Imperial College Healthcare NHS
NHS Trust

Class II anti-HLA IgG2 and IgG3 DSAs Predict Poorer Outcomes in Chronic Antibody Mediated Rejection of Renal Allografts

Alexander Gueret-Wardle[1], Gaetano Lucisano[1], Sevda Hassan[1] Paul Brookes[2], Eva Santos-Nunez[2], Rachel Wilson[2], Fiona Powell[2], Dawn Goodall[1], Candice Clarke[1], Jack Galliford[1], Candice Roufosse[1], Michelle Willicombe[1], David Taube[1]

[1] *Renal and Transplant Centre, Imperial College Healthcare NHS Trust, London (UK)*

[2] *Histocompatibility and Immunogenetics, Imperial College Healthcare NHS Trust, London (UK)*

Imperial College
RENAL & TRANSPLANT CENTRE

Imperial College Healthcare NHS
NHS Trust

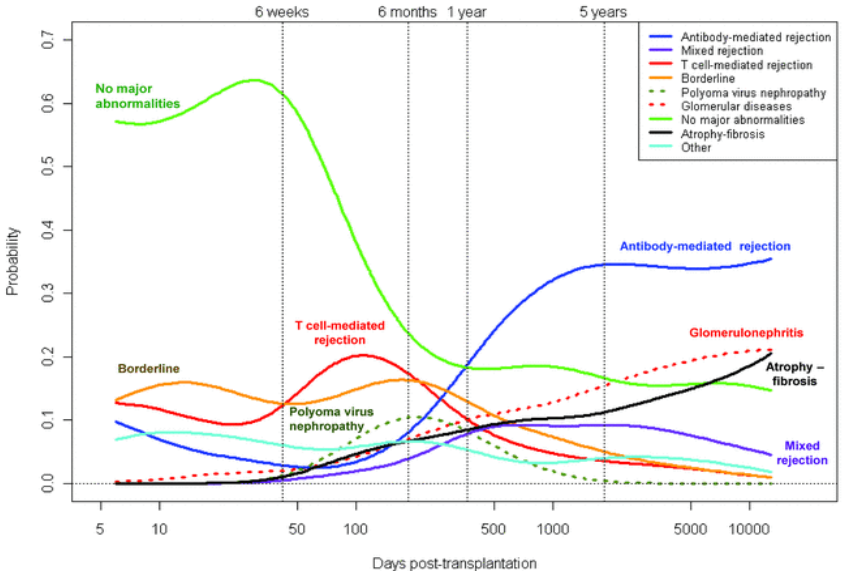
Authors have no disclosures

Imperial College
RENAL & TRANSPLANT CENTRE

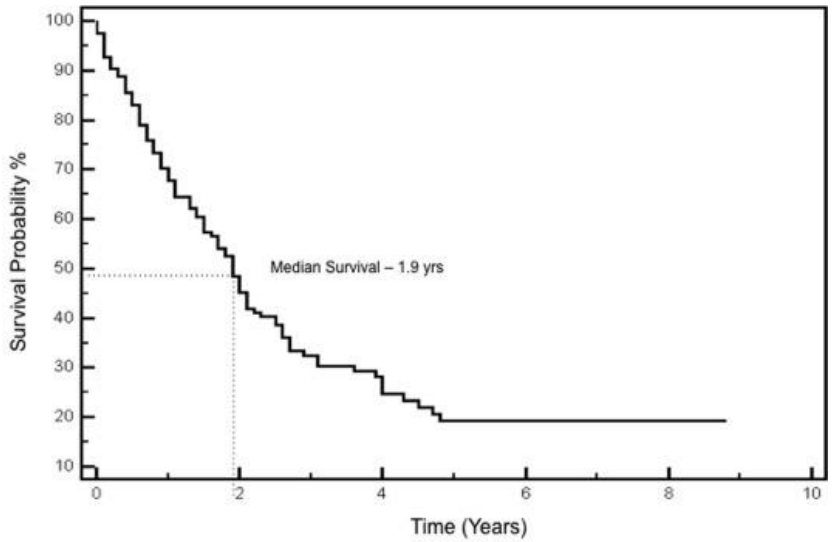
The Problem

The leading cause of late graft failure is chronic antibody mediated rejection [cAMR]

Sellares et al. Am J Transplant 2012



Redfield et al. Human Immunology 2016



Imperial College
RENAL & TRANSPLANT CENTRE

Background

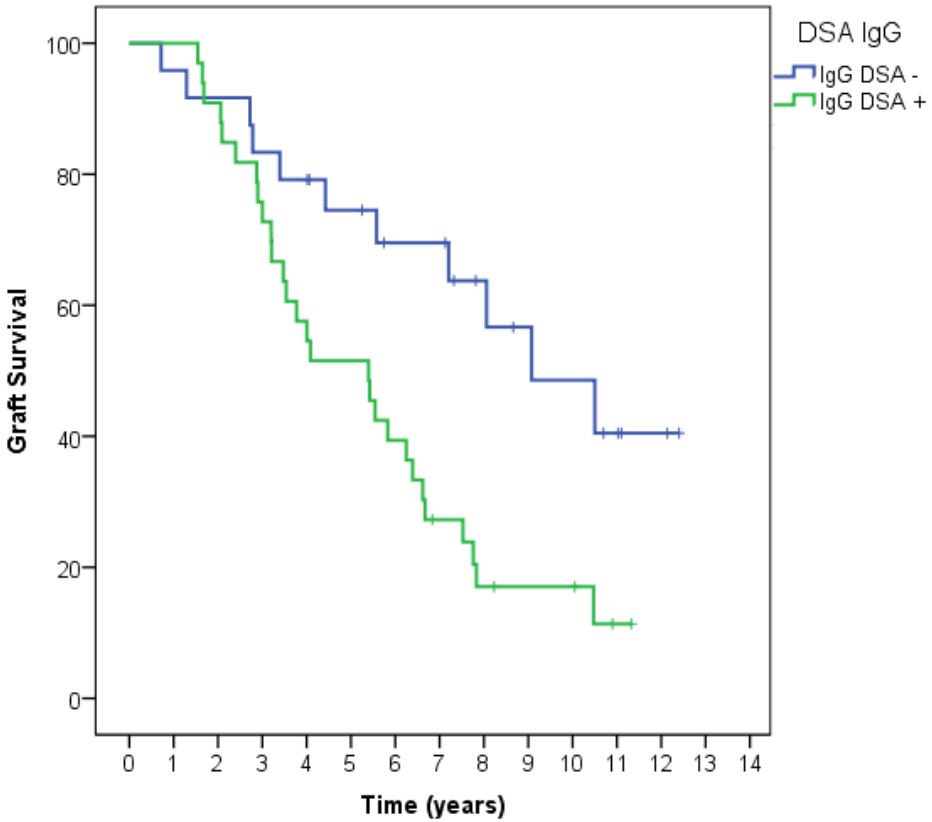
1. Clear association of DSA with the development of cAMR and Transplant glomerulopathy [TG]
- Although many cases of antibody negative cAMR (30-50%)

2. cAMR and TG present significant problems that are often unresponsive to current standard of care therapies.

