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HLA DSA properties assessment in the real life: what does it add

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Thomas Starzl MD PhD
1926-2017

"Tom Starzl was a man of unsurpassed intellect passion and courage whose work open up a new frontier in science and for ever changed modern medicine" UPMC

2006 Fellows Reunion

Paul I Terasaki PhD
1929-2016

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Special Article

Humoral Theory of Transplantation

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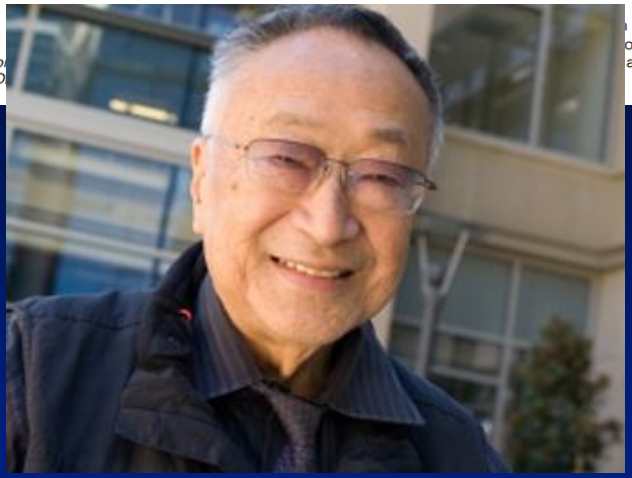
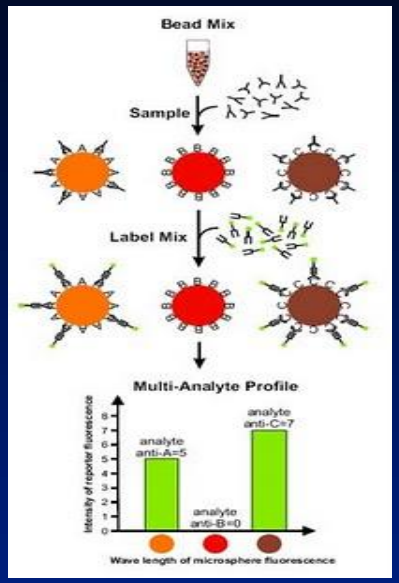
we do not
ow 'KNOW'
are involved



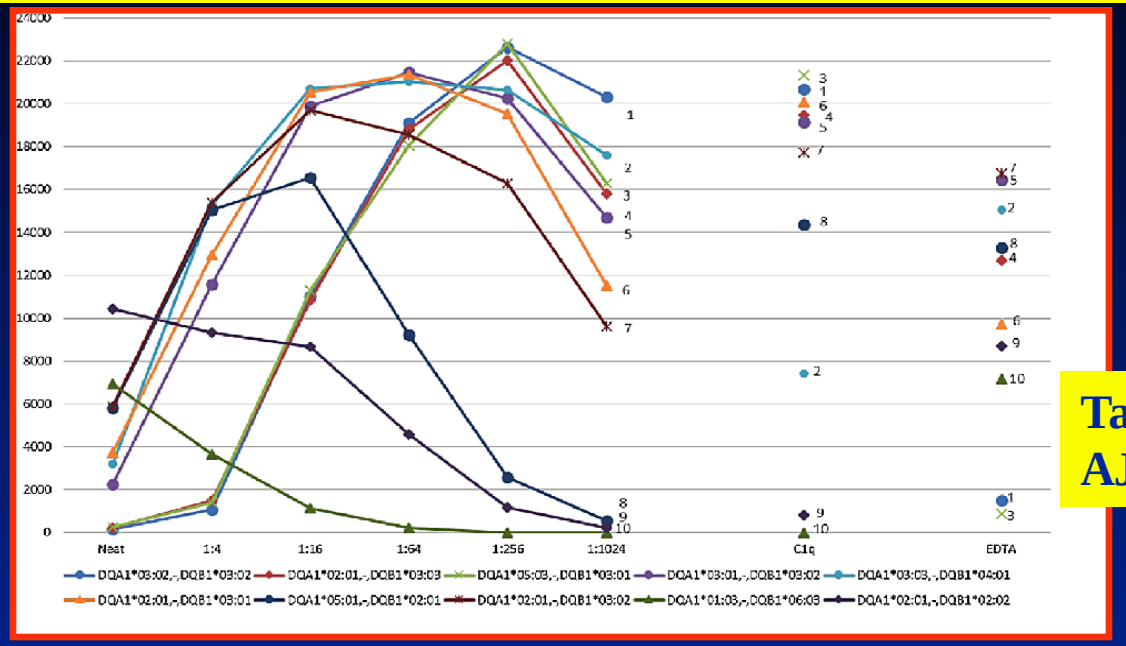
Outline

- Characterization of donor-specific HLA antibody (DSA) level (titer vs. strength)
- Review of the characteristics of complement binding and IgG subtype testing.
- Why one test does not fit all: DSA MFI for pre- and post-transplant patient management

DSA Evaluation – Beyond MFI

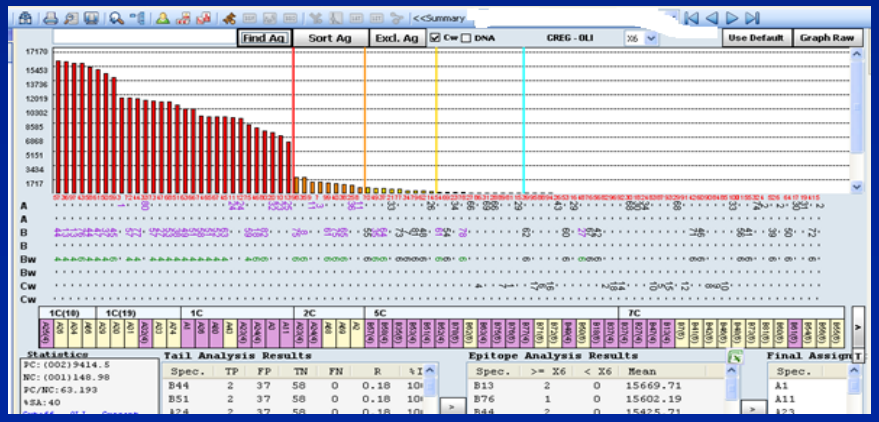


Estimation of Level TITER (NOT MFI)



Tambur AJT 2015

Specificity and Strength MFI



Complement fixing ability

C1q
C3d
C4d

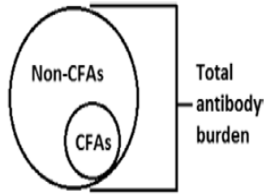


Figure. The total antibody burden present in a sensitized patient is composed of non–complement fixing antibodies (Non-CFAs) and complement fixing antibodies (CFAs).

