Desensitization: Increasing Access to Transplantation for Highly-HLA Sensitized Patients

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Life Sciences Symposium 2018

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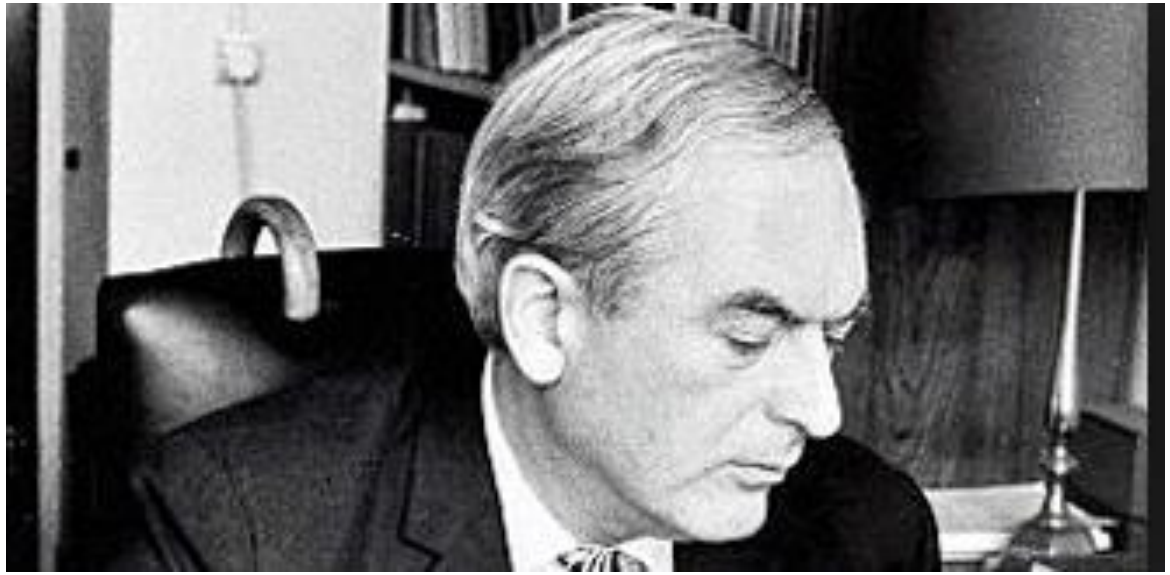
I have grants from Hansa Medical, Roche-Genentech, Vitaeris , Novartis and CSL-Behring. I am a consultant for Hansa Medical, Roche-Genentech, Vitaeris and CSL-Behring

AND

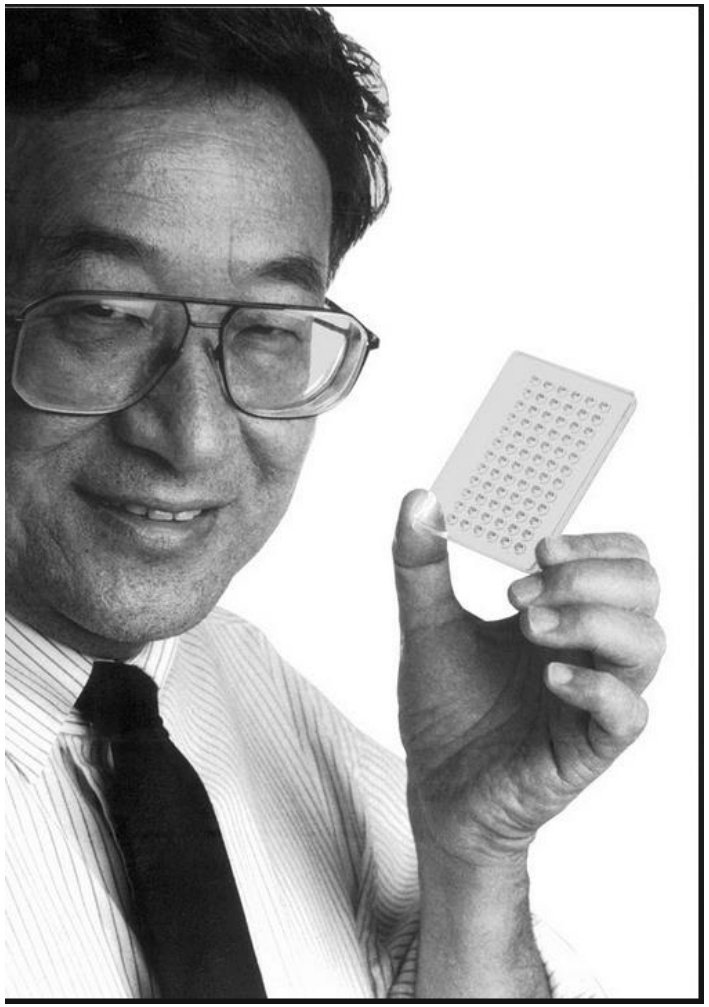
My presentation does include discussion of off-label or investigational use.

Sir Peter Medwar, Nobel Prize in Medicine 1960



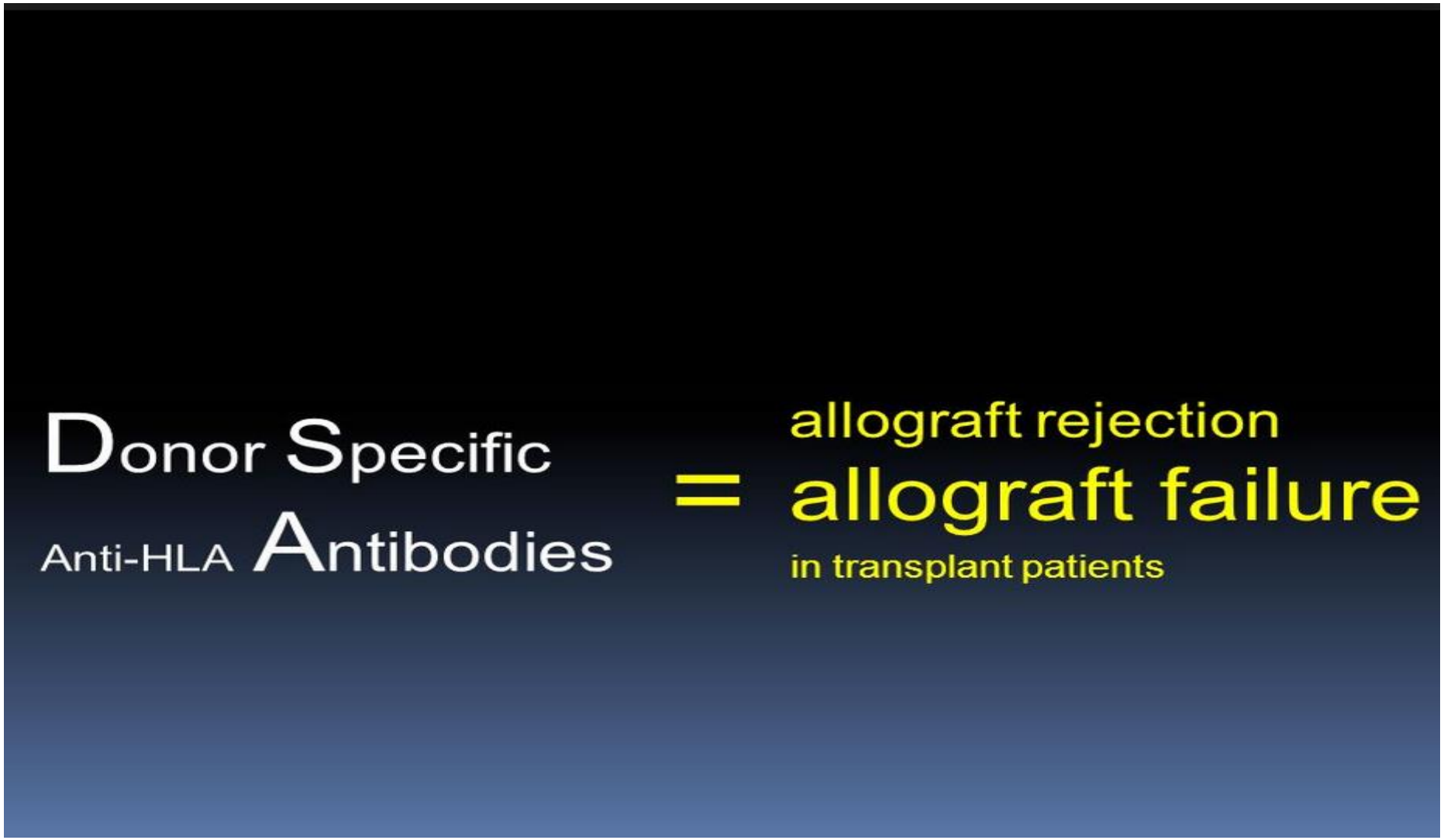
Champion of T-cell centric view of transplantation
And proponent of Tolerance

Paul Terasaki, PhD



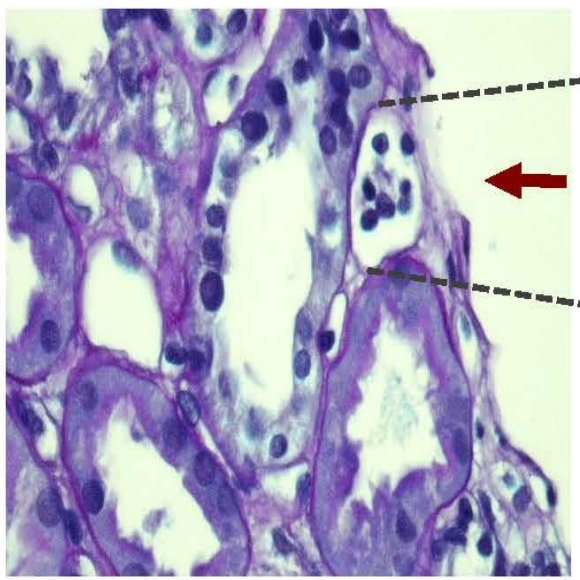
Champion of the Humoral Theory of Transplantation

Allo-sensitization & Donor Specific Antibodies: Enemies of Allograft Survival



Patients With De Novo DSA Have Early (0-6 mo) TCMR With More Intense PTC Inflammation[1-3]

TCMR PTC Score		
2.0	de novo DSA	$P < .05$
1.0	No DSA	

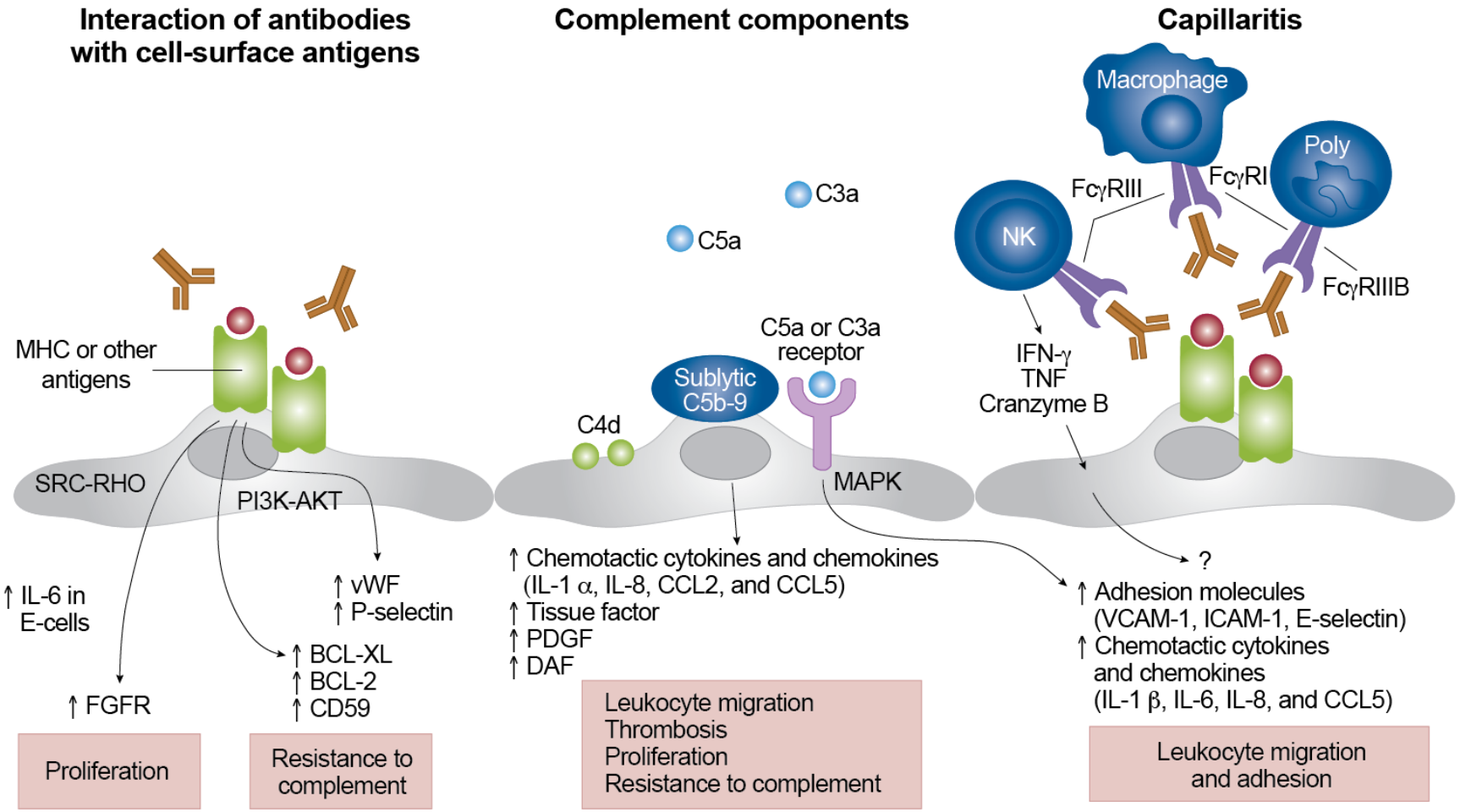


IFN: interferon.

1. Wiebe C et al. *Am J Transplant*. 2012;12:1157-1167.
2. Gibson IW et al. *Am J Transplant*. 2008;8:819-825.
3. Muczynski KA et al. *J Am Soc Nephrol*. 2003;14:1336-1348.

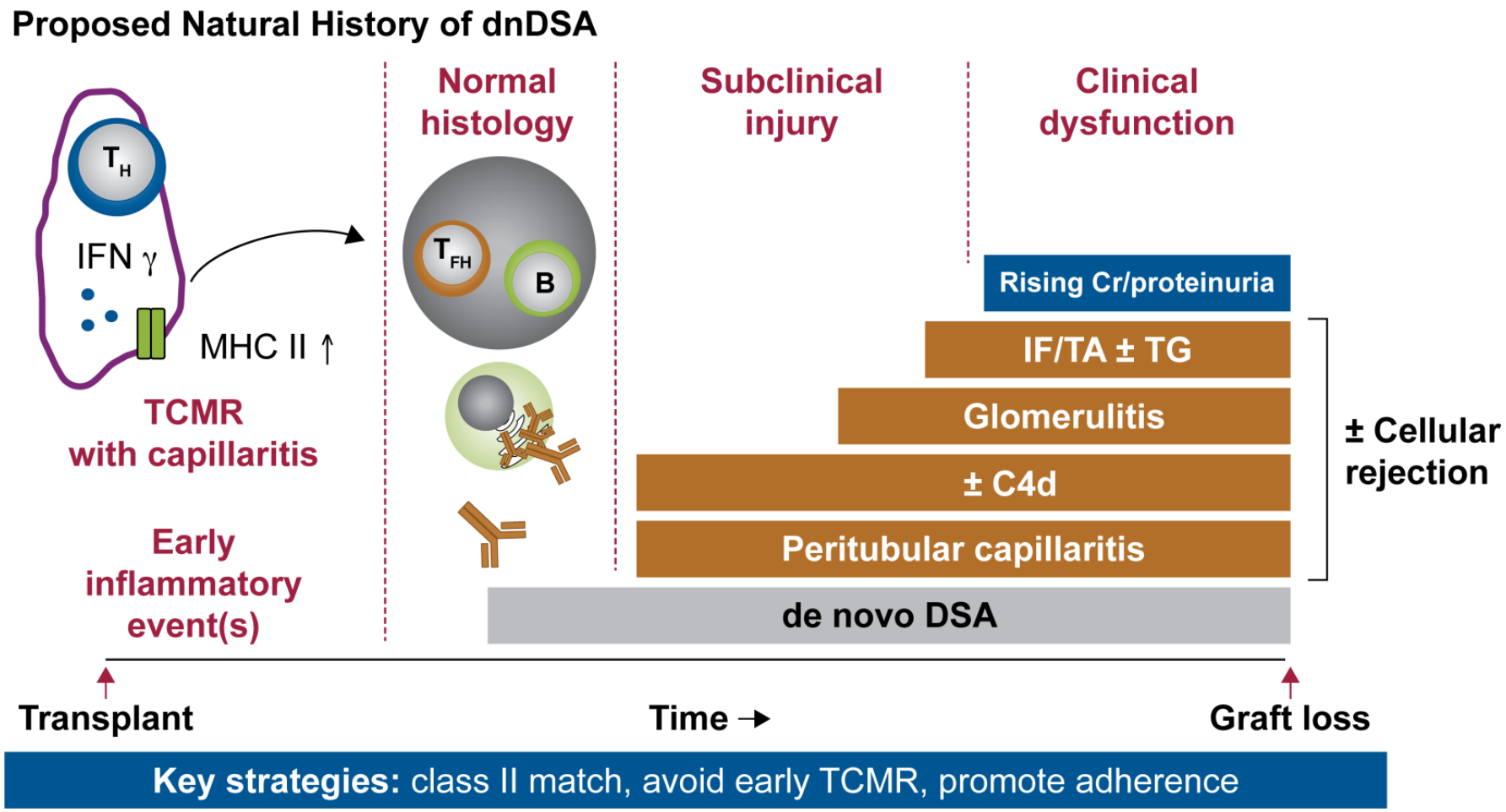
Source: Peter Nickerson

Mechanisms of Donor-Specific Antibody-Mediated Endothelial Injury in Renal Allografts[1]



1. Farkash EA, Colvin RB. *Nat Rev Nephrol*. 2012;8:255-257.

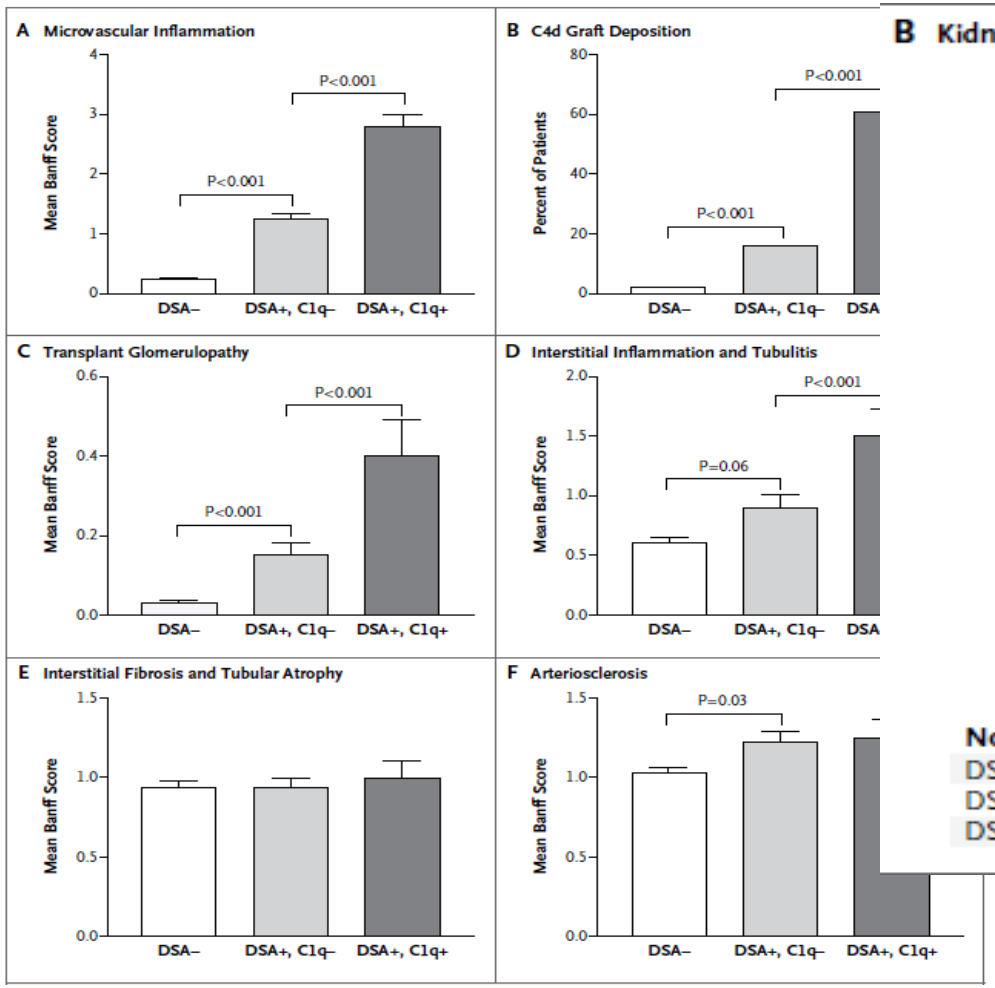
Model Linking TCMR, dnDSA, and AMR With Graft Loss[1]

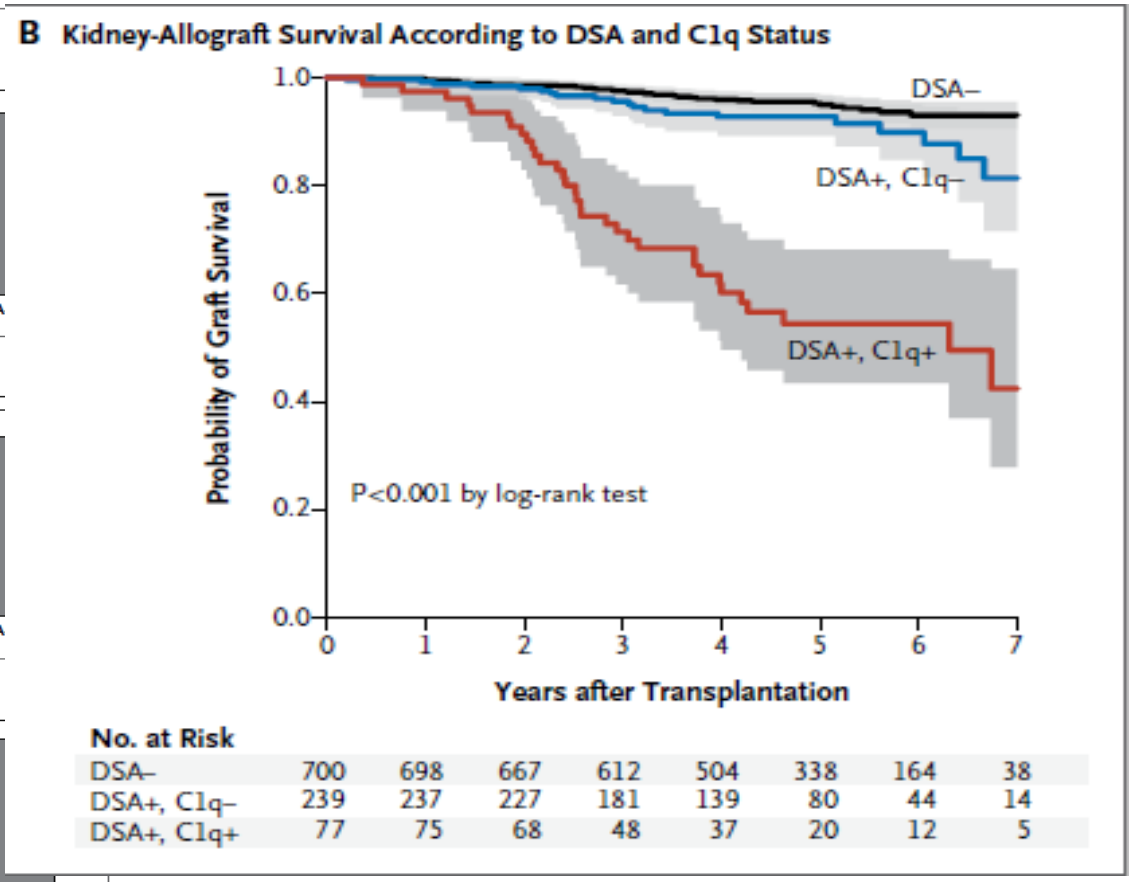


Source: Peter Nickerson

1. Wiebe C et al. *Am J Transplant*. 2012;12:1157-1167.

Complement Fixing (C1q+) DSAs Have the Greatest Potential to Injure and Destroy Allografts





Loupy A. N Engl J Med. 2013;369(13):1215-26.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Rituximab and Intravenous Immune Globulin for Desensitization during Renal Transplantation

Ashley A. Vo, Pharm.D., Marina Lukovsky, Pharm.D., Mieko Toyoda, Ph.D., Jennifer Wang, M.D., Nancy L. Reinsmoen, Ph.D., Chih-Hung Lai, Ph.D., Alice Peng, M.D., Rafael Villicana, M.D., and Stanley C. Jordan, M.D.

ABSTRACT

BACKGROUND

Few options for transplantation currently exist for patients highly sensitized to HLA. This exploratory, open-label, phase 1–2, single-center study examined whether intravenous immune globulin plus rituximab could reduce anti-HLA antibody levels and improve transplantation rates.

METHODS

Between September 2005 and May 2007, a total of 20 highly sensitized patients (with a mean [±SD] T-cell panel-reactive antibody level, determined by use of the complement-dependent cytotoxicity assay, of 77±19% or with donor-specific antibodies) were enrolled and received treatment with intravenous immune globulin and rituximab. We recorded rates of transplantation, panel-reactive antibody levels, cross-matching results at the time of transplantation, survival of patients and grafts, acute rejection episodes, serum creatinine values, adverse events and serious adverse events, and immunologic factors.

RESULTS

The mean panel-reactive antibody level was 44±30% after the second infusion of intravenous immune globulin (P<0.001 for the comparison with the pretreatment level). At study entry, the mean time on dialysis among recipients of a transplant from a deceased donor was 144±89 months (range, 60 to 324). However, the time to transplantation after desensitization was 5±6 months (range, 2 to 18). Sixteen of the 20 patients (80%) received a transplant. At 12 months, the mean serum creatinine level was 1.5±1.1 mg per deciliter (133±97 μmol per liter), and the mean survival rates of patients and grafts were 100% and 94%, respectively. There were no infusion-related adverse events or serious adverse events during the study. Long-term monitoring for infectious complications and neurologic problems revealed no unanticipated events.

CONCLUSIONS

These findings suggest that the combination of intravenous immune globulin and rituximab may prove effective as a desensitization regimen for patients awaiting a transplant from either a living donor or a deceased donor. Larger and longer trials are needed to evaluate the clinical efficacy and safety of this approach. (ClinicalTrials.gov number, NCT00642655.)

From the Comprehensive Transplant Center, Transplant Immunology Laboratory, and HLA Laboratory, Cedars–Sinai Medical Center, Los Angeles. Address reprint requests to Dr. Vo at the Comprehensive Transplant Center, Cedars–Sinai Medical Center, 8635 W. 3rd St., Suite 590W, Los Angeles, CA 90048, or at ashley.vo@cshs.org.

N Engl J Med 2008;359:242-51.

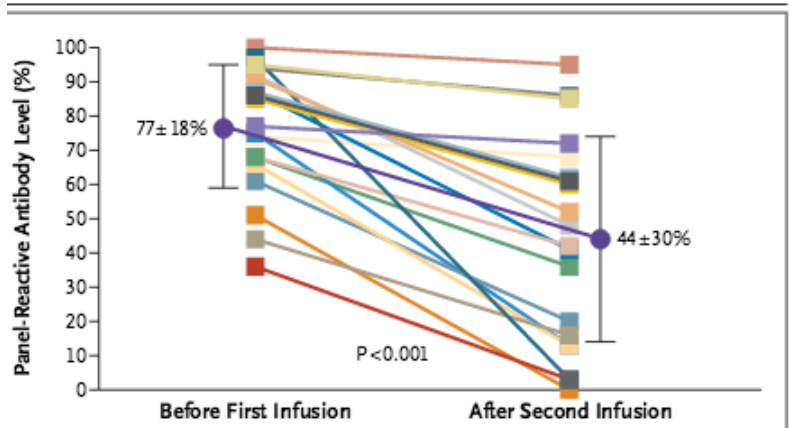


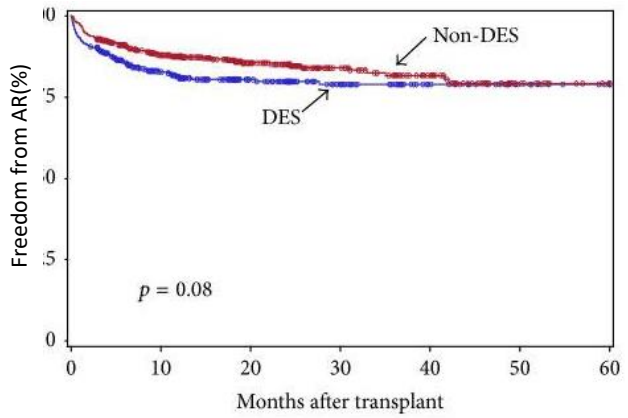
Figure 1. Panel-Reactive Antibody Levels in the 20 Study Patients.
Individual data are shown for patients before the first infusion of intravenous immune globulin and after the second infusion. The pretreatment and post-treatment means are also shown, as determined with the T-cell complement-dependent cytotoxicity panel-reactive antibody assay. The means were significantly different (P<0.001). I bars denote standard deviations.

Rituximab +IVIG significantly lowered PRA levels and improved transplant rates For HS patients.

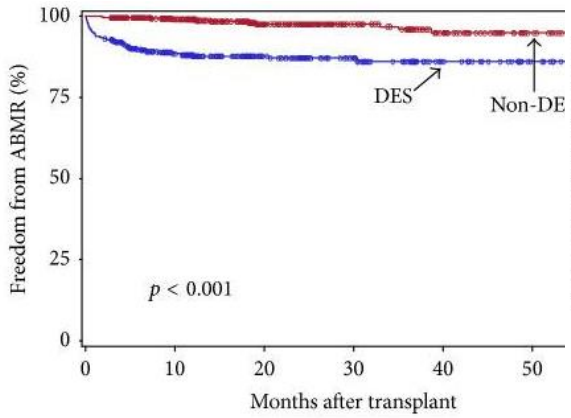
Desensitization for Kidney Transplantation

- Patient underwent desensitization with IVIG + Rituximab without successful reduction of DSAs. After 6M, the pati /IG/Rituximab and was transpla TCMX: 200, BCMX 283. Patient ith Campath 1H and maintained c
- At 1M post-t SA present was a weak DQ7. DSAs have subsequently disappeared. Patient is now 5 years post-transplant with SCr 0.9mg/dl. Biopsy showed no evidence of ABMR or TG.