3. No clinically licensed treatment for cAMR

4. cAMR is one of the main barriers to improving long term graft survival

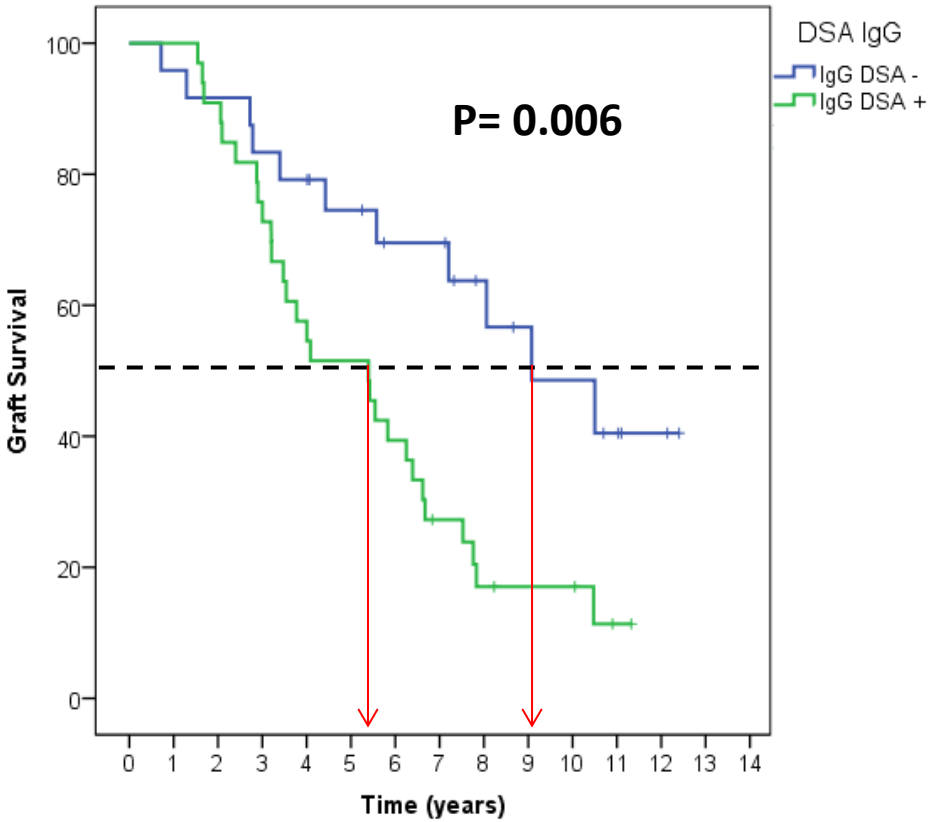
Significance of DSAs in cAMR at ICRTC



90 patients with cAMR
March 2005 – Nov 2015

Imperial College
RENAL & TRANSPLANT CENTRE

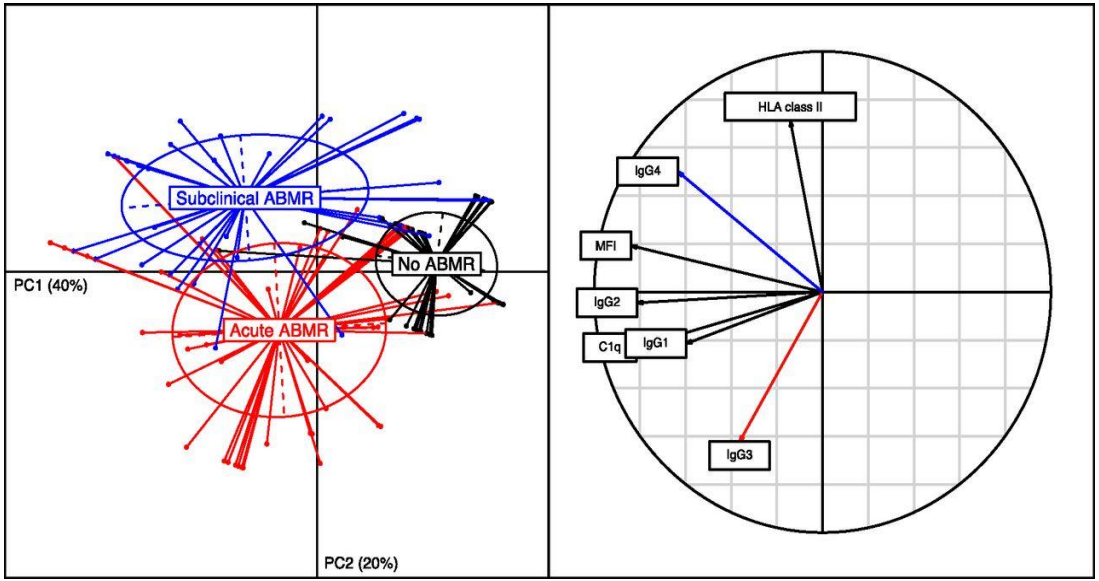
Significance of DSAs in cAMR at ICRTC



90 patients with cAMR
March 2005 – Nov 2015

Imperial College
RENAL & TRANSPLANT CENTRE

Background



Lefaucheur et al. JASN 2016

1. 125/635 pts developed a dn DSA at one year
2. IgG3 was associated with greater C1q binding in patients with acute AMR
3. Higher MI C4d deposition
4. Worse prognosis