Castleberry Circulation 2014

SAB Testing
Limitation / Resolution

Problem	Interpretation	Resolution	References
HLA-Ab to denatured antigen	**False positive Clinically irrelevant Abs**	Repeat testing after denaturation with acetic acid treatment	Otten 2013 Visentin 2014 Pereira 2011
Variable protein concentration on beads	**HLA-C, DQ and DP higher MFI on SAB than on cellular targets**	Potential discrepant results high MFI and weak/ negative CXM Compare to PRA beads	
Inhibition of MFI on SAB "prozone"effect	**Blocking factors cause low/ false negative results**	Dilution, Inhibition C1q, EDTA*, Heat	Schnaid 2011 Zeevi 2013 Zachary 2009
False negative or low results	**Epitope shared by multiple SAB (public)**	Adequate analysis of specificity /epitope level	

* EDTA treatment may not be effective with high titer >1024 HLA-Ab
Tait 2013, Tambur 2014, Tinckam 2015, Eckels 2014

Review Article

From Humoral Theory to Performant Risk Stratification in Kidney Transplantation

C. Lefaucheur,[1,2] D. Viglietti,[1,2] M. Mangiola,[3] A. Loupy,[1,4] and A. Zeevi[3]

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

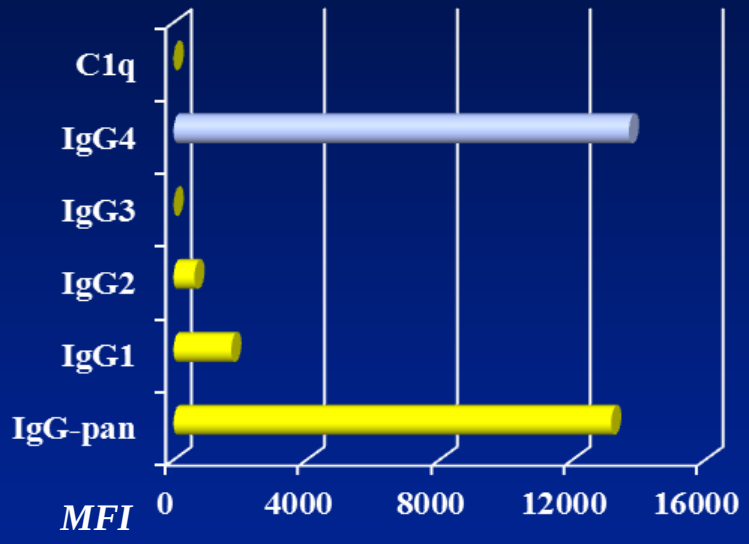
Table 1: Correlation between different approaches currently used to assign antibody strength, for different HLA loci

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

Pediatric Liver Transplant Recipients Similar Total IgG MFI with Different IgG subtype distribution and C1q reactivity

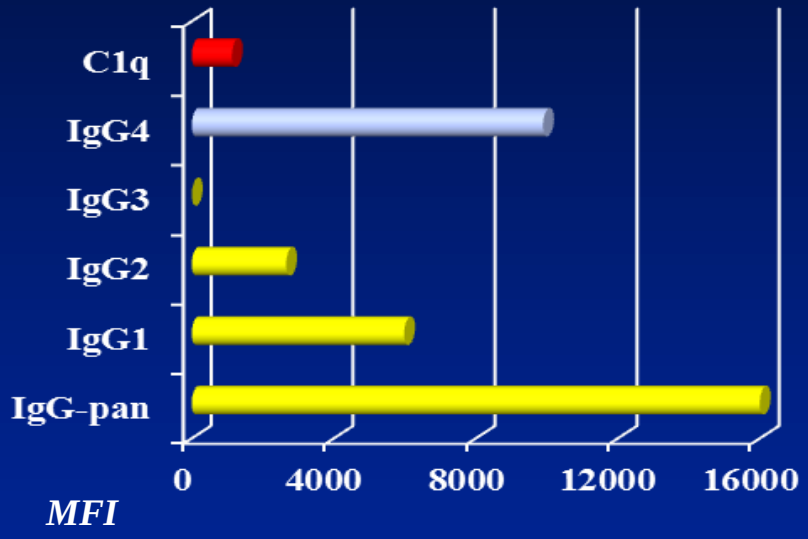
Pt 1218: DSA DQ7/DQA05



Total IgG >10,000 MFI
Weak IgG1 and IgG2, Strong IgG4

C1q Negative

Pt 1015: DSA DQ2



Total IgG>10,000 MFI
Moderate IgG1, IgG2 and IgG4

C1q Positive

Complement Binding HLA-Ab Characteristics

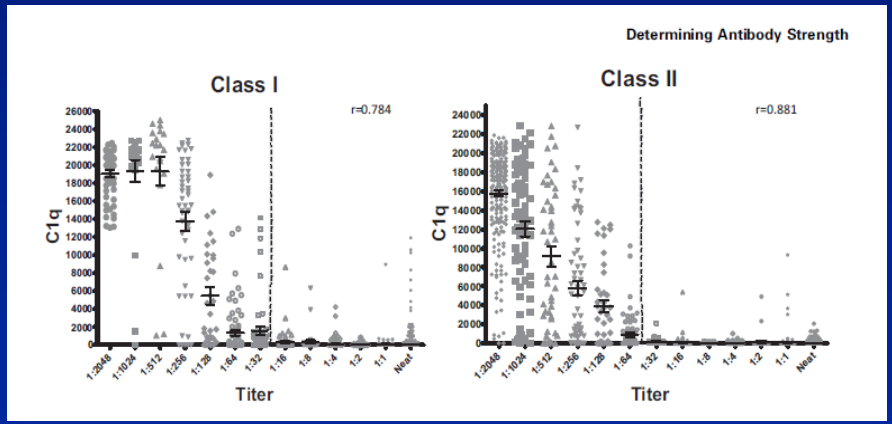
C1q Screen Positive

Relative Ratio of IgG subtype:
CF: IgG1 and IgG3
NCF: IgG2, IgG4

Level of IgG:
Titer > 1:16 to 1:32 Class I
Titer >1:32 to 1:64 Class II

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

Hidalgo



Saturation SAB High Titer IgG/ C1q

Tambur AJT 2015

Changes in DSA level pre and post-Tx impact on patient management

- Renal Tx. 1/2017: Flow CXM Negative for T and B cells. Historical DSA: Class I HLA-B13 positive following transfusion 2y prior Tx.
- Post-Tx -Memory response: increase in DSA B13 early post Tx. (low titer and C1q negative). Indication biopsy to rule out acute rejection.