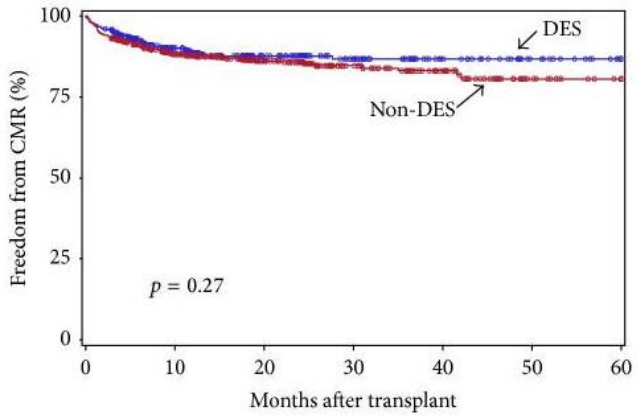
Freedom from Allograft Rejection & Infection Post-Desensitization(#372) v. Normal(#578)



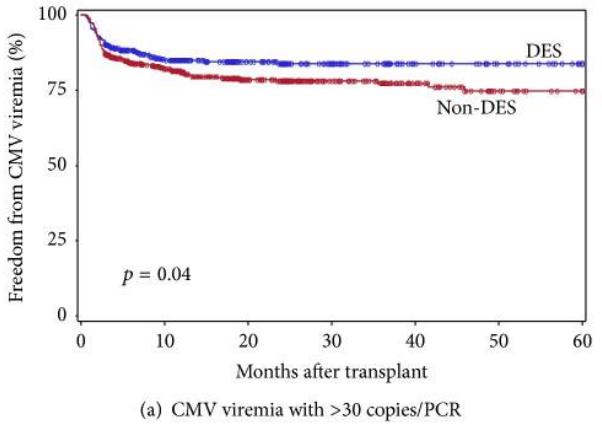
(a) Allograft rejection (ABMR or CMR)



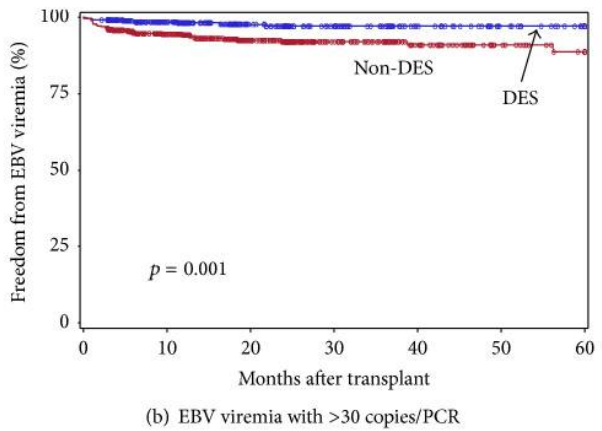
(b) ABMR



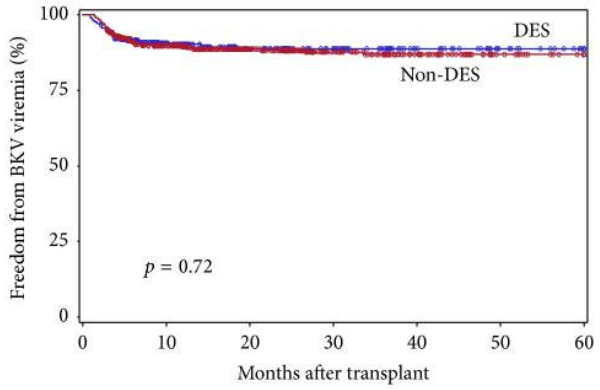
(c) CMR



(a) CMV viremia with >30 copies/PCR

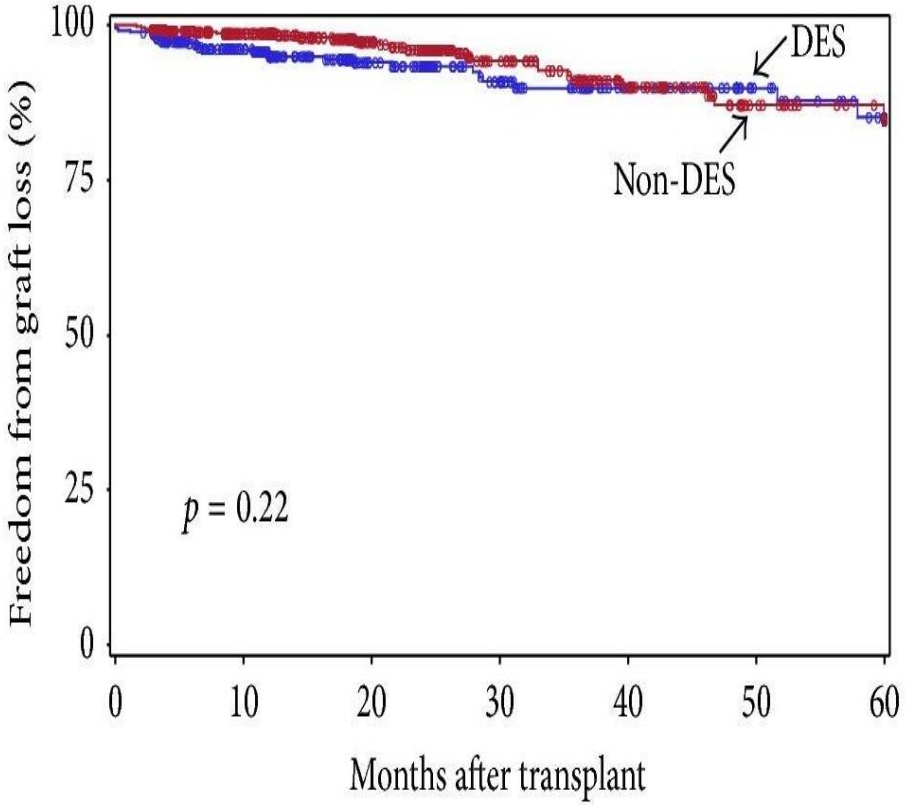


(b) EBV viremia with >30 copies/PCR

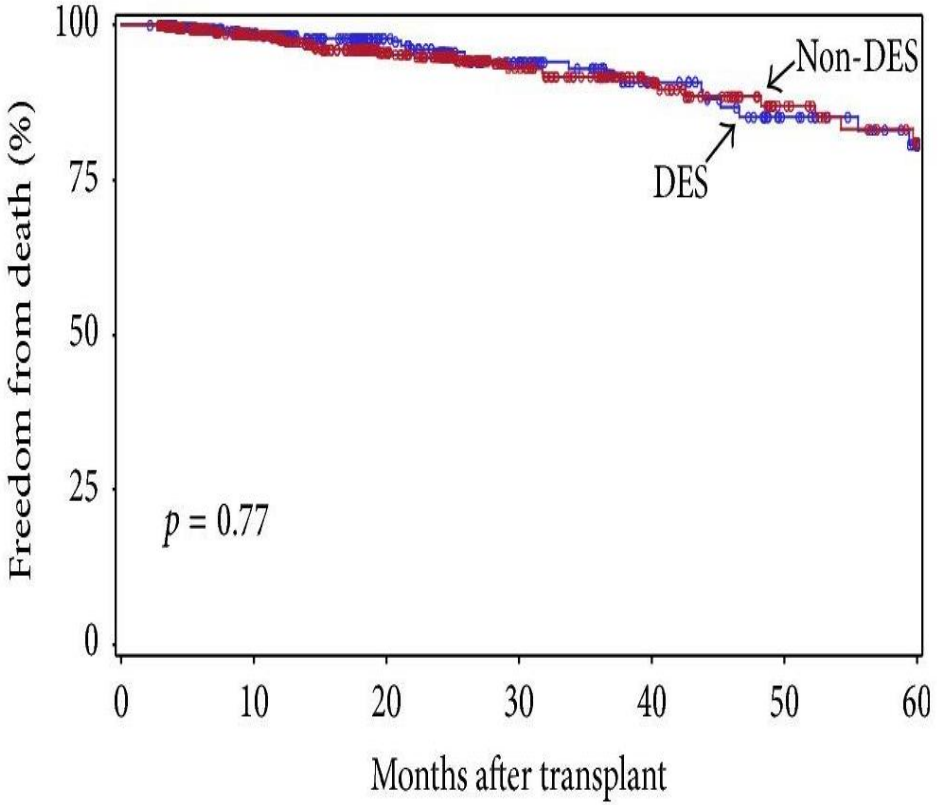


(c) BKV viremia with >1500 copies/ml

Patient & Graft Survival for Desensitized (#372) v. Normal(#578)at 5 Years



(a) Graft loss (death-censored)



(b) Patient survival

Original Clinical Science

Factors Predicting Risk for Antibody-mediated Rejection and Graft Loss in Highly Human Leukocyte Antigen Sensitized Patients Transplanted After Desensitization

Ashley A. Vo,[1] Aditi Sinha,[2] Mark Haas,[3] Jua Choi,[1] James Mirocha,[4] Joseph Kahwaji,[1] Alice Peng,[1] Rafael Villicana,[1] and Stanley C. Jordan[1]

Background. Desensitization with intravenous immunoglobulin and rituximab (I+R) significantly improves transplant rates in highly sensitized patients, but antibody-mediated rejection (ABMR) remains a concern. **Patients and Methods.** Between July 2006 and December 2012, 226 highly sensitized patients received transplants after desensitization. Most received alemtuzumab induction and standard immunosuppression. Two groups were examined: $ABMR^-$ (n = 181) and $ABMR^+$ (n = 45, 20%). Risk factors for ABMR, pathology, and outcomes were assessed. **Results.** Significant risks for ABMR included previous transplants and pregnancies as sensitizing events, donor-specific antibody (DSA) relative intensity scores greater than 17, presence of both class I and II DSAs at transplant and time on waitlist. The $ABMR^-$ showed a significant benefit for graft survival and glomerular filtration rate at 5 years ($P < 0.0001$). Banff pathology characteristics for $ABMR^+$ patients with or without graft loss did not differ. $C4d^+$ versus $C4d^-$ ABMR did not predict graft loss ($P = 0.086$). Thrombotic microangiopathy (TMA^+) significantly predicted graft failure ($P = 0.045$). The ABMR episodes were treated with I+R (n = 25), or, in more severe $ABMR^+$, plasma exchange (PLEX)+I+R (n = 20). Graft survival for patients treated with I+R was superior ($P = 0.028$). Increased mortality was seen in $ABMR^+$ patients experiencing graft loss after ABMR treatment ($P = 0.004$). The PLEX + Eculizumab improved graft survival for TMA^+ patients ($P = 0.036$). **Conclusion.** Patients desensitized with I+R who remain $ABMR^-$ have long-term graft and patient survival. The $ABMR^+$ patients have significantly reduced graft survival and glomerular filtration rate at 5 years, especially TMA^+. Severe $ABMR^+$ episodes benefit from treatment with PLEX + Eculizumab. The DSA-relative intensity scores at transplant was a strong predictor

DSA Number & Strength are Strong Predictors of Risk for ABMR

DSA Relative Intensity Scale (RIS) at Transplant by ABMR Status

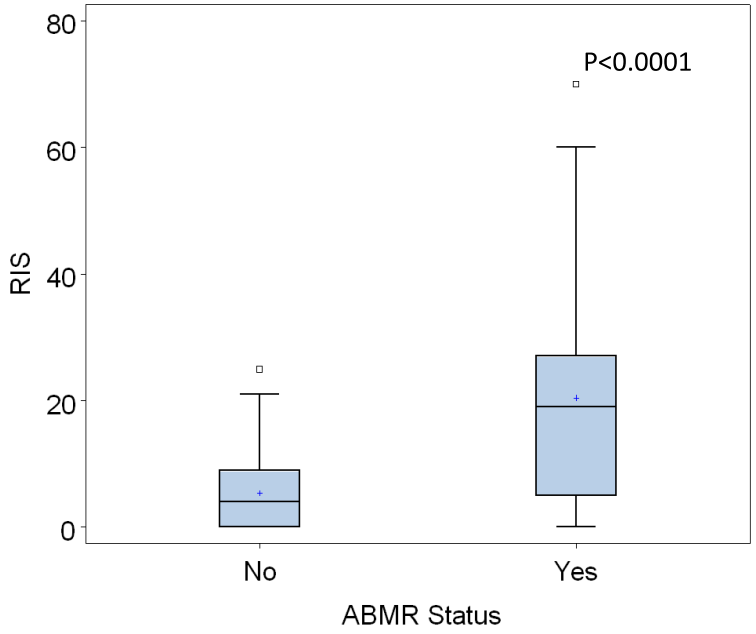


Figure 1C

Positive Predictive Value (PPV) of RIS for ABMR Episodes

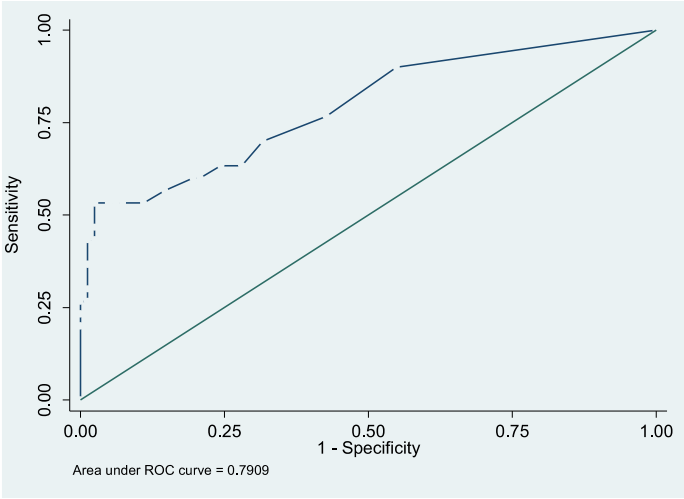
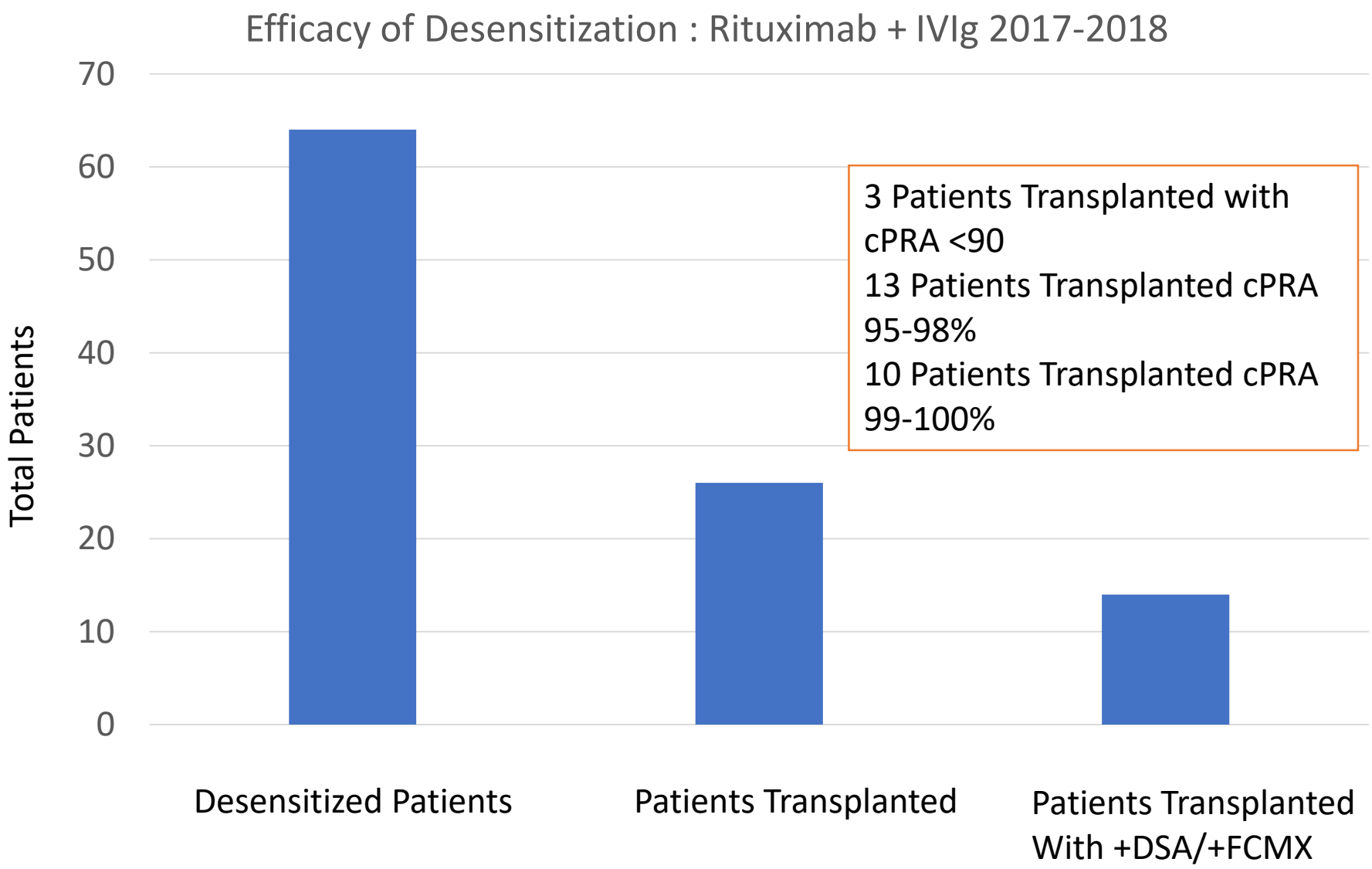


Figure 1D

Vo et al Transplantation 2014


Efficacy of Desensitization : Rituximab + IVIg 2017-2018
Total Patients
70
60
50
40
30
20
10
0
3 Patients Transplanted with cPRA <90
13 Patients Transplanted cPRA 95-98%
10 Patients Transplanted cPRA 99-100%
Desensitized Patients
Patients Transplanted
Patients Transplanted With +DSA/+FCMX

Survival Benefits of HLAi Transplantation After Desensitization

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Survival Benefit with Kidney Transplants from HLA-Incompatible Live Donors

B.J. Orandi, X. Luo, A.B. Massie, J.M. Garonzik-Wang, B.E. Lonze, R. Ahmed, K.J. Van Arendonk, M.D. Stegall, S.C. Jordan, J. Oberholzer, T.B. Dunn, L.E. Ratner, S. Kapur, R.P. Pelletier, J.P. Roberts, M.L. Melcher, P. Singh, D.L. Sudan, M.P. Posner, J.M. El-Amm, R. Shapiro, M. Cooper, G.S. Lipkowitz, M.A. Rees, C.L. Marsh, B.R. Sankari, D.A. Gerber, P.W. Nelson, J. Wellen, A. Bozorgzadeh, A.O. Gaber, R.A. Montgomery, and D.L. Segev

ABSTRACT

BACKGROUND

A report from a high-volume single center indicated a survival benefit of receiving a kidney transplant from an HLA-incompatible live donor as compared with remaining on the waiting list, whether or not a kidney from a deceased donor was received. The generalizability of that finding is unclear.

METHODS

In a 22-center study, we estimated the survival benefit for 1025 recipients of kidney transplants from HLA-incompatible live donors who were matched with controls who remained on the waiting list or received a transplant from a deceased donor (waiting-list-or-transplant control group) and controls who remained on the waiting list but did not receive a transplant (waiting-list-only control group). We analyzed the data with and without patients from the highest-volume center in the study.

RESULTS

Recipients of kidney transplants from incompatible live donors had a higher survival rate than either control group at 1 year (95.0%, vs. 94.0% for the waiting-list-or-transplant control group and 89.6% for the waiting-list-only control group), 3 years (91.7% vs. 83.6% and 72.7%, respectively), 5 years (86.0% vs. 74.4% and 59.2%), and 8 years (76.5% vs. 62.9% and 43.9%) (P<0.001 for all comparisons with the two control groups). The survival benefit was significant at 8 years across all levels of donor-specific antibody: 89.2% for recipients of kidney transplants from incompatible live donors who had a positive Luminex assay for anti-HLA antibody but a negative flow-cytometric cross-match versus 65.0% for the waiting-list-or-transplant control group and 47.1% for the waiting-list-only control group; 76.3% for recipients with a positive flow-cytometric cross-match but a negative cytotoxic cross-match versus 63.3% and 43.0% in the two control groups, respectively; and 71.0% for recipients with a positive cytotoxic cross-match versus 61.5% and 43.7%, respectively. The findings did not change when patients from the highest-volume center were excluded.

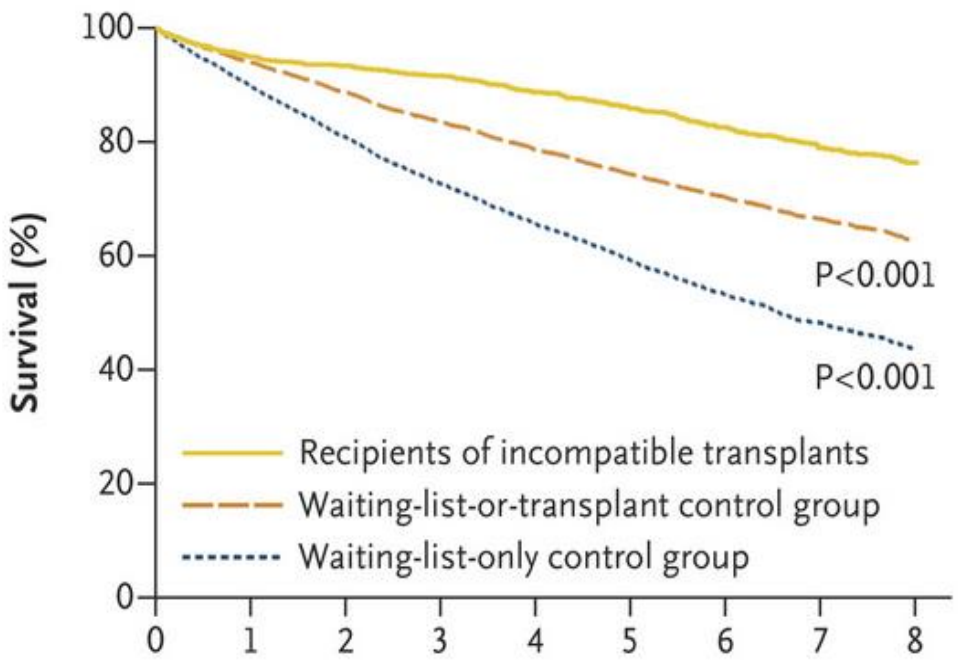
CONCLUSIONS

This multicenter study validated single-center evidence that patients who received kidney transplants from HLA-incompatible live donors had a substantial survival benefit as compared with patients who did not undergo transplantation and those who waited for transplants from deceased donors. (Funded by the National Institute of Diabetes and Digestive and Kidney Diseases.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Segev at the Department of Surgery, Johns Hopkins University, 720 Rutland Ave., Turner 034, Baltimore, MD 21287, or at dorry@jhmi.edu.

Drs. Montgomery and Segev contributed equally to this article.

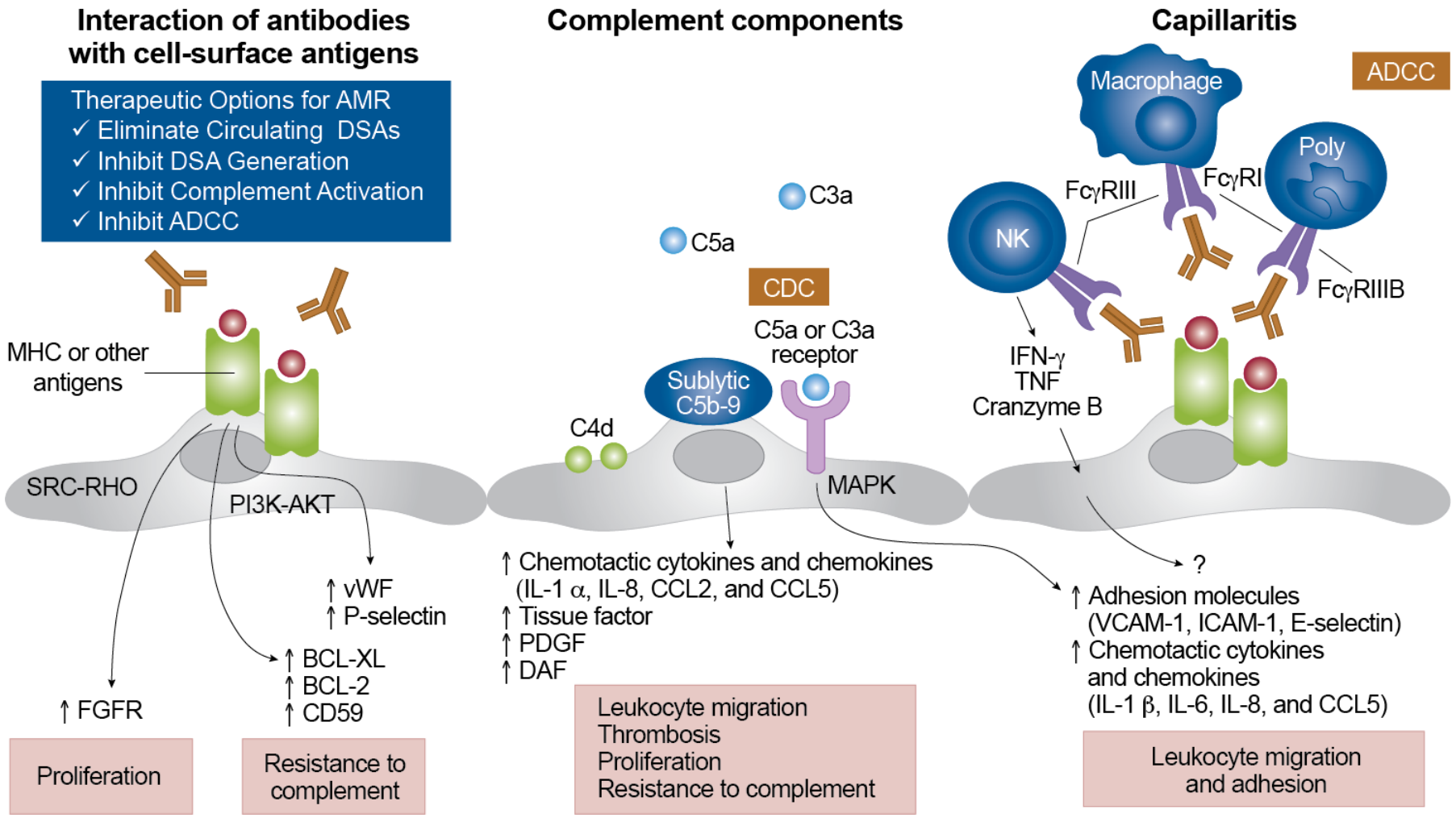
N Engl J Med 2016;374:940-50.
DOI: 10.1056/NEJMoa1508380



No. at Risk

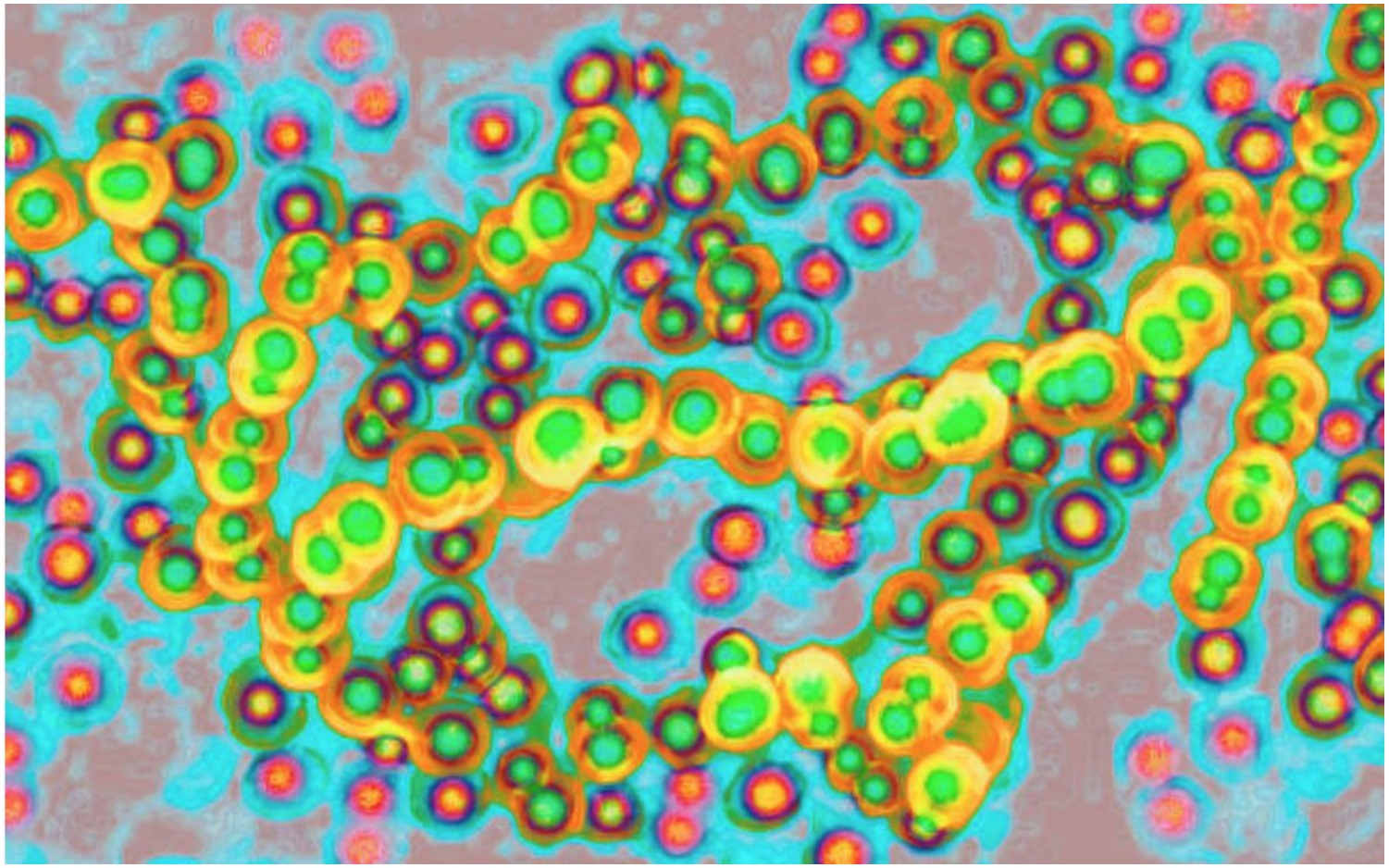
Recipients of incompatible transplants	1025	958	832	584	327
Waiting-list-or-transplant control group	5125	4546	3673	2493	1414
Waiting-list-only control group	5125	4141	3024	1810	916

Therapeutic Options for AMR[1]

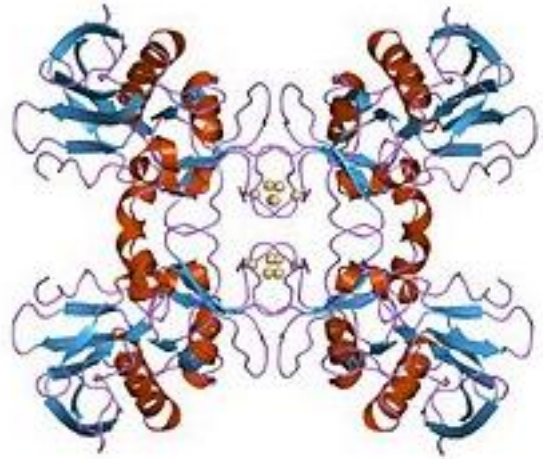


1. Farkash EA, Colvin RB. *Nat Rev Nephrol*. 2012;8:255-257.

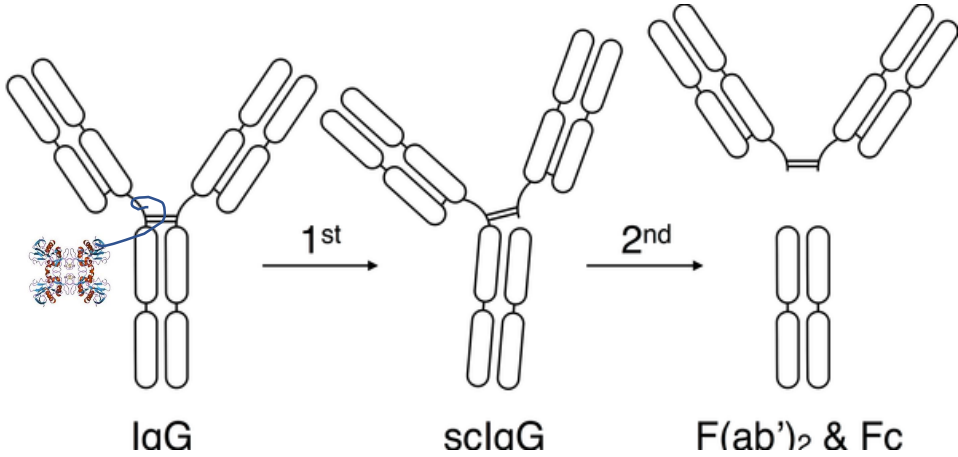
IdeS (IgG Endopeptidase): A Potent IgG Degrading Enzyme



Steptococcus Pyogenes

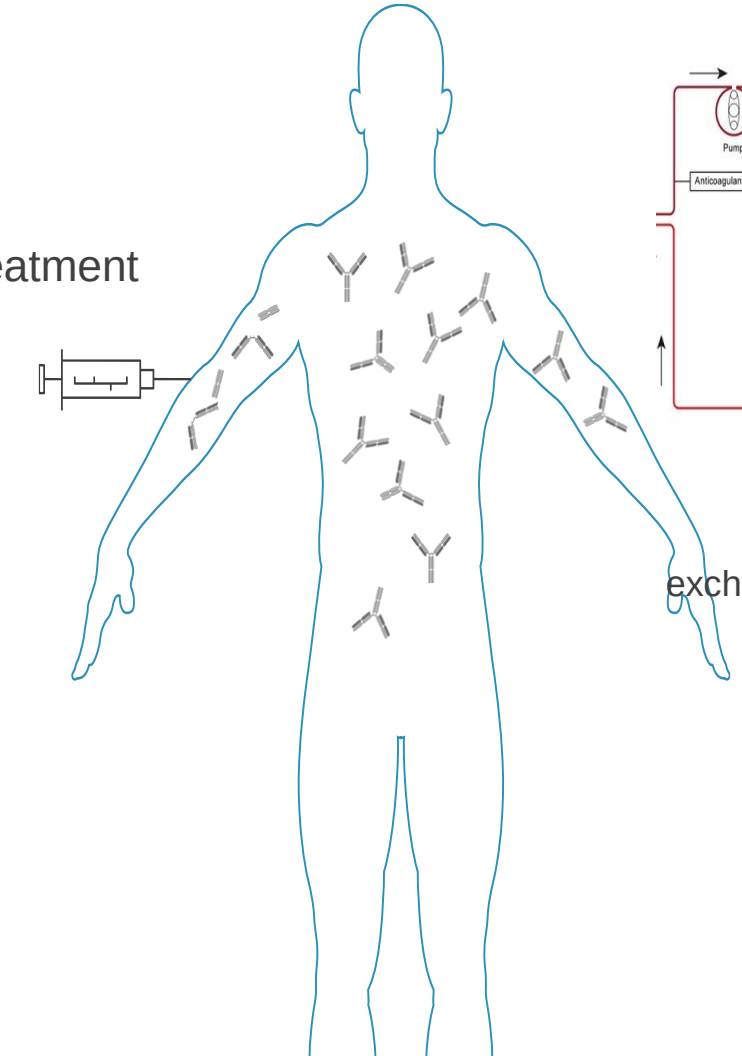


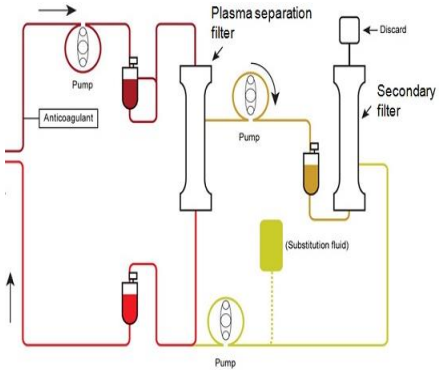
IgG Endopeptidase



Solutions to remove pathogenic IgG in Patients

IdeS treatment





Therapeutic plasma exchange/Immunoadsorption

ORIGINAL ARTICLE

IgG Endopeptidase in Highly Sensitized Patients Undergoing Transplantation

S.C. Jordan, T. Lorant, J. Choi, C. Kjellman, L. Winstedt, M. Bengtsson, X. Zhang, T. Eich, M. Toyoda, B.-M. Eriksson, S. Ge, A. Peng, S. Järnum, K.J. Wood, T. Lundgren, L. Wennberg, L. Bäckman, E. Larsson, R. Villicana, J. Kahwaji, S. Louie, A. Kang, M. Haas, C. Nast, A. Vo, and G. Tufveson

ABSTRACT

BACKGROUND

Donor-specific antibodies create an immunologic barrier to transplantation. Current therapies to modify donor-specific antibodies are limited and ineffective in the most highly HLA-sensitized patients. The IgG-degrading enzyme derived from *Streptococcus pyogenes* (IdeS), an endopeptidase, cleaves human IgG into $F(ab')_2$ and Fc fragments inhibiting complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, which suggests that IdeS might be useful for desensitization. We report on the combined experience of two independently performed open-label, phase 1–2 trials (conducted in Sweden and the United States) that assessed the efficacy of IdeS with regard to desensitization and transplantation of a kidney from an HLA-incompatible donor.

METHODS

We administered IdeS to 25 highly HLA-sensitized patients (11 patients in Uppsala

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Jordan at the Comprehensive Transplant Center, Cedars–Sinai Medical Center, 8900 Beverly Blvd., Los Angeles, CA 90048, or at sjordan@cshs.org.

Drs. Jordan and Lorant, and Drs. Vo and Tufveson, contributed equally to this article.

