Supplementary Materials Table of Contents

Study Population and Design

This single-center, observational case series included adult kidney transplant recipients (age 18-75 age) with refractory cABMR who were followed for one year. Refractory cABMR was defined as persistent microcirculation inflammation in patients with biopsy confirmed cABMR after receiving our institutions standard treatment of one dose of rituximab (375 mg/m^2 BSA), IVIG (200 mg/kg every two weeks for three months) and dexamethasone (100 mg and taper)[S5]. These patients who required further treatment and follow up, including biopsies and blood draws at the time of index biopsy and three, six, and 12 months after diagnosis. Participants were enrolled between August 2016 and February 2019. Patients receiving multiorgan transplants or those with contraindication to renal biopsy were excluded. No other exclusion criteria were applied. Demographic and clinical data were collected for all patients including age, race, sex, body mass index (BMI), history of diabetes and hypertension, date of kidney transplantation, human leukocyte antigen (HLA) mismatches, DSA, history of previous transplants and rejection episodes, treatment regimens, graft loss, and death. Parameters of kidney function were collected at index biopsy, three, six, and 12 months after diagnosis. The most recent serum creatinine and estimated glomerular filtration rate for patients with a viable graft were also collected (time from index biopsy 1.8-4.3 years). This study was approved by the University of Wisconsin School of Medicine and Public Health Institutional Review Board (IRB# 2014-0076).

Kidney Allograft Biopsy

Patients underwent a total of four biopsies over the course of one year unless the graft failed. Index biopsies were performed for new-onset allograft dysfunction, or de novo DSA. Standard of care follow up biopsies were performed at three, six and 12 months after index biopsy because patients required additional therapy for refractory rejection. Each biopsy was assessed by a single renal pathologist (blinded to clinical tests) using light microscopy and C4d immunofluorescence. Biopsies were scored according to the Banff 2017 criteria. [S9]

Luminex Screening for Anti-HLA Antibodies and HLA typing

Donor specific HLA antibodies (DSA) against HLA Class I and Class II were measured at the time of index and all subsequent biopsies using Luminex single antigen beads (One Lambda, West Hills, CA) as per manufacturer's instructions. The strength of DSA were represented as the sum of the mean fluorescence intensity (MFI_{sum}) of positive individual beads representing each donor antigen. Our virtual crossmatch uses an MFI signal >500 and/or a clear epitope reactivity pattern to assign a positive reactivity as reported previously[S5]. HLA typing was performed using real-time PCR sequence-specific primer technology (LinkSeq™, OneLambda, West Hills, CA) for deceased donors starting in April 2017. Other donor and recipient typings were performed using reverse sequence-specific oligonucleotide probe technology (LABType™, OneLambda, West Hills, CA). All donors and recipients were genotyped across 11 HLA loci including HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1, -DQB1, -DPA1, and –DPB1.

Treatment of chronic active ABMR

After index biopsy and confirmation of cABMR, patients were treated with one dose of rituximab (375 mg/m^2 BSA), IVIG (200 mg/kg every two weeks for three months) and dexamethasone (100 mg and taper).[S5] Additional treatment was administered after each subsequent biopsy with persistent active rejection (Table S3). This included IVIG (500 mg/kg weekly, then reduced to bi-

monthly or monthly), pulse steroids (dexamethasone, 100 mg and taper) and an additional dose of rituximab (375 mg/m^2 BSA). Improvement in DSA levels between the first and second biopsy was observed in most patients although active rejection persisted. Thus, IVIG and corticosteroids were continued after subsequent biopsies. Patients who showed no improvement in *mvi* score or persistent DSA were given additional doses of rituximab. Differences in corticosteroid use between patients was primarily due to individual clinical characteristics and concern for repeat steroid use in patients at higher risk for infection or glycemic dysregulation. Antimicrobial prophylaxis consisted of Valganciclovir (900 mg daily for three months) or Acyclovir (400 mg twice daily for three months), TMP/Sulfa (160/800 mg daily for three months), and Nystatin (twice daily for one month). Maintenance immunosuppression consisted of tacrolimus (5-10 ng/dL), prednisone (5 mg/day), and mycophenolate mofetil (500-750 mg twice daily).

Statistical analysis

The D'Agostino and Pearson normality test was performed to determine Gaussian distribution before data were assessed using an ANOVA mixed-effects analysis with the Geisser-Greenhouse correction or Friedman test as appropriate. For Class I DSA, missing values due to graft loss were input as zeros because the Friedman test does not allow for missing values. Analysis between time points was conducted using the Student's paired-T test or Wilcoxon Test. Comparisons between patients with or without graft loss were conducted using the Mann-Whitney test. The Pearson correlation coefficient was used to assess relationship between changes in DSA and improvement in ABMR. Analyses were performed using GraphPad Prism version 8.2 (GraphPad Software, San Diego, CA) and p-values ≤0.05 were considered statistically significant. Figures and tables are presented as mean ± standard deviation (SD).