DSA	5/2015	8/2015	7/2016	Tx 1/2017	2/2017	3/2017
B13 A26	559 <100	11,647 1,377	653 <100	854 <100	7,903 1,756	7,456 1,096

Blood transfusion

1:16 dilution
B13- 2,700 MFI
A26 <100
C1q Negative

Minimal interstitial Inflammation, no ACR
No AMR
g0, i1, t0, v0

A Case of Desensitization
Pediatric HTx cPRA 70%

Antibody	Pre-Bortezomib (BTZ)			1 month post-BTZ			2 months post-BTZ			Day of transplant (Virtual XM)			12 days post-Transplant		
	Neat	1:16	C1q	Neat	1:16	C1q	Neat	1:16	C1q	Neat	1:16	C1q	Neat	1:16	C1q
DQ7/DQA1*05 (DSA)	6269	2416	12,832	11,655	1764	<500	3882	<500	<500	3934	<500	<500	882	<500	<500
DR53 (non-DSA)	7191	12,116	23,808	6364	11,769	23,356	14,727	4156	8765	14,617	4772	13,356	10,711	1467	<500

1) DQ7 DSA dropped to <2000 MFI at 1:16 and C1q-negative 1 month post BTZ and remained at <4000 MFI ever since.
2) the non-DSA DR53 remained strong (>10,000 MFI) at 1:16 titer and C1q-positive up to transplant day.
3) At day +12 the patient did not have DSA DQ7 above 1000 MFI or C1q-positive.
4) Efficacy of BTZ treatment : by 1 month DSA DQ7 drop titer and C1q (not by neat MFI)
5) B cell Flow CXM Weak / B cell CDC CXM Strong Positive
6) Based on: Flow CXM, DSA level (<1:16) and C1q- the B cell CXM was proven to be false positive.

Persistent DSA/C1q+ pre AMR

Pediatric Renal Tx 2011, No DSA 2011-2013

DSA Class I+II	Pre AMR June 2014 (MFI)	Pre AMR May 2015 (MFI)	Pre AMR Dec 2015 (MFI)	AMR May 2016 (MFI)	Post AMR Sept 2016 (MFI)	Post AMR Dec 2016 (MFI)
DQB1*06:03 DQA1*01:01 IgG IgG 1:16 IgG1:64 C1q	 6,433 <100	 5,036 <100	 847* 19,840	 2,134* 19,055 17,960 27,375	 1,626 1,859 <100 1,924	 3,674 <100
B44 IgG	1,044	3,428	1,988	3,221	Neg	Neg
C5 IgG	Neg	4,885	2,187	3,104	1,030	3,075
A1 IgG	Neg	4,585	2,649	3,553	Neg	Neg
A11 IgG	Neg	Neg	1,766	2,566	Neg	Neg

Class I DSA C1q Neg

* Prozone

Cr 0.6 – Current 1.3
GFR 103 – Current 53

T cell rejection Banff 2A
Active ABMR, Diffuse C4d
Mild interstitial fibrosis/tubular athrophy
Treated:14 day Bortezomib Protocol

Dynamic changes DSA properties from active AMR, treated AMR and re-current AMR

Pediatric Renal 14 y Tx 2010 , No DSA 4 years

DSA Class II	3/2015 AMR C4d +	6/2015 Post Treat.	8/2015	10/2015	12/2015 AMR C4d+	1/2016 Post Treat.	4/2016
DQB1*05:01 DQA1*01:01							
IgG	2,086*	4,127	2,860	2,930	429*	2,171*	1,100*
C1q	27,861	6,898	<100	<100	28,356	27,904	29,559
DQB1*03:03 DQA1*02:01							
IgG	4,340	<100	<100	300	3,025*	3,303	4420
C1q	3,545	<50	<50	<50	19,158	79	3,845

* Prozone

Plasma cell rich inflammation
Mild interstitial fibrosis, Diffuse C4d
Sol/ Thymo/ Bortez protocol

Cr 1.3 – Current 2.5
GFR 70 – Current 30

T cell rejection Banff 2A
Active ABMR, Mild interstitial fibrosis/tubular athrophy
Chronic rejection (arteritis and obliterative artheriopathy)
Bortez protocol

Folllow-up 1/2016: ACR with ABMR

Contents lists available at SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Human leukocyte antigen epitope analysis to assess complement- and non-complement-binding donor-specific antibody repertoire in a pediatric heart transplant recipient

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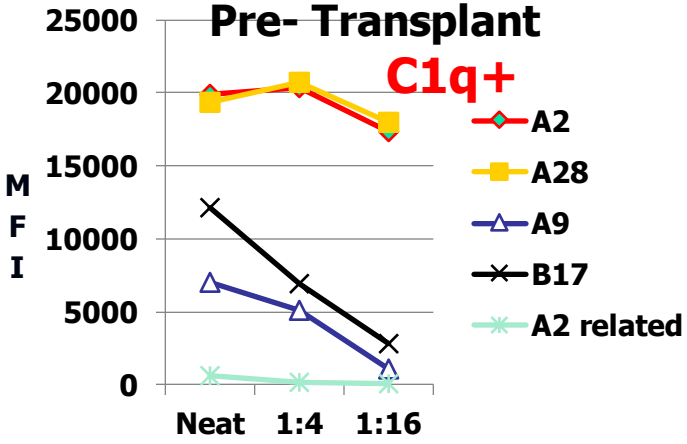
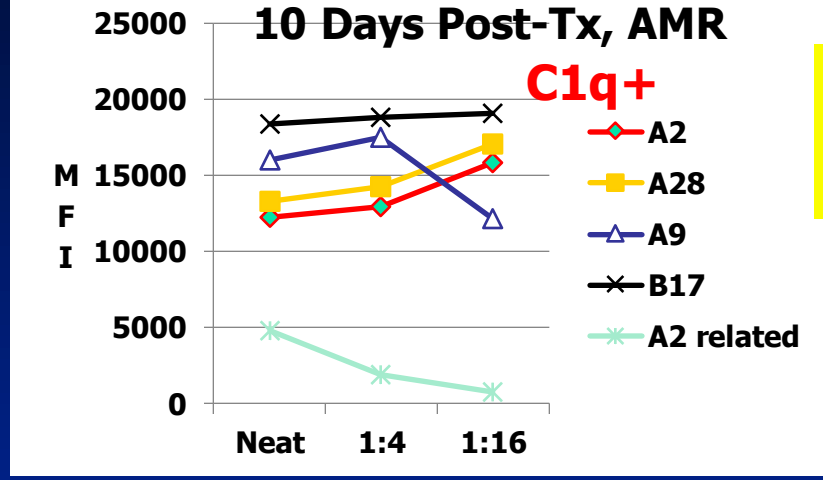
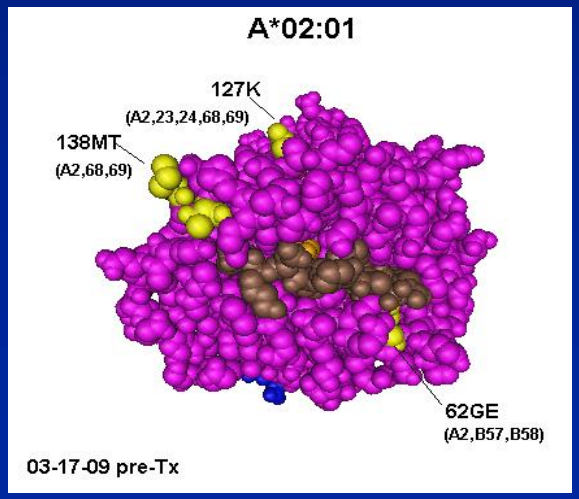
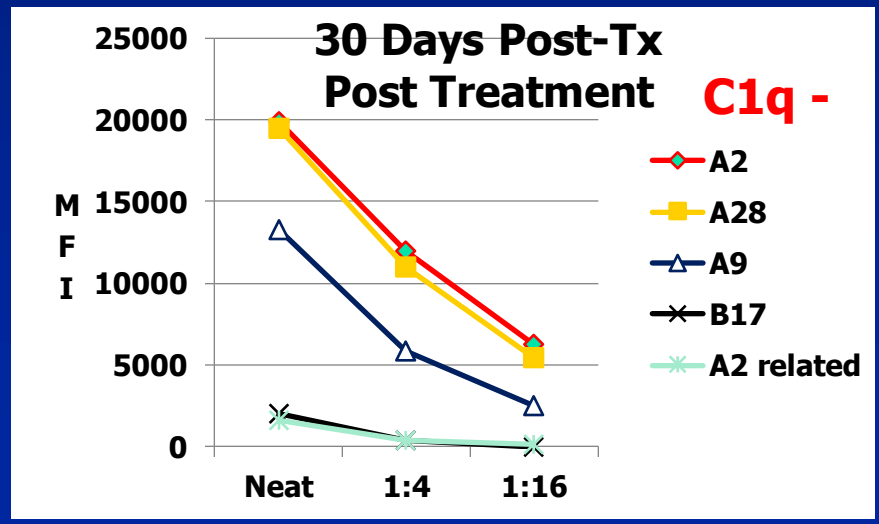








