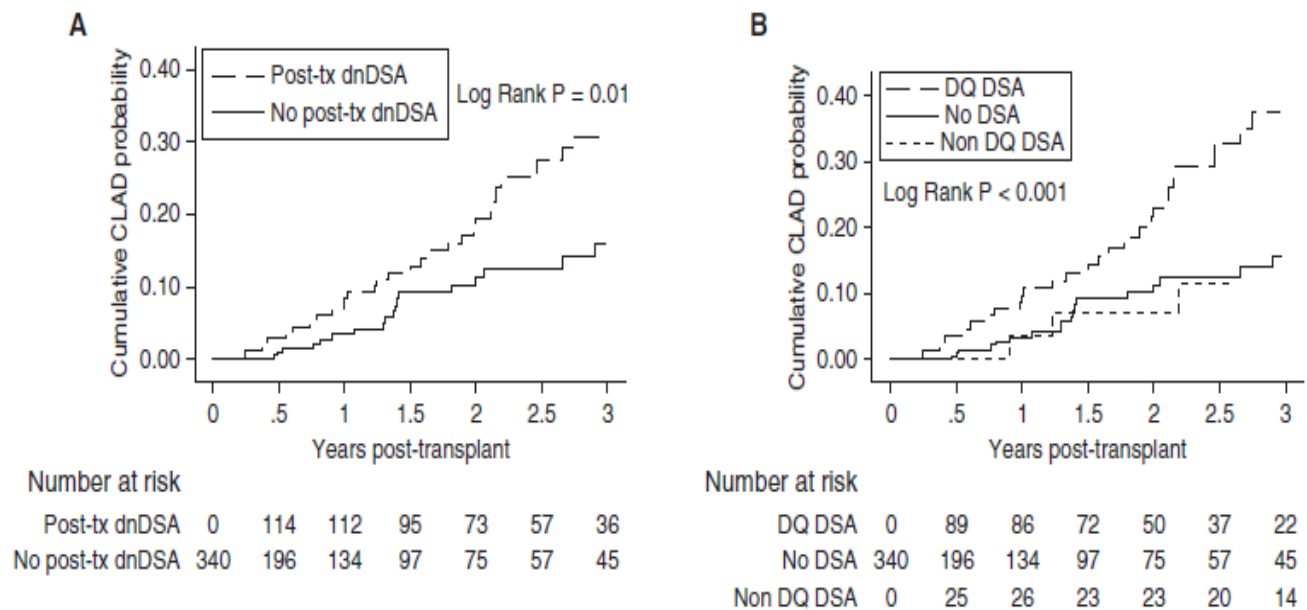
Jussi M. Tikkanen<sup>1</sup>, Lianne G. Singer<sup>1</sup>, S. Joseph Kim<sup>2</sup>, Yanhong Li<sup>2</sup>, Matthew Binnie<sup>1</sup>, Cecilia Chaparro<sup>1</sup>, Chung-Wai Chow<sup>1</sup>, Tereza Martinu<sup>1</sup>, Sassan Azad<sup>1</sup>, Shaf Keshavjee<sup>1</sup>, and Kathryn Tinckam<sup>2,3</sup>

## At a Glance Commentary

**Scientific Knowledge on the Subject:** Increasing evidence suggests that donor-specific antibodies may play a role in development of chronic lung allograft dysfunction.

**What This Study Adds to the Field:** This study demonstrates that post-transplant *de novo* donor-specific human leukocyte antigen (HLA) antibodies (dnDSA) are common and occur early after lung transplantation and before chronic lung allograft dysfunction (CLAD). The number of HLA DQ mismatches between donor and recipient is the strongest independent predictor of dnDSA development. HLA-DQ dominates dnDSA specificity and independently drives the association with CLAD.