Supplementary Tables

Table S1: Patient Characteristics at Index Biopsy	
Average age, mean (SD) (years)	40 (14.5)
Sex	
Male, n (%)	7 (88%)
Female, n (%)	1 (12%)
Race	
Caucasian, n (%)	8 (100%)
Body mass index, mean (SD)	27.7 (10.2)
Diabetes, n (%)	3 (38%)
Hypertension, n (%)	7 (88%)
De novo DSA, n (%)	7 (88%)

DSA, donor specific antibody

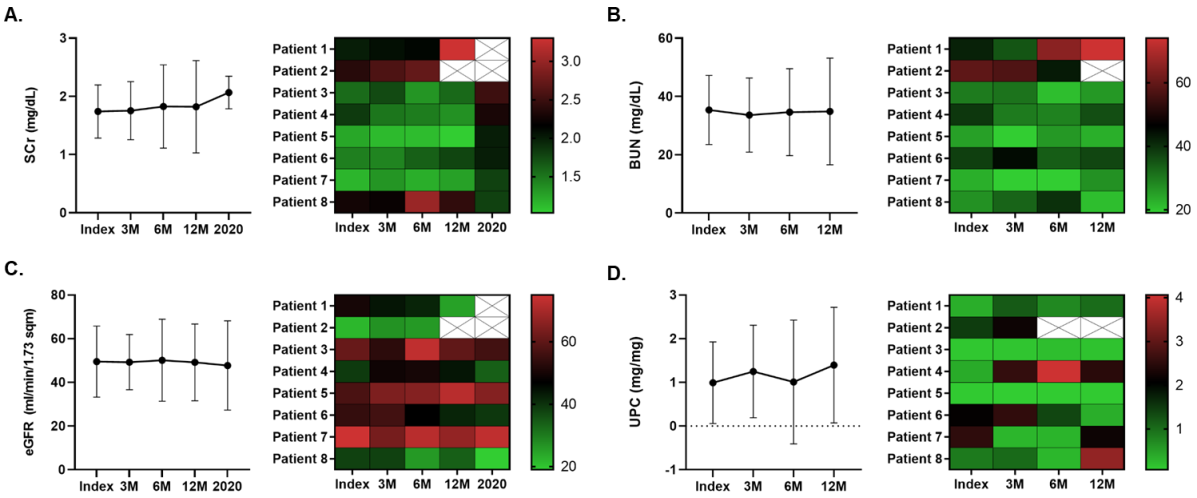
Table S2: Transplant and Clinical Characteristics	
Donor Type	
Living donor, (%)	38%
Deceased donor, (%)	62%
MHC Mismatch	
MHC Class I, mean (SD)	3.2 (2.2)
MHC Class II, mean (SD)	3.3 (1.8)
DSA at Transplant, n (%)	1 (12%)
Previous Rejection Episodes, n (%)	3 (38%)
Previous Transplants, n (%)	
Kidney Transplant, n (%)	3 (38%)
Liver Transplant, n (%)	1 (12%)
Transplant to ABMR Interval, mean (SD) (years)	4.4 (3.8)

MHC, major histocompatibility complex
DSA, donor specific antibody
ABMR, antibody mediated rejection

Table S3: Treatment Modalities after Biopsy				
	Patients Treated (n)	*IVIG*	*Corticosteroids*	*rituximab*
1st Biopsy, n (%)	8	8 (100%)	8 (100%)	8 (100%)
2nd Biopsy, n (%)	7	7 (100%)	5 (71%)	1 (14%)
3rd Biopsy, n (%)	7	7 (100%)	5 (71%)	4 (57%)
4th Biopsy, n (%)	6	6 (100%)	3 (43%)	1 (14%)