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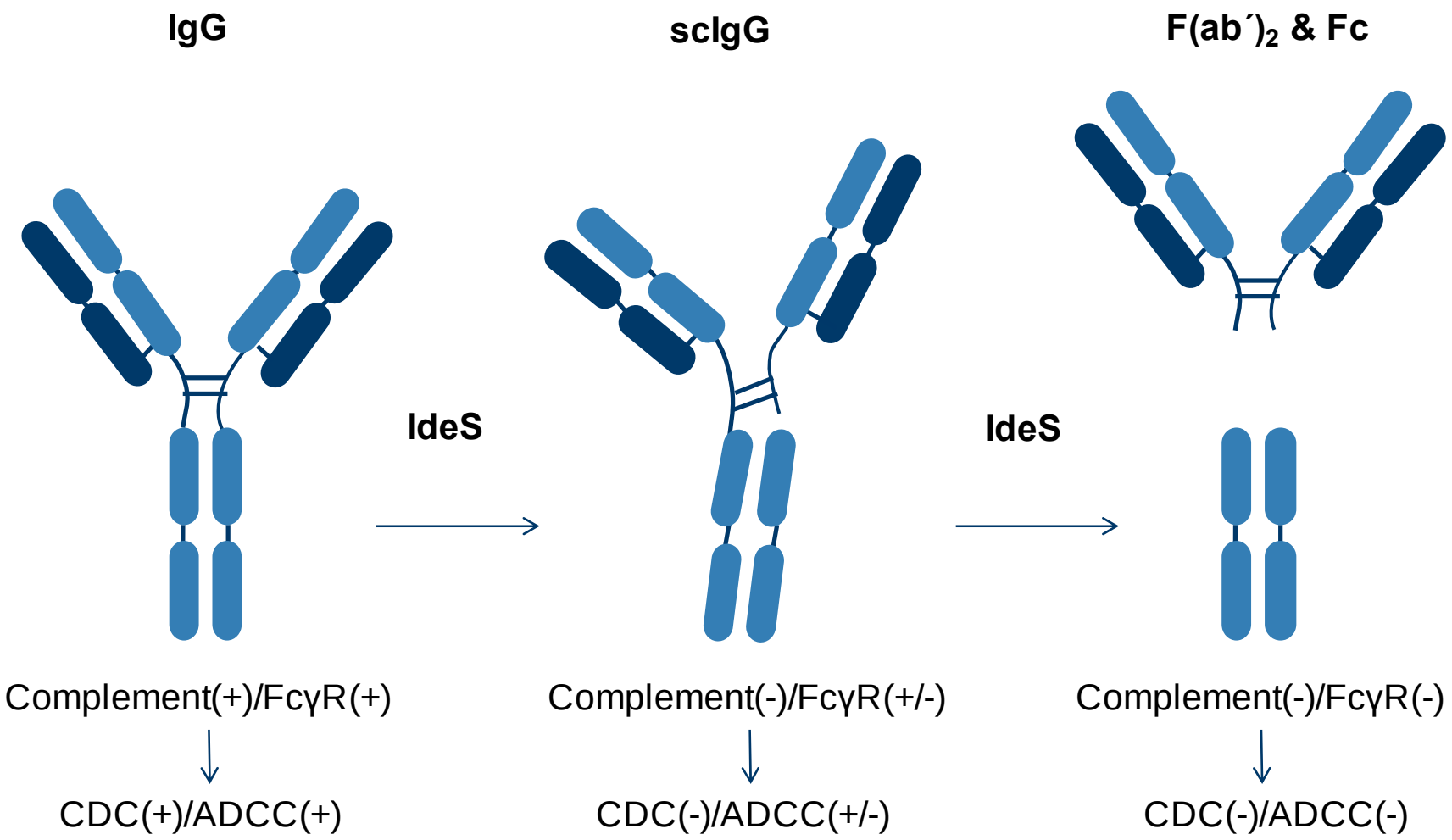
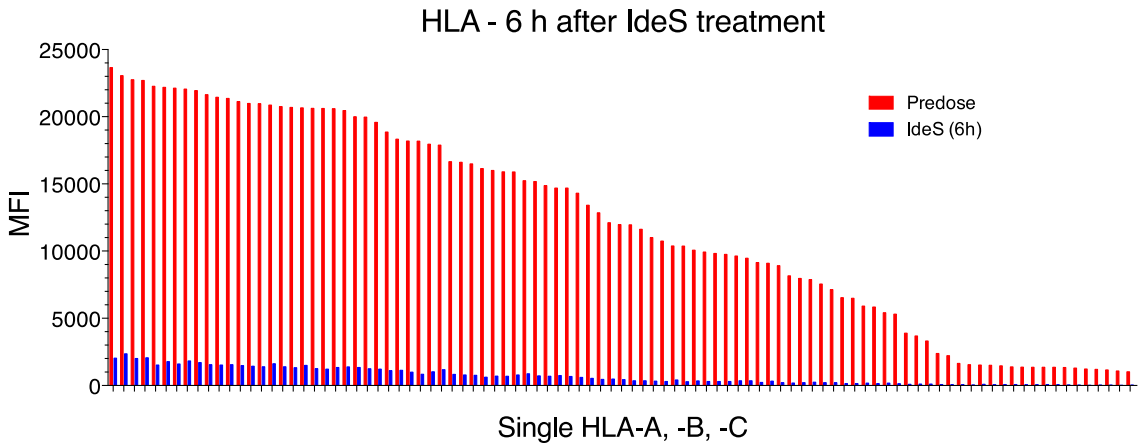
Mechanism of Action of IdeS with Implications for CDC and ADCC[1]



1. Jordan SC et al. 2017. In press.

Impact of IdeS on Luminex SAB and C1q Assays



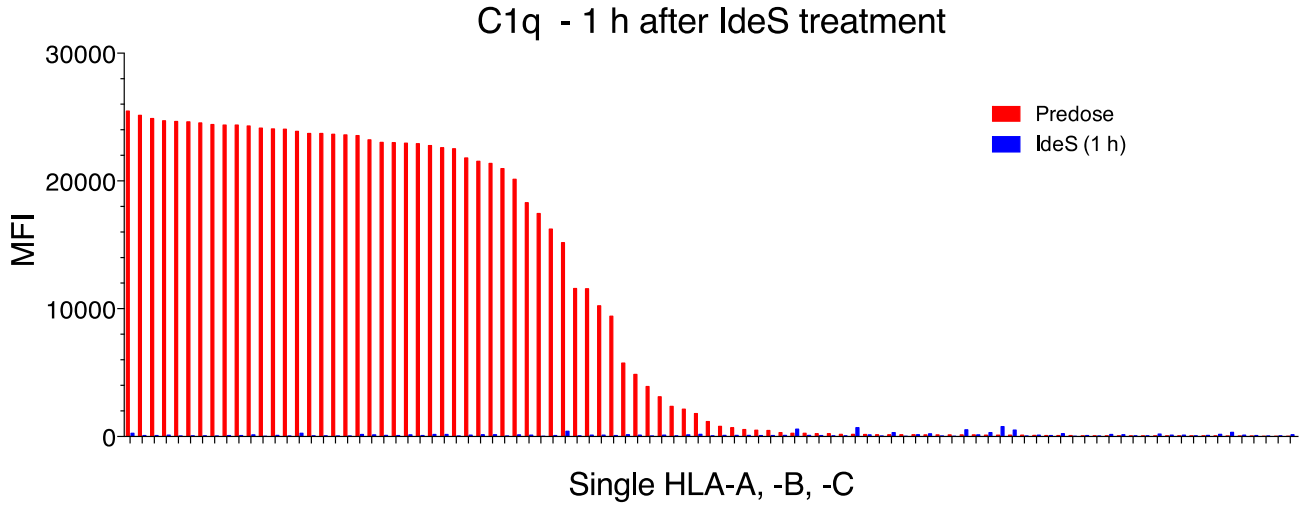
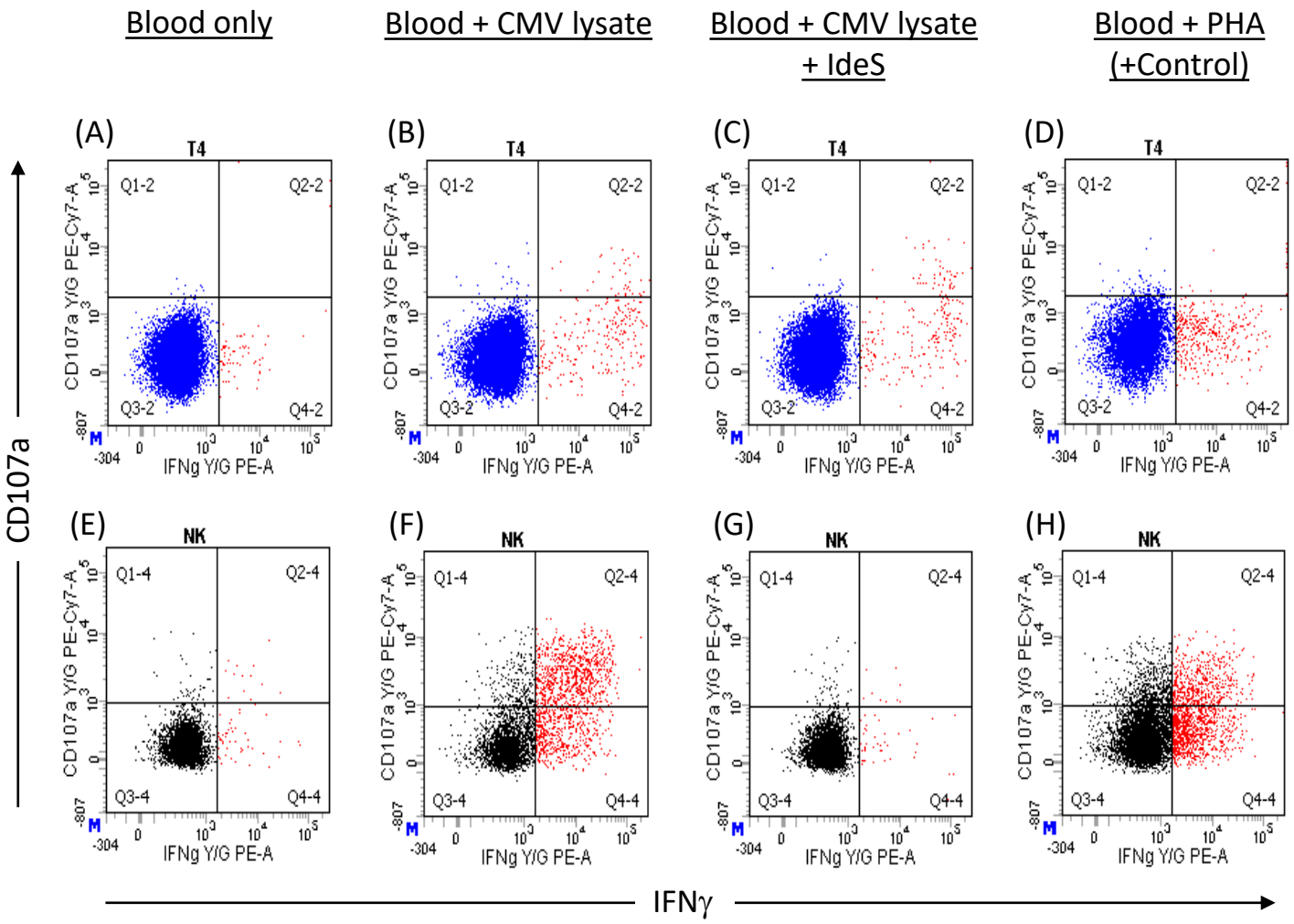
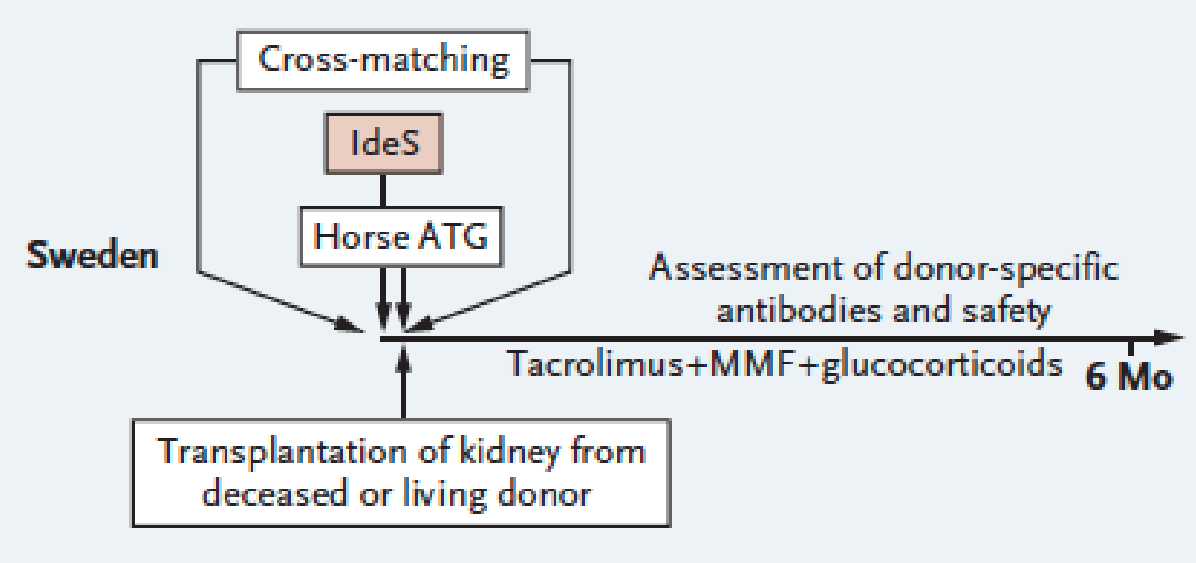
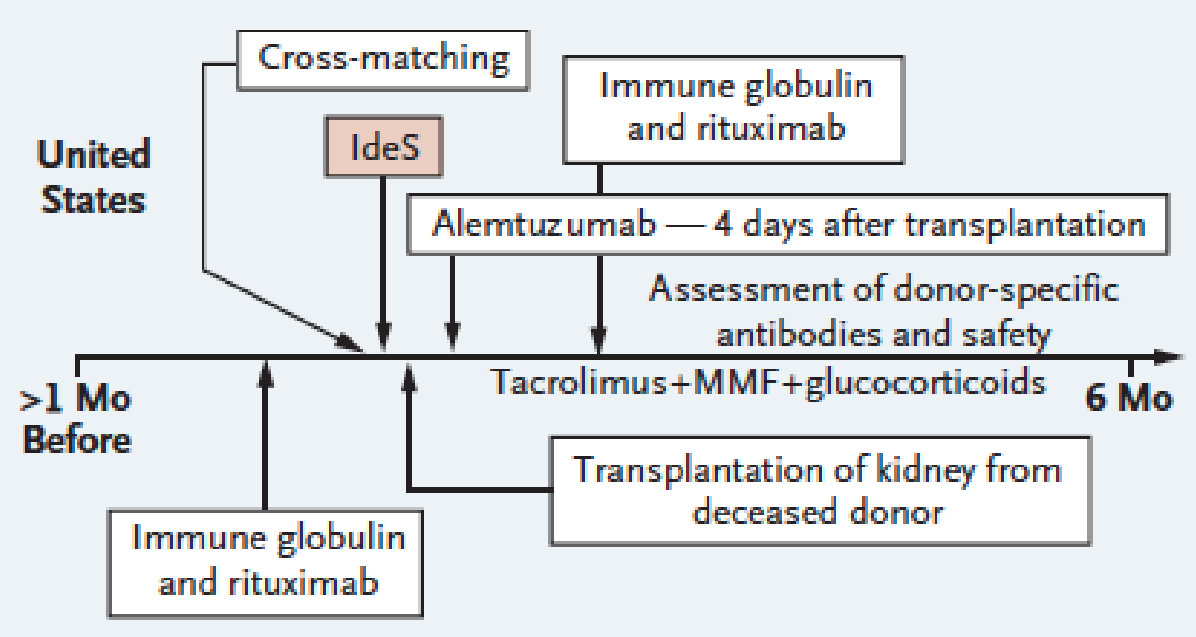


Fig. 6


Blood only
Blood + CMV lysate
Blood + CMV lysate + IdeS
Blood + PHA (+Control)
CD4+ T Cells
CD56+NK Cells
CD107a
IFNγ
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
T4
NK
Q1-2
Q2-2
Q3-2
Q4-2
Q1-4
Q2-4
Q3-4
Q4-4
CD107a Y/G PE-Cy7-A
IFNg Y/G PE-A

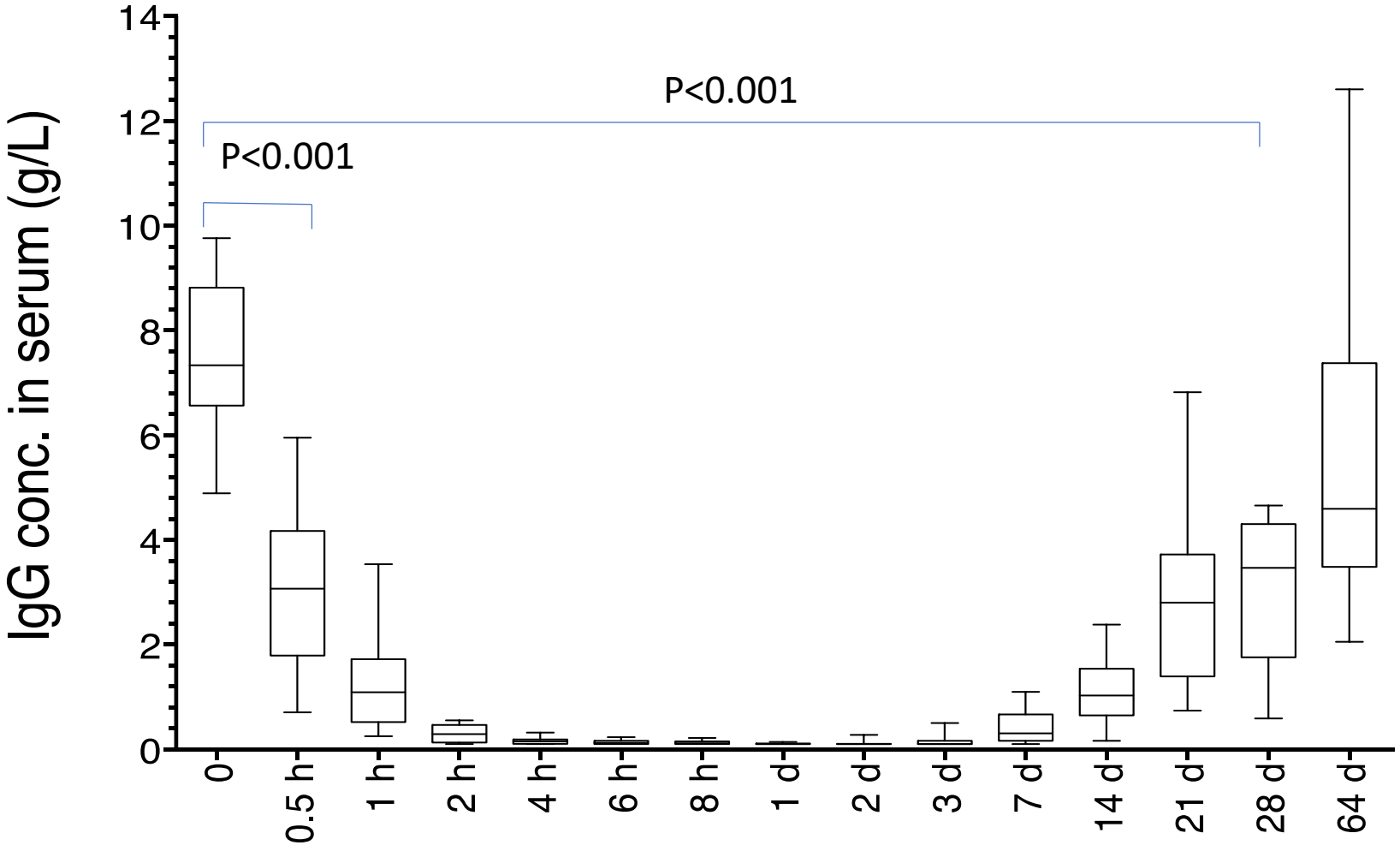
Protocol



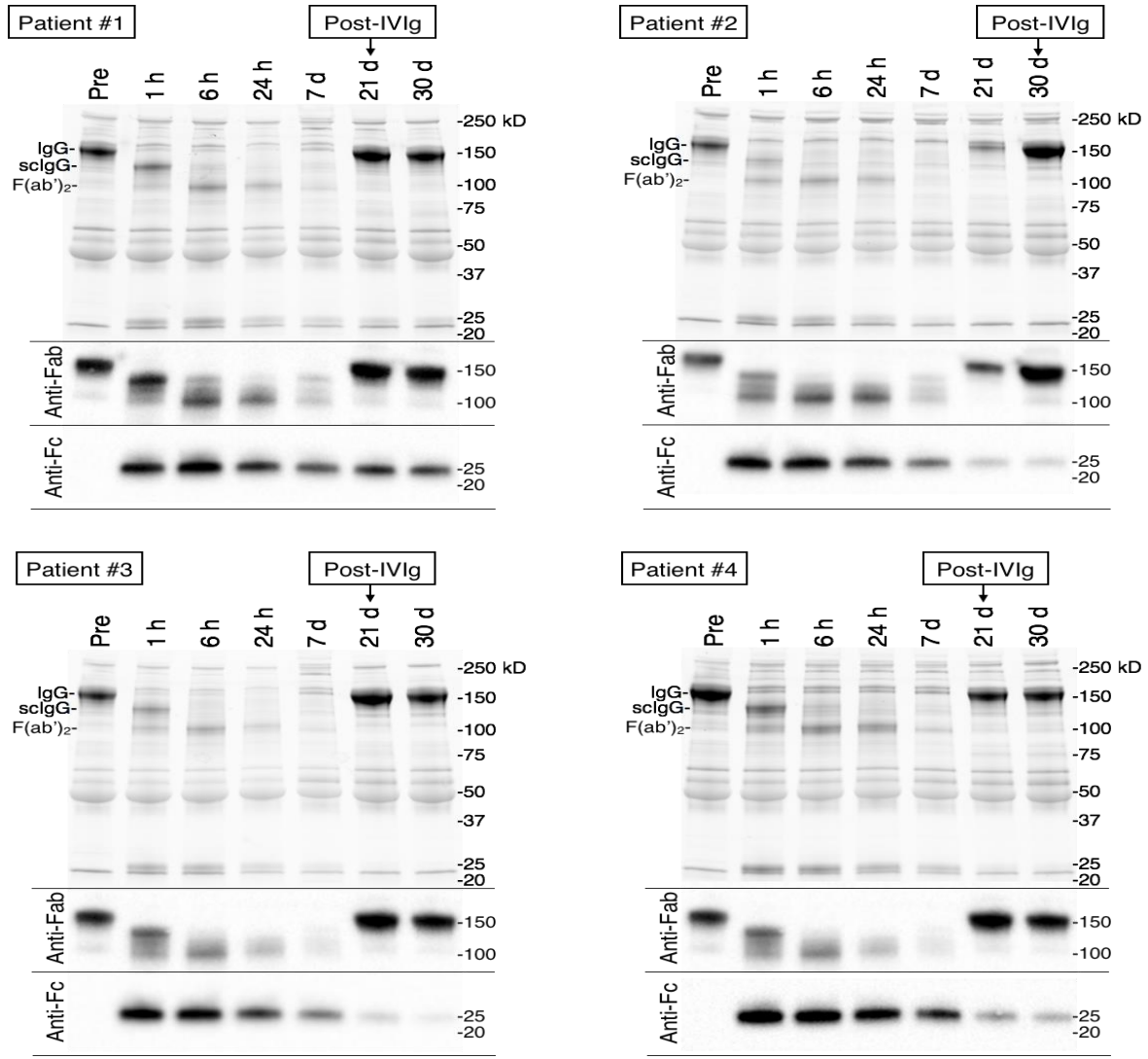


- 11 patients in Swedish cohort and 14 in US
- DSA detected with solid-phase assay
- Samples obtained before IdeS administration
  - DSA and flow-cytometric cross-match
- Cross-match and DSA tests done at 6 hours, 24 hours, and days 7, 30, 60, 90 (US only), 180 (US only)
- Biopsies performed to assess ABMR when allograft dysfunction noted
- Protocol biopsy at 6 months
  - C4d staining and Banff 2013 criteria
- IdeS and IgG levels assessed with SDS-PAGE and Western blot
- Cleavage and clearance of Fc and $F(ab')_2$ fragments analyzed with ELISA
- Routine lab tests, vital signs, adverse events

Impact of IdeS on Circulating IgG Levels in HS Patients

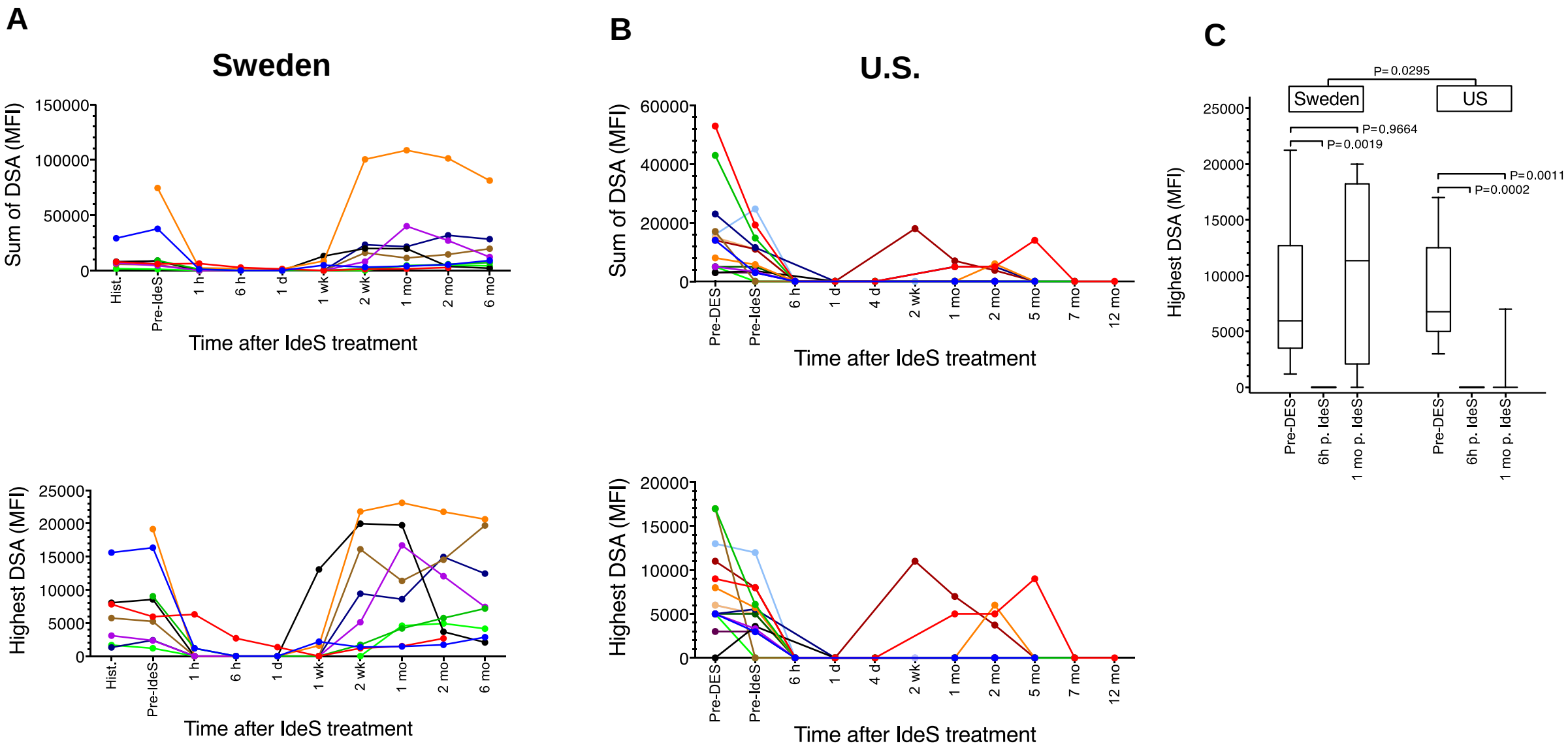


SDS-Page and Western Blot Analysis of Serum Pre and Post IdeS Treatment[1]

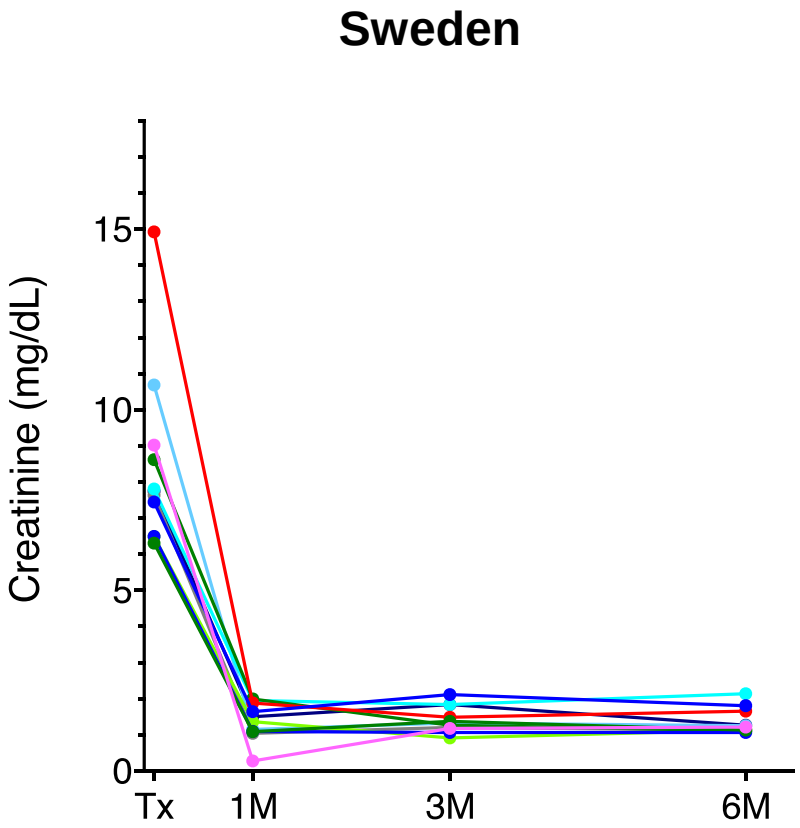


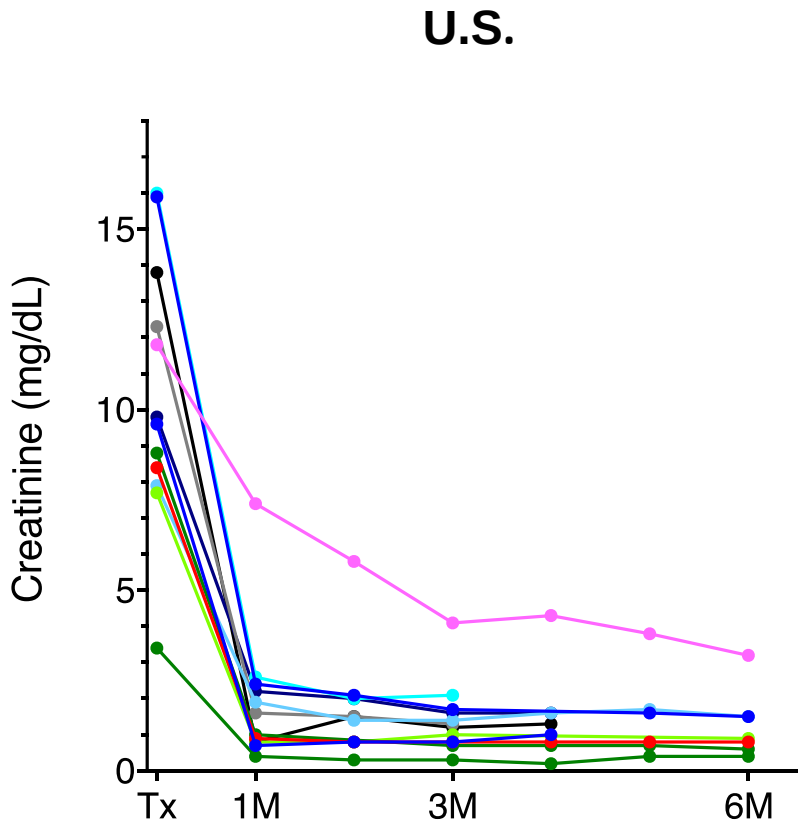
1. Jordan SC et al. 2017. In press.

Figure 4


A
Sweden
Sum of DSA (MFI)
150000
100000
50000
0
Hist.
Pre-IdeS
1 h
6 h
1 d
1 wk
2 wk
1 mo
2 mo
6 mo
Time after IdeS treatment
Highest DSA (MFI)
25000
20000
15000
10000
5000
0
Hist.
Pre-IdeS
1 h
6 h
1 d
1 wk
2 wk
1 mo
2 mo
6 mo
Time after IdeS treatment
B
U.S.
Sum of DSA (MFI)
60000
40000
20000
0
Pre-DES
Pre-IdeS
6 h
1 d
4 d
2 wk
1 mo
2 mo
5 mo
7 mo
12 mo
Time after IdeS treatment
Highest DSA (MFI)
20000
15000
10000
5000
0
Pre-DES
Pre-IdeS
6 h
1 d
4 d
2 wk
1 mo
2 mo
5 mo
7 mo
12 mo
Time after IdeS treatment
C
P=0.0295
Sweden
US
P=0.9664
P=0.0019
P=0.0011
P=0.0002
Highest DSA (MFI)
25000
20000
15000
10000
5000
0
Pre-DES
6h p. IdeS
1 mo p. IdeS
Pre-DES
6h p. IdeS
1 mo p. IdeS

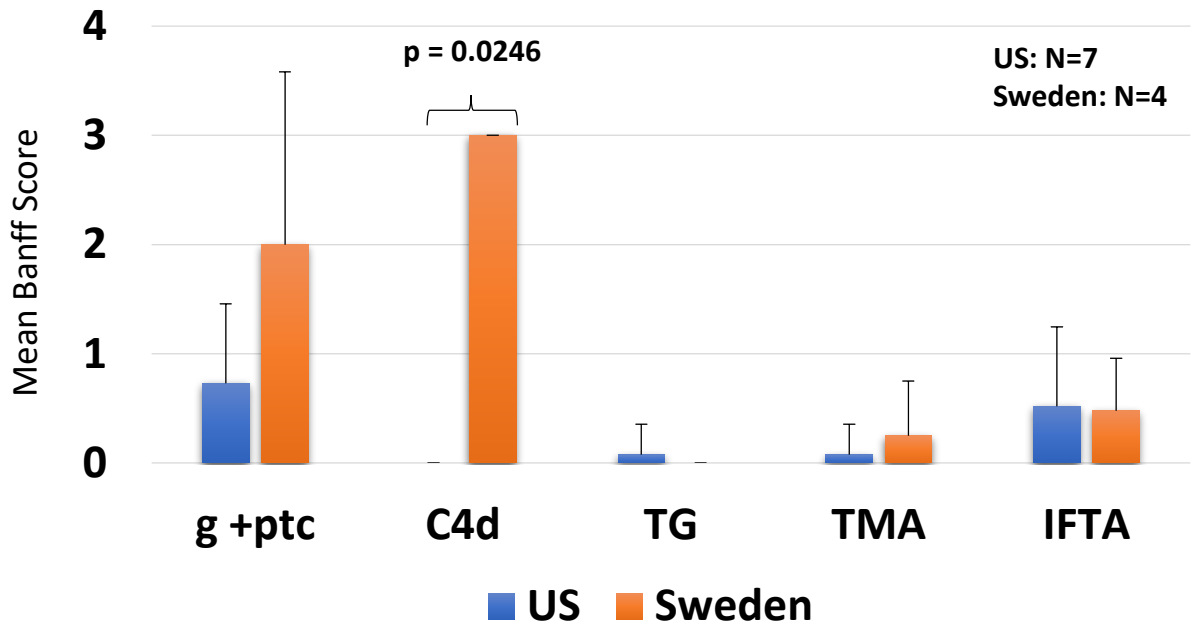
Serum Cr Values Over the First 6 Months Post-Transplant



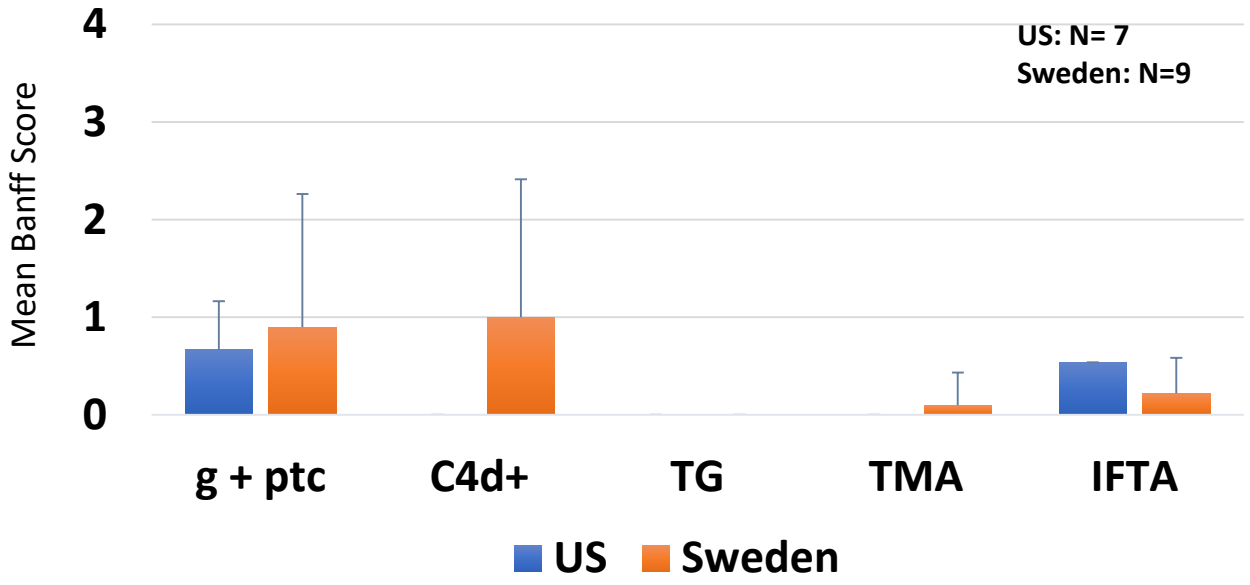


Banff 2013 Biopsy Scores

(1) ABMR Biopsy Banff Scoring US vs Sweden



(2) Protocol Biopsy Banff Scoring US vs Sweden



Is Desensitization Necessary in the KAS Era?