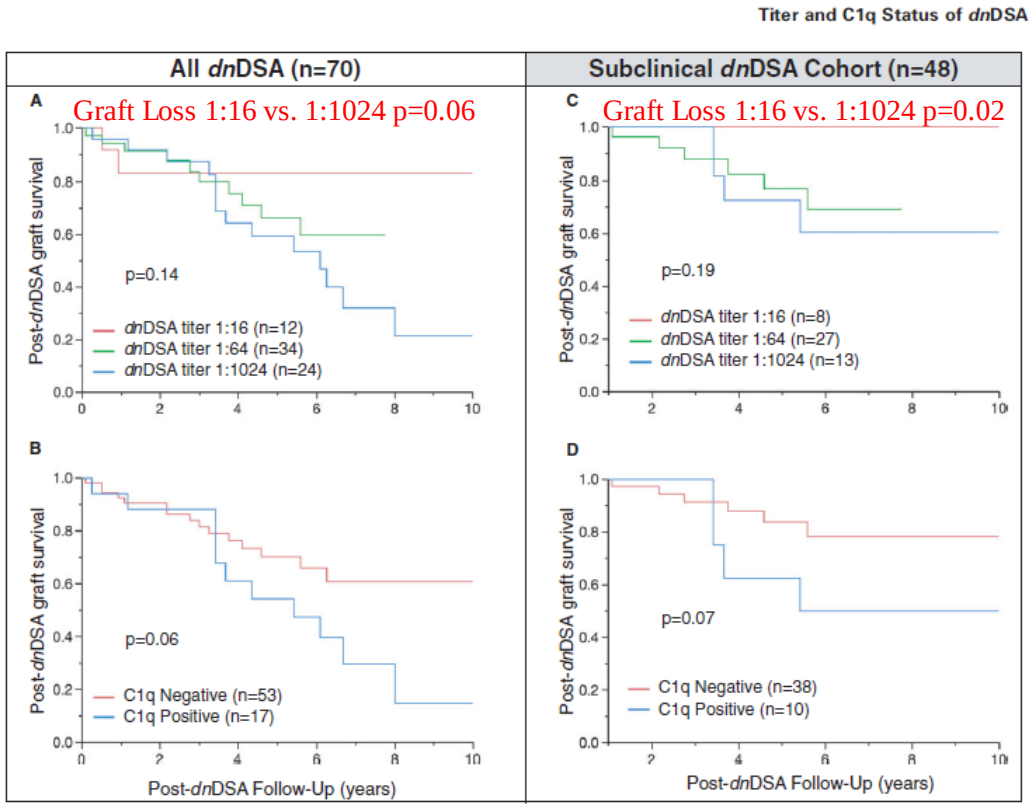
*HLA-A*02:01 Epitope*

Zeevi et al 2012

Evaluation of C1q Status and Titer of *De Novo* Donor-Specific Antibodies as Predictors of Allograft Survival

Wiebe et al AJT 2017

Titer and C1q Status of dnDSA



C1q+ DSA more likely graft loss (71% vs. 28% p<0.01)
At high titer >1:1024 there is a saturation of SAB (IgG/C1q)
Greater DSA titer correlated with TCMR and ABMR

Banff histology

Table 2: Banff histology by C1q status

Banff scores/diagnosis	C1q status: Negative	C1q status: Positive	p-value
g ≥ 1	14/31 (45%)	4/12 (33%)	0.22
i ≥ 1	20/31 (65%)	10/12 (83%)	0.15
t ≥ 1	14/31 (45%)	10/12 (83%)	0.02
v ≥ 1	1/31 (3%)	1/12 (8%)	0.41
ptc ≥ 1	21/31 (68%)	11/12 (92%)	0.09
C4d positive	12/30 (40%)	8/10 (80%)	0.03
cg ≥ 1	3/31 (10%)	3/12 (25%)	0.16
ci ≥ 1	22/31 (71%)	9/12 (75%)	0.28
ct ≥ 1	28/31 (90%)	11/12 (92%)	0.43
cv ≥ 1	16/31 (52%)	8/12 (67%)	0.19
T cell–mediated rejection	15/31 (48%)	8/12 (67%)	0.15
Antibody-mediated rejection	22/31 (71%)	10/12 (83%)	0.23