## The impact and temporal relationship of dnDSA and DQ specific DSA on chronic allograft dysfunction (CLAD)

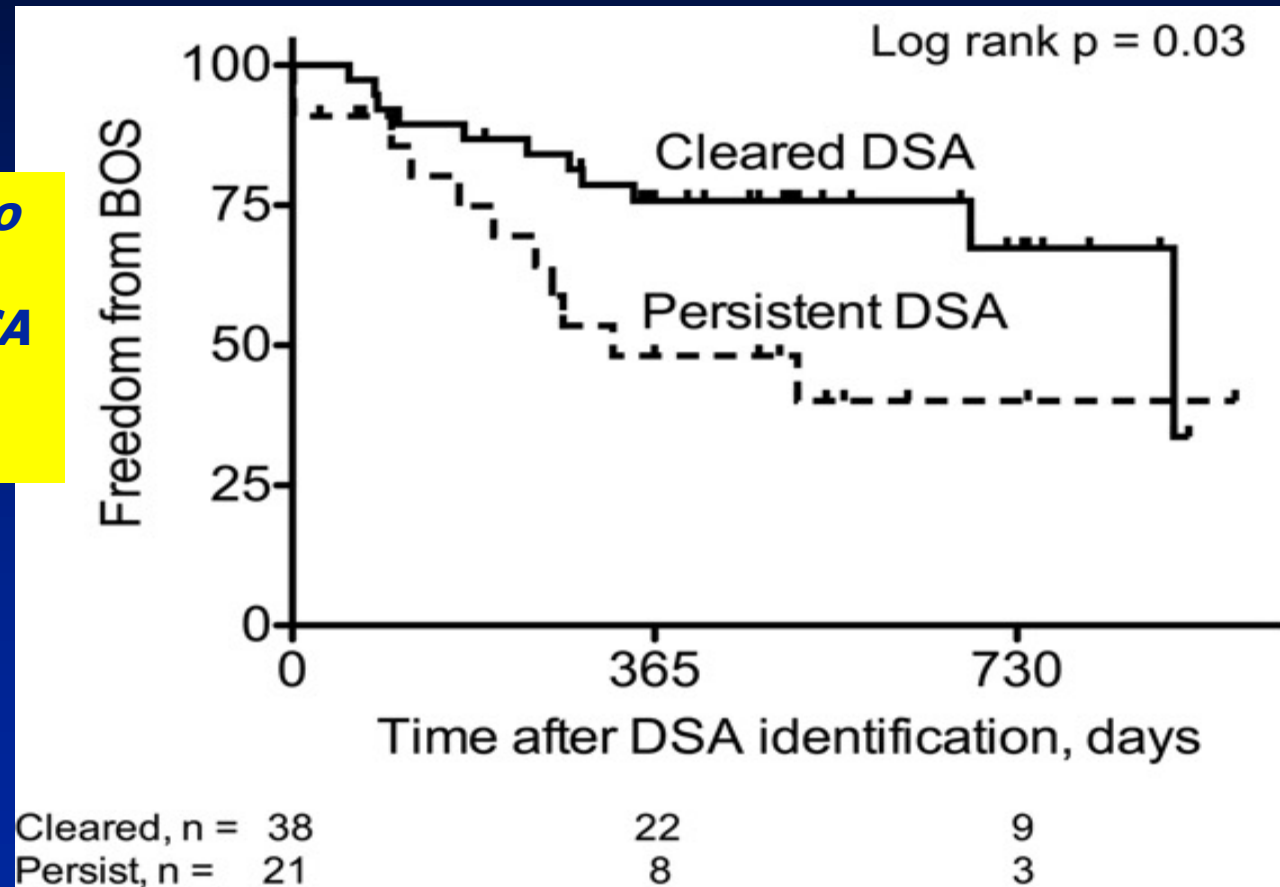


# **Implications for Human Leukocyte Antigen Antibodies After Lung Transplantation A 10-Year Experience in 441 Patients**

- HLA antibodies after lung transplant are associated with
  - increased risk for BOS and worse survival,
  - DSA is associated with worse survival.
- HLA antibodies appear to be an integral part of the immune response to the allograft both preceding graft dysfunction and after allograft dysfunction.
- **A key question to address in further analysis is if the decrease or elimination of these antibodies correlates with improved outcomes**

# ***Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation***

***BOS was more likely to develop in recipients who had persistent DSA than in those who cleared the DSA.***



*Ramsey R. Hachem, MD, et al J Heart Lung Transplant 2010;29:973–80*

# Proteasome Inhibitor Carfilzomib-Based Therapy for Antibody-Mediated Rejection of the Pulmonary Allograft: First Use and Short-Term Findings