Imperial College
RENAL & TRANSPLANT CENTRE

Background

However

1. Heterogenous group histologically (AMR free, Acute AMR, S-AMR)

2. Looked at total subclasses combining class I+II immunodominant DSA

3. DSAs at 1 year post transplant

4. No specific group investigated histologically and serologically

Aims of our study

Focus on cAMR and antibody phenotypes

1. Identify which antibody characteristics predict poor outcomes in cAMR

2. Identify correlations between subclass profiles and histology

3. Identify whether C1q binding influences allograft outcomes in cAMR

4. Identify the most high risk patient groups with cAMR

5. Potential to individualise future treatment

Imperial College
RENAL & TRANSPLANT CENTRE

Methods

1657 CDC/FXCM negative transplant recipients investigated between March2005 – November 2015
All patients received monoclonal antibody induction, with a tacrolimus based, steroid sparing maintenance immunosuppressive protocol

ABOi/HLAi transplants were excluded

90 cases of biopsy proven cAMR

Only patients with an IgG DSA post transplant at the time of diagnosis of cAMR were included
57/90 (63.3%) IgG DSA positive

Diagnosis of chronic active AMR was based on Banff 2015 criteria

Median follow up was 5.5 years (IQR 3.2-7.3)

Imperial College
RENAL & TRANSPLANT CENTRE

Methods

Sera were tested for class I HLA (A/B/Cw) and class II (DR/DQ) HLA antibodies at the time of diagnostic biopsy using the single antigen Luminex assay.

- Each sample was tested replacing the PE conjugated anti-pan IgG antibody with monoclonal antibodies specific for IgG1-4
- Each sample was also tested for C1q-fixing anti-HLA DSAs using SAg beads

Mean fluorescence intensity value of >500 was considered positive

Statistical and graphical analysis: IBM SPSS Statistics ver. 20.0

Imperial College
RENAL & TRANSPLANT CENTRE

Demographics 1

Demographics	DSA+ cAMR n=57, (%)
Male Female	37 (64.9) 20 (35.1)
Age at Tx, years	45.3± 11.8
Caucasian Asian Afro-Caribbean Other	29 (50.8) 20 (35.2) 4 (7.0) 4 (7.0)
Pre-emptive	9 (15.7)
Live donor	21 (36.8)
HLA-A/B MM	2.3 ± 1.1
HLA-DR MM	1.3 ± 0.7
Total MM	3.6 ± 1.6
Induction Anti-CD52 mab Anti-IL-2R mab	 44 (77.2) 13 (22.8)

Imperial College
RENAL & TRANSPLANT CENTRE

DSA Characteristics

	Number of Cases (%)	Median MFI	IQR MFI	p Value
Class I	7 (12.2)	1170	500-2554	-
Class II	20 (35.1)	1596	923-4964	**<0.001**
Class I + II	30 (52.7)	1572	900-4188	

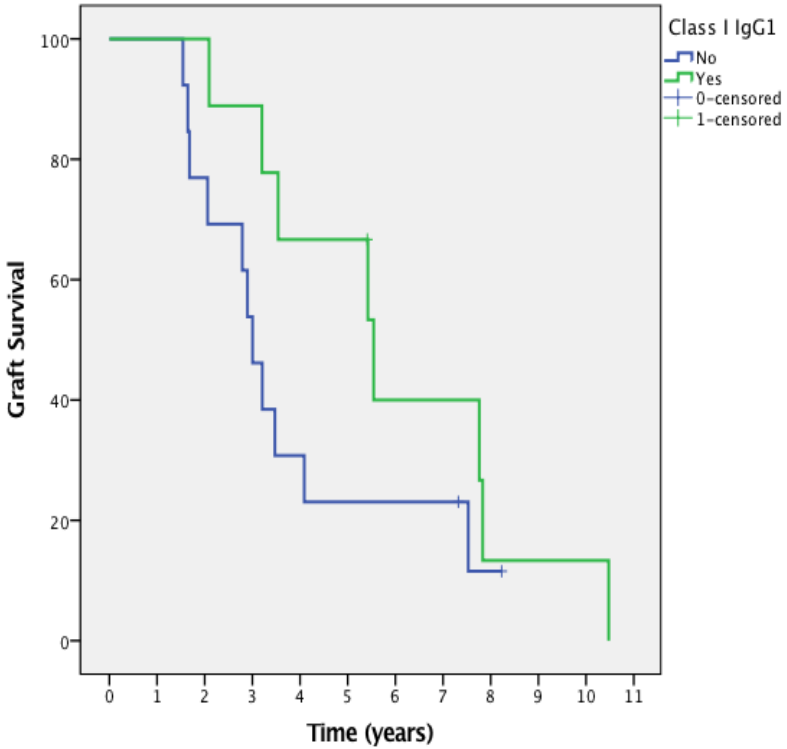
DSA Characteristics

	Number of Cases (%)	Median MFI	IQR MFI	p Value
Class I	7 (12.2)	1170	500-2554	-
Class II	20 (35.1)	1596	923-4964	**<0.001**
Class I + II	30 (52.7)	1572	900-4188	

	IgG1 (%)	IgG2 (%)	IgG3 (%)	IgG4 (%)
Class I	9 (15.8)	0 (0)	1 (1.8)	2 (3.5)
Class II	36 (63.1)	22 (38.6)	7 (12.2)	15 (26.3)

Imperial College
RENAL & TRANSPLANT CENTRE

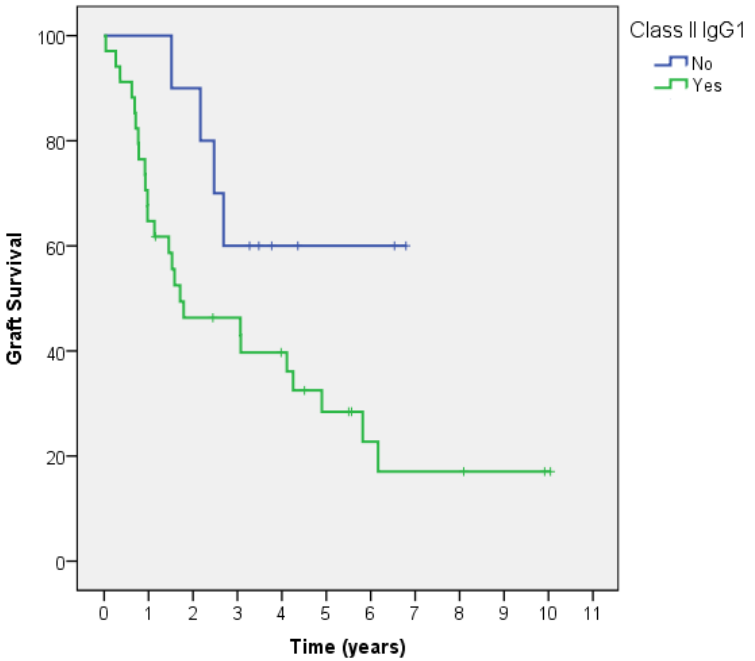
Results – Death Censored Allograft Survival – Class I IgG Subclasses