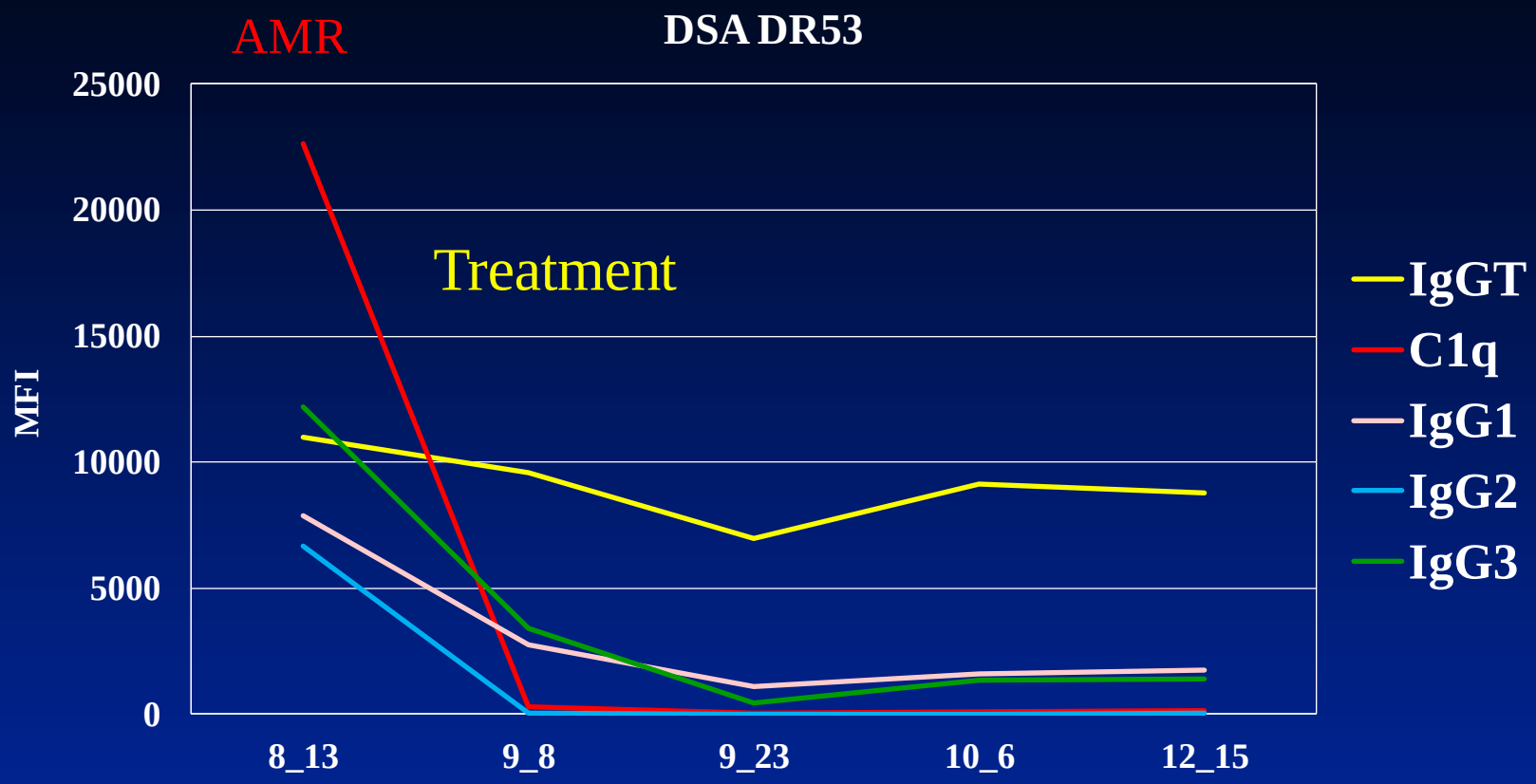
C1q reactivity (>500 MFI) associated:

1. 50% in dnDSA with 1:64 to 1:1024 titer
2. with tubulitis (p=0.02)
3. C4d positivity (p=0.03)

Case of Late AMR Liver Tx

- The 29 year-old male underwent OLTx at age 8 months because of biliary atresia and developed low-grade de novo HCV several years after transplantation with minimal liver injury test elevations.
- In year 26 s/p OLTx he was treated with an IFN/Ribavirin regimen, which resulted in HCV clearance, but within weeks developed cholestatic liver injury tests.
- A biopsy showed recurrent HCV, biliary strictures, and focally to diffusely positive C4d. DSA testing was requested.

DSA Phenotype in Liver Tx Recipient



AMR

Total IgG>10,000 MFI,
C1q >20,000 MFI
IgG1+2+3+> 5,000 MFI

Post-Treatment

Total Ig 10,000 MFI
C1q Negative
Low level IgG1+2+3

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

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Recent literature confirms donor-specific HLA alloantibodies (DSA) impair 5-year survival in some but not all liver transplant recipients. In an effort to improve DSA testing's association with rejection and death, we retrospectively evaluated 1270 liver transplant recipients for the presence of IgG3 and C1q-fixing DSA. In patients with preformed DSA, 29 and 51% had IgG3 and C1q-fixing DSA, respectively. In patients with *de novo* DSA, 62% and 67% had IgG3 and C1q-fixing DSA, respectively. When different types of DSA positive patients were compared to DSA negative patients, multivariable analysis showed that IgG3 DSA positivity had the highest numerical hazard ratio for death (IgG3: HR = 2.4, p < 0.001; C1q: HR = 1.9, p < 0.001; standard DSA: HR = 1.6, p < 0.001). Similarly, multivariable analysis demonstrated *de novo* IgG3 DSA positivity compared to no DSA had the highest hazard ratio for death (IgG3: HR = 2.1, p = 0.004; C1q: HR = 1.9, p = 0.02; standard DSA: HR = 1.8, p = 0.007). Preformed C1q-fixing class II DSA showed the strongest correlation with early rejection. In conclusion, preformed and *de novo* IgG3 subclass DSA positive patients had the highest absolute HR for death in side-by-side comparison with C1q and standard DSA positive versus DSA negative patients; however, IgG3 negative DSA positive patients still had inferior outcomes compared to DSA negative patients.

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; AMR, antibody-mediated rejection; CMV, cytomegalovirus; CNI, calcineurin inhibitor; DSA, donor-specific HLA antibodies; HCV, hepatitis C virus; MELD, model for end-stage liver disease; MFI, mean fluorescence intensity

Received 04 June 2014, revised 08 October 2014 and accepted for publication 29 October 2014

Introduction

Studies in different types of solid organ transplants have shown that donor-specific HLA alloantibodies (DSA) are a risk factor for the development of acute and chronic rejection, graft loss, and patient death (1–5). Moreover, antibody-mediated rejection (AMR) is now recognized as a cause of allograft loss in all solid organ transplants despite significant differences in incidence and outcome (6–11). Historically, it has been recognized that the liver is relatively resistant to the pathologic effects of DSA compared to other solid organs (12–15). However, with improved short- and long-term outcome in liver transplant recipients there has been a resurgence of interest in understanding the role of DSA in liver transplantation (16–24).