Perspective

Immunoglobulin G–Degrading Enzyme of *Streptococcus pyogenes* (IdeS), Desensitization, and the Kidney Allocation System

Complementary Approaches to Increase Transplantation in Highly HLA Sensitized Patients

Edmund Huang and Stanley C. Jordan

Clin J Am Soc Nephrol 13: 799–801, 2018. doi: https://doi.org/10.2215/CJN.12031017

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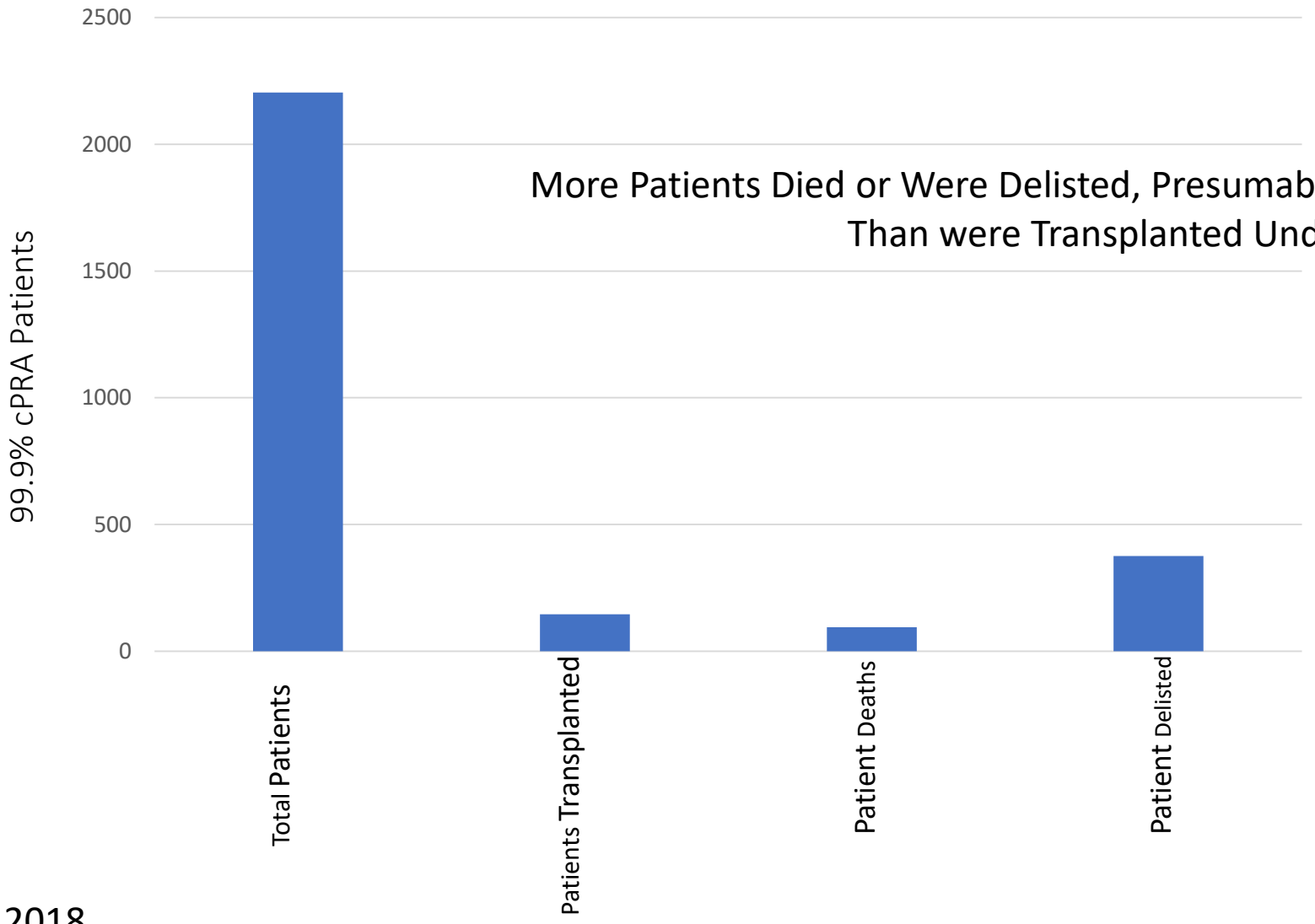
In a recent Perspectives article in the *Clinical Journal of the American Society of Nephrology*, Formica and Kulkarni (1) contextualize the use of the IgG-degrading enzyme of *Streptococcus pyogenes* (IgG endopeptidase) for desensitization to the era of the new kidney allocation system (KAS). Since the KAS, more highly sensitized kidney candidates have received transplants than before. Therefore, it was suggested that the allocation priority for highly sensitized candidates in the new KAS diminishes the need for desensitization and that waiting for a suitably matched donor is preferable to undergoing an incompatible transplant.

As previously reported, the median waiting time for patients with calculated panel reactive antibodies 98%–100% has fallen from >19 years pre-KAS to 3.2 years post-KAS (2). Therefore, it was stated that, if a sensitized patient can receive a compatible transplant quickly, there is "no clinical justification for desensitization" (1). Although it is true that more patients with calculated panel reactive antibodies of 98%–100% were transplanted since the KAS, not all highly sensitized patients benefited to the same degree. Patients with calculated panel reactive antibodies ≥99.95% accounted for 34.0% of candidates with calculated panel reactive antibodies ≥99% (approximately 2700 candidates in the United States) but received only 8% of the transplants for those with calculated panel reactive antibodies ≥99% in the first year after the KAS was implemented (3). For any given calculated panel reactive antibodies percentage, the probability of finding an acceptable match can be estimated with the following formula: 1− (calculated panel reactive antibodies percentage)^n, where n is the number of potential donors (4). Using this formula, candidates with calculated panel reactive antibodies of 99.95% would need approximately 6000 match runs to have a 95% probability of an acceptable crossmatch. This estimate increases approximately 12,000 donors; however, clearly, a significant number of highly sensitized candidates will not benefit from the KAS and are unlikely to receive a transplant without desensitization.

Although highly sensitized candidates have received transplants more frequently after the KAS, it is unknown how many received transplants with a negative crossmatch. One cannot assume that the increased number of transplants was performed with a negative crossmatch and without donor-specific antibodies. Houp *et al.* (5) reported that 58% of transplants performed among candidates with calculated panel reactive antibodies of 99%–100% at Johns Hopkins after the KAS was implemented were in the presence of donor-specific antibodies (18 of 30 in 2015 and eight of 15 in 2016), and they noted that 40%–47% of their highly HLA–sensitized list could not be transplanted under the KAS and required desensitization. Here, the authors noted that, before KAS implementation, patients with calculated panel reactive antibodies of 50% determined by cytotoxicity assays could be "converted" to calculated panel reactive antibodies of 100% by using more sensitive Luminex assays and listing mean fluorescence intensities at or below the threshold of detection, thus increasing their chances for early transplantation in the KAS. These patients are not as immunologically challenging as the highly and broadly sensitized patients who have calculated panel reactive antibodies of 100% and are less likely to need desensitization.

The Perspectives article cautioned against the use of desensitization, because HLA-incompatible transplants have lower graft survival compared with compatible transplants. This may not be a valid comparison. As discussed above, a large number of patients are so broadly sensitized that their chances of finding a compatible donor are remote. Additionally, it was

Outcomes of cPRA 99.9% ESRD Patients Awaiting Transplant in KAS Era



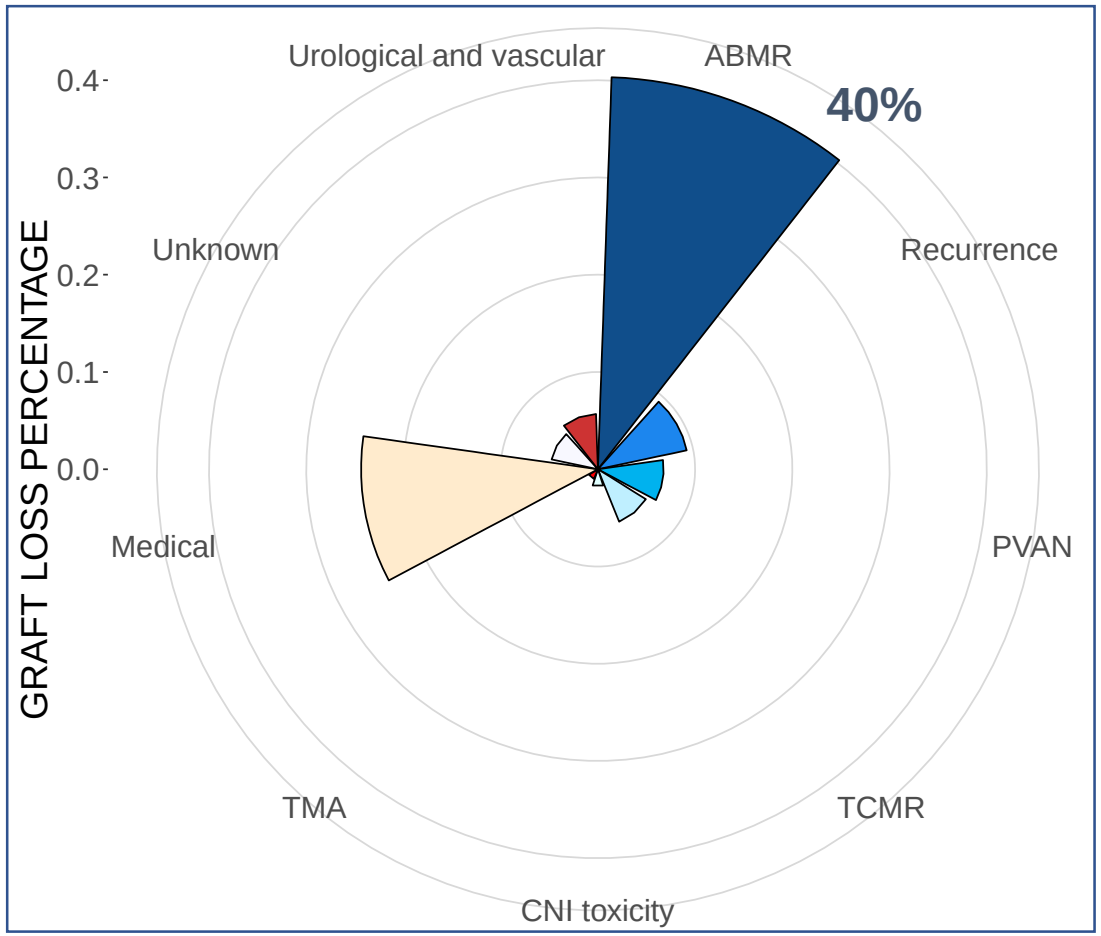
More Patients Died or Were Delisted, Presumably Due to Health Reasons, Than were Transplanted Under KAS

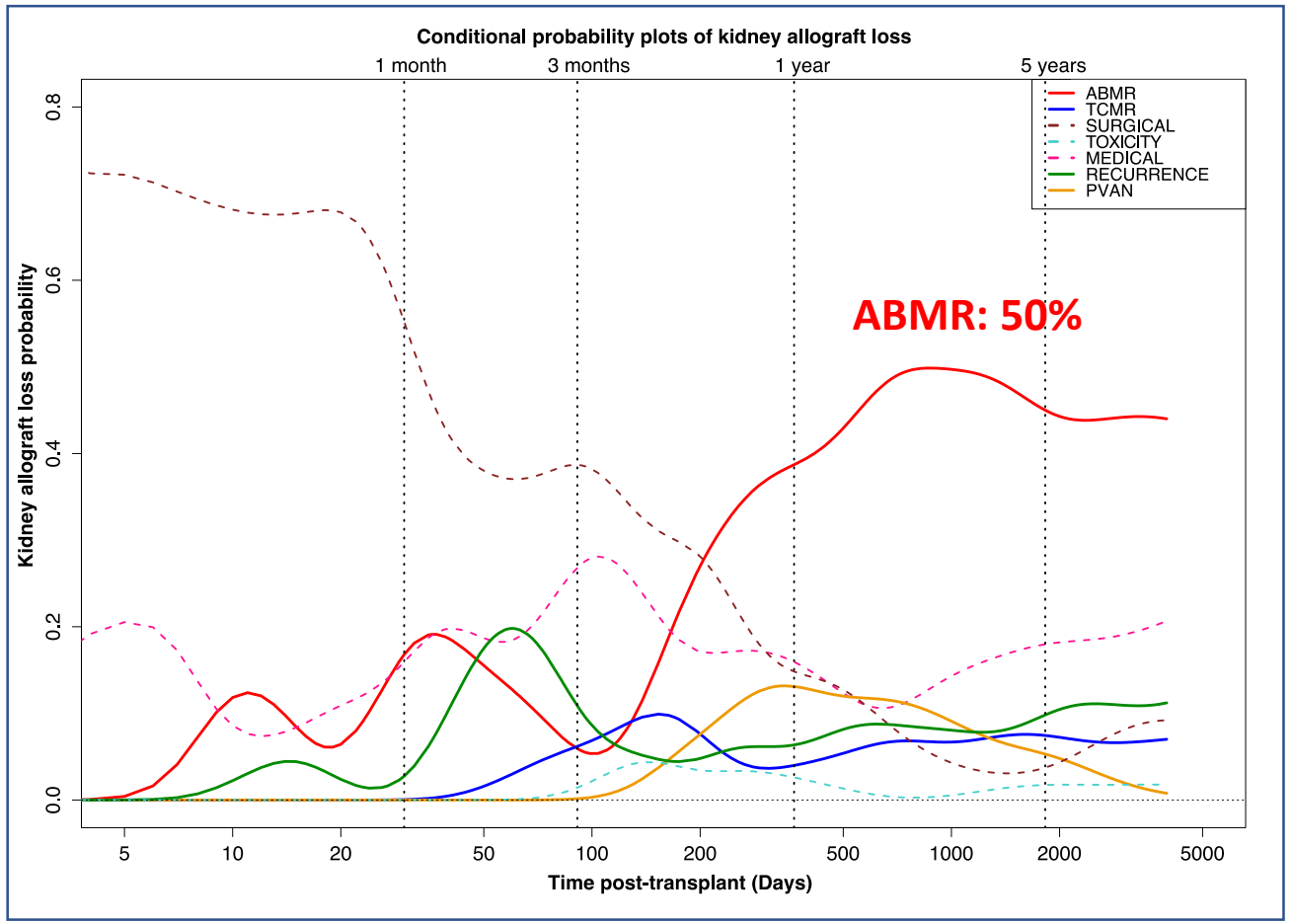
SRTR STAR File 2018

AMR: STILL THE MAIN CAUSE OF ALLOGRAFT LOSS

4 French centers: 4,921 kidney recipients and 10,293 kidney allograft biopsies

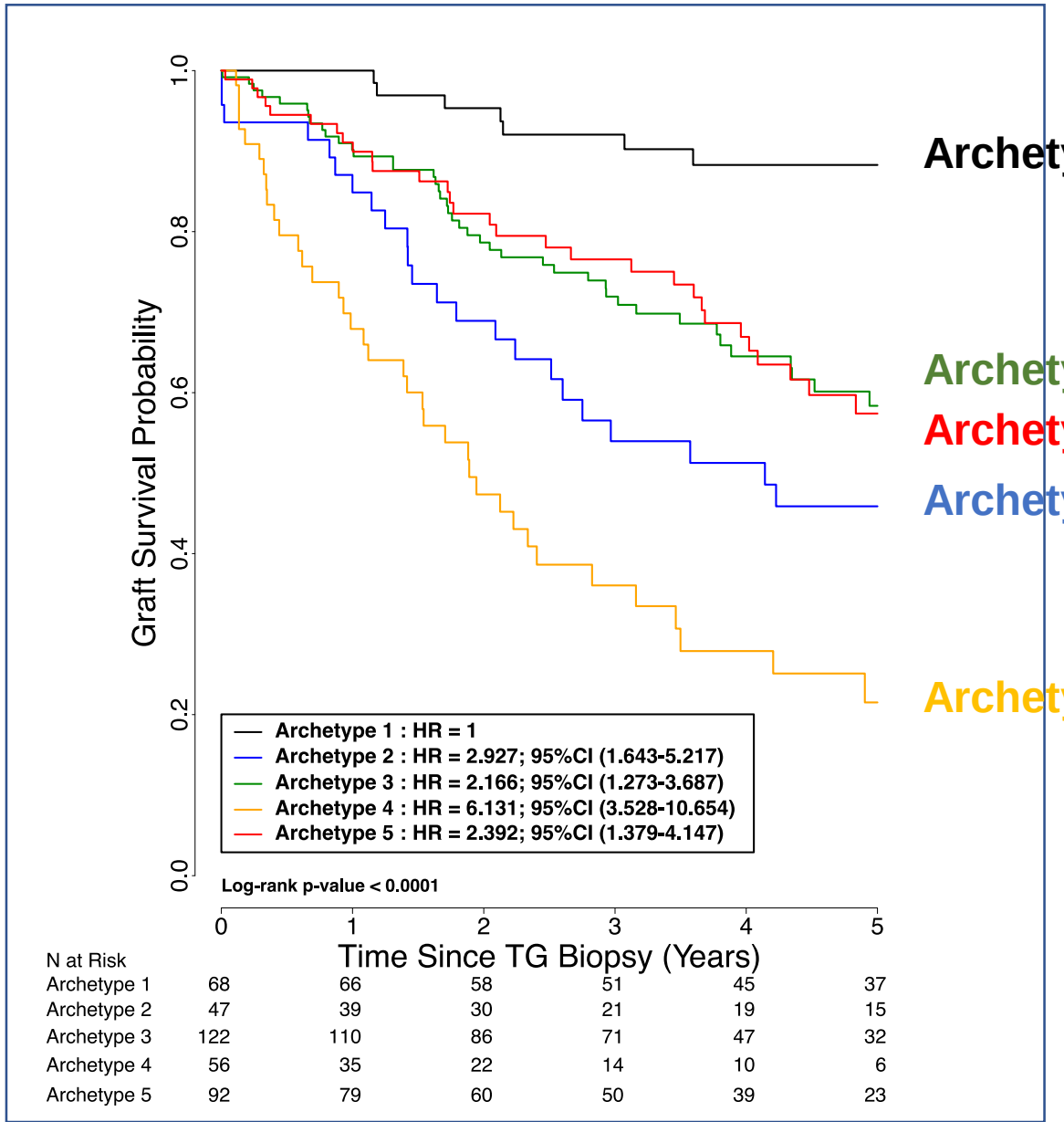
739 graft losses





Unpublished results

HETEROGENEITY OF CHRONIC AMR: ARCHETYPES IDENTIFY DISTINCT ALLOGRAFT SURVIVALS



N at Risk						
Archetype 1	68	66	58	51	45	37
Archetype 2	47	39	30	21	19	15
Archetype 3	122	110	86	71	47	32
Archetype 4	56	35	22	14	10	6
Archetype 5	92	79	60	50	39	23

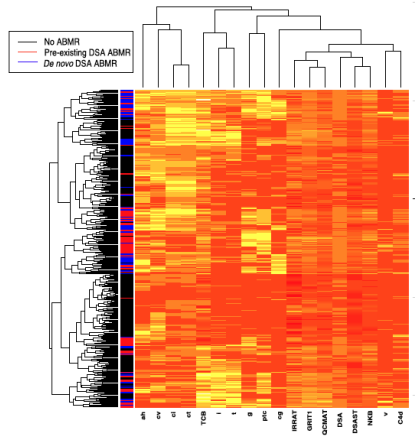
Abstract FR-OR136

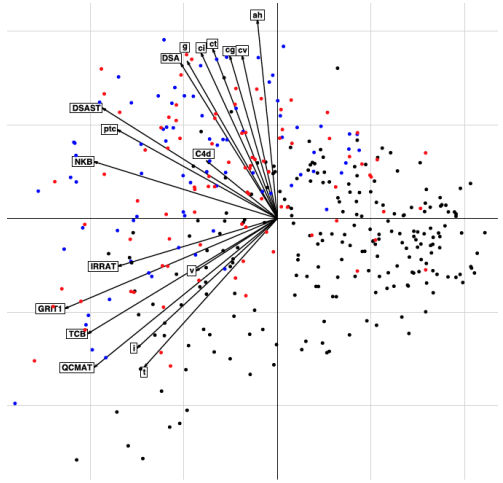
PRE-EXISTING / RECURRENT VERSUS DE NOVO DSA : HOW THESE PROCESSES COMPARE?

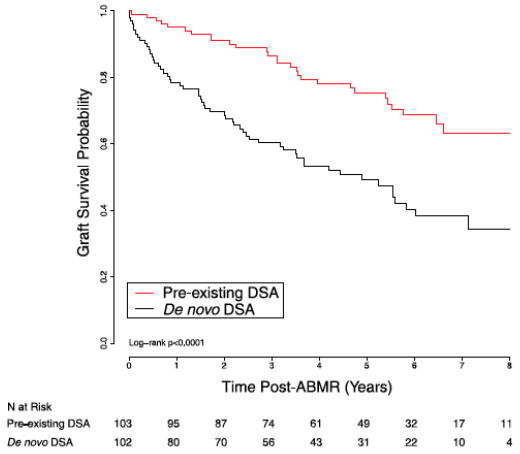
CLINICAL RESEARCH www.jasn.org

Antibody-Mediated Rejection Due to Preexisting versus *De Novo* Donor-Specific Antibodies in Kidney Allograft Recipients

Olivier Aubert,* Alexandre Loupy,*†‡ Luis Hidalgo,§‖ Jean-Paul Duong van Huyen,¶ Sarah Higgins,** Denis Viglietti,*†† Xavier Jouven,* Denis Glotz,*†† Christophe Legendre,*†‡ Carmen Lefaucheur,*†† and Philip F. Halloran‖‡‡







Pre-existing DSA AMR

***De novo* DSA AMR**

Aubert O, et al. *J Am Soc Nephrol.* 2017

Differences in pathologic features and graft outcomes in antibody-mediated rejection of renal allografts due to persistent/recurrent versus *de novo* donor-specific antibodies

Mark Haas[1], James Mirocha[2], Nancy L. Reinsmoen[3], Ashley A. Vo[4], Jua Choi[4], Joseph M. Kahwaji[4], Alice Peng[4], Rafael Villicana[4,5] and Stanley C. Jordan[4]

[1]*Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, California, USA;* [2]*Biostatistics Core, Research Institute and General Clinical Research Center, Cedars-Sinai Medical Center, Los Angeles, California, USA;* [3]*HLA and Immunogenetics Laboratory, Cedars-Sinai Medical Center, Los Angeles, California, USA;* [4]*Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, California, USA; and* [5]*Transplantation Institute, Loma Linda University Medical Center, Loma Linda, California, USA*

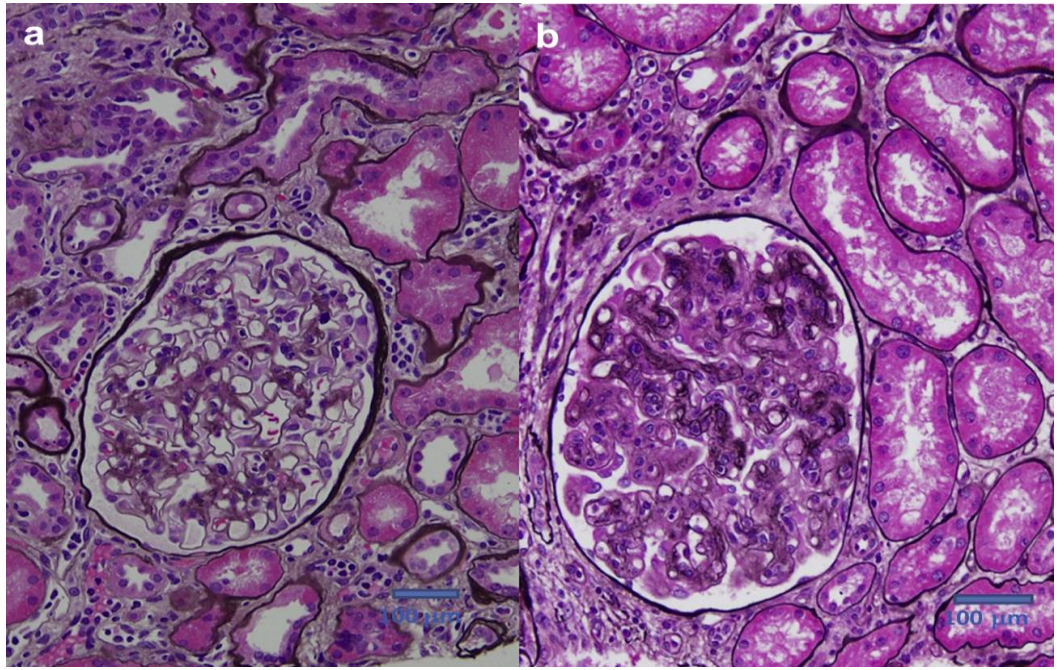
Antibody-mediated rejection (ABMR) of renal allografts occurs in two forms. Type 1 ABMR results from persistence and/or a rebound of preexisting donor-specific antibodies in sensitized patients and usually occurs early post-transplantation. Type 2 ABMR is associated with *de novo* donor-specific antibodies and usually occurs over one year post-transplantation. It is generally accepted that types 1 and 2 also differ with regard to certain pathologic features including the frequencies of C4d positivity and concurrent cell-mediated rejection. However, direct comparison of pathologic, serologic, and clinical features of types 1 and 2 ABMR is lacking. Here we compared these features in 80 cases of ABMR (37 type 1, 43 type 2) diagnosed at our center. Compared with type 1, type 2 ABMR occurred later post-transplantation, was more often associated with donor-specific antibodies against Class II HLA, and was associated with more interstitial fibrosis/tubular atrophy and more frequent cell-mediated rejection, although these did not differ with respect to C4d positivity. By univariate analysis, graft survival was lower with type 2 than type 1 ABMR with borderline significance. Still, among these 80 patients, all but one treated for ABMR following diagnosis, the only two independent predictors of graft failure were at least moderate interstitial fibrosis/tubular atrophy and failure of the donor-specific antibody relative intensity scale score, a measure of the combined strength of all donor-specific antibodies present, to decrease in response to therapy.

Kidney International (2017) **91,** 729–737; http://dx.doi.org/10.1016/j.kint.2016.10.040

KEYWORDS: antibody-mediated rejection; Banff classification; C4d; cell-mediated rejection; donor-specific antibodies; renal transplant

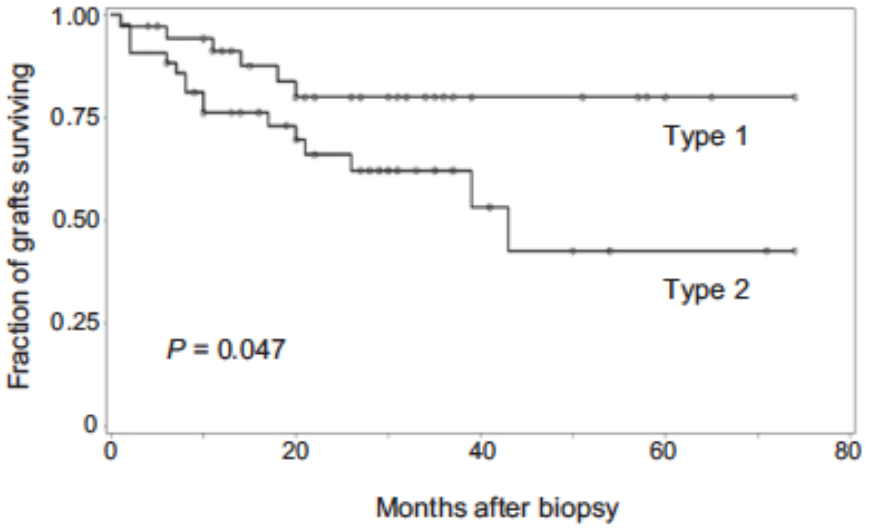
Antibody-mediated rejection (ABMR) is a major cause of renal allograft failure.[1–4] Active ABMR is manifest morphologically as microvascular inflammation (MVI), primarily glomerulitis and peritubular capillaritis.[1–3,5–12] If unrecognized or not successfully treated by measures including the removal of donor-specific antibodies (DSAs), acute ABMR leads to chronic allograft damage, including transplant glomerulopathy (TG), arterial intimal fibrosis, and interstitial fibrosis/tubular atrophy (IF/TA).[11–16] TG in particular is strongly associated with increased rates of graft loss.[17–19] Historically, ABMR has been under-recognized in renal allografts for 2 reasons. First, it may be subclinical and lead to chronic damage, including TG, before a detectable rise in serum creatinine occurs.[9,12,20,21] Second, it was not until 2009 that evidence began to appear indicating that ABMR may occur in the absence of complement deposition in the microcirculation,[12,22] and prior to the most recent (2013) Banff classification for ABMR[5] complement deposition, in the form of C4d staining within peritubular capillaries (ptc), was a requirement for diagnosis of ABMR in renal allograft biopsies.[23] Furthermore, acute/active ABMR may occur at any time after transplantation, and late-onset ABMR due to *de novo* DSA is a major determinant of late renal allograft failure.[1–4]

ABMR of renal allografts occurs in the following 2 forms: type 1, resulting from persistence and/or a rebound of pre-existing DSA in sensitized patients, and type 2, associated with *de novo* DSA. It is generally accepted that type 1 ABMR usually occurs early after transplantation, whereas type 2 ABMR most often occurs at least 1 year after transplantation

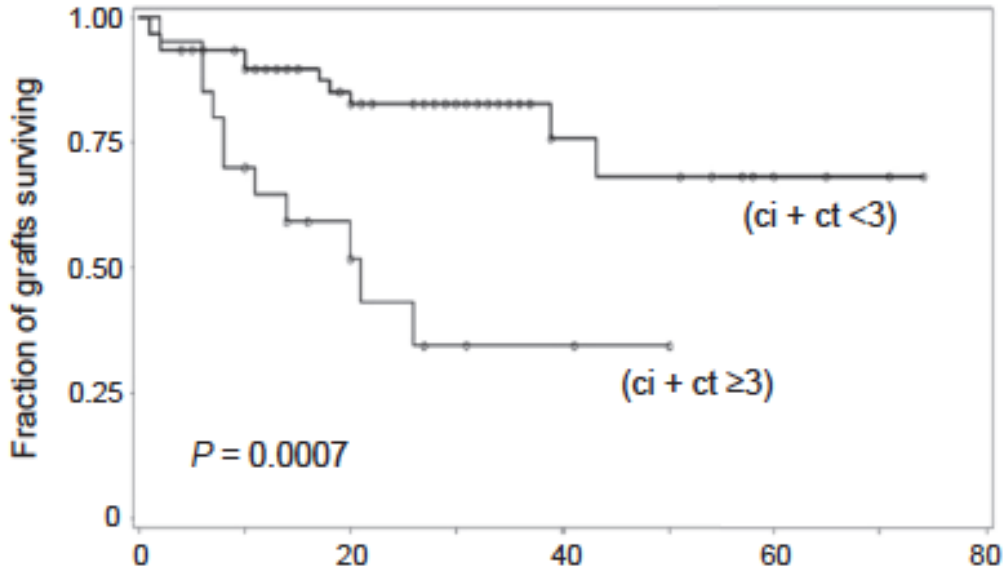


AMBR with Pre-Existing DSA (type1) AMBR with dnDSA (type2)

Graft Survival Post-ABMR by Type of DSA



Factors Associated with Graft Loss in ABMR Patients



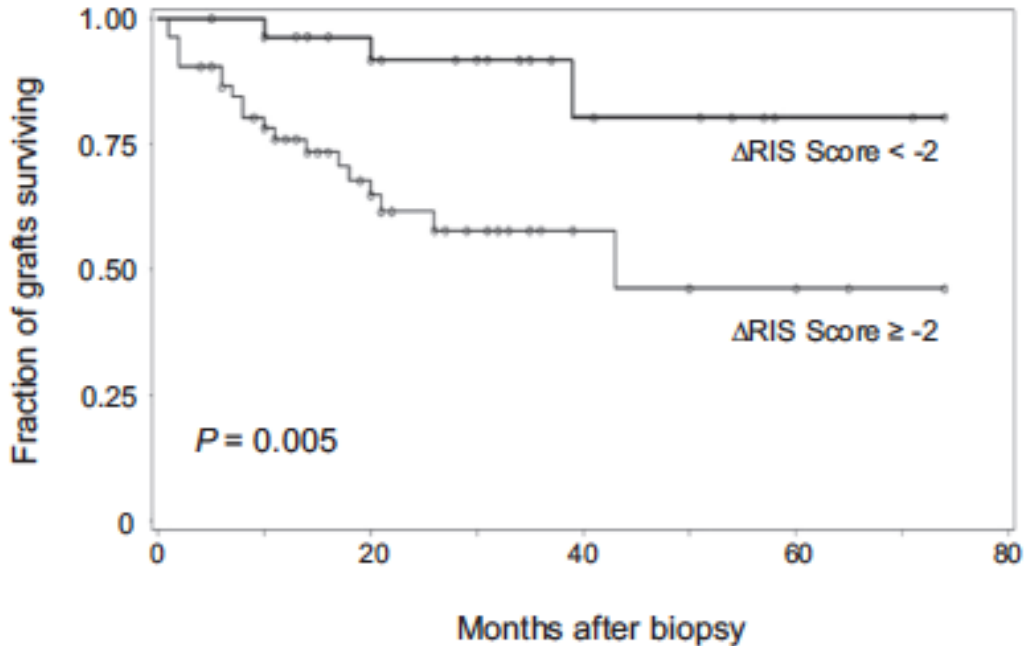


Table 3 | Predictors of death-censored graft loss

A. Univariate analysis

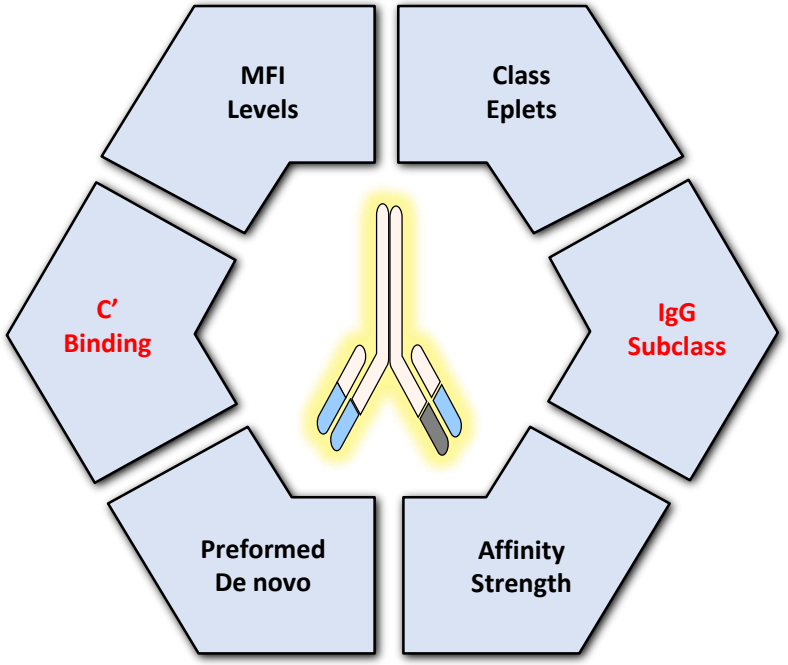
Predictor	Hazard Ratio (95% CI)	*P* Value
Age	1.01 (0.99–1.04)	0.33
Male gender	0.74 (0.32–1.70)	0.33
Live donor	1.97 (0.85–4.56)	0.12
Biopsy indication: progressive dysfunction	1.48 (0.64–3.46)	0.36
Interval transplant to biopsy ≥ 84 months	2.56 (1.05–6.22)	0.038
Type 2 versus type 1 ABMR	2.51 (0.98–6.43)	0.054
C4d score 2–3 versus 0–1	1.16 (0.43–3.15)	0.77
cg score ≥ 1	2.31 (0.98–5.42)	0.054
Chronic, active versus acute/active ABMR	1.97 (0.84–4.63)	0.12
(ci + ct) ≥ 3	3.88 (1.67–9.05)	0.002
CMR, Banff grade 1a or higher	2.48 (1.07–5.75)	0.037
TMA	2.58 (0.75–8.84)	0.13
Presence of anti-HLA DQ DSA	1.53 (0.62–3.76)	0.36
Decrease in RIS score > 2	0.21 (0.06–0.70)	0.012