Ensor, Zeevi , McDyer AJT 2017

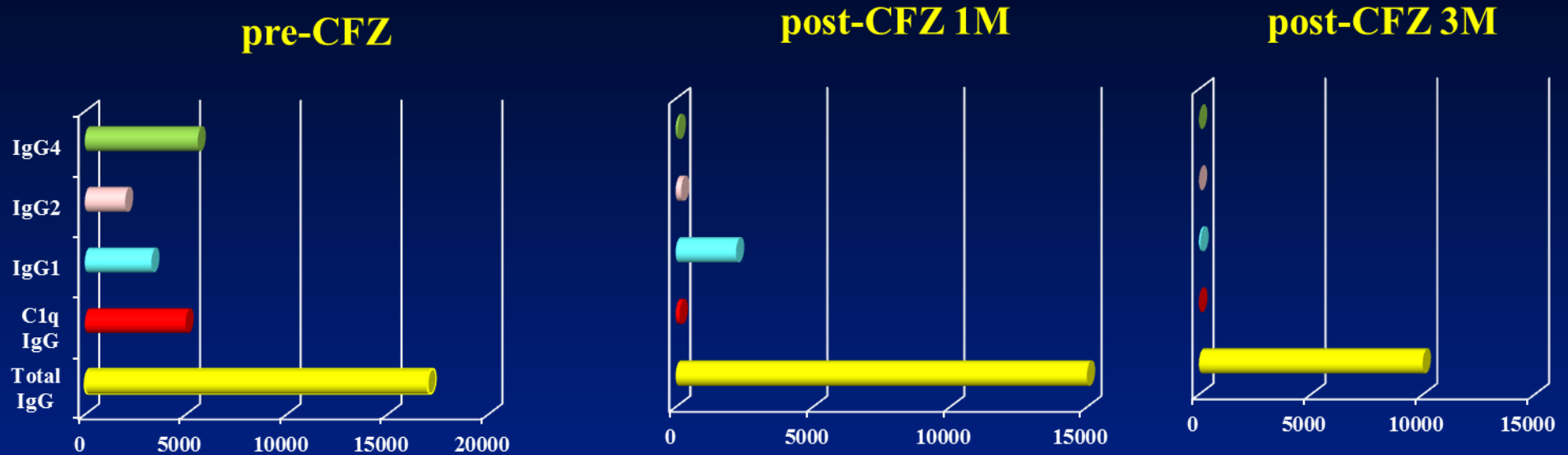
16pts (23 iDSA) : 69% DQ, 19% DQ+DR, 12% DR  
6 patients had C4d+ AMR, 10 patients C4d- probably AMR

CFZ-based therapy resulted in profound depletion of circulating iDSA, removal of DSA C1q-fixing ability *in vitro*, a high degree of responsiveness, and stabilized or recovered lung allograft function.