DSA evaluations in different populations of liver transplant recipients documented that, although there is a clear association between the presence of DSA in serum and reduced patient survival, not all patients with DSA in serum experience acute or chronic rejection or graft loss. In fact, many patients with preformed DSA in serum and some patients with posttransplant DSA in serum survive for a prolonged time with normal liver function tests (25). This dichotomy was clearly seen in a study of chronic rejection versus comparator patients: almost all patients with biopsy-proven chronic rejection had DSA in serum, but 61% of comparator patients also had DSA in serum at some point (18). Of note, comparator patients had normal liver function tests, but some chronic rejection patients maintained high mean fluorescence intensity (MFI) DSA for as long as 15 years before chronic rejection was diagnosed. This later point highlights that long-term prospective trials with serial protocol biopsies are needed before one can determine if overt or covert histologic injury occurs in all patients with DSA in serum. However, current data demonstrated that some antibodies are associated with more rapid graft injury and loss than others; DSA in chronic rejection patients was found to be more often of multiple IgG subclasses including IgG3 compared to the control group where most DSA was of a single IgG subclass and without IgG3 according to the testing method used (26).

In addition, a retrospective evaluation of a large single center liver transplant cohort demonstrated that preformed DSA and *de novo* DSA are both independently associated

Inferior survival associated with IgG3$^+$ DSA, and C1q$^+$ DSA compared to DSA negative patients.

Risk Factors for IgG3 and C1q binding De-novo DSA (CsA vs. Tac)

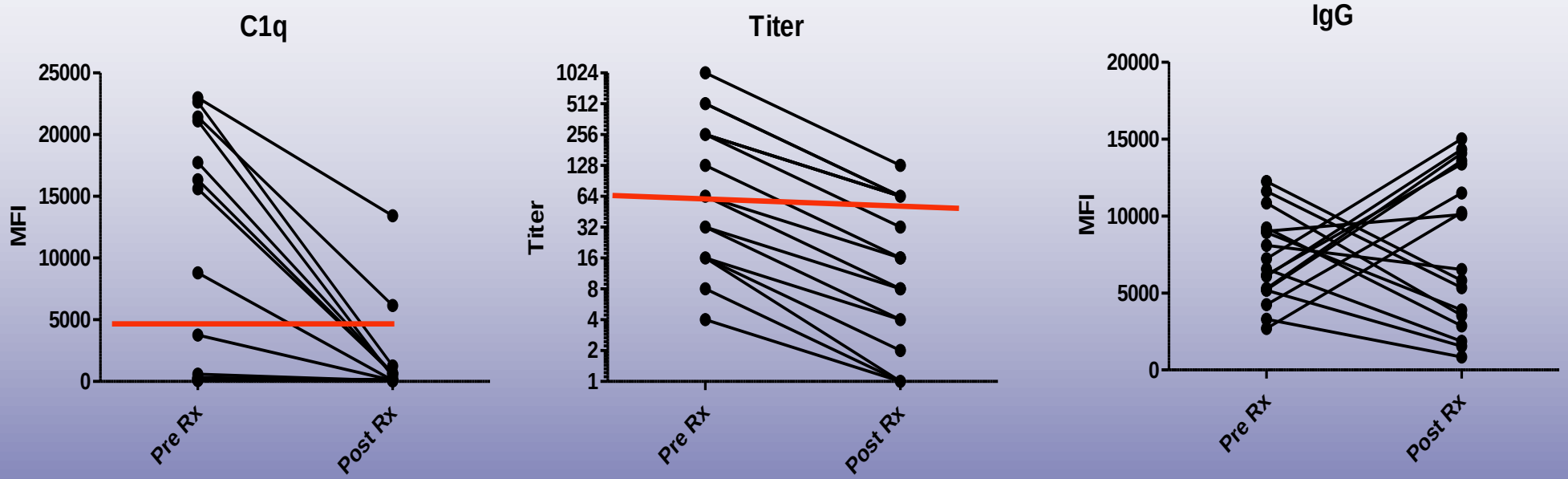
Table 3: Multivariable logistic regression analysis of risk factors for development of *de novo* IgG3 and C1q-fixing DSA

	OR	95% CI	p-value
De novo IgG3 DSA			
Cyclosporine at 1 year (compared to tacrolimus)	2.5	1.2–5.2	0.02
Low CNI levels	2.5	1.1–5.9	0.04
MELD score >15	0.4	0.2–0.8	0.02
De novo C1q-fixing DSA			
Cyclosporine at 1 year (compared to tacrolimus)	2.6	1.3–5.3	0.008

OR, odds ratio; CI, confidence interval; DSA, donor-specific HLA alloantibodies; Low calcineurin inhibitor (CNI) levels, a tacrolimus level <3ng/mL or a cyclosporine level <75ng/mL before the posttransplant serum sample; MELD, model for end-stage liver disease.

Can solid phase assays be better utilized to measure efficacy of antibody removal therapies?

Anat R. Tambur [a,*], Denis Glotz [b], Nancy D. Herrera [a], Erik N. Chatroop [a], Tal Roitberg [a], John J. Friedewald [c], David Gjertson [d]



Monitoring Responses to Ritux/PP/IVIg Desensitization

Multiple Antibodies – One Patient

Majority of HLA-Ab became C1q negative and dropped <1:64 dilution in response to therapy

Human Immunol 2016

Northwestern University Feinberg School of Medicine
Comprehensive Transplant Center

Northwestern University Comprehensive Transplant Center

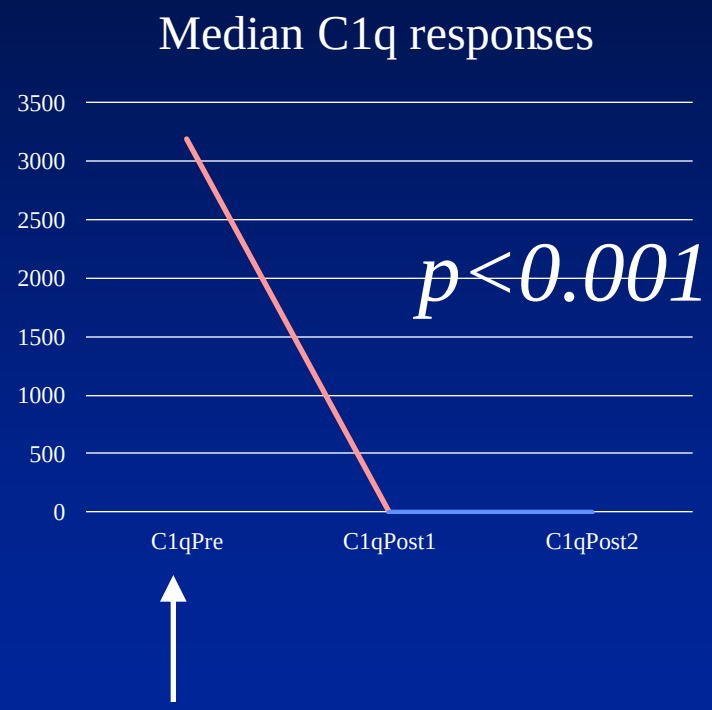
Proteasome Inhibitor Carfilzomib-Based Therapy for Antibody-Mediated Rejection of the Pulmonary Allograft: First Use and Short-Term Findings