B. Multivariable analysis

Predictor	Hazard ratio	95% CI	*P* value
(ci + ct) ≥ 3	2.98	1.26–7.06	0.013
Decrease in RIS score > 2	0.23	0.07–0.79	0.020
CMR, Banff grade 1a or higher	2.19	0.93–5.15	0.074

HETEROGENEITY, THE ANTIBODY PROBLEM

Characterization of anti-HLA DSA to better assess:

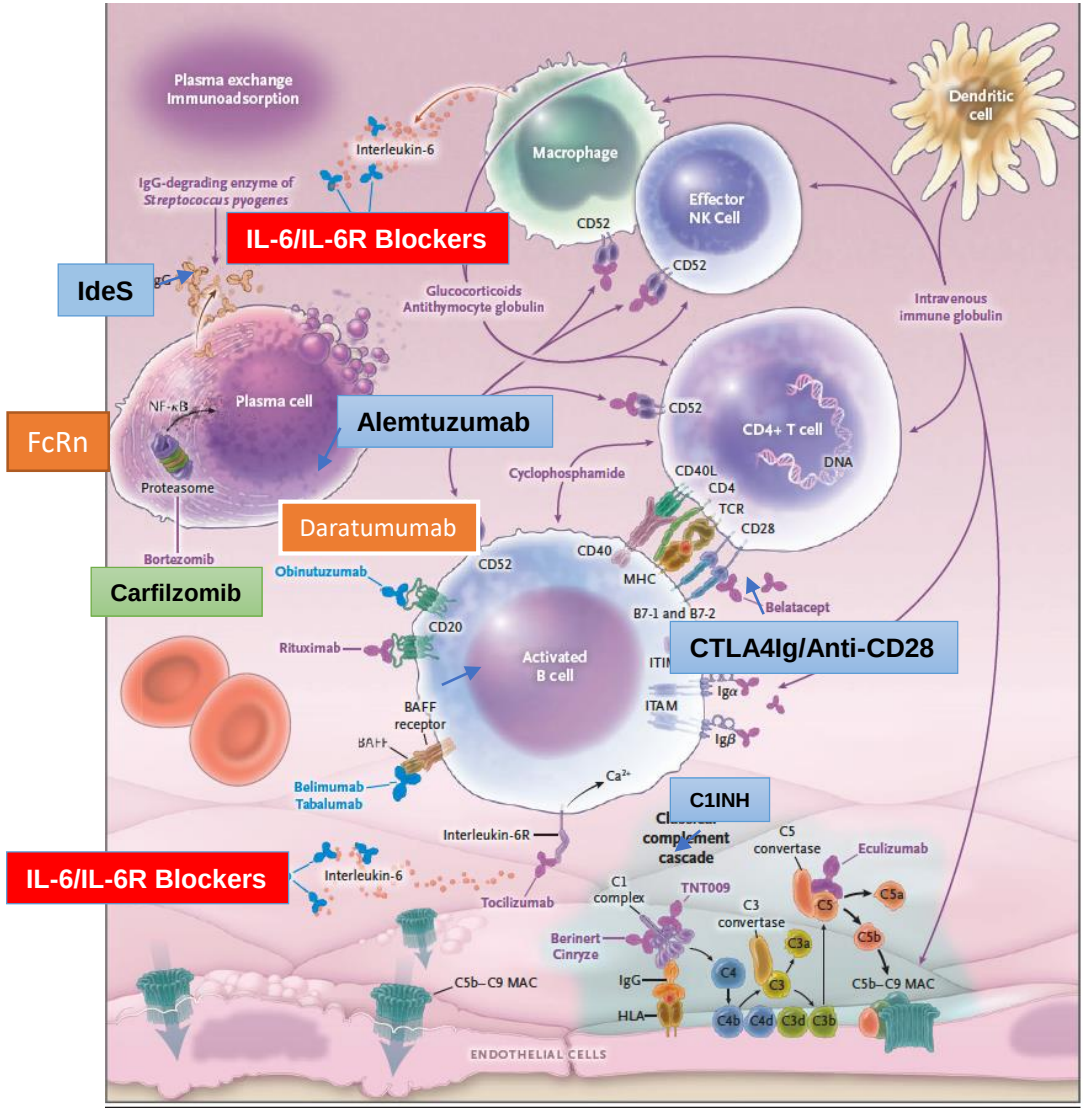


- **Rejection phenotypes**
- **Operating biological processes**
- **Allograft loss profiles**
- **Response to therapy**

Tambur AR, et al. *Am J Transplant.* 2015;15(9):2421-2430; Duquesnoy RJ. *Transplantation.* 2017;101(8):1755-1765; Sutherland SM, et al. *Pediatr Transplant.* 2012:16(1):12-17; Sicard A, et al. *J Am Soc Nephrol.* 2014;ASN-2013101144; Smith JD, et al. *J Heart Lung Transplant.* 2014;33(10):1074-1082; Loupy A, et al. *N Engl J Med.* 2013;369(13):1215-1226; Viglietti D, et al. *J Am Soc Nephr.* 2016;ASN-2016030368.

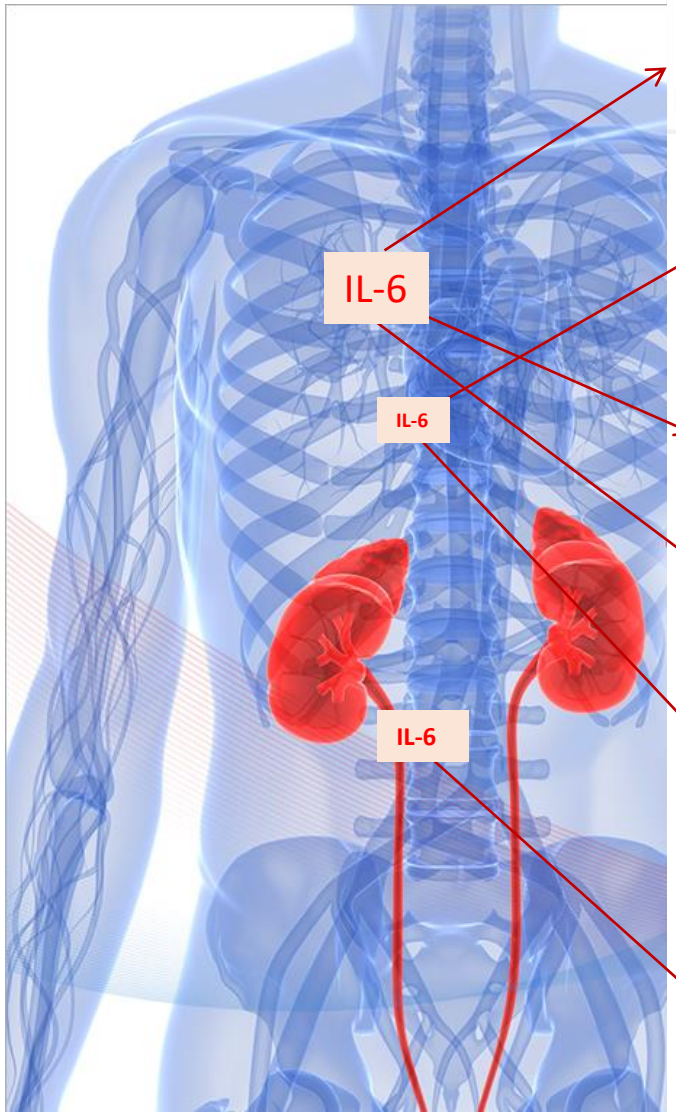
ABMR TREATMENT OPTIONS: WHAT'S NEW?

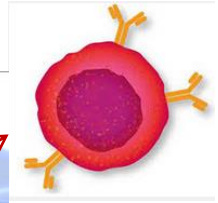
B-cell Therapeutics



Loupy et al. NEJM 2018.

IL-6: A Pleiotropic Cytokine Impacting Multiple Organ Systems



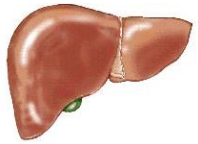


Immune cells

1. Stimulate B- cells differentiation to antibody producing plasma cells
2. Plasma cell & myeloma cell growth factor
3. Maintains Th1 & Th2 cell activities
4. Increases Th17 and Tfh cells
5. Decreases Tregs

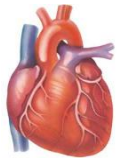
Skin

1. Increase Keratinocytes
2. Increase dermal fibroblast collagen
3. May be primary factor in scleroderma



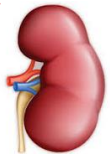
Liver

1. Increase CRP
2. Increase Serum Amyloid A
3. Increase Fibrinogen
4. Increase Hepcidin (anemia of chronic disease)
5. Decreases albumin& transferrin



Cardiac

1. Increase VEGF to increase angiogenesis
2. Increase risk for coronary artery disease
3. Increases mortality from cardiovascular disease in ESRD patients



Kidney

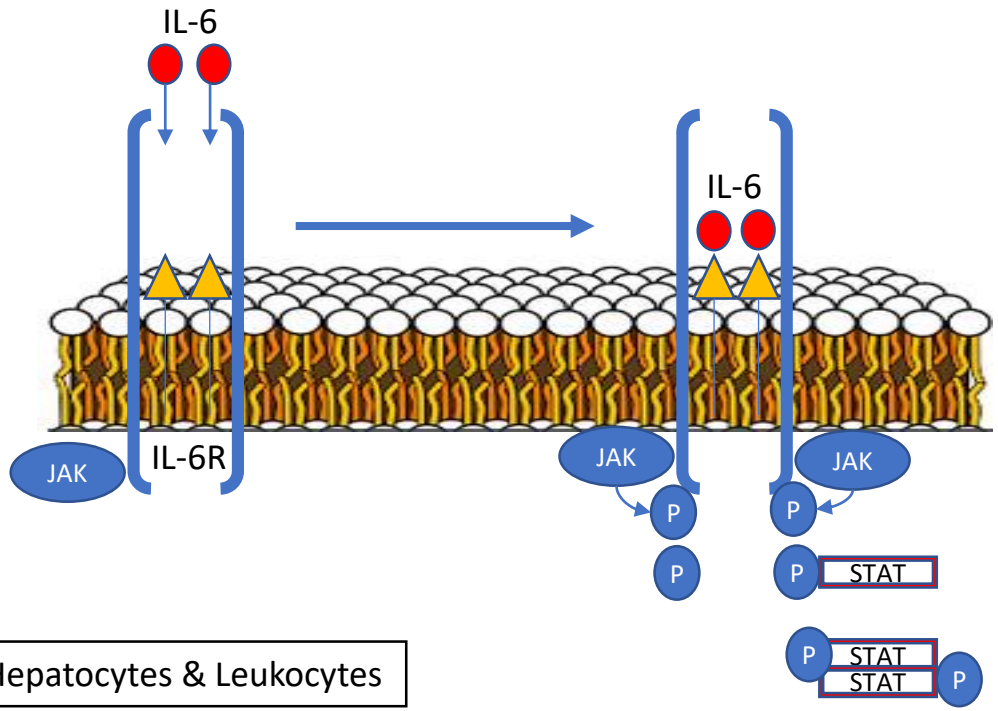
1. Increase mesangial cell proliferation
2. Contributes to glomerular crescent formation
3. Contributes to chronic antibody rejection of kidney allografts

Bone Marrow

Synovial Fibroblast

1. Increase osteoclast differentiation
2. Increase angiogenesis
3. Increase platelet
4. Responsible for joint damage in RA

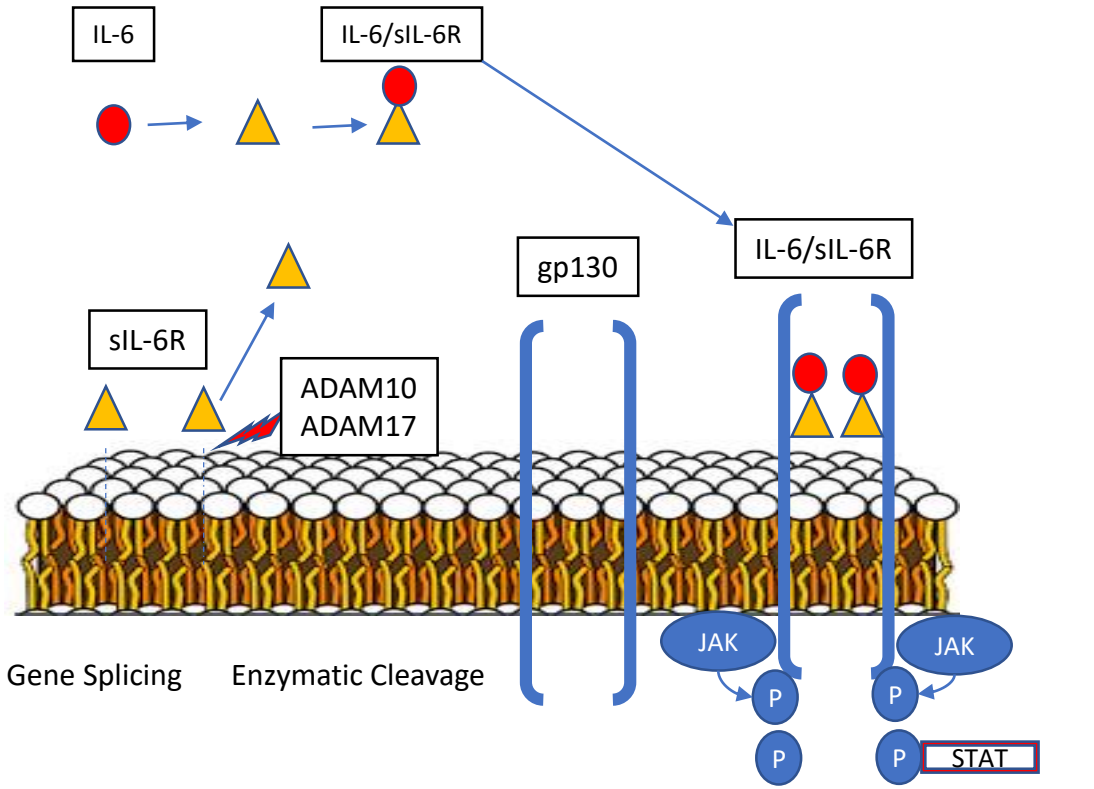
Classic Membrane Bound IL-6R Signaling



Hepatocytes & Leukocytes

Gene Transcription

IL-6/sIL-6R Trans-signaling



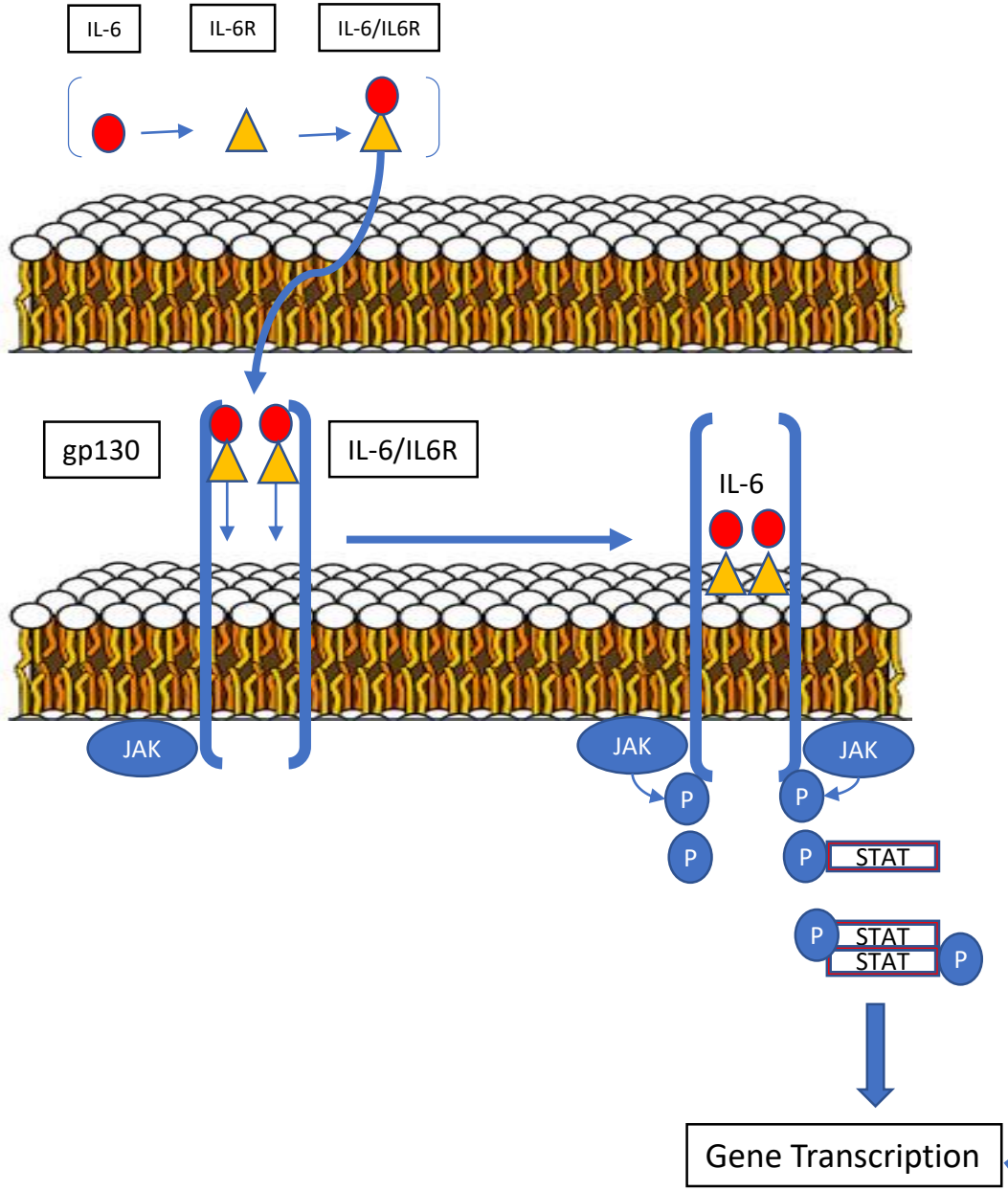
All gp130+ Cells

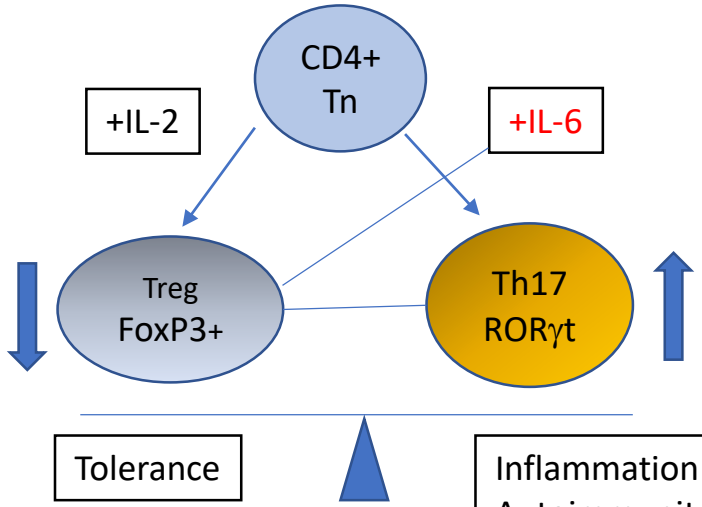
Gene Transcription

IL-6/IL-6R Trans-Presentation

Dendritic Cell (APC)

Naïve CD4+ T-cell

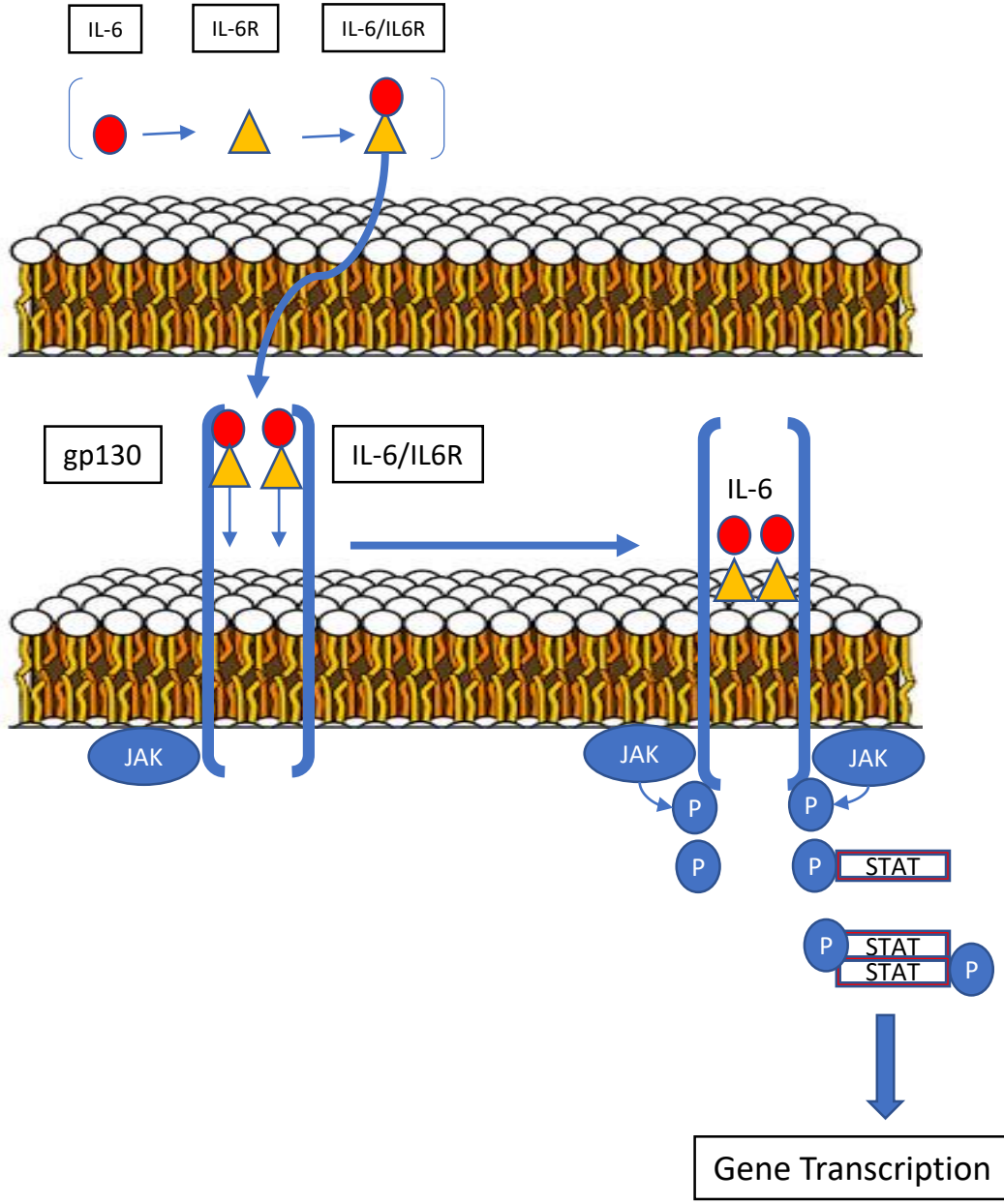


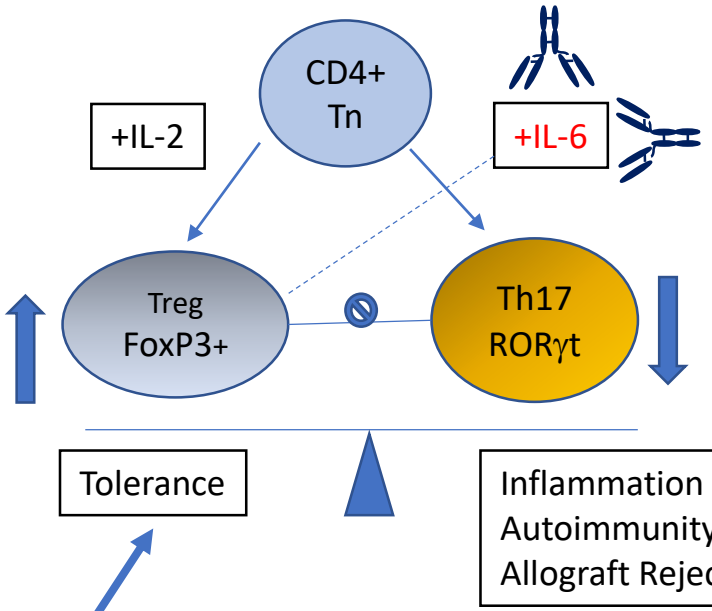


IL-6/IL-6R Trans-Presentation

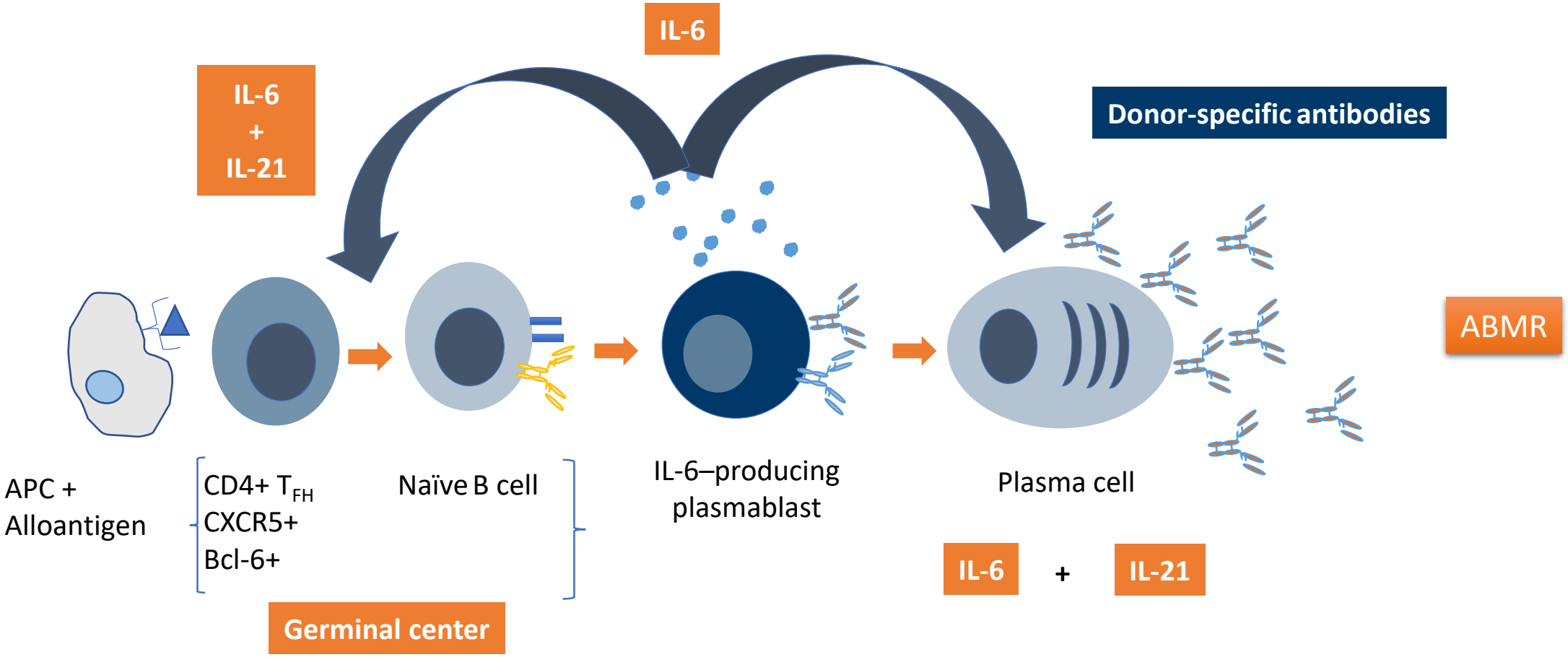
Dendritic Cell (APC)

Naïve CD4+ T-cell





IL-6 DRIVES B-CELL ACTIVATION AND DIFFERENTIATION TO ANTIBODY-PRODUCING PLASMA CELLS



Jordan SC et al. *Transplantation.* 2017

What is the current evidence of the potential of anti-IL-6 Ab for the prevention and the treatment of AMR?

Review

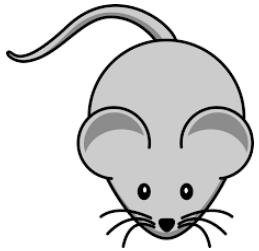
Interleukin-6, A Cytokine Critical to Mediation of Inflammation, Autoimmunity and Allograft Rejection: Therapeutic Implications of IL-6 Receptor Blockade

Stanley C. Jordan, MD,[1] Jua Choi, PharmD,[1] Irene Kim, MD,[1] Gordon Wu, PhD,[1] Mieko Toyoda, PhD,[1] Bonga Shin, PhD,[1] and Ashley Vo, PharmD[1]

Abstract: The success of kidney transplants is limited by the lack of robust improvements in long-term survival. It is now recognized that alloimmune responses are responsible for the majority of allograft failures. Development of novel therapies to decrease allosensitization is critical. The lack of new drug development in kidney transplantation necessitated repurposing drugs initially developed in oncology and autoimmunity. Among these is tocilizumab (anti–IL-6 receptor [IL-6R]) which holds promise for modulating multiple immune pathways responsible for allograft injury and loss. Interleukin-6 is a cytokine critical to proinflammatory and immune regulatory cascades. Emerging data have identified important roles for IL-6 in innate immune responses and adaptive immunity. Excessive IL-6 production is associated with activation of T-helper 17 cell and inhibition of regulatory T cell with attendant inflammation. Plasmablast production of IL-6 is critical for initiation of T follicular helper cells and production of high-affinity IgG. Tocilizumab is the first-in-class drug developed to treat diseases mediated by IL-6. Data are emerging from animal and human studies indicating a critical role for IL-6 in mediation of cell-mediated rejection, antibody-mediated rejection, and chronic allograft vasculopathy. This suggests that anti–IL-6/IL-6R blockade could be effective in modifying T- and B-cell responses to allografts. Initial data from our group suggest anti–IL-6R therapy is of value in desensitization and prevention and treatment of antibody-mediated rejection. In addition, human trials have shown benefits in treatment of graft versus host disease in matched or mismatched stem cell transplants. Here, we explore the biology of IL-6/IL-6R interactions and the evidence for an important role of IL-6 in mediating allograft rejection.

(*Transplantation* 2017;101: 32–44)

BASIC AND EXPERIMENTAL RESEARCH



Anti–Interleukin 6 Receptor Antibodies Attenuate Antibody Recall Responses in a Mouse Model of Allosensitization

Irene Kim,[1] Gordon Wu,[1,2] Ning-ning Chai,[1] Andrew S. Klein,[1] and Stanley Jordan[1]

Background. Interleukin (IL)-6 is a regulatory cytokine for T helper type 17 (Th17) and Treg cells and a potent stimulus for B/plasma cells. The current study evaluated the effect of IL-6 receptor (IL-6R) blockade with an anti–IL-6R monoclonal (mMR16-1) in alloantibody recall responses.

Methods. A mouse model of human leukocyte antigen (HLA).A2 sensitization was used for studies to evaluate the efficacy of anti–IL-6R on alloantibody recall responses and to examine the impact of IL-6R blockade on Th17, Treg, follicular T helper (Tfh) and plasma cells using multiparameter flow cytometry, flow antibody binding, and enzyme-linked immunospot (ELISpot) assay.

Results. Re-exposure of C57BL/6 mice to HLA.A2$^+$ skin allografts resulted in a surge of donor-specific (anti–HLA.A2) immunoglobulin (Ig)G antibodies. Anti–IL-6R treatment significantly decreased but did not eliminate alloantibody responses (IgG mean fluorescence intensity, 486 ± 153 vs. control 792 ± 193, $P = 0.0076$). Flow cytometry analysis showed that anti–IL-6R treatment resulted in reduction of IL-21$^+$CD4$^+$ (Th17) cells ($P = 0.006$ vs. control) and CXCR5$^+$CD4$^+$ Tfh cells ($P = 0.04$), but increased foxp3$^+$CD4$^+$ (Treg) cells in the CD4$^+$ population ($P = 0.04$ vs. control). The IgG ELISpot experiments showed a significant reduction of IgG spots in the bone marrow and the spleen cells from the anti–IL-6R–treated mice. In vitro treatment of mouse hybridoma (PA2.1) cultures with anti–IL-6R decreased IgG spot formation but had limited effect on cell proliferation.