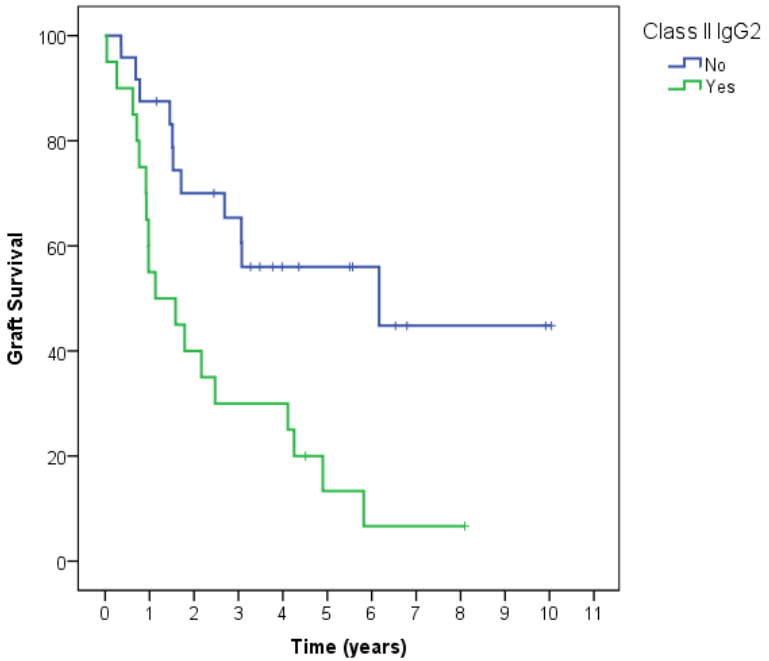
p= 0.841

Imperial College
RENAL & TRANSPLANT CENTRE

Results – Death Censored Allograft Survival – Class II IgG Subclasses



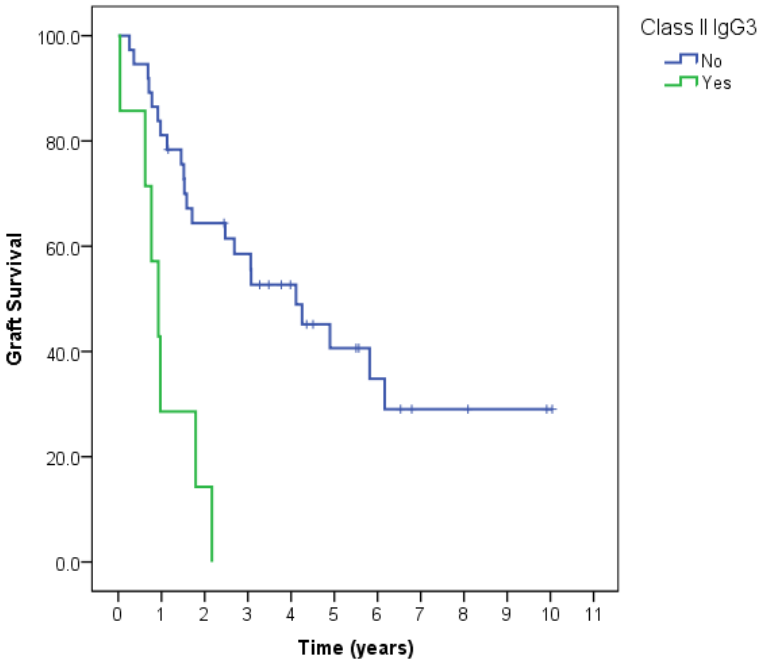
p= 0.068



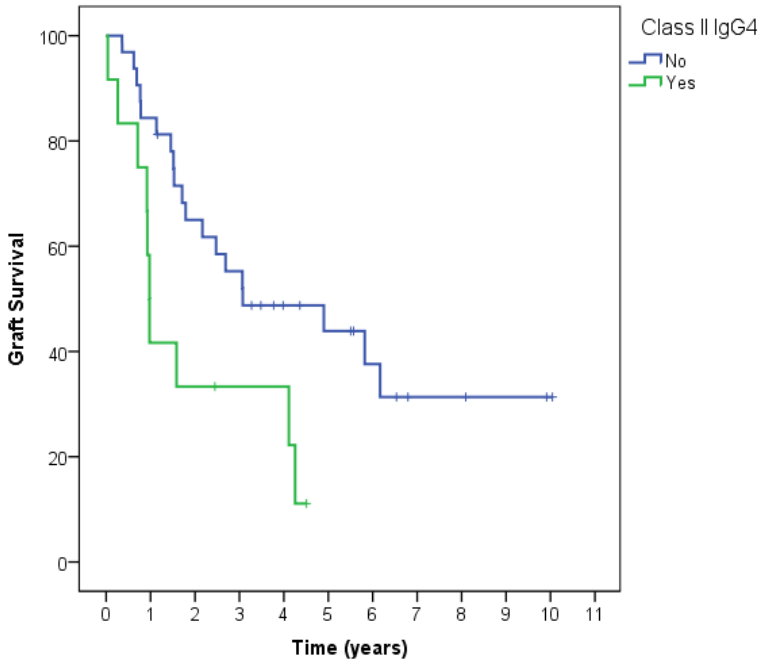
p= 0.004

Imperial College
RENAL & TRANSPLANT CENTRE