# Carfilzomib Responder :Loss of DSA C1q Reactivity

## Pt1 DSA DQB1\*04:02/DQA1\*04:01

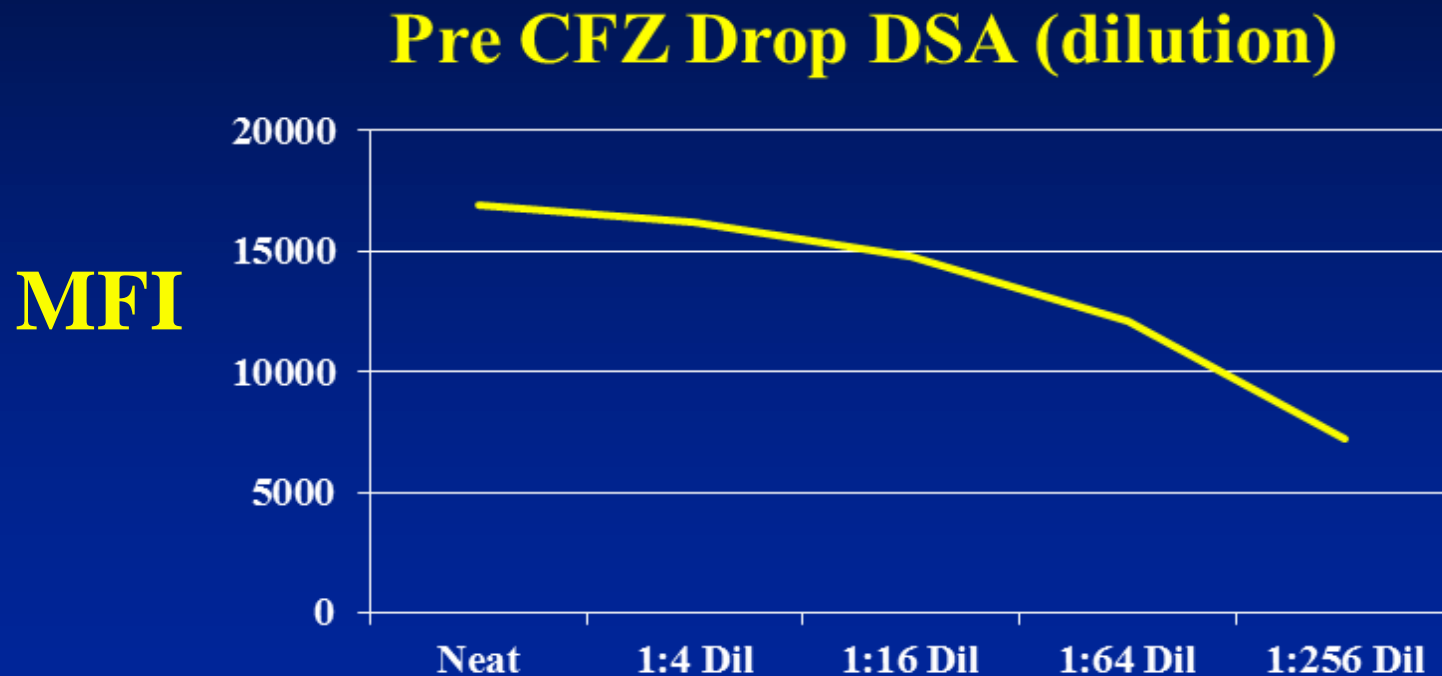


1. Total IgG MFI Persisted (yellow bar)
2. Loss of C1q Reactivity (red bar)
3. Drop in level of IgG Subtypes (IgG1 blue, IgG2 pink and IgG4 green)