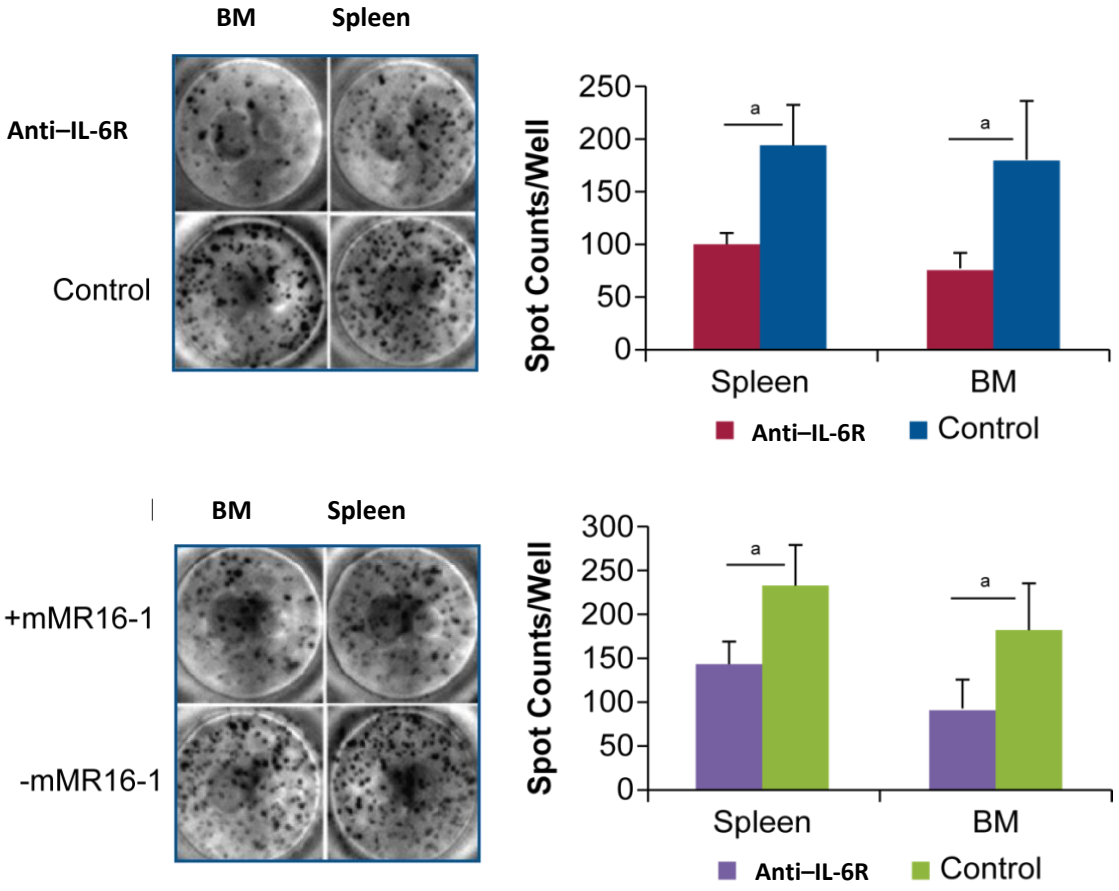
Conclusion. The data indicate that anti–IL-6R therapy attenuates alloantibody recall responses by modulating a number of immune regulatory and effector cells, including Th17, Tfh, Treg, and importantly, the long-lived plasma cells in the bone marrow.

Keywords: Alloantibody recall response, Anti–IL-6R, B cell, ELISpot, Flow cytometry, Mice, Plasma cell, Tfh, Th17, Treg.

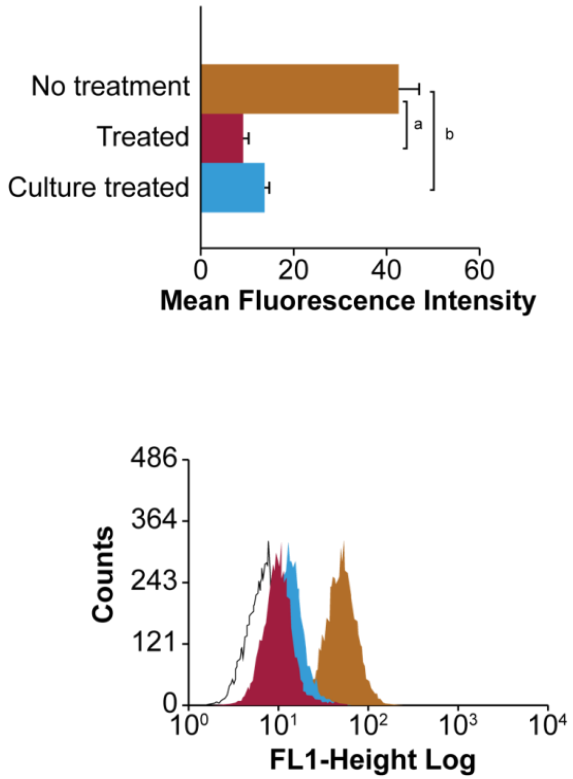
(*Transplantation* 2014;98: 1262–1270)

ANTI–IL-6R INHIBITS PLASMA CELL IgG PRODUCTION AND ANTI–HLA-A2 ANTIBODY

Anti–IL-6R Significantly Suppressed IgG+ Plasma Cells in the Bone Marrow and Spleens Demonstrated in an ELISpot Assay



Detection of Anti–HLA-A2 IgG Antibodies in Conditioned Media of BM Cell Cultures by Flow-Antibody Binding Assay

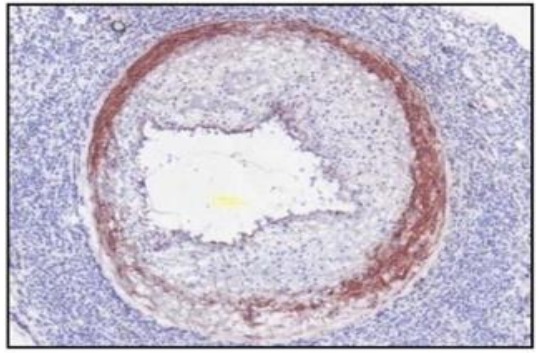


[a] $P < .01$. [b] $P < .05$.

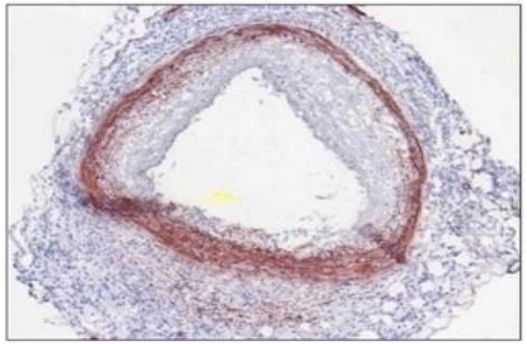
Kim I et al. *Transplantation*. 2014

ANTI-IL-6 INHIBITS CORONARY ARTERY VASCULOPATHY IN A HUMANIZED MOUSE MODEL

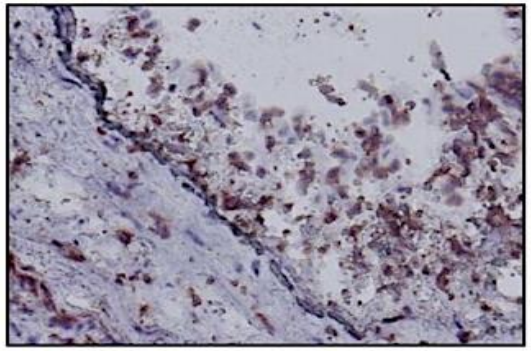
No Treatment



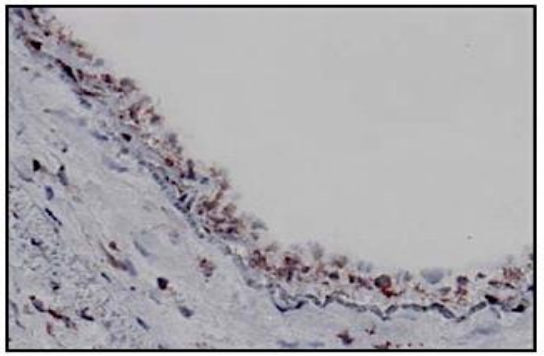
Rx anti-IL-6



Endothelial Cell IL-6 Production



Rx anti-IL-6



Pober et al J. Immunology 2012

A Phase I/II Trial of the Interleukin-6 Receptor Specific Humanized Monoclonal (Tocilizumab) + Intravenous Immunoglobulin in Difficult to Desensitize Patients

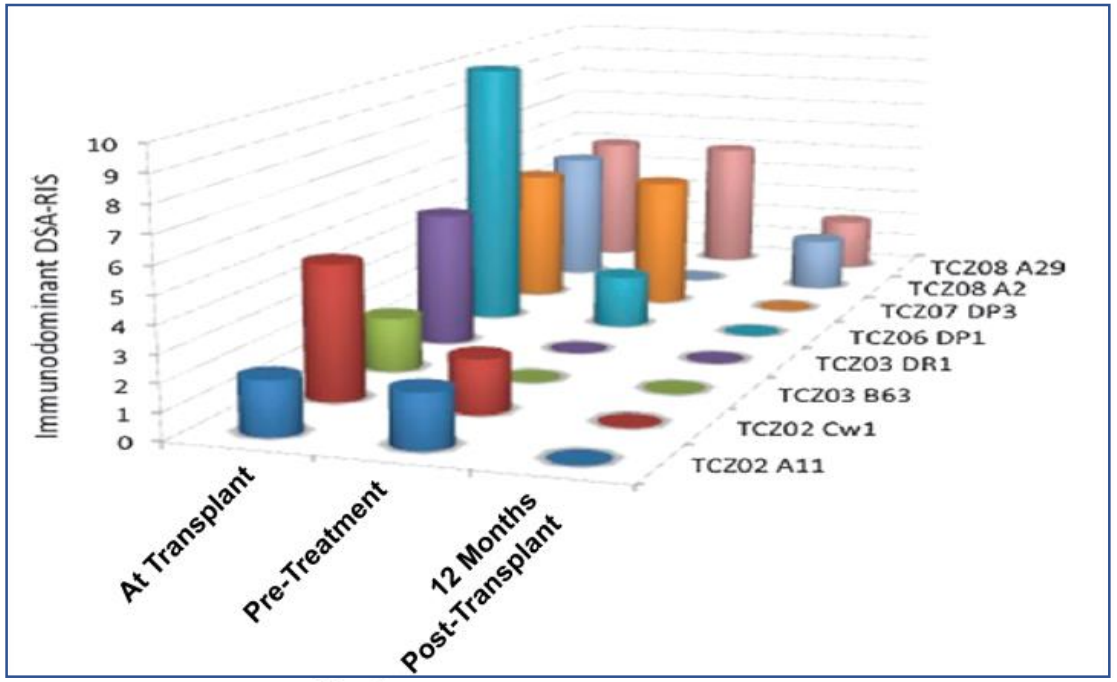
Ashley A. Vo, PharmD,[1] Jua Choi, PharmD,[1] Irene Kim, MD,[1] Sabrina Louie, MPH,[1] Kristen Cisneros, RN,[1] Joseph Kahwaji, MD,[1] Mieko Toyoda, PhD,[2] Shili Ge, PhD,[2] Mark Haas, MD,[3] Dechu Puliyanda, MD,[1] Nancy Reinsmoen, PhD,[4] Alice Peng, MD,[1] Rafael Villicana, MD,[1] and Stanley C. Jordan, MD[1]

Background. Current desensitization (DES) methods are not always effective. Thus, novel, more effective approaches are desirable. Interleukin (IL)-6 is an attractive target as it promotes B-cell differentiation to plasma cells, is important for immunoglobulin production, and induces Th_{17} cells. Here, we undertook a phase I/II pilot study of DES using a novel drug (anti–IL-6 receptor (IL-6R),Tocilizumab [TCZ]) + intravenous Ig (IVIg) to assess safety and limited efficacy. **Methods.** From July 2012 to November 2013, 10 patients unresponsive to DES with IVIg + Rituximab were treated with IVIg + TCZ. Patients received IVIg on days 0 and 30 at 2 g/kg and TCZ 8 mg/kg on day 15 then monthly for 6 months. If transplanted, patients received IVIg once and TCZ monthly for 6 months. **Results.** No differences in baseline characteristics were seen in patients not transplanted versus transplanted. Two patients in each group developed serious adverse events: not transplanted- pulmonary congestion with epilepticus (likely not related) versus transplanted infective colitis with colonic perforation and Bell Palsy (both possibly related). Five of 10 patients were transplanted. Mean time to transplant from first DES was 25 ± 10.5 months but after TCZ was 8.1 ± 5.4 months. Six-month protocol biopsies showed no antibody-mediated rejection. Donor-specific antibody strength and number were reduced by TCZ treatment. Renal function at 12 months was 60 ± 25 mL/min. **Conclusions.** Tocilizumab and IVIg appear to be safe. From this pilot trial, we are cautiously optimistic that targeting the IL-6/IL-6R pathway could offer a novel alternative for difficult to desensitize patients. Larger controlled studies are essential to prove efficacy.

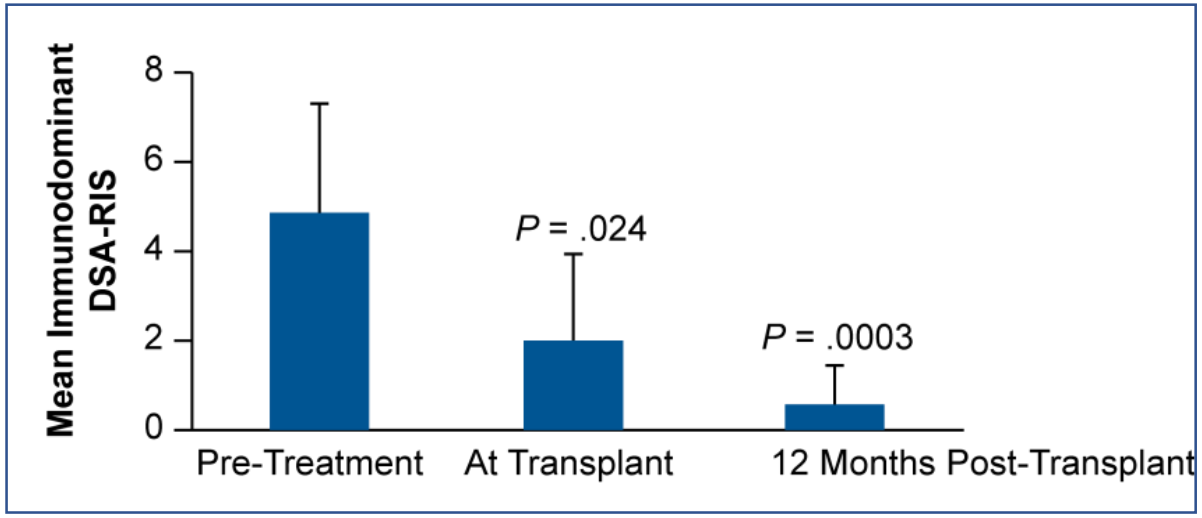
(*Transplantation* 2015;99: 2356–2363)

ANTI-IL-6R: DECREASE IN DSA LEVEL AFTER TRANSPLANTATION

Course of Immunodominant DSAs



Mean Immunodominant DSA levels for TCZ-Treated and Transplanted Patients



Vo AA et al. *Transplantation*. 2015

ANTI-IL-6R IN THE TREATMENT OF CHRONIC AMR

American Journal of Transplantation 2017; XX: 1–9
Wiley Periodicals Inc.

doi: 10.1111/ajt.14228

Assessment of Tocilizumab (Anti–Interleukin-6 Receptor Monoclonal) as a Potential Treatment for Chronic Antibody-Mediated Rejection and Transplant Glomerulopathy in HLA-Sensitized Renal Allograft Recipients

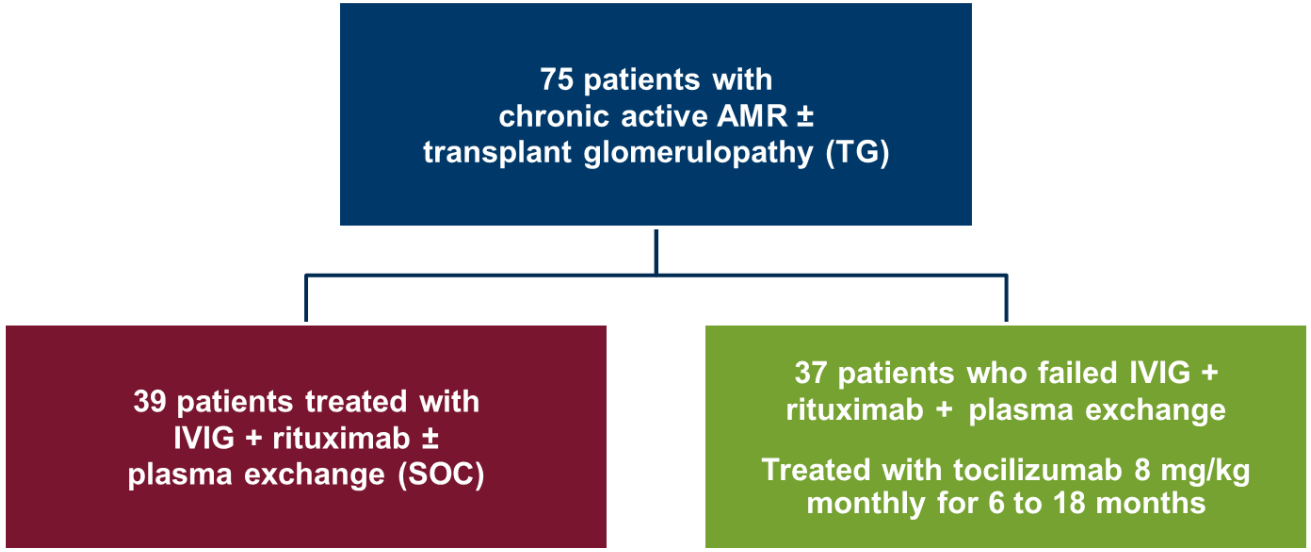
J. Choi[1,*], O. Aubert[2], A. Vo[1], A. Loupy[2], M. Haas[3], D. Puliyanda[1], I. Kim[1], S. Louie[1], A. Kang[1], A. Peng[1], J. Kahwaji[1], N. Reinsmoen[3], M. Toyoda[4] and S. C. Jordan[1]

[1]Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA
[2]Paris Translational Research Center for Organ Transplantation, INSERM U970, Biostatistics Department, Paris, France
[3]Department of Pathology, Cedars-Sinai Medical Center, Los Angeles, CA
[4]HLA Laboratory, Cedars-Sinai Medical Center, Los Angeles, CA
*Corresponding author: Jua Choi, jua.choi@cshs.org

Abbreviations: AE, adverse event; AMR, antibody-mediated rejection; cAMR, chronic active antibody-mediated rejection; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; FDA, US Food and Drug Administration; iDSA, immunodominant donor-specific antibody; IF/TA, interstitial fibrosis/tabular atrophy; IL-6R, interleukin-6 receptor; IL, interleukin; IQR, interquartile range; NSTEMI, non–ST-segment elevation myocardial infarction; PLEX, plasma exchange; SAE, severe adverse event; Tfh, T follicular helper cell; TG, transplant glomerulopathy; Th17, T helper 17 cell; Treg, T regulatory cell

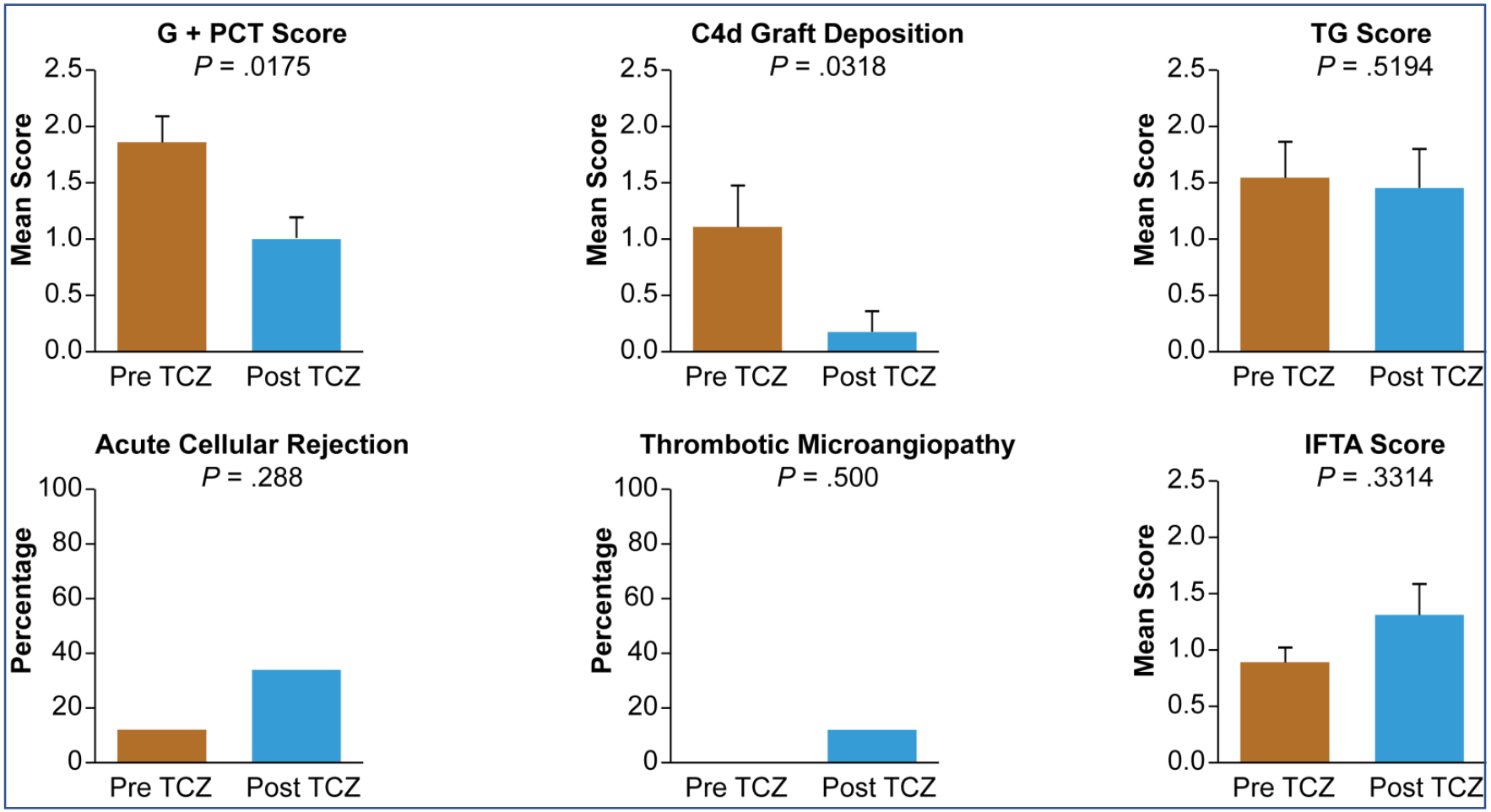
Received 14 September 2016, revised 01 February 2017 and accepted for publication 08 February 2017

Tocilizumab Treatment of cAMR and TG: Treatment Protocol



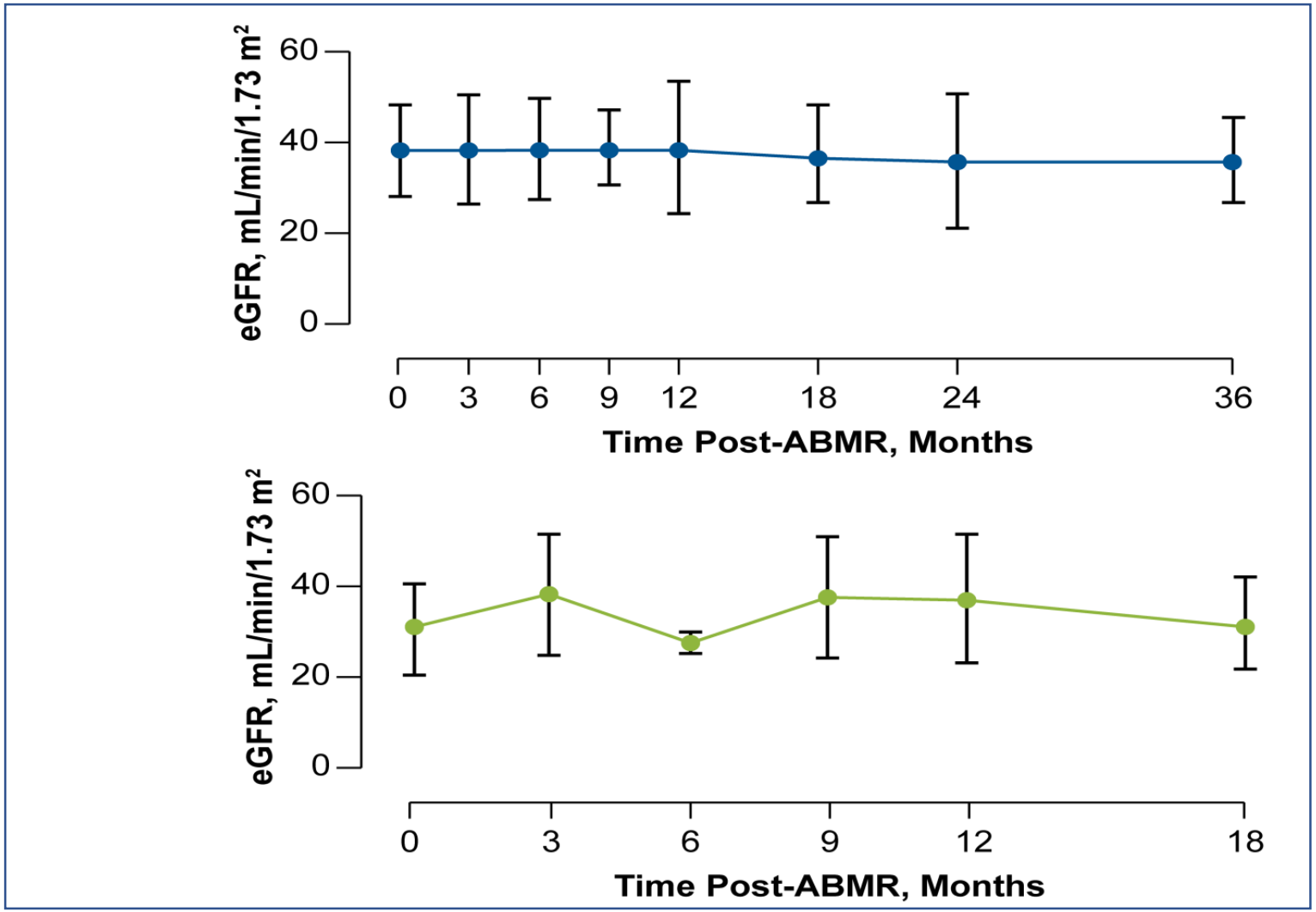
Choi J, Aubert O, et al. *Am J Transplant*. 2017

ALLOGRAFT PHENOTYPE IN PATIENTS TREATED WITH TOCILIZUMAB FOR CHRONIC AMR



Choi J, Aubert O, et al. *Am J Transplant*. 2017

eGFR IN ADULT AND PEDIATRIC PATIENTS TREATED WITH TOCILIZUMAB FOR CHRONIC AMR

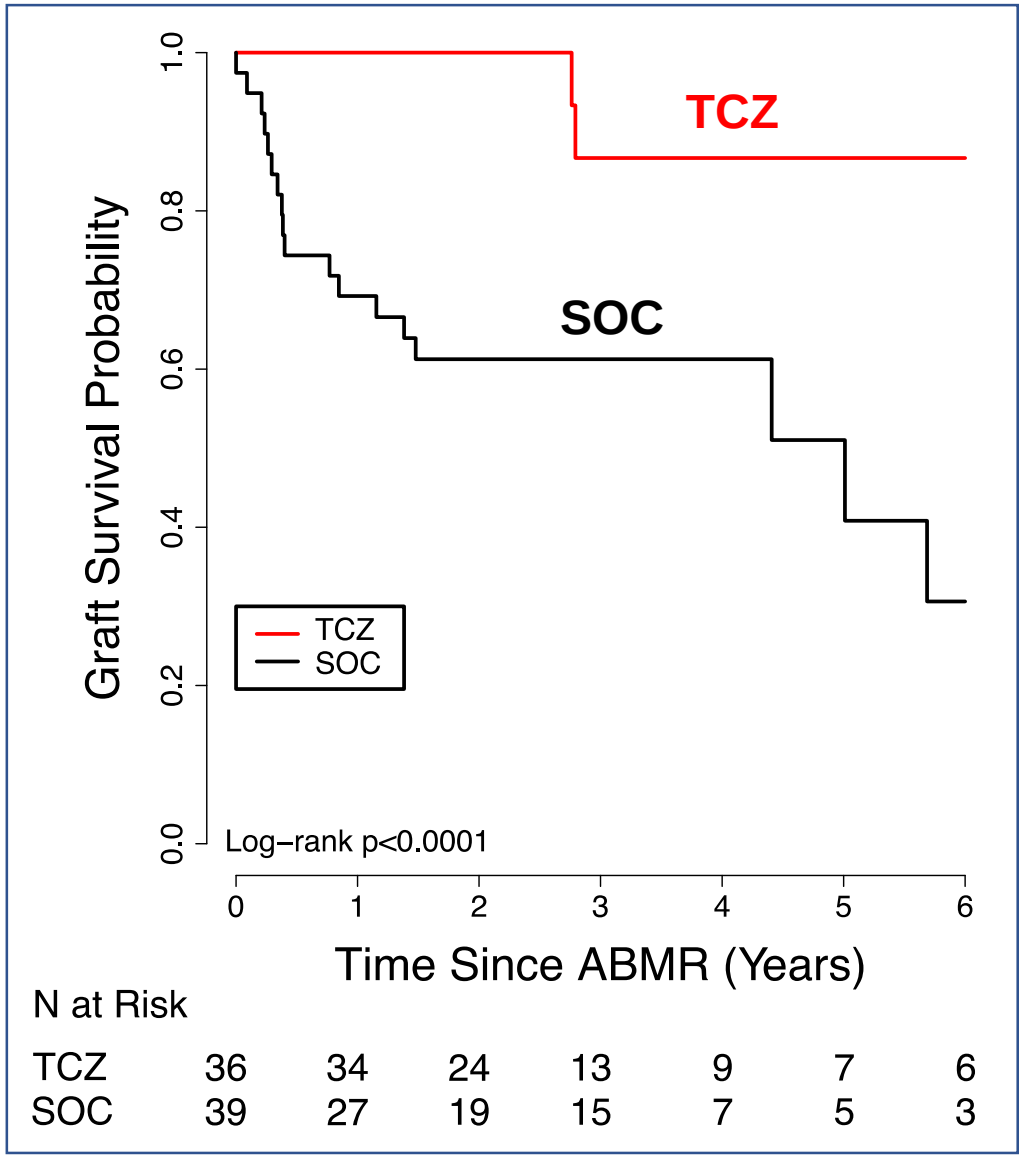


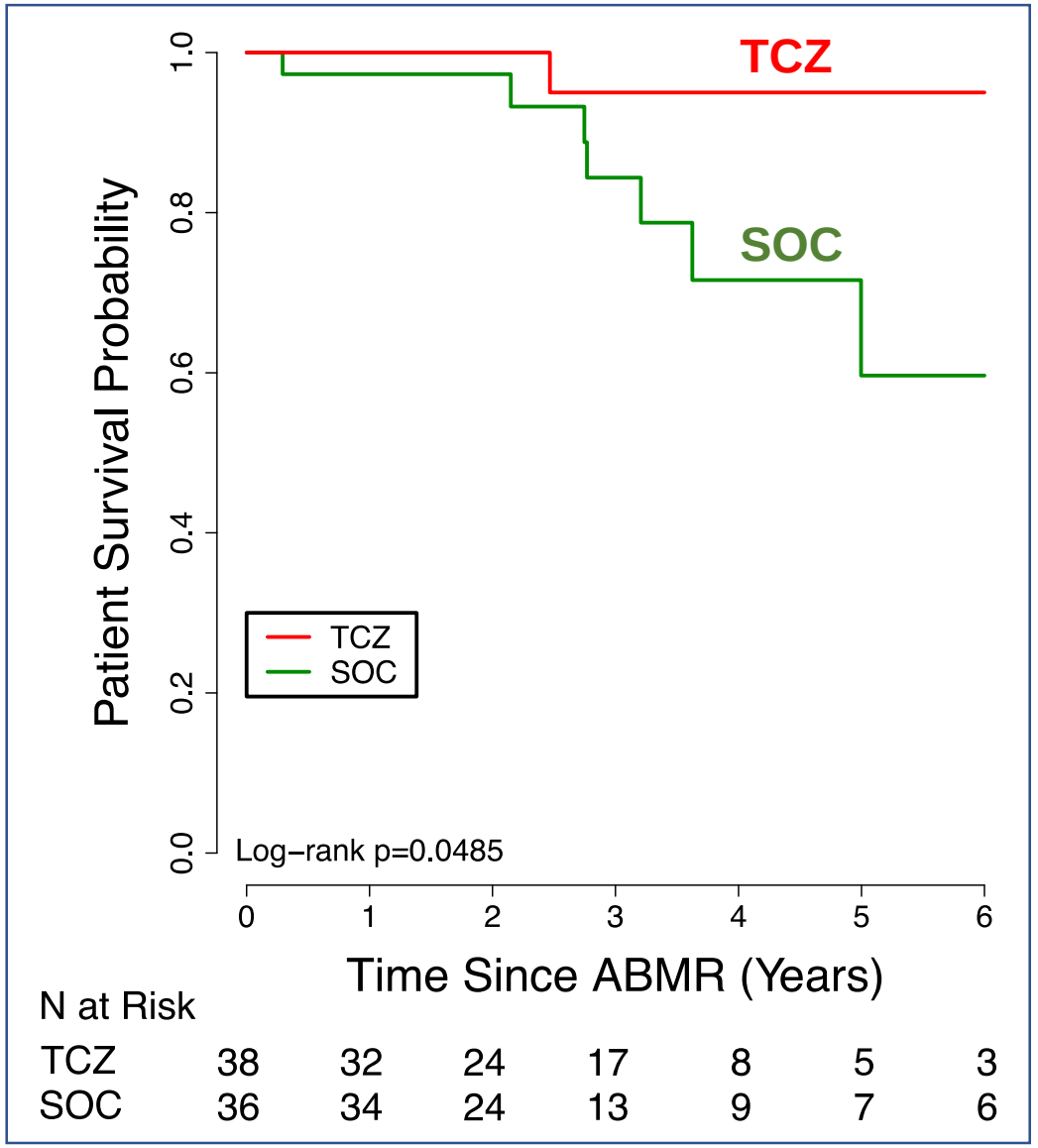
ADULT PATIENTS

PEDIATRIC PATIENTS

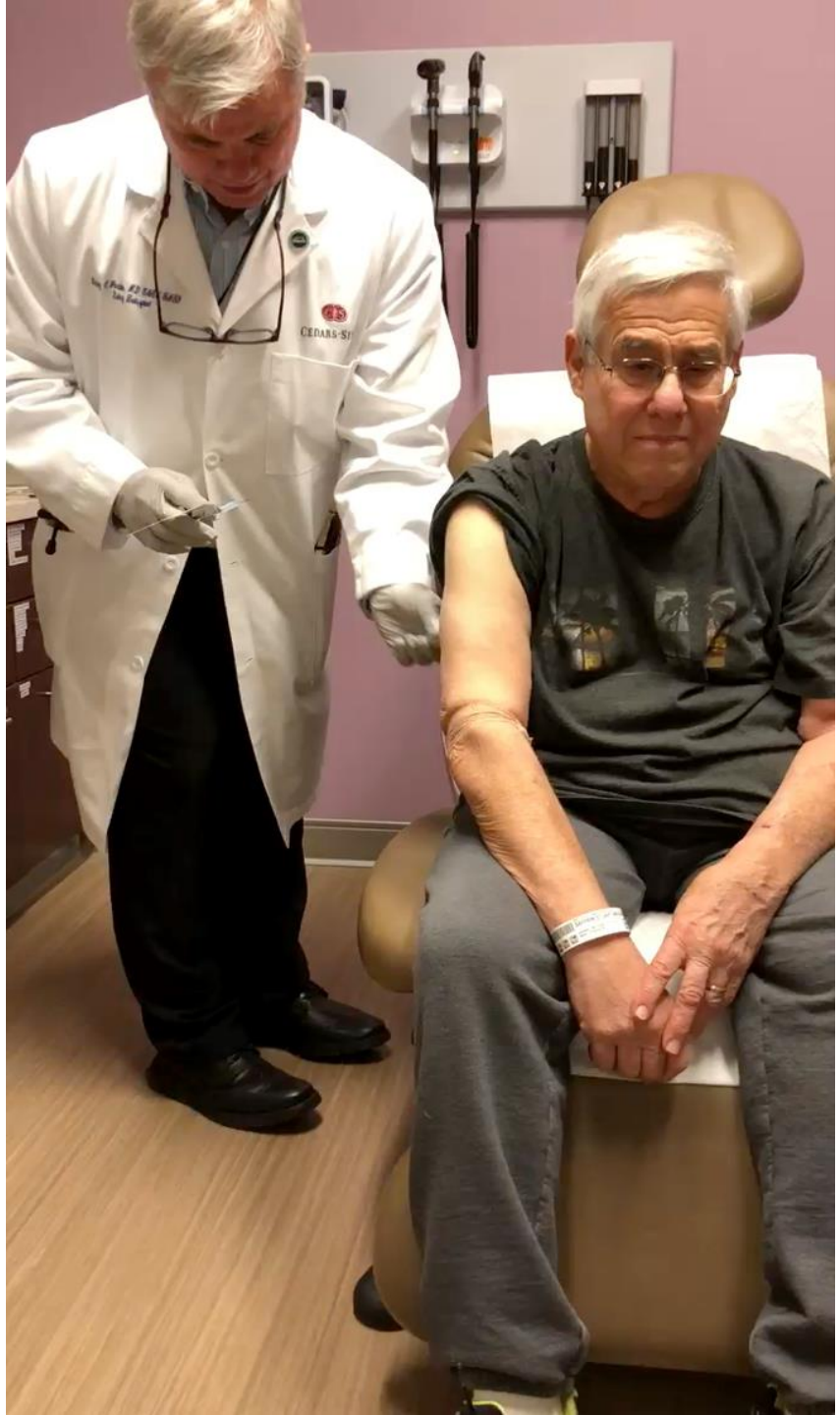
Choi J, Aubert O, et al. *Am J Transplant*. 2017