Results – Death Censored Allograft Survival – Class II IgG Subclasses



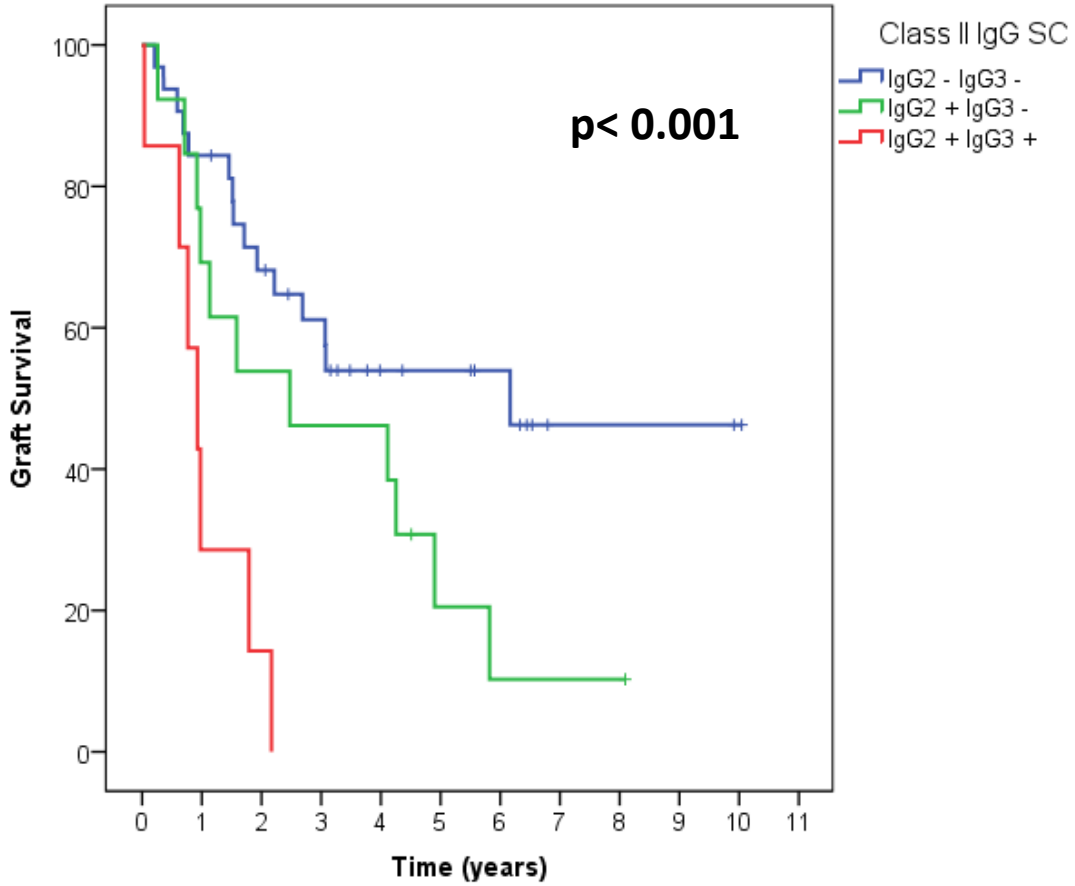
p< 0.001



p= 0.017

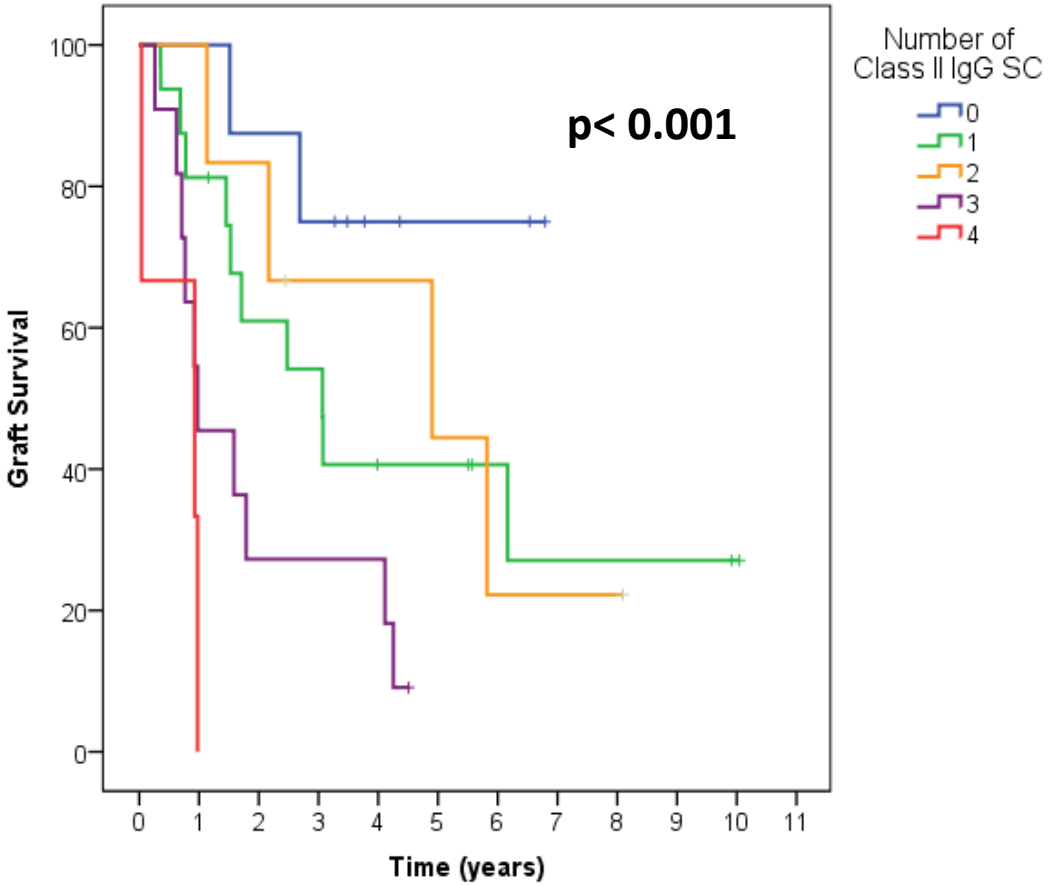
Imperial College
RENAL & TRANSPLANT CENTRE

Results – Death Censored Allograft Survival



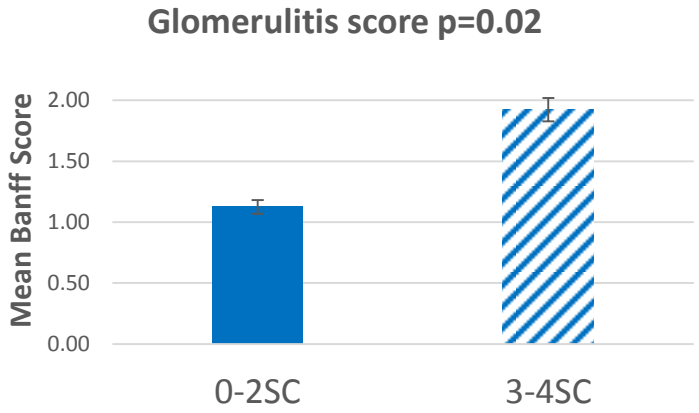
Imperial College
RENAL & TRANSPLANT CENTRE