# DSA Titer Pre AMR Treatment

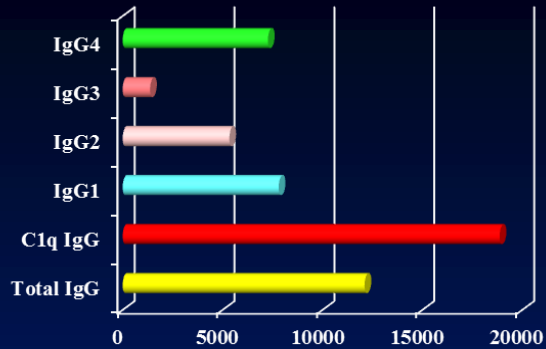
## Immuno-dominant DSA

### DQB1\*04:02/DQA1\*04:01



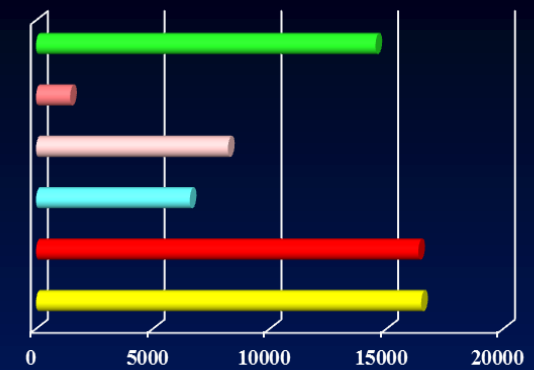
*iDSA was <2000 MFI at 1:2048 titer*

Pre CFZ



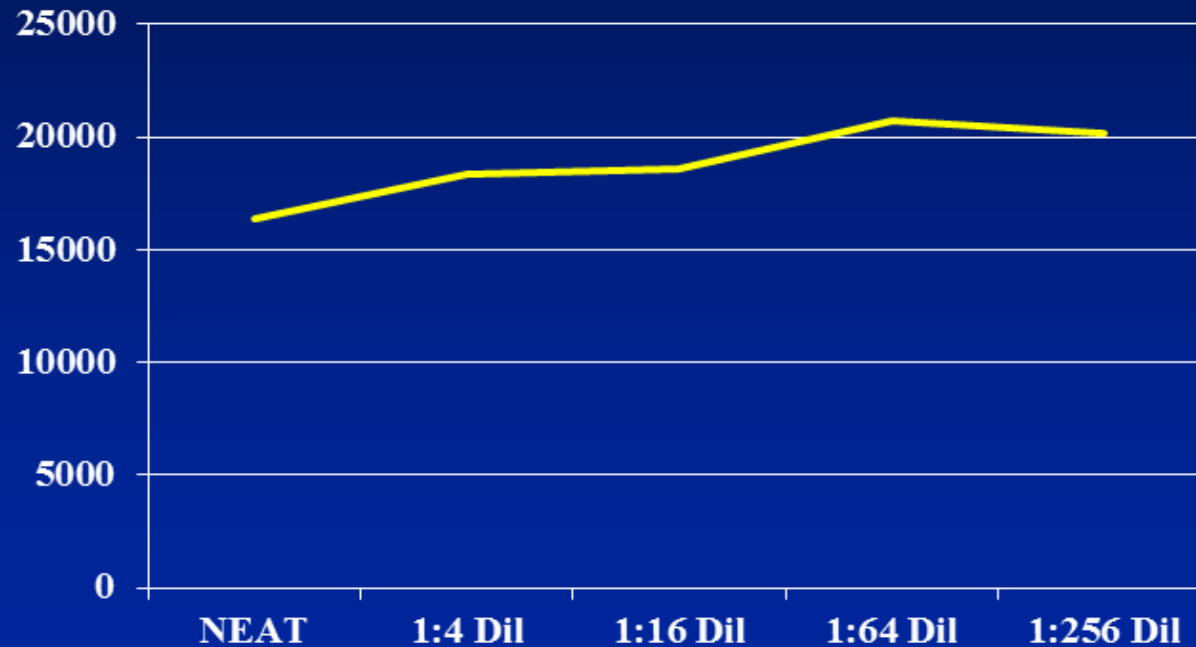
LTx Non Responder  
High Titer DSA Pre-AMR  
Persisted post Treatment