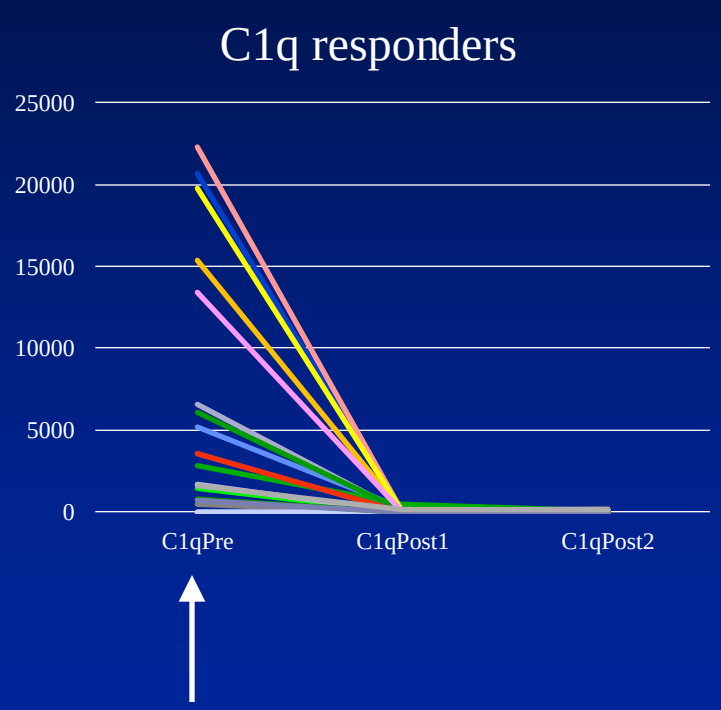
Ensor, Zeevi , McDyer AJT 2017

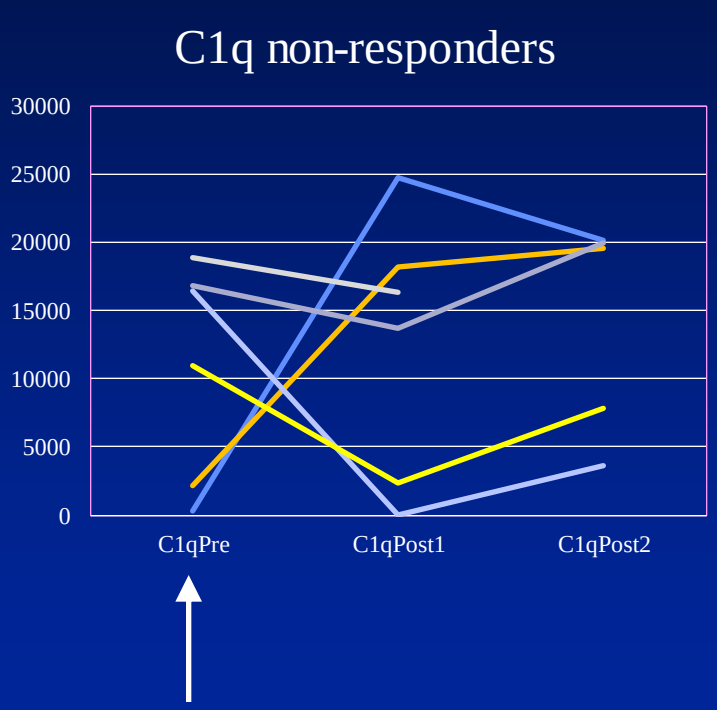
16pts (23 iDSA) : 69% DQ, 19% DQ+DR, 12% DR
6 patients had C4d+ AMR, 10 patients C4d- probably AMR

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.