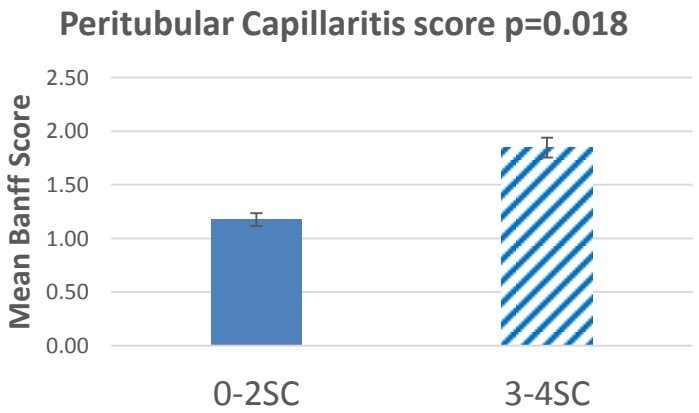
Results – Death Censored Allograft Survival

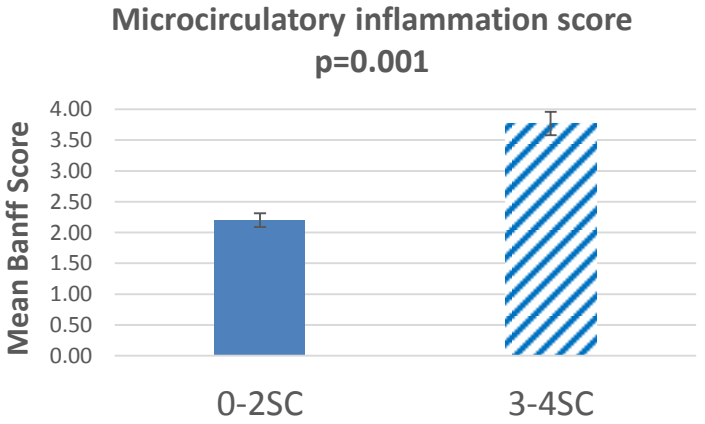


Imperial College
RENAL & TRANSPLANT CENTRE

Results – Class II Subclasses and microcirculatory inflammation

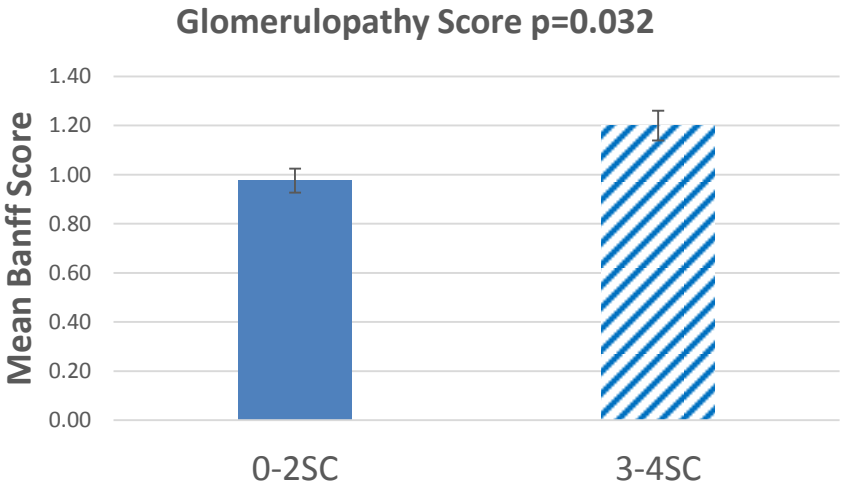


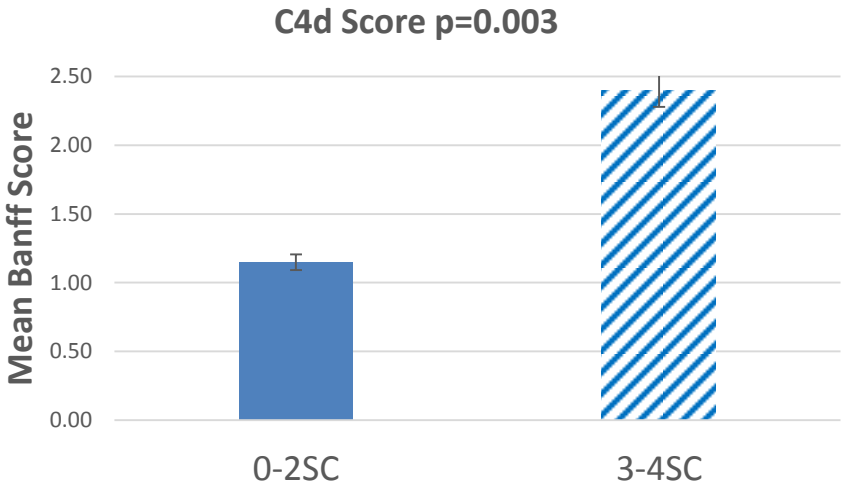




Imperial College
RENAL & TRANSPLANT CENTRE

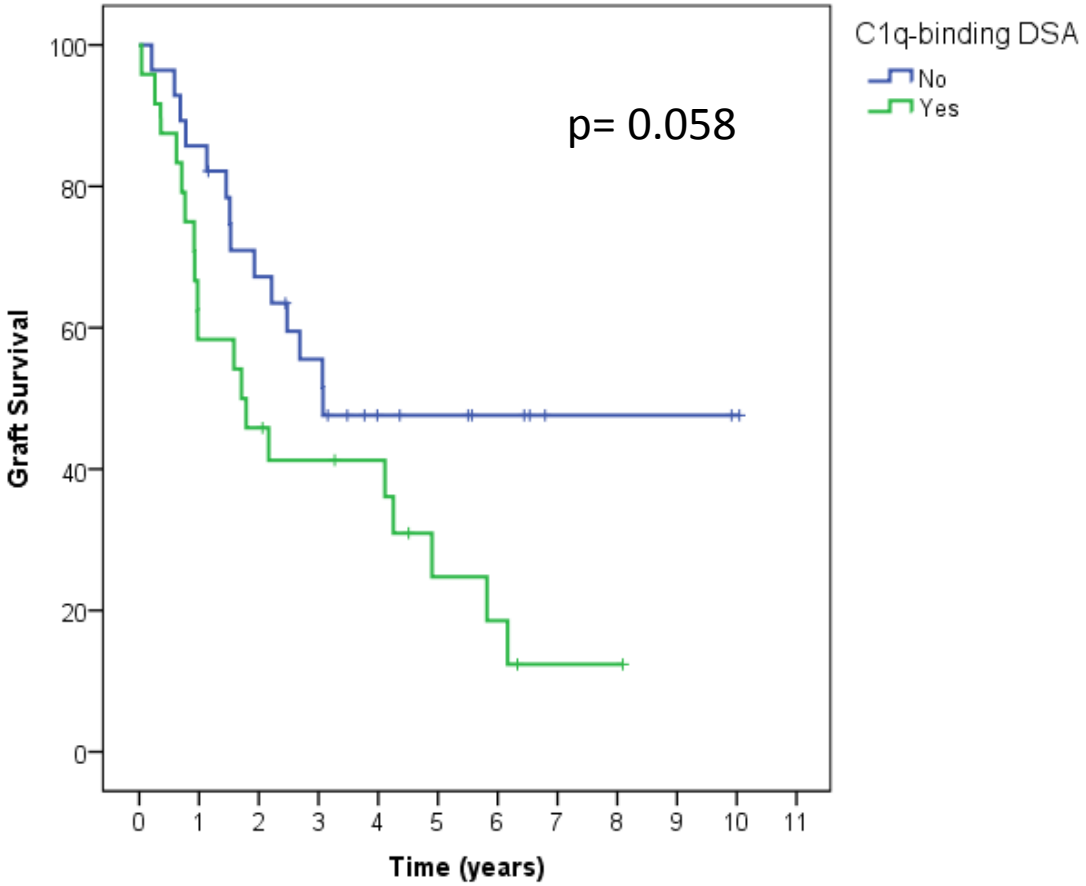
Results – Class II subclasses - Cg and C4d scores





Imperial College
RENAL & TRANSPLANT CENTRE

Results – C1q binding



Imperial College
RENAL & TRANSPLANT CENTRE

Summary

1. Class I IgG DSA subclasses do not affect allograft outcomes in cAMR

2. Class II IgG2 and IgG3 DSAs predict poor outcomes in cAMR

3. IgG2 and 3 combined have even poorer outcomes
    - IgG3 has a strong affinity for fixing complement and binding Fc receptors
    - IgG2 can bind complement although with less affinity than IgG1 and IgG3
    - IgG2 canonically stimulates production of IgG1 and IgG3
    - The presence of IgG2 may potentiate IgG3

4. The presence of multiple class II IgG subclasses predicts the worst outcome
- No grafts with 4 subclasses survived beyond 1 year

Imperial College
RENAL & TRANSPLANT CENTRE

Summary