ALLOGRAFT AND PATIENT SURVIVAL IN PATIENTS TREATED WITH TOCILIZUMAB FOR CHRONIC AMR





First Patient Treated with Anti-IL-6 Clazakizumab for CABMR



…ON GOING TRIALS USING Ani-IL-6

Anti-IL-6	Trial ID	Design	Clinical scenario	Target population
CLAZAKIZUMAB	NCT 03380962	Single center, phase I/II open label single arm	Desensitization	Sensitized recipients of renal transplants
CLAZAKIZUMAB	NCT 03380377	Single center, phase I/II open label single arm	Treatment of cAMR	Recipients of renal transplants with biopsy-proven cAMR
CLAZAKIZUMAB	NCT 03444103	Prospective, Randomized, placebo-controlled, bi-center trial	Treatment of cAMR	Recipients of renal transplants with biopsy-proven cAMR

NIH U.S. National Library of Medicine

ClinicalTrials.gov

Anti-IL-6 : TAKE HOME MESSAGES

- **Anti-IL-6 to prevent and treat chronic AMR seems promising by :**
  - Inhibiting plasma cell IgG production
  - Decreasing DSA level
  - Reducing microvascular inflammation and C4d deposition
  - Maintaining a stable renal function
  - Improving graft and patient survival
- **Results are limited by :**
  - Absence of published randomized studies
  - Small populations
  - Limited follow-up
  - Phase II/III studies planned for Clazakizumab (anti-IL-6) in 2019

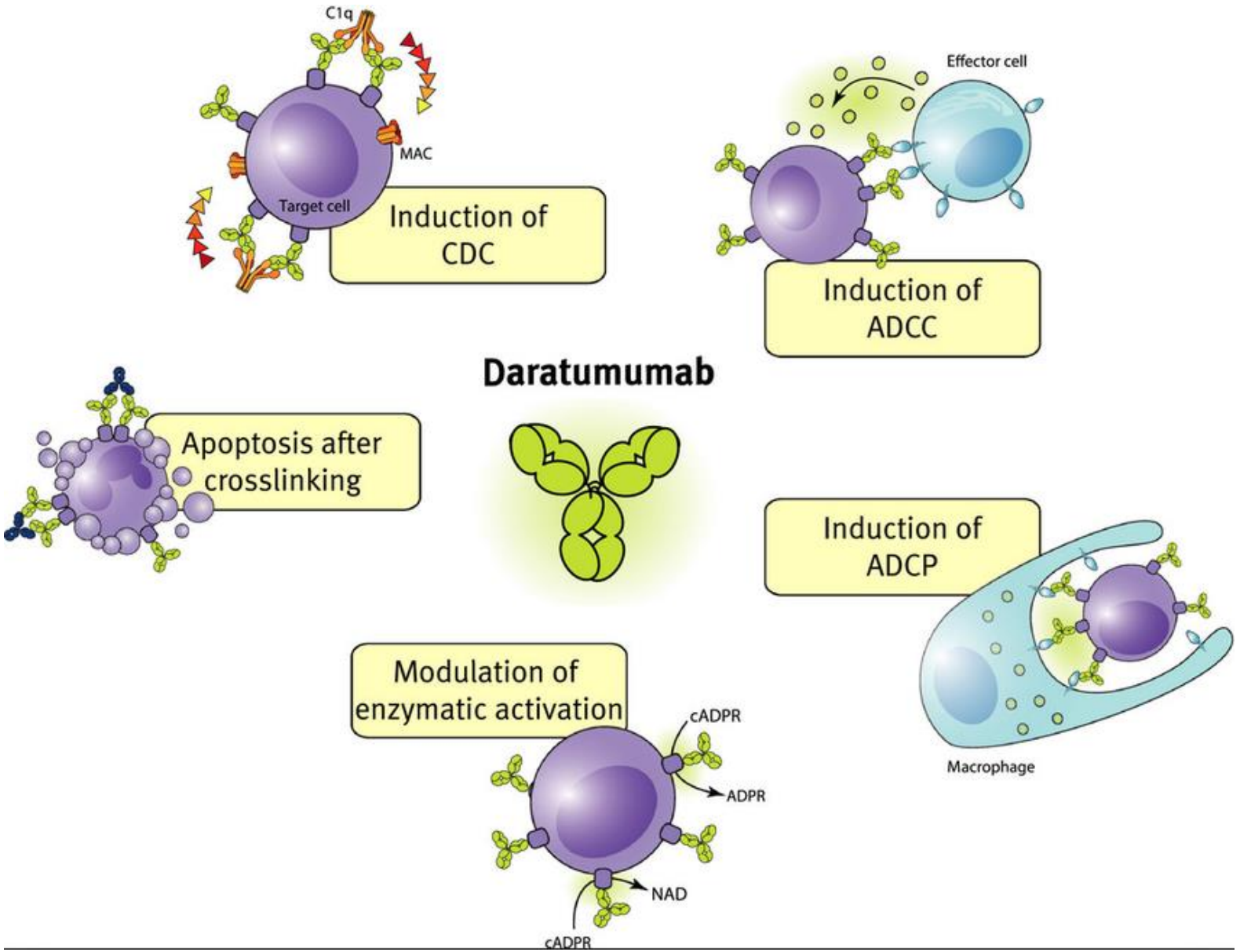
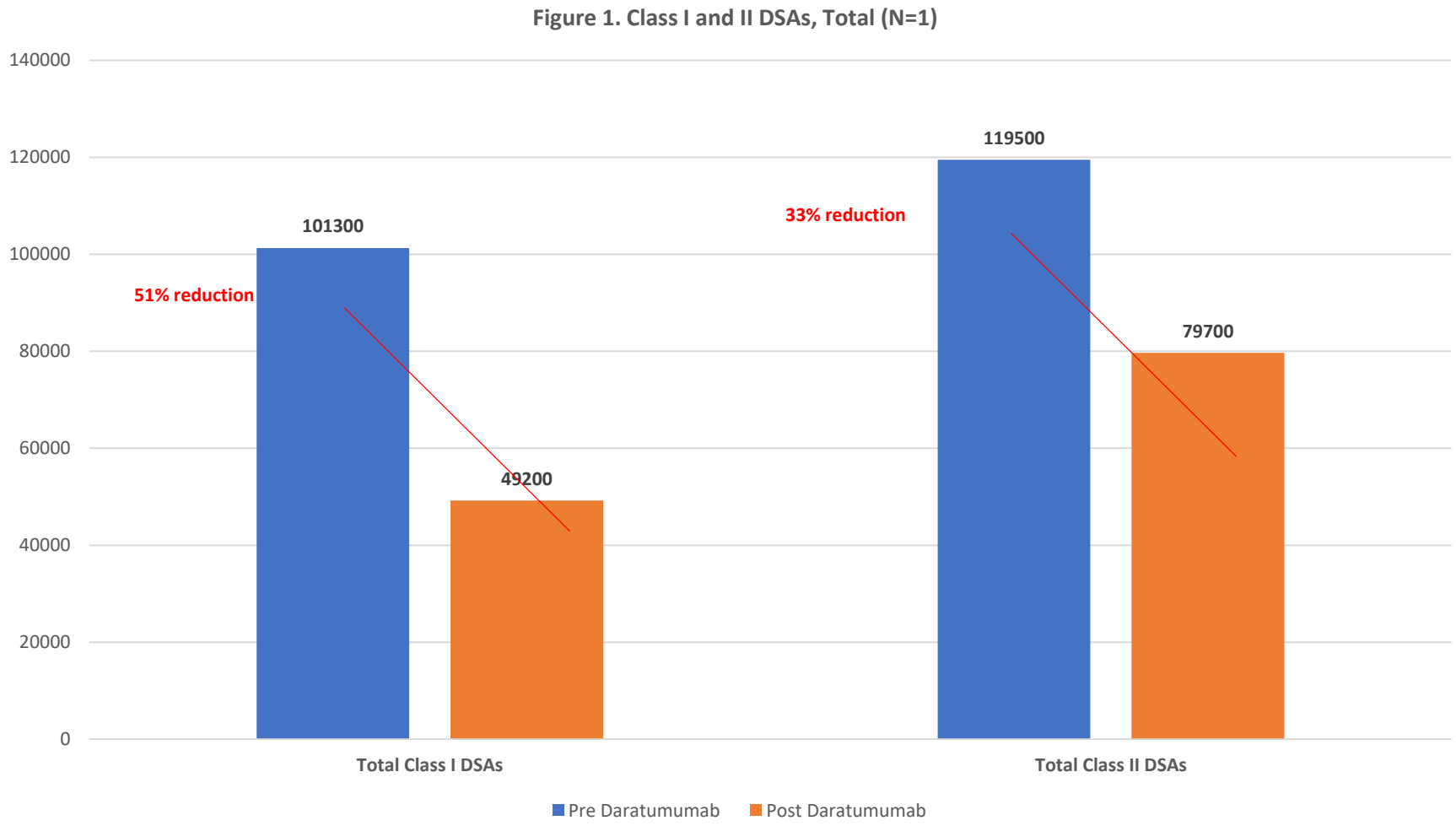
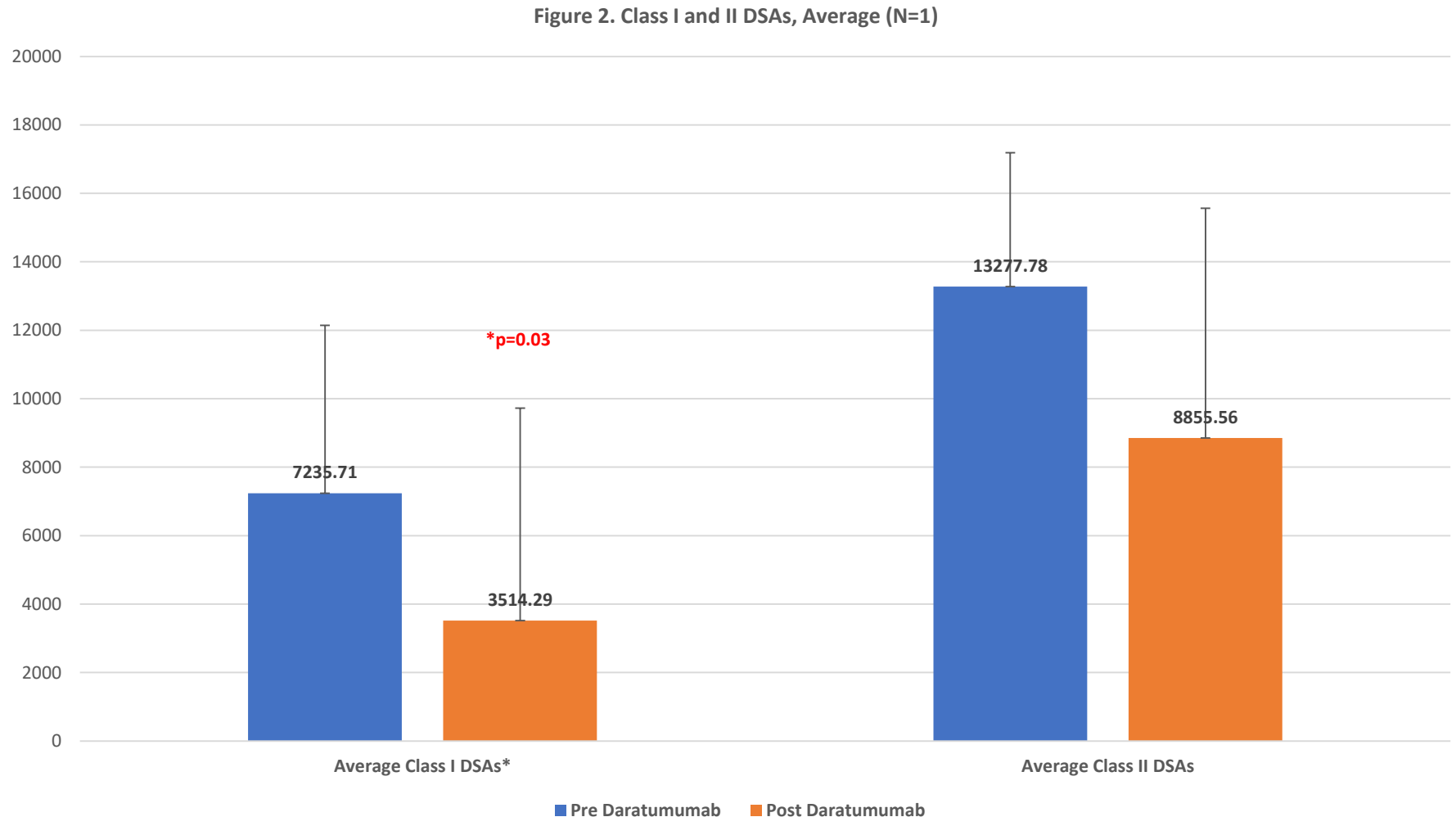

C1q
MAC
Target cell
Induction of CDC
Effector cell
Induction of ADCC
Daratumumab
Apoptosis after crosslinking
Induction of ADCP
Macrophage
Modulation of enzymatic activation
cADPR
ADPR
NAD
cADPR

Figure 1. Total Class I and II DSAs, baseline vs after completion of 4 doses of daratumumab



CEDARS-SINAI MEDICAL CENTER
Comprehensive Transplant Center

Figure 2. Average Class I and II DSAs, baseline vs after completion of 4 doses of daratumumab



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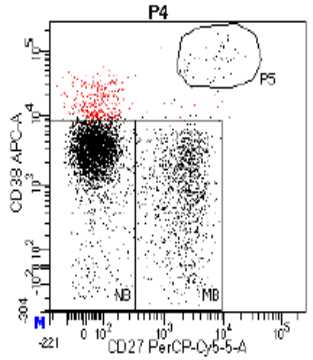
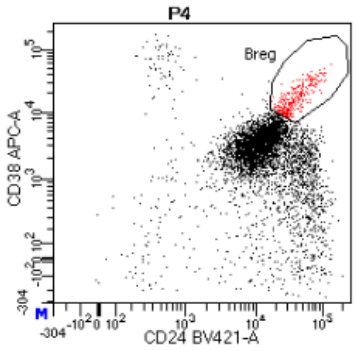
Normal Control

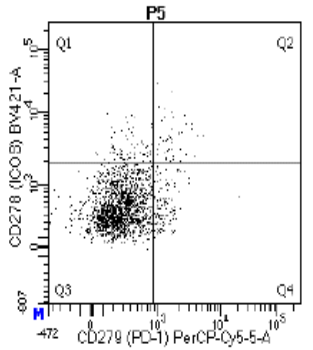
Plasmablasts & Plasma Cells



Regulatory B Cells



Follicular Th Cells



Patient: SV

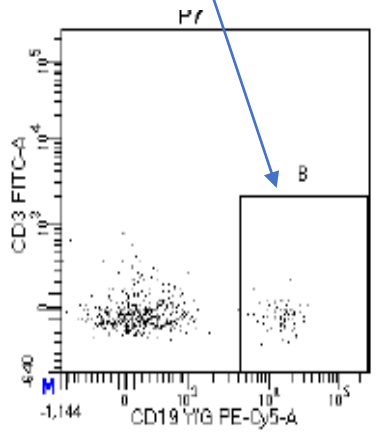
DARATUMUMAB (ANTI-CD38) FOR TREATMENT OF ABMR

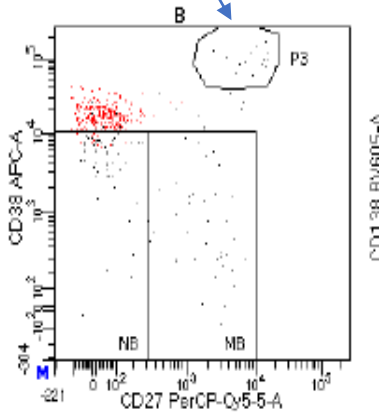
B cells

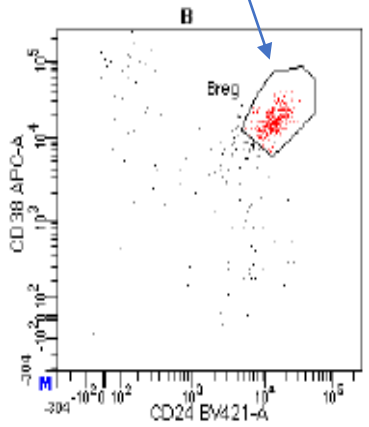
Plasmablast & Plasma cells

Breg

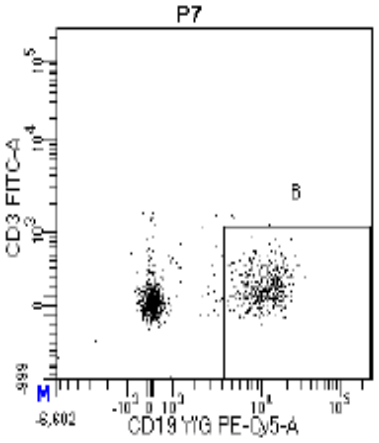
Pre-anti-CD38 (3/1/18)

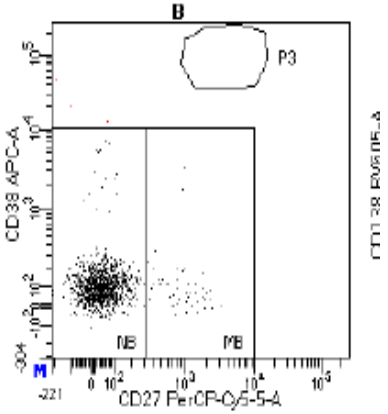


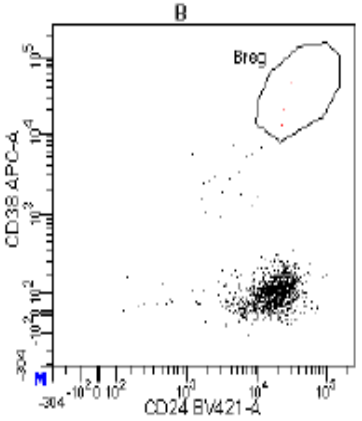




Post-anti-CD38 (3/22/18)





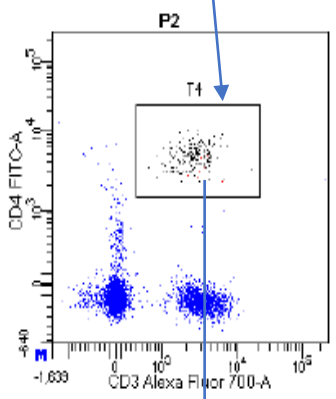


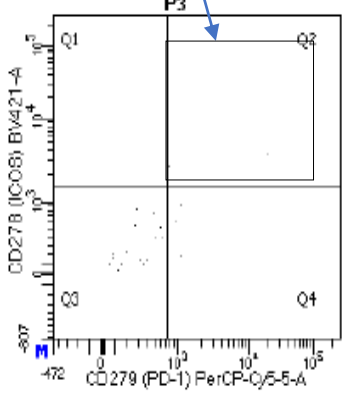
Patient: SV

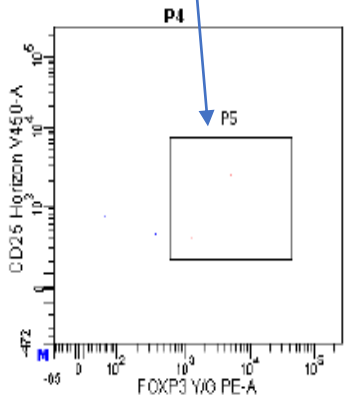
DARATUMUMAB (ANTI-CD38) FOR TREATMENT OF ABMR

CD4+ cells | **Tfh cells** | **Treg cells**

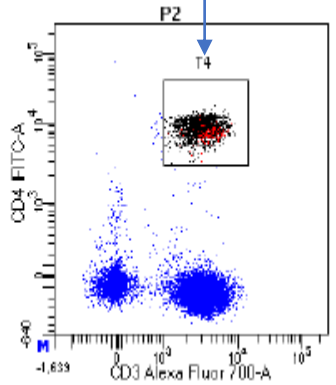
Pre-anti-CD38

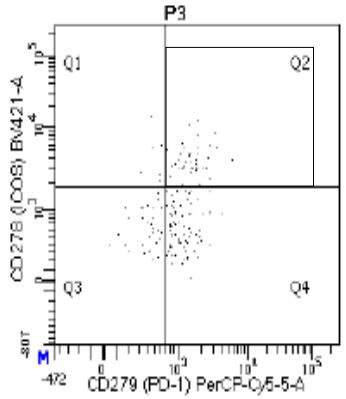


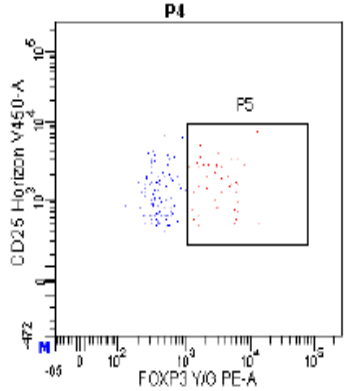




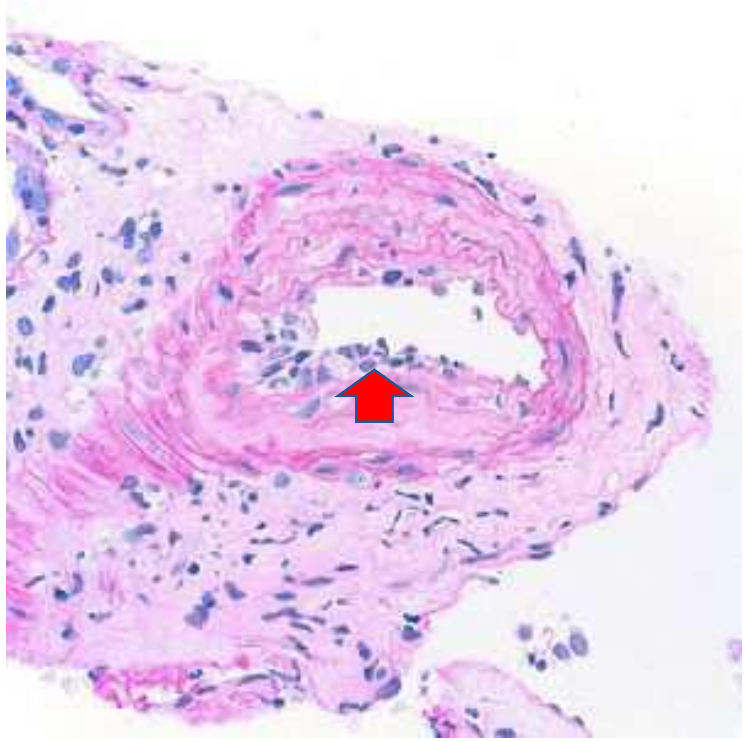
Post-anti-CD38



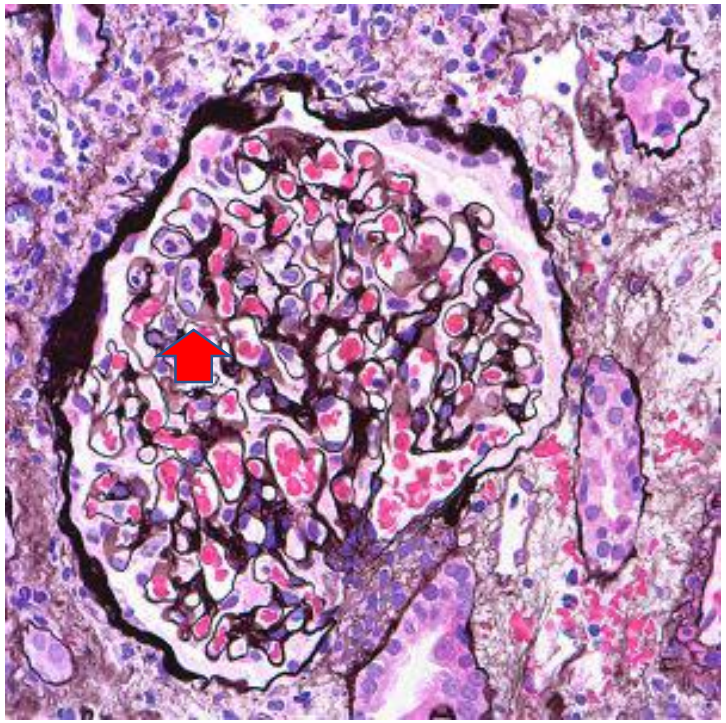




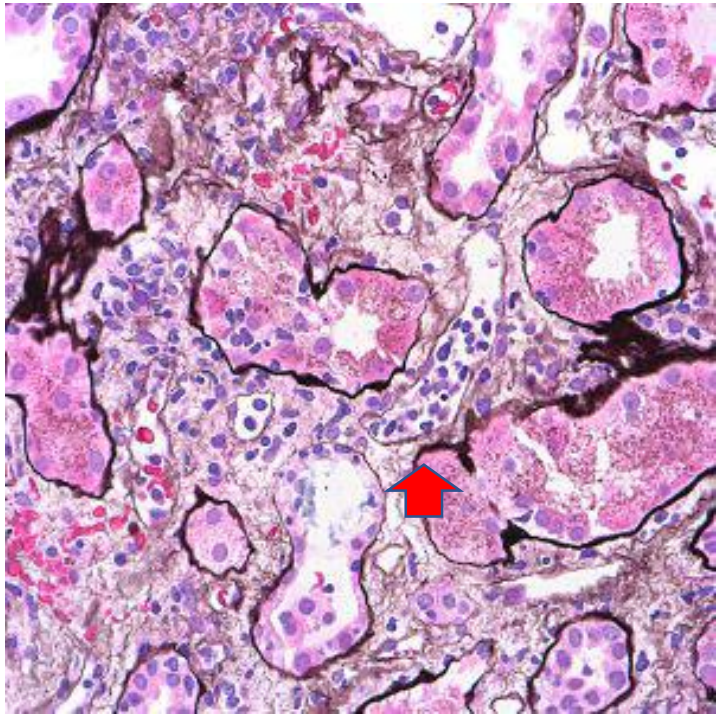
Renal Transplant Biopsy Prior to Daratumumab Therapy



Severe Arteritis

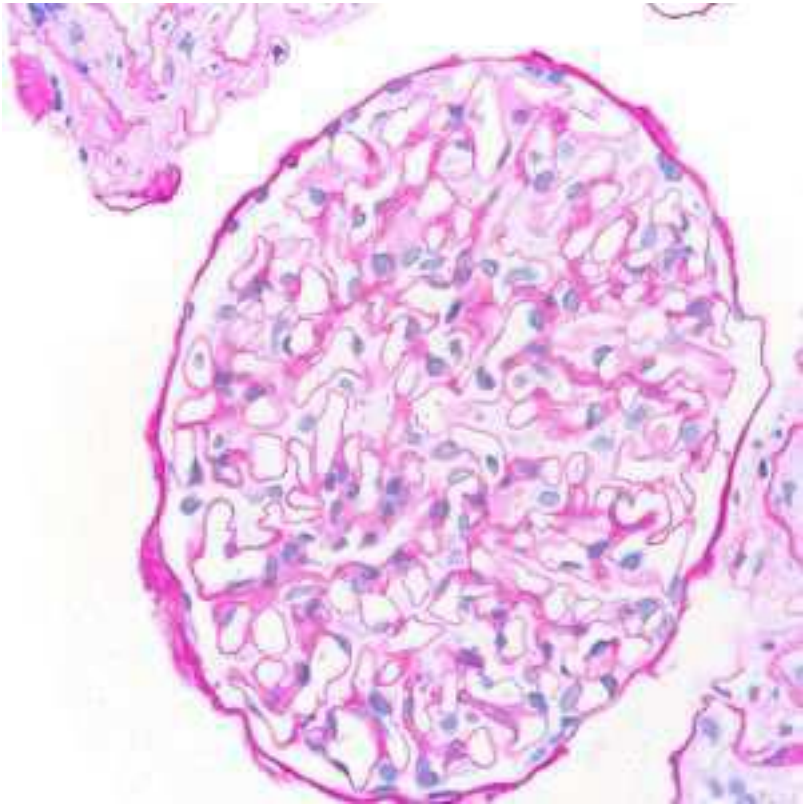


Severe Glomerulitis

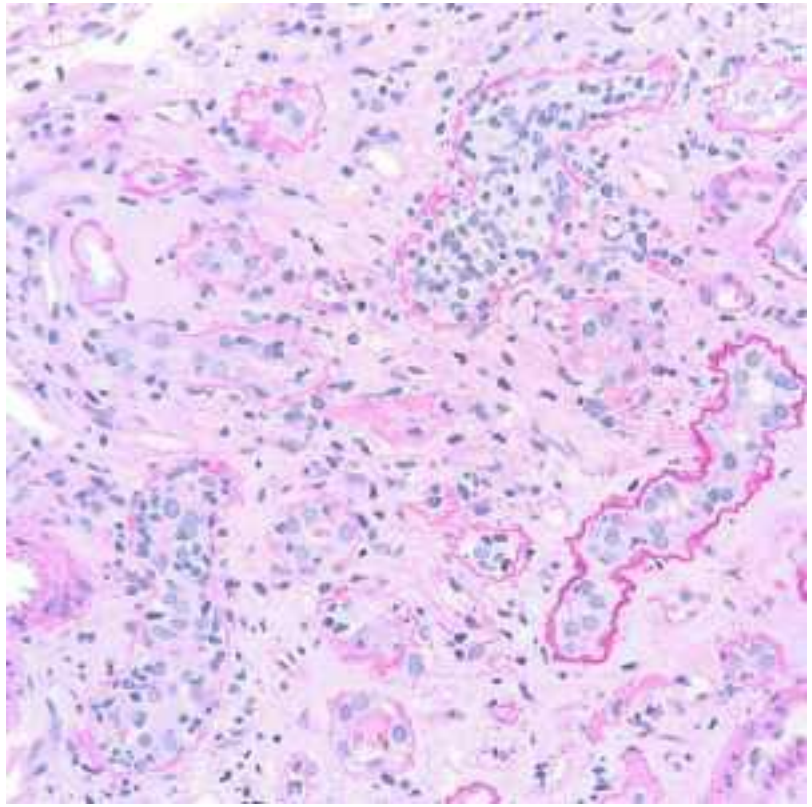


Severe PTCitis

Biopsy Immediately Post-Daratumumab (16mg/kg X 4 doses)



Normal Glomeruli



Intense T-cell Mediated Rejection

Did We Remove Tregs??

Regular Article

blood

IMMUNOBIOLOGY

Daratumumab depletes CD38$^+$ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma

Jakub Krejcik,[1,2,*] Tineke Casneuf,[3,*] Inger S. Nijhof,[1] Bie Verbist,[3] Jaime Balc
Niels W. C. J. van de Donk,[1] Brendan M. Weiss,[6] Tahamtan Ahmadi,[4] Henk M
A. Kate Sasser[4,†]

[1]Department of Hematology, VU University Medical Center, Amsterdam, The Netherlands; [2]Institute
Hematology, Sections of Internal Medicine, Vejle Hospital and University of Southern Denmark, Vejl
Beerse, Belgium; [4]Janssen Research & Development, LLC, Spring House, PA; [5]Janssen Research
Hematology-Oncology, Department of Medicine, Abramson Cancer Center and Perelman School of M

Key Points

- CD38-expressing immunosuppressive regulatory T and B cells and myeloid-derived suppressor cells were sensitive to daratumumab treatment.
- Cytotoxic T-cell number, activation, and clonality increased after daratumumab treatment in heavily pretreated relapsed and refractory MM.

Daratumumab targets CD38-expressing
mediated mechanisms (complement-de
mediated cytotoxicity, and antibody-depe
with crosslinking. These mechanisms ma
which prompted evaluation of daratumun
lations. Peripheral blood (PB) and bone m
myeloma from 2 daratumumab monothe
therapy and at relapse. Regulatory B cells
shown to express CD38, were evaluated f
sensitivity in the myeloma setting. A nc
expressing CD38 was identified. These T
CD38-negative Tregs and were reduce
daratumumab induced robust increases i
PB and BM, daratumumab induced sign
ratios, and increased memory T cells v
patients demonstrated these broad T-
response or better showed greater maximum effector and helper T-cell increase
responses, and significantly greater increases in T-cell clonality as measured by T
clonality positively correlated with increased CD8$^+$ PB T-cell counts. Depletion of CD3
with an increase in T-helper cells, cytotoxic T cells, T-cell functional response, an
mechanisms of action for daratumumab and deserves further exploration. (*Blood.* 20

Introduction

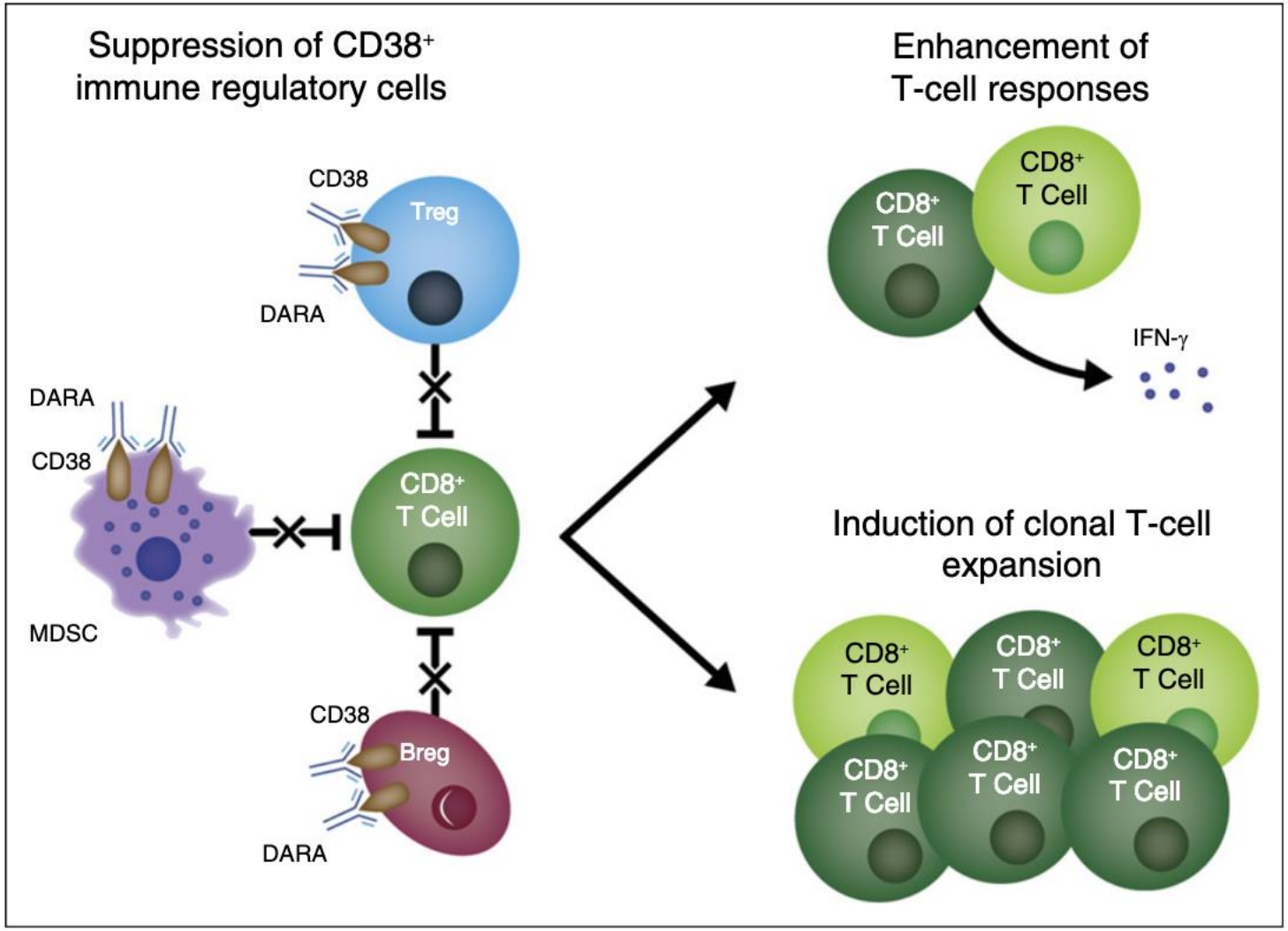
Proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) have improved outcomes in patients with multiple myeloma (MM).[1-3] Despite these advances, prognosis for patients with relapsed MM remains poor, particularly for those who have relapsed after PI and IMiD treatment.[4] New therapies with novel mechanisms of action are needed for resistant patient populations.