Post CFZ 3M



High Titer DSA Pre-CFZ

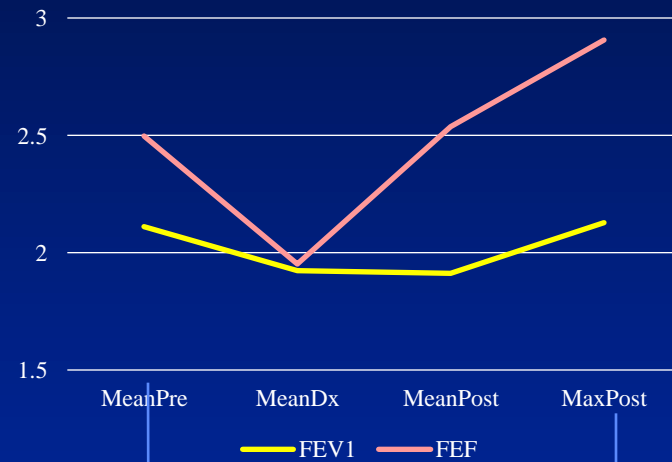
MFI



*iDSA was >10,000 MFI at 1:2048 titer*

# PFT Recovery after Carfilzomib-Based AMR Therapy

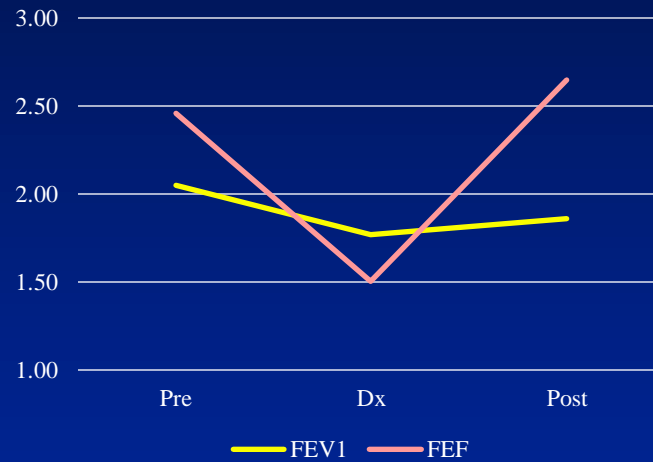
PFTs mean change



*FEV1*  $p=0.4$

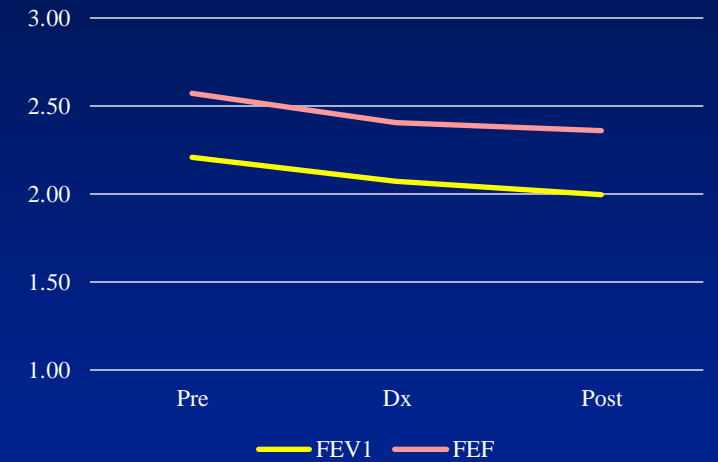
*AMR*

PFTs in C1q responders



*AMR*

PFTs in C1q non-responders

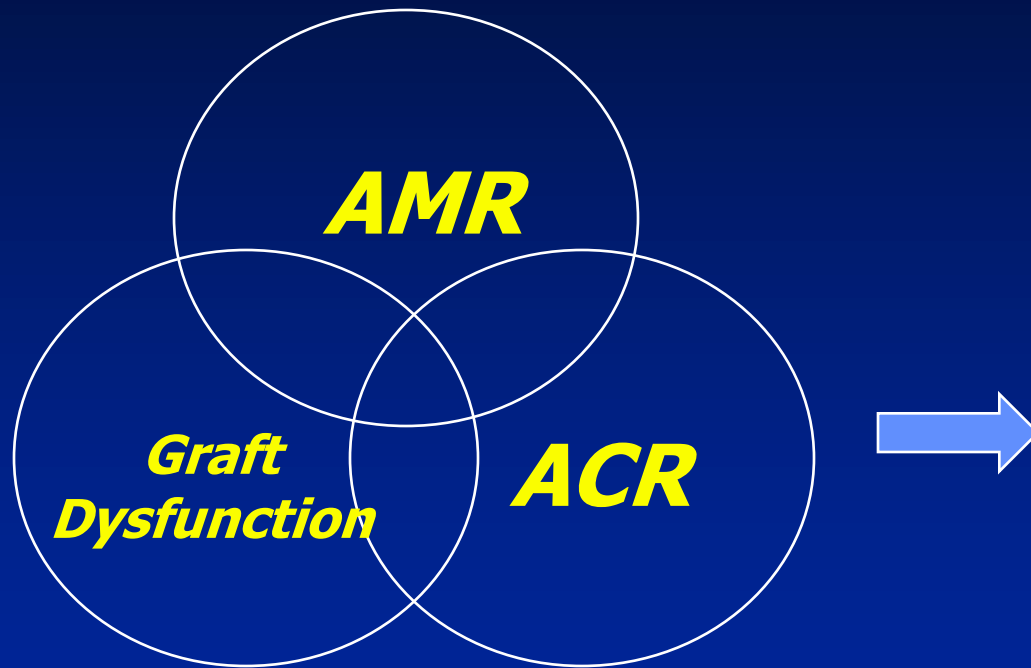


*AMR*

*Courtesy of Chris Ensor PharmD*

# Impact of DSA in LTx

Antibody rejection in lung transplantation: Myth or reality?



Allan Glanville JHLT 2010; 29

## Consensus Report AMR in LUNG Tx



2017

The Journal of  
Heart and Lung  
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<http://www.jhltonline.org>

ISHLT CONSENSUS REPORT

### Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation



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From the <sup>a</sup>Pulmonary Disease and Critical Care Medicine, University of Texas Health Science Center San Antonio, San Antonio, Texas, USA; <sup>b</sup>The Lung Transplant Unit, St. Vincent's Hospital, Sydney, New South Wales, Australia; <sup>c</sup>Pulmonary Disease and Critical Care Medicine, University of California, Los Angeles, California, USA; <sup>d</sup>Division of Pulmonary Medicine, University Hospital Zurich, Zurich, Switzerland; <sup>e</sup>Division of Pathology, Stanford University Medical Center, Palo Alto, California, USA; <sup>f</sup>Division of Pulmonology, Washington University, St. Louis, Missouri, USA; <sup>g</sup>Department of Pulmonology, The Ohio State University, Columbus, Ohio, USA; <sup>h</sup>Department of Pathology, Queen Elizabeth Hospital, Birmingham, UK; <sup>i</sup>Department of Immunology, Cedars-Sinai Hospital, Los Angeles, California, USA; <sup>j</sup>Department of Pulmonology, Duke University, Durham, North Carolina, USA; <sup>k</sup>Department of Pulmonology, University Hospitals Leuven, Leuven, Belgium; <sup>l</sup>Department of Pulmonology, Alfred Hospital, Melbourne, Victoria, Australia; <sup>m</sup>Department of Pulmonology, University of Colorado, Denver, Colorado, USA; and the <sup>n</sup>Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.



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**Renal, Heart, Lung, Liver Team**  
**Adult and Pediatric**