5. The greater the number of subclasses the more severe phenotype of cAMR histologically

- Higher MI, C4d and Cg scores

6. Patients with class II IgG2 and IgG3 DSAs or multiple subclasses may benefit from enhanced or novel treatment

- Syk inhibition, IL-6R blockade (Tocilizumab)

Acknowledgements

1. Patients and Families

2. Transplant Team at Imperial College Renal and Transplant Centre

3. H&I Scientists at Imperial College Healthcare NHS Trust

4. National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London

Imperial College
RENAL & TRANSPLANT CENTRE

Demographics 2 – Treatment groups

1. Optimisation - 51/57 (89.4%)
- Tacrolimus [8-12 ng/ml]
- MMF [1.2-2.4 mg/l]
- Addition of steroids

2. Maintenance - 6/57 (10.5%)
- Maintenance of Tacrolimus based immunosuppression [8-12ng/ml]

3. PEX/IVIg - 24/57 (42.1%)
- 10 rounds of plasma exchange with a total of 4g/Kg of IVIg
- Plasma exchange era based with all treatments occurring between 2009-11
- 23/24 patients had a class II DSA

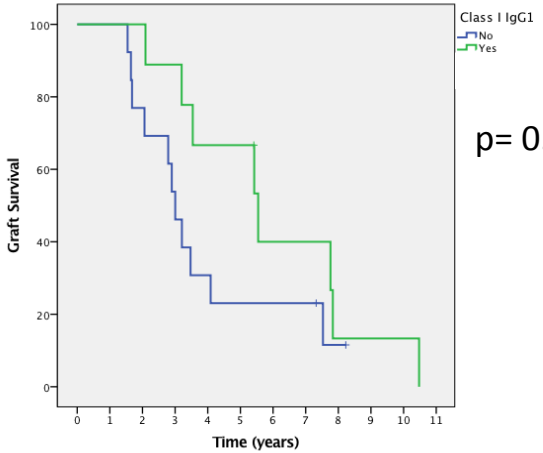
4. Other – 2/57 (3.5%)
- Rituximab

Outcomes – Causes of graft failure

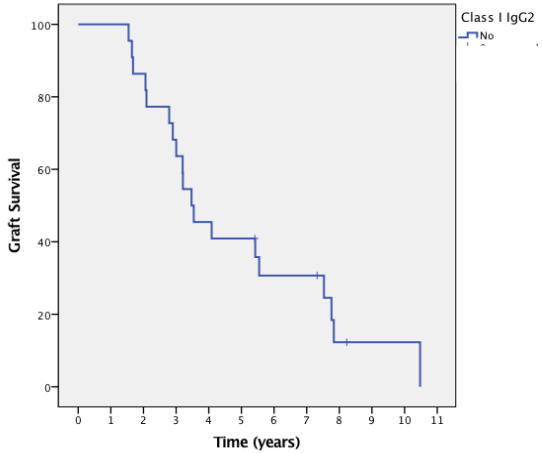
1. Number of failed grafts - 37/57 (64.9%)

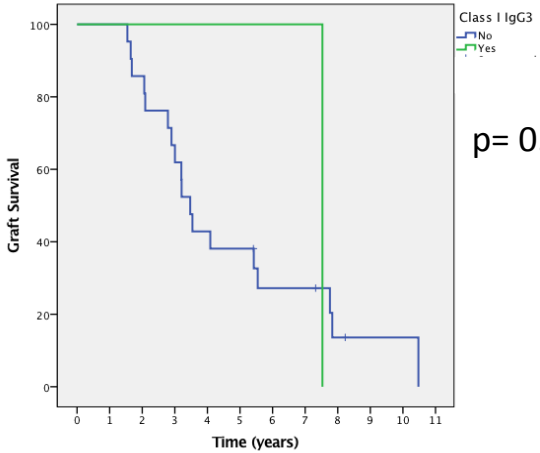
- 36/37 – Rejection
- 1/37 – Malignancy requiring nephrectomy