Myeloma is associated with immune dysfunction,[5] including immune evasion through the expression of immune checkpoint ligands on plasma cells,[6] elevated adenosine receptor and adenosine activity,[7,8] and immune suppression through myeloid-derived suppressor cells (MDSCs) and regulatory T cell (Treg) activity.[9-11] CD38 is ubiquitously expressed on MM cells,[12,13] but is also present

(Bregs).[14,15] The
cell populations a
disease progressi
cell biology may

Daratumumab
antibody that targ
mechanisms, incl
ity (ADCC), cor
dependent cellul

shown promising antimyeloma activity in 2 clinical studies (GEN501 and SIRIUS) in patients with relapsed and refractory MM, resulting in remarkable response rates that include stringent complete responses



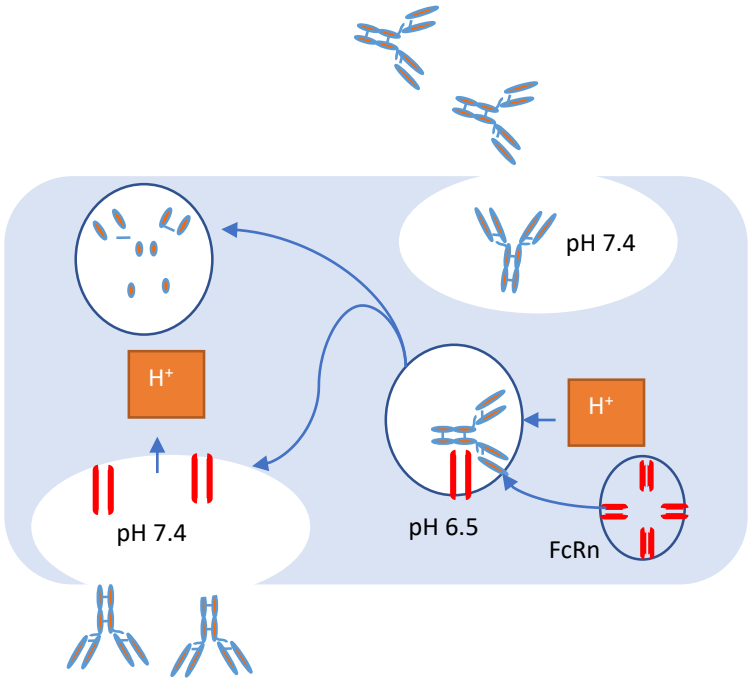
Breastfeeding: A Natural Process with Many Benefits for Mother & Baby

What Does This Have to do with an Immunology Lecture?

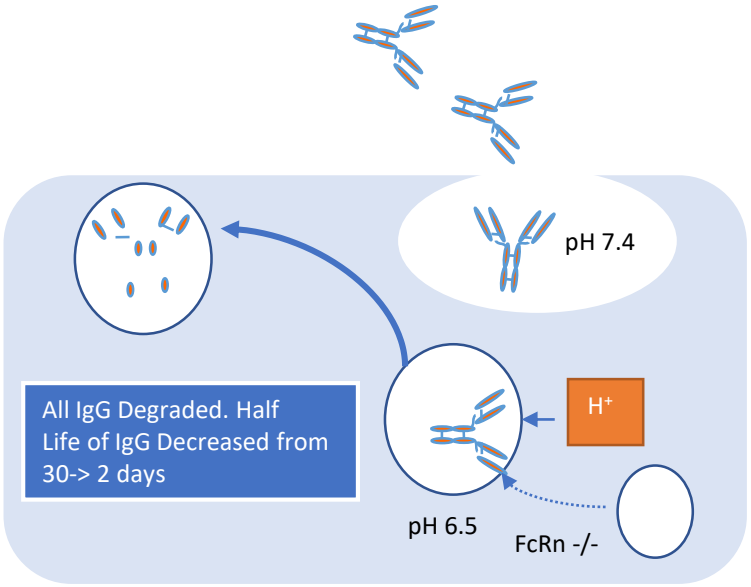
The Fc Neonatal Receptor: An Interesting Odyssey

- It has been 52 years since F.W.R. Brambell hypothesized the existence of a specific receptor in neonates responsible for transport of IgG molecules from maternal milk to infants.
- Originally described in rats, the FcRn provided a shuttle service for IgG molecules from maternal milk to the infant circulation across the gastrointestinal epithelium
- Subsequently, the FcRn was also found to be expressed in placental villi where maternal blood pools and allows for extraction of maternal IgG -> fetal circulation beginning in the 3rd trimester.

FcRn Enhances Half-Life of Circulating IgG: Inhibition of FcRn Drastically Reduces Half-Life of Circulating IgG Molecules



Normal FcRn Function Half-Life 21 days



FcRn (-/-) Half-Life 2-3 days

High Dose IVIg Enhances Clearance of Pathogenic IgG by Saturating FcRn

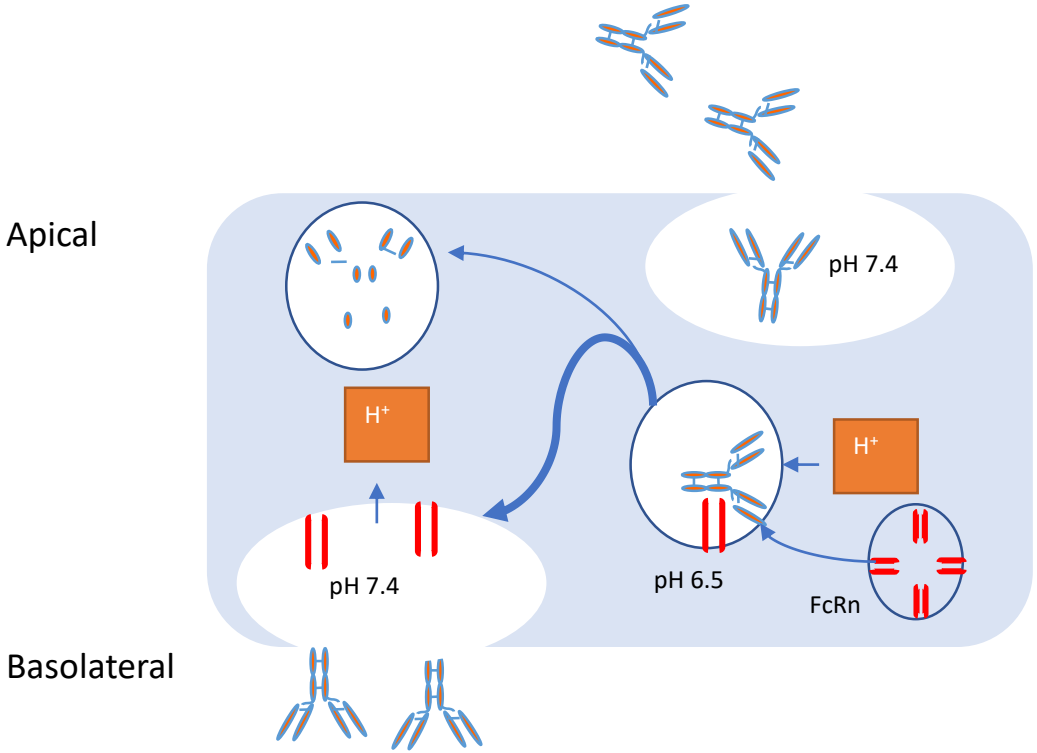


Figure 1A

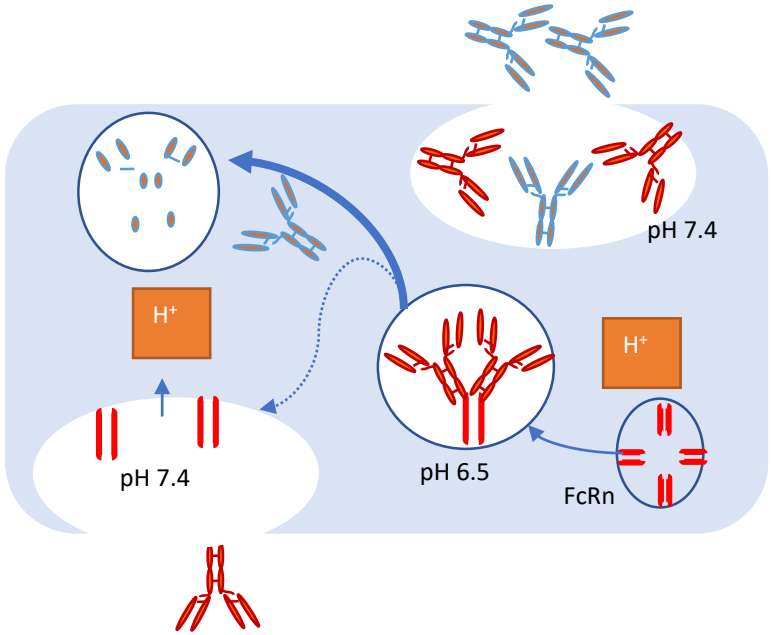
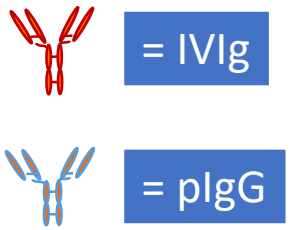
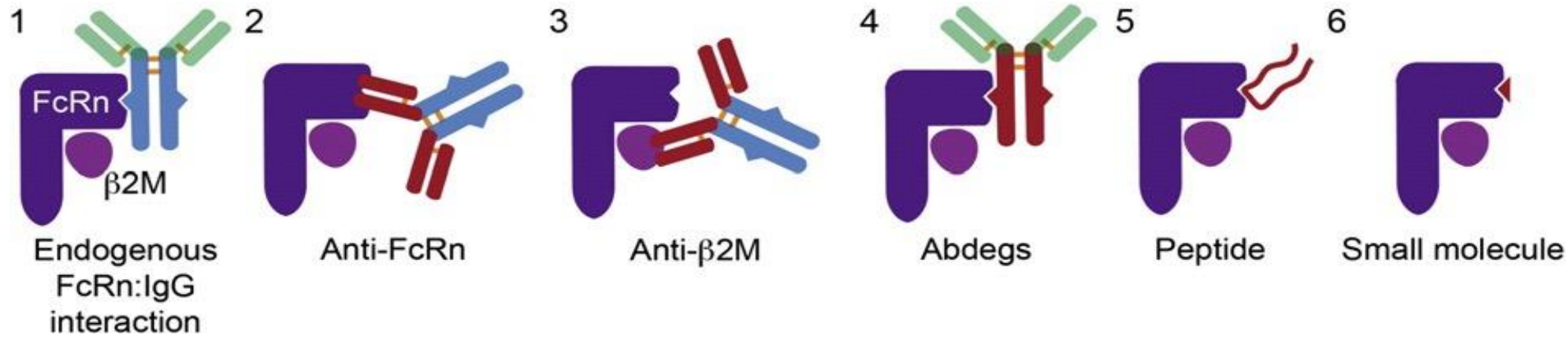


Figure 1C



Potential Therapeutic Approaches to Inhibit IgG/FcRn Interactions

Figure 4



Inhibitors of the FcRn–IgG-Fc interaction. From left to right: 1) High dose IVIg can saturate FcRn and accelerate the clearance of endogenous IgG, 2) anti-FcRn heavy chain antibodies and 3) anti-β2m light chain antibodies bind FcRn epitopes, inhibiting FcRn function and accelerating degredation of circulating IgG 4) Fc-engineered IgGs that that have increased, pH-independent affinity for FcRn (Abdegs), and 5) peptides and 6) small molecules that compete with IgG for binding to FcRn. To date, anti-FcRn and synthetic peptides that block IgG-Fc/FcRn interactions are now in clinical trials. (From [17] with permission)

AUTOIMMUNITY

The FcRn inhibitor rozanolixizumab reduces human serum IgG concentration: A randomized phase 1 study

Peter Kiessling,[1] Rocio Lledo-Garcia,[2]* Shikiko Watanabe,[3] Grant Langdon,[4] Diep Tran,[2] Muhammad Bari,[2] Louis Christodoulou,[2] Emma Jones,[5] Graham Price,[2] Bryan Smith,[2] Frank Brennan,[2] Ian White,[2] Stephen Jolles[6]

Pathogenic immunoglobulin G (IgG) autoantibodies characterize some human autoimmune diseases; their high concentration and long half-life are dependent on recycling by the neonatal Fc receptor (FcRn). Inhibition of FcRn is an attractive new treatment concept for IgG-mediated autoimmune diseases. Rozanolixizumab (UCB7665; CA170_01519.g57 IgG4P) is an anti-human FcRn monoclonal antibody. In cynomolgus monkeys, rozanolixizumab reduced IgG (maximum 75 to 90% by about day 10), was well tolerated, and did not increase risk of infection. We also report a first-in-human, randomized, double-blind, placebo-controlled, dose-escalating study of intravenous (IV) or subcutaneous (SC) rozanolixizumab in healthy subjects (NCT02220153). The primary objective was to evaluate safety and tolerability. Secondary objectives were assessment of rozanolixizumab pharmacokinetics and pharmacodynamics, including effects on circulating IgG concentrations. Forty-nine subjects were randomized to receive rozanolixizumab (n = 36) or placebo (n = 13) across six cohorts. The first three cohorts received IV doses, and the subsequent three cohorts received SC doses, of rozanolixizumab 1, 4, or 7 mg/kg (n = 6 for each cohort; plus n = 7 or 6 for placebo, respectively). The most frequent treatment-emergent adverse event [TEAE; headache, 14 of 36 (38.9%) subjects] was dose-dependent and more prominent after IV administration. Severe TEAEs occurred in four subjects, all in the highest-dose IV group [headache (n = 3) and back pain (n = 1)]. Rozanolixizumab pharmacokinetics demonstrated nonlinear increases with dose. There were sustained dose-dependent reductions in serum IgG concentrations (IV and SC rozanolixizumab). These data provide clinical evidence for the therapeutic potential of rozanolixizumab.

INTRODUCTION

Autoimmune and alloimmune diseases, such as anti–glomerular basement membrane antibody disease, immune thrombocytopenia (ITP), myasthenia gravis (MG), hemolytic anemia, and pemphigus vulgaris, are characterized by the presence of pathogenic autoantibodies, commonly of the immunoglobulin G (IgG) isotype. A number of strategies currently exist to reduce pathogenic autoantibodies; these include treatments aimed at reducing autoantibody production (immunosuppression with corticosteroids and second-line agents such as azathioprine, cyclophosphamide, mycophenolate mofetil, and B cell ablation) (*1*) or increasing autoantibody removal [plasma exchange, immunoadsorption, or immunomodulatory doses of intravenous immunoglobulin (IVIg)] (*2*). However, these treatments can be associated with side effects, accessibility issues, patient inconvenience, and overall time and cost implications (*3*–*6*).

Therapeutic plasma exchange involves the filtration of venous blood to remove high–molecular weight components, including immunoglobulins (both pathogenic and normal), albumin and proinflammatory factors that are involved in the pathogenesis of numerous autoimmune diseases (*3*). Although plasma exchange offers a potentially efficacious treatment option for autoimmune disorders, it is associated with numerous disadvantages including adverse reactions, exposure to blood products, and reduction of circulating plasma concentrations of all immunoglobulin isotypes (including IgM and IgA, not just IgG) (*7*). An alternative treatment option is immunoadsorption, which specifically removes IgG and no other plasma component, thus reducing the breadth of impact on the patient's humoral immune system; however, immunoadsorption is also associated with adverse reactions and the disadvantages associated with hospital-based therapies (*8*).

IVIg comprises human immunoglobulin (95 to 99% IgG and varying trace amounts of IgM, IgA, IgD, and IgE) prepared from large numbers of healthy donors (*4*). The mechanisms of action of IVIg are multiple and may include functional blockade of Fc receptors, autoantibody neutralization, inhibition of autoantibody production, complement inhibition, and modulation of cytokine and cytokine antagonist production (*5*). Administration of immunomodulatory doses of IVIg can reduce endogenous (including pathogenic) IgG concentrations as a result of saturation of the neonatal Fc receptor (FcRn) (*9*–*11*). Although IVIg is generally considered to have an acceptable safety profile, adverse systemic reactions are common, occurring in 20 to 50% of patients (*12*). In most chronic autoimmune diseases in which IVIg is used for immunomodulation (rather than replacement doses in antibody deficiency), a long-term dose (1 to 2 g/kg per cycle) may be required (*13*, *14*).

Another common treatment option for many autoimmune diseases is corticosteroids, used either as stand-alone therapy or in combination with second-line immunosuppressive agents, plasma exchange, or IVIg (*1*). Corticosteroids are known to modestly reduce IgG concentrations in plasma (*15*); however, long-term steroid treatment is often limited by significant dose-dependent toxicities and lack of effect over time (*6*). Despite the universally accepted efficacy of corticosteroids in autoimmune conditions such as MG, the long-term adverse events (AEs) make the availability of other treatment options highly desirable (*1*).

IgG and albumin have half-lives of 3 to 4 weeks, the longest of any plasma proteins (*16*, *17*). Their high concentrations (IgG, 7 to 17 g/liter in humans) and long half-lives are critically dependent on salvage and

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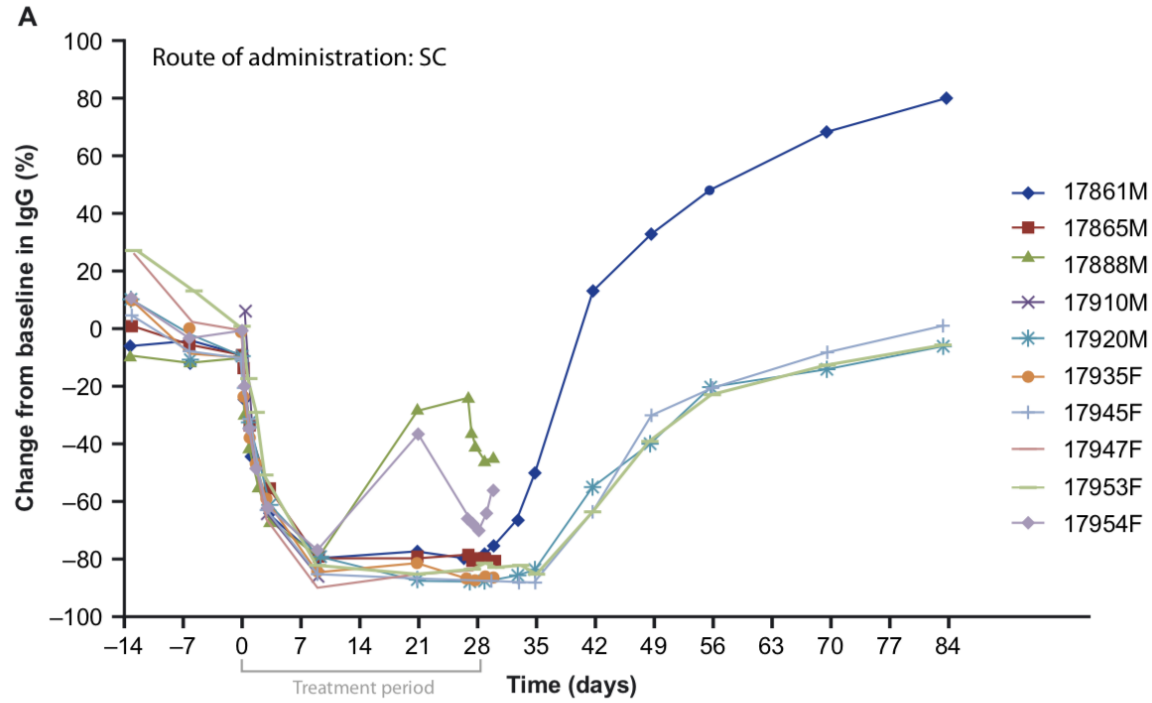
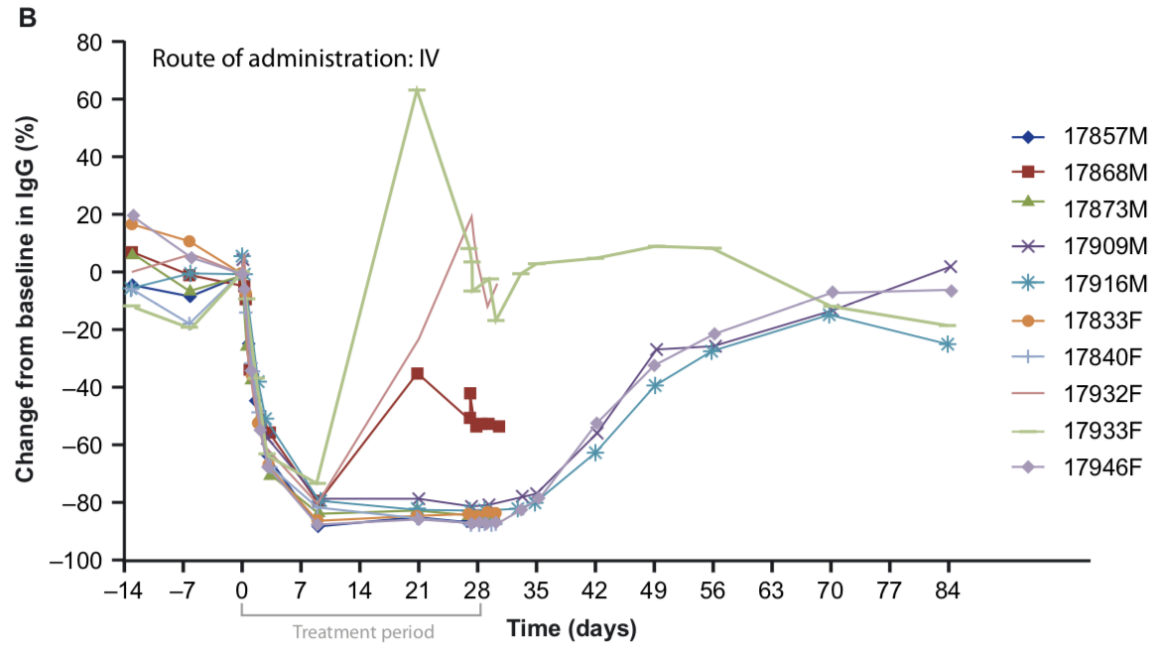
*Corresponding author. Email: rocio.lledo-garcia@ucb.com





AUTOIMMUNITY

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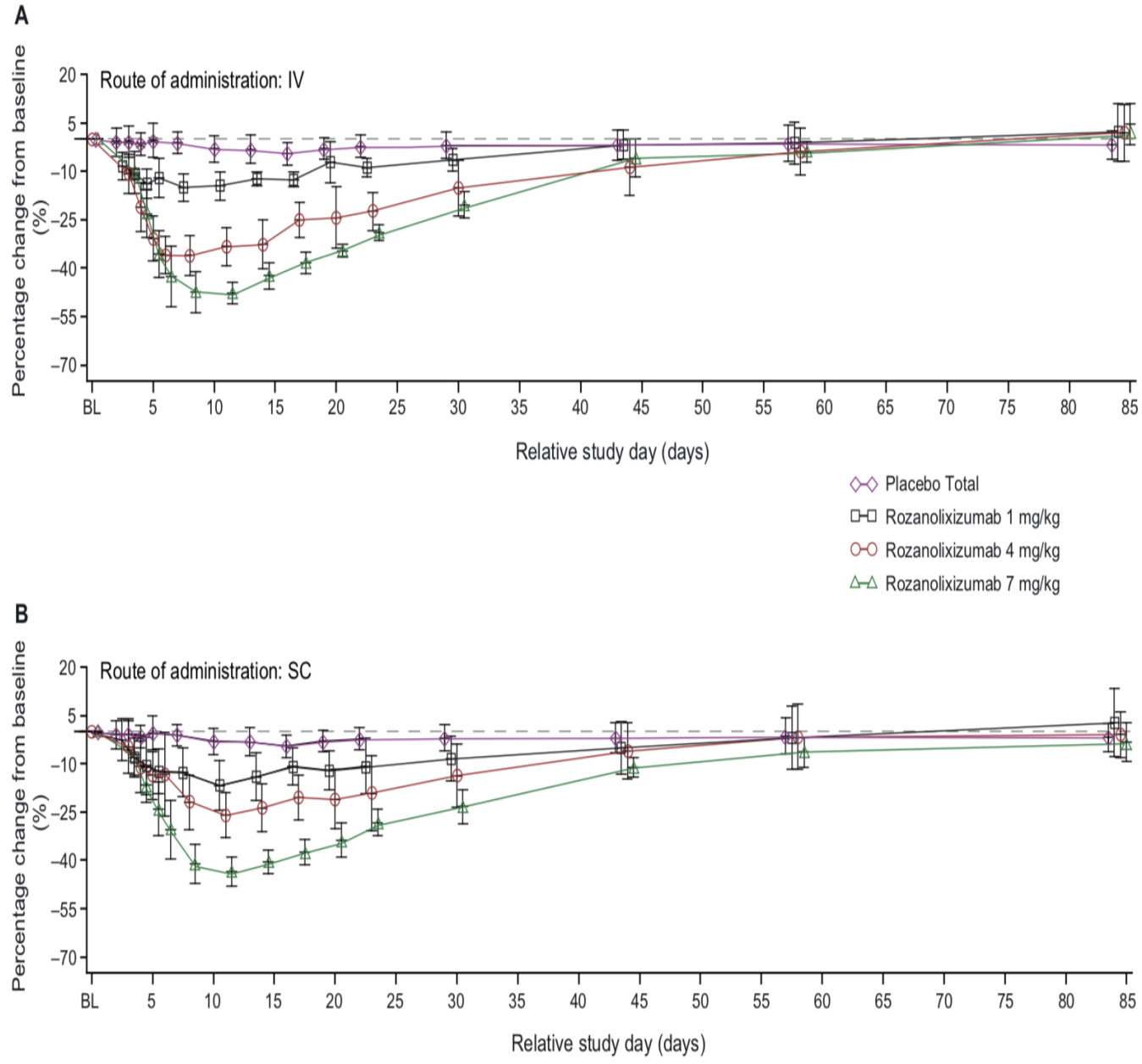

SCIENTIFIC REPORTS

OPEN

In vivo depletion of serum IgG by an affibody molecule binding the neonatal Fc receptor

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Johan Seijsing[1,3], Shengze Yu[1], Fredrik Y Frejd[2], Ingmarie Höiden-Guthenberg[2] & Torbjörn Gräslund[1]

Lowering the total level of Immunoglobulin G (IgG) in circulation is a promising general treatment option for many autoimmune diseases driven by pathogenic autoantibodies. The half-life of IgG in circulation is unusually long as a consequence of its interaction with the neonatal Fc receptor (FcRn), which protects it from lysosomal degradation by cells in contact with blood. Blocking the IgG/FcRn interaction prevents FcRn-mediated rescue, which may lead to increased catabolism and a lowering of the total IgG level. Here, we find that an engineered alternative scaffold protein, an affibody molecule, interacting specifically with FcRn, is able to block the IgG/FcRn interaction *in vitro*. The affibody molecule (Z_{FcRn}) was expressed alone or as a fusion to an albumin binding domain (ABD), to extend its half-life in circulation, in both cases with retained affinity and blocking potential. Repeated i.v. injections in mice of Z_{FcRn} and Z_{FcRn}-ABD were found to result in an up to 40% reduction of the IgG serum-level after 5 days. Potential applications of Z_{FcRn} as a general treatment modality for autoimmune diseases are discussed.

Pathogenic immunoglobulin G (IgG) autoantibodies are responsible for driving pathogenesis in a number of autoimmune diseases[1]. Compared to other serum proteins, IgG have an unusually long half-life in circulation due to interaction with the neonatal Fc receptor, which protects it from lysosomal catabolism by cells in contact with blood. In humans, the average half-life of IgG in circulation is approximately 3 weeks[2] and in mice it is 6–8 days[3]. However, in FcRn$^{-/-}$mice the half-life of IgG in circulation is reduced to 1 day[4] and the mice cannot maintain IgG homeostasis, resulting in a 70–80% reduction of the total level of IgG.

FcRn is a hetero-dimeric receptor, consisting of an α-chain and β2-microglobulin (β_2m), of which it has the latter in common with the class I Major histocompatibility complex[5]. It resides predominantly in the endosomes, where it can bind to IgG in the slightly acidic environment (pH < 6.5). FcRn together with its bound cargo is then sorted from the endosomes, followed by transport to the cell surface, where the cargo is released upon encountering the higher pH (>7) in the blood. This rescue mechanism is responsible for the long serum circulation half-life of IgG. With a similar mechanism but with a binding site that is separate from the IgG-binding site, FcRn can rescue serum albumin from lysosomal catabolism, also leading to a long residence time in circulation[6].

Convincing evidence suggests that blocking FcRn-mediated rescue of IgG can ameliorate the symptoms of many different autoimmune diseases[7–9]. In addition, FcRn$^{-/-}$mice have been found to be protected from induction of e. g. autoimmune arthritis, which suggest that FcRn may also play an important role in the development of different autoimmune diseases[10]. This was further supported by the finding that FcRn deficiency could protect animals in a model of the IgG-driven autoimmune disease Epidermolysis bullosa acquisita[11]. Several strategies have been evaluated for blocking the FcRn/IgG interaction towards the goal of increasing IgG catabolism in order to treat different autoimmune diseases[12]. Intravenous Ig (IVIg) is the administration of large amounts of donor-derived polyclonal IgG, and has been found to be efficient for treatment of e.g. Guillain–Barré Syndrome and is used clinically[13]. The mechanism of IVIg action is partly to increase catabolism of pathogenic IgG by blocking IgG-mediated rescue by FcRn through saturation of the rescue machinery[14]. However, IVIg treatment requires a large amount of protein making it expensive and is derived from a limited human donor source. ABDEGs (antibodies that enhance IgG degradation) are IgG molecules, where the Fc-part has been engineered to

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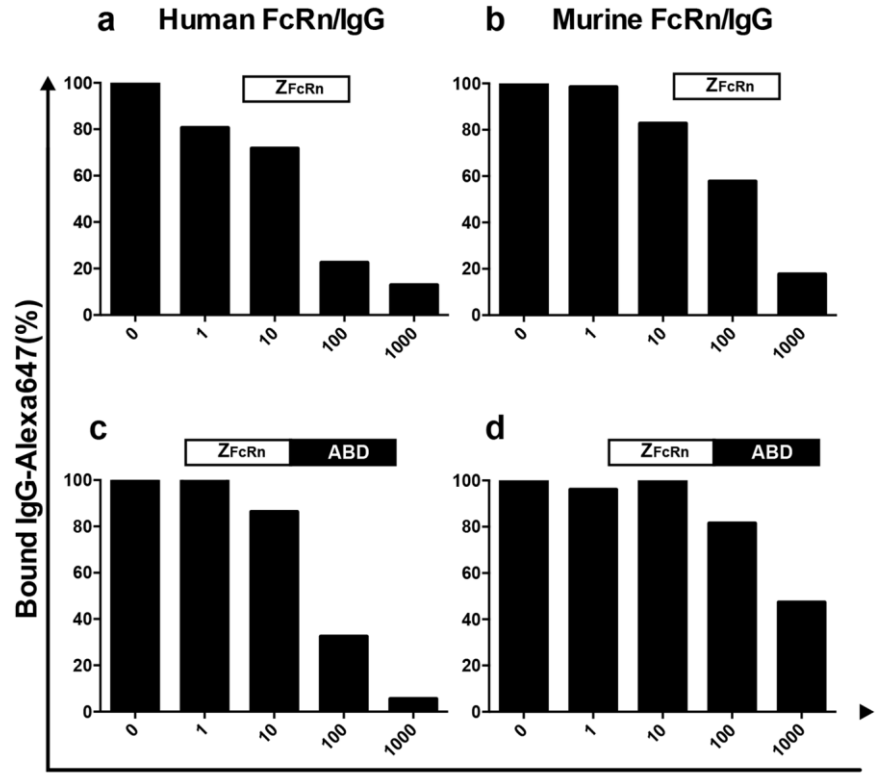

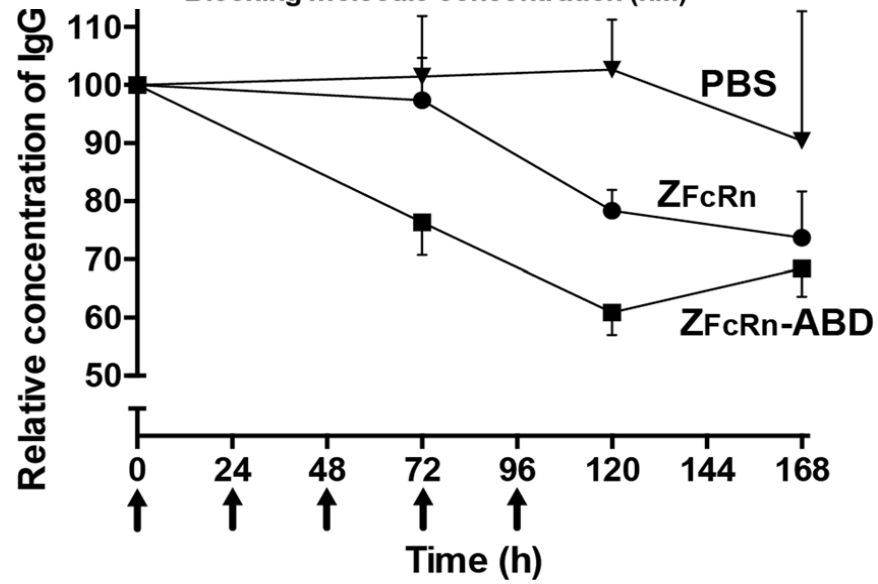

Therapeutic Approaches to Reducing Alloantibody Injury to Allografts