Antibody Responses After Carfilzomib-Based AMR Therapy



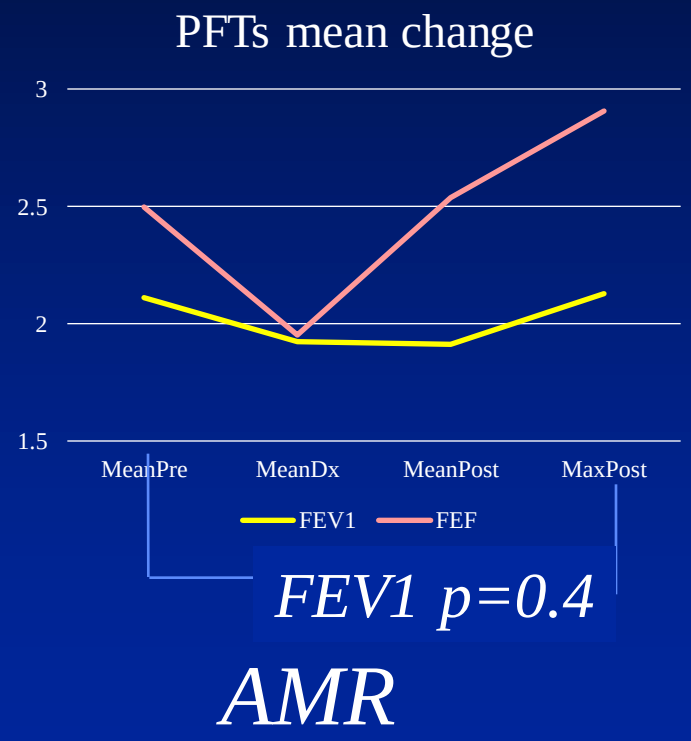


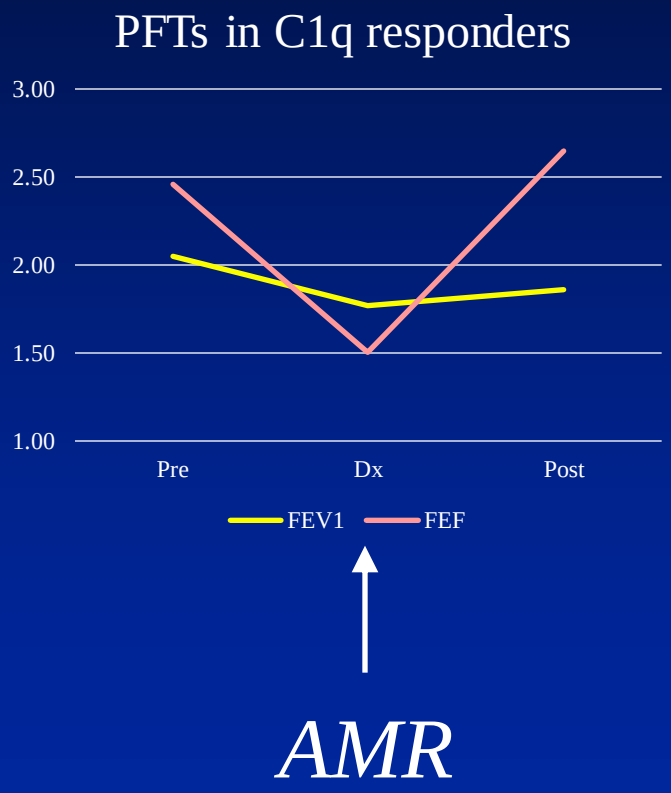


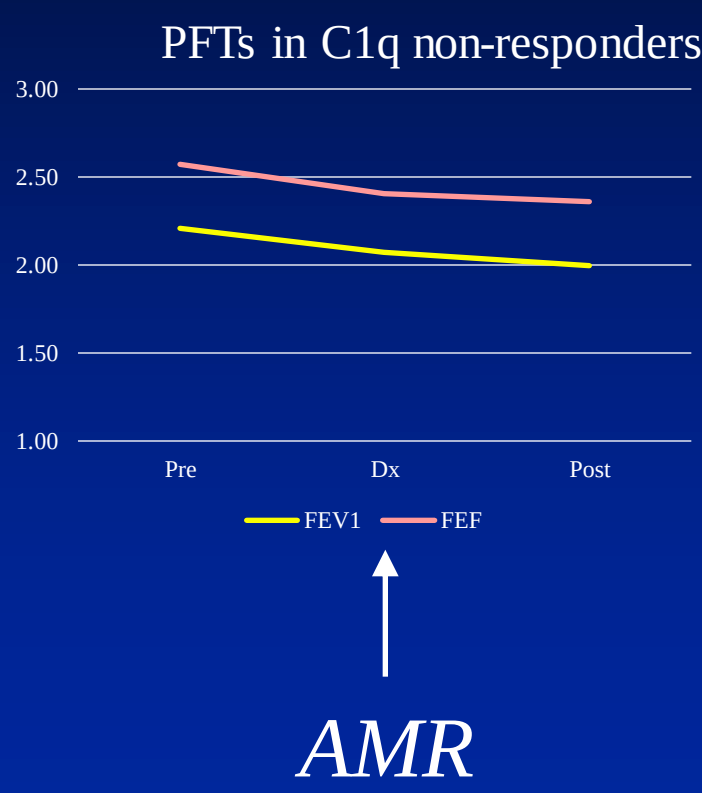
AMR *AMR* *AMR*

Courtesy of Chris Ensor PharmD

PFT Recovery after Carfilzomib-Based AMR Therapy

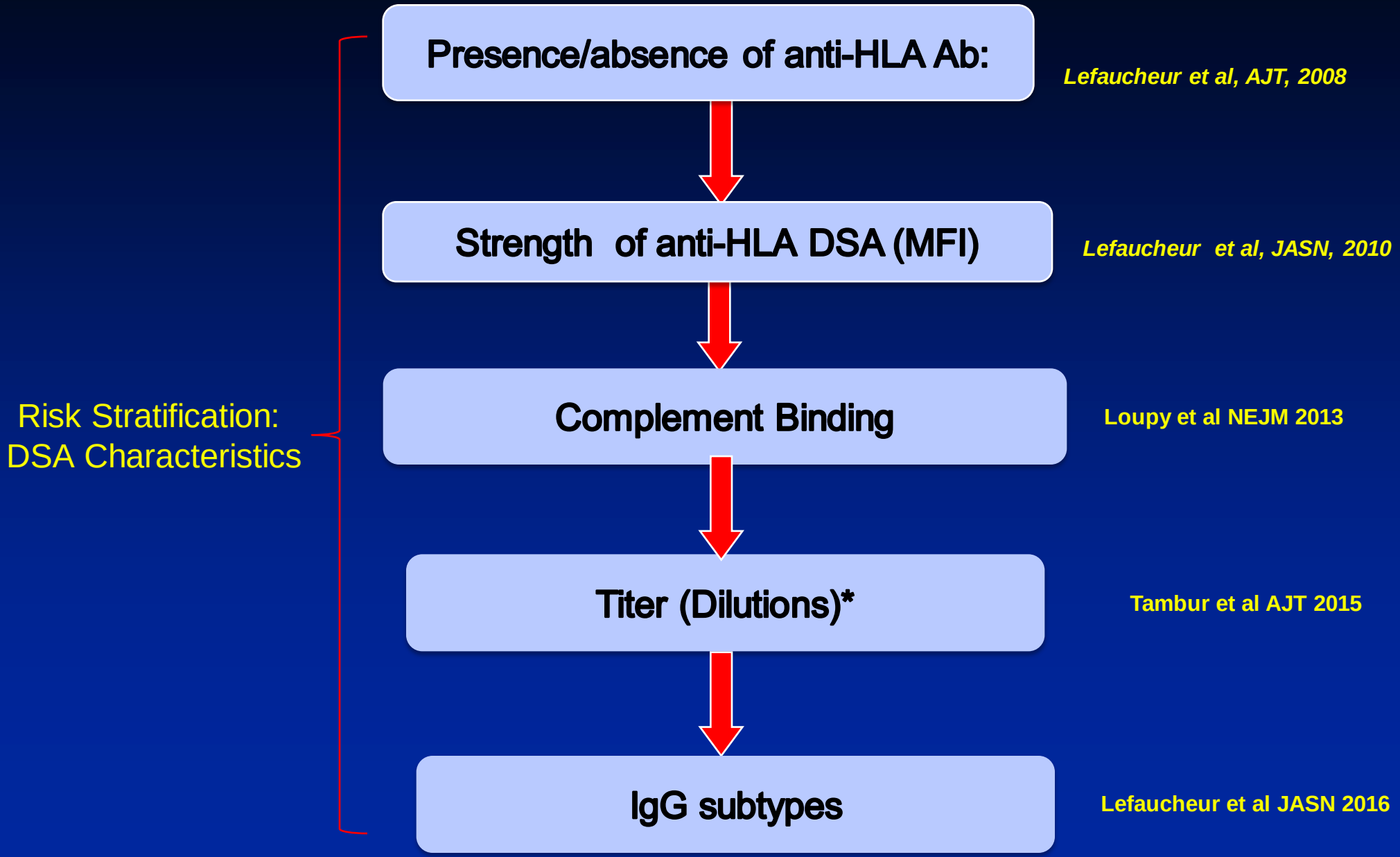






Courtesy of Chris Ensor PharmD

Linking circulating Ab/allograft injury



DSA Assessment
Precision Medicine

One Size
Fits All
MFI

Titer
Complement Binding
IgG subtypes

Acknowledgments

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Transplant Pathology

Clinical Associates
Renal, Heart, Lung, Liver Team

Adult and Pediatric

Néphrologie
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