Results – Death Censored Allograft Survival – Class I IgG Subclasses

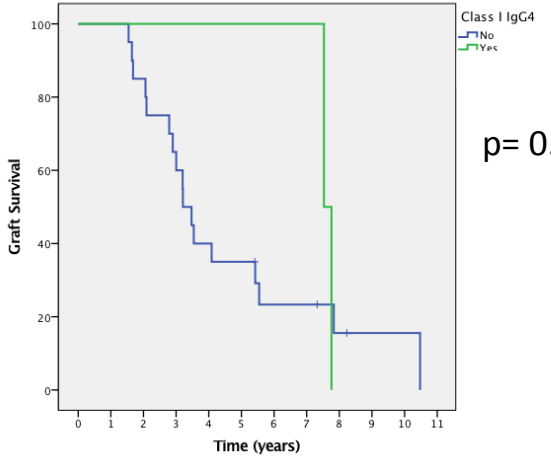


p= 0.841





p= 0.874



p= 0.629

Imperial College
RENAL & TRANSPLANT CENTRE

Results – MFI ranges according to total subclasses

Number of subclasses	MFI range
0SC	500 - 12500
1SC	850 - 11000
2SC	900 - 20500
3SC	600 - 8360
4SC	650 - 15250

Banff 2015 criteria for the diagnosis of cAMR

Chronic active ABMR[2]

All three features must be present for diagnosis. As with acute/active ABMR, biopsies showing histological features plus evidence of current/recent antibody interaction with vascular endothelium or DSA, but not both, may be designated as suspicious, and it should be noted if the lesion is C4d-positive or C4d-negative, based on the criteria listed:

1 Histologic evidence of chronic tissue injury, including one or more of the following:
   - TG (cg >0), if no evidence of chronic thrombotic microangiopathy; includes changes evident by EM only (cg1a; Table 4)
   - Severe peritubular capillary basement membrane multilayering (requires EM)[3]
   - Arterial intimal fibrosis of new onset, excluding other causes; leukocytes within the sclerotic intima favor chronic ABMR if there is no prior history of biopsy-proven TCMR with arterial involvement but are not required

2 Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d >0 by IHC on paraffin sections)
   - At least moderate microvascular inflammation ([g + ptc] ≥2), although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥2 alone is not sufficient and g must be ≥1
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated

3 Serologic evidence of DSAs (HLA or other antigens):
   - Biopsies suspicious for ABMR on the basis of meeting criteria 1 and 2 should prompt expedited DSA testing

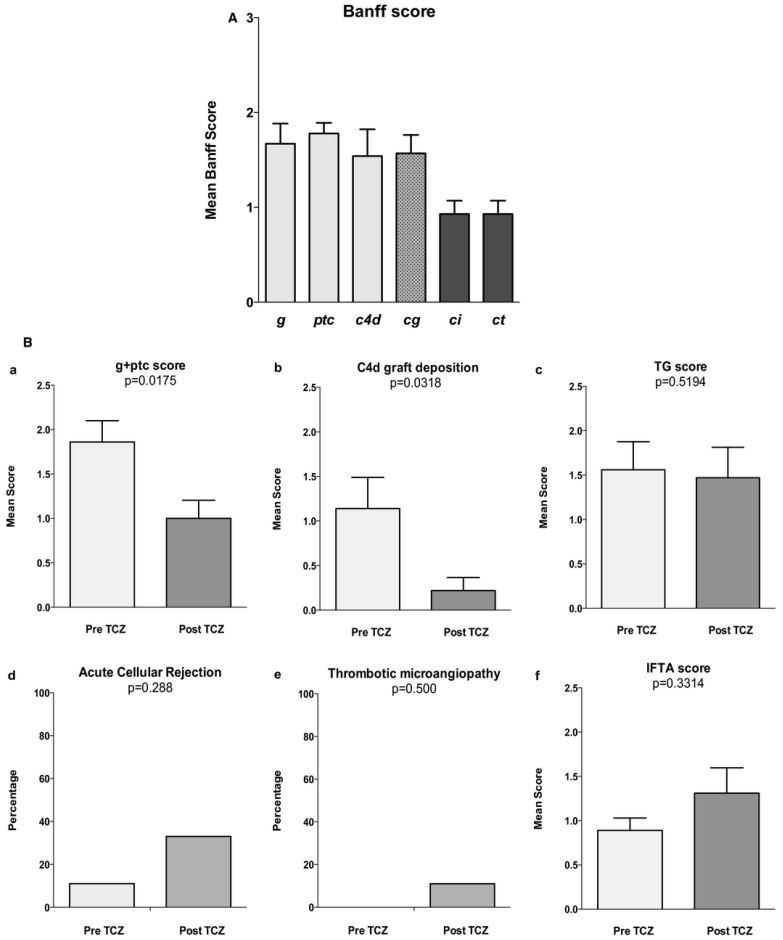
SYK Inhibition

- SYK-kinase is an important component of signalling system that activates both Fc and B cell receptor
- Activation culminates in the production of inflammatory cytokines
- Immunostaining of SYK in renal transplant biopsies with ABMR shows a role for SYK in the pathogenesis of DSA

- Rodent models of antibody mediated GN – the inhibition of SYK was shown to prevent and in some cases reverse renal injury
    - McAdoo et al

- Currently undertaking a phase I open label trial of SYK inhibition in patients with cAMR

Choi et al. Am J Transplant 2017; 17:2381-2389



Blockade of IL-6R shows a reduction of alloantibodies

Tocilizumab (IL-6R blocker)

Single centre open labelled study - 75 cases cAMR

36 who had SOC (RTX/ IVIg/ PLEX) received IL-6RB (Tocilizumab for 6-18/12)

Reduced mean MCI and C4d scores

Better allograft survival compared to 80% predicted survival at 6yrs

Phase 1/2 open label desensitisation study

Patients who failed RTX, IVIg and PLEX

Reduction in DSA in 8/10 patients

After Tx - protocol biopsy at 6 months – no ABMR or TG

Imperial College
RENAL & TRANSPLANT CENTRE

DSA CHARACTERISTICS (2)

	Total Sc = 0 (%)	Total Sc = 1 (%)	Total Sc = 2 (%)	Total Sc = 3 (%)	Total Sc = 4 (%)
Class I	24 (71)	9 (26.5)	1 (5.9)	0 (0)	0 (0)
Class II	10 (20.4)	16 (32.7)	8 (16.3)	12 (24.4)	3 (6.1)