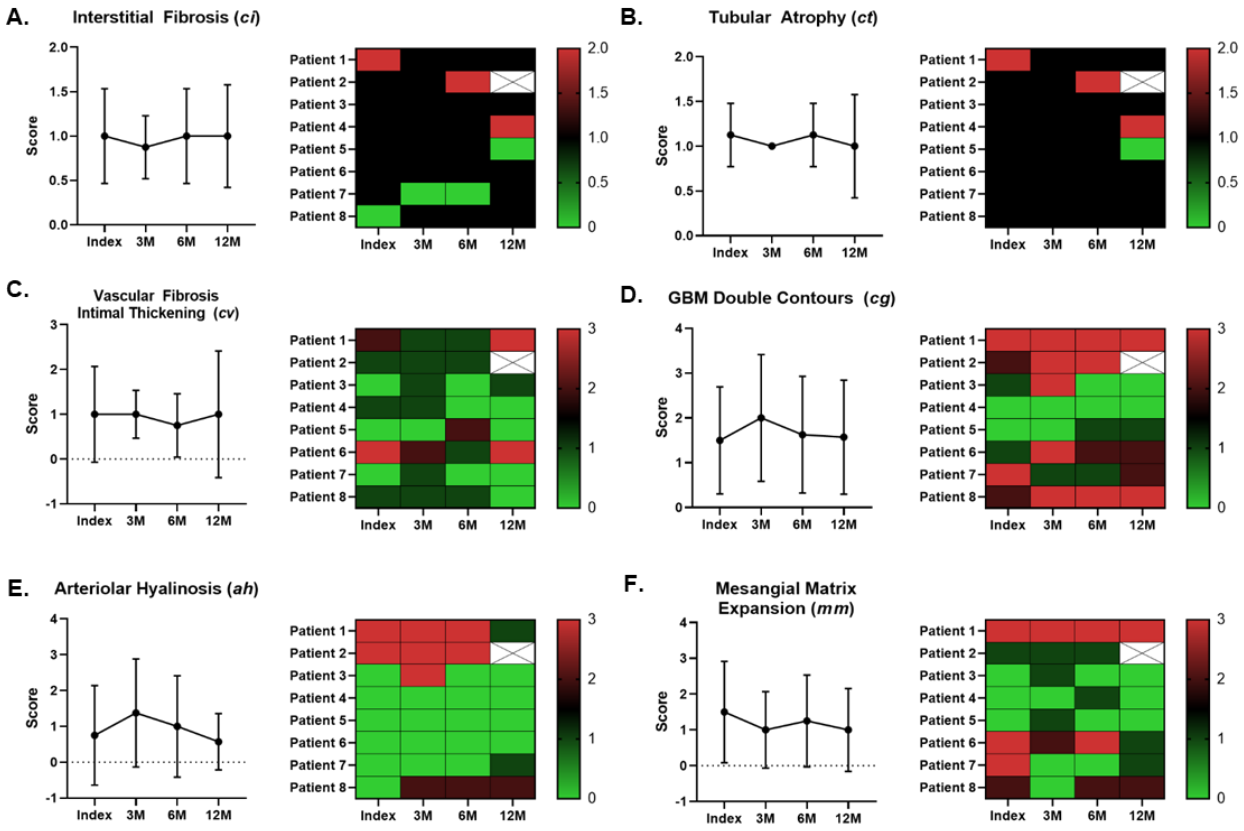
IVIG, intravenous immunoglobin

Figure S1: Kidney function in patients treated for refractory cABMR



Kidney function was assessed by serum creatinine (SCr) (A), blood urea nitrogen (BUN) (B), estimated glomerular filtration rate (eGFR) (C) and urine protein creatinine ratio (UPC) (D). There were no significant differences between SCr (p=0.6), BUN (p=0.7), eGFR (p=0.6) and UPC (p=0.8) at index, three month, six month, and 12 month follow up biopsy. Patient 2 lost their graft within one year after index biopsy so missing lab values are designated by an X. The most current SCr and eGFR are shown in the "2020" column. The most recent eGFR was not significantly different from values from index biopsy (p=0.2), but SCr trended higher (p=0.06). Patient 1 lost their allograft ~17 months after index biopsy. Data were assessed for normality using the D'Agostino and Pearson test, then analyzed using the ANOVA mixed-effects analysis. Data are expressed as the mean ± SD.

Figure S2: Chronic allograft pathology in the first year after diagnosis of cABMR



Graft pathology was assessed using the Banff 2017 criteria at index, three month, six month, and 12 month follow up biopsies. There were no significant differences in the chronicity scores *ci* (p=0.9), *ct* (p=0.8), *cv* (p=0.7), *cg* (p=0.8), *ah* (p=0.6), or *mm* (p=0.8) between biopsies. Missing values for Patient 2 are due to graft loss and are designated with an X. Data were assessed for normality using the D'Agostino and Pearson test, then analyzed using the ANOVA mixed effects test or Friedman test. Follow up analysis was conducted using the Wilcoxon test. Data are expressed as the mean ± SD.

Supplementary References

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