A Randomized Trial of Anti-Interleukin-6 Antibody Clazakizumab in Late Antibody-Mediated Kidney Transplant Rejection

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Supplementary Table 1. Single lesions and molecular classifiers at baseline.

Parameter	Total (n=20)	Clazakizumab (n=10)	Placebo (n=10)
Morphological ABMR lesions and scores			
Glomerulitis (g score ≥1), n (%)[a]	17 (89.5)	8 (80)	9 (100)
g score, median (IQR)	2 (1-3)	2 (1-3)	3 (1-3)
Peritubular capillaritis (ptc score ≥1), n (%)[b]	13 (68.4)	6 (60)	7 (77.8)
ptc score, median (IQR)	2 (0-2)	2 (0-2)	2 (1-3)
Transplant glomerulopathy (cg score ≥1), n (%)	17 (89.5)	8 (80)	9 (100)
cg score, median (IQR)	3 (2-3)	3 (1-3)	3 (3-3)
C4d in peritubular capillaries (C4d score ≥1), n (%)	7 (35)	4 (40)	3 (30)
C4d score, median (IQR)	0 (0-2)	0 (0-2)	0 (0-1)
MLPTC, n (%)[c]	6 (42.9)	2 (25)	4 (66.7)
High-grade MLPTC, n (%)[c]	2 (14.3)	1 (12.5)	1 (16.7)
Interstitial fibrosis (ci score ≥1), n (%)	17 (85)	8 (80)	9 (90)
ci score, median (IQR)	2 (1-3)	2 (1-2)	2 (1-3)
Tubular atrophy (ct score ≥1), n (%)	17 (85)	8 (80)	9 (90)
ct score, median (IQR)	1 (1-2)	1 (1-2)	2 (1-2)
Vascular fibrous intimal thickening (cv score ≥1), n (%)[d]	15 (88.2)	6 (75)	9 (100)
cv score, median (IQR)	1 (1-2)	1 (0-2)	2 (1-2)

Molecular classifiers of rejection and injury			
ABMR score, median (IQR)	0.65 (0.35-0.81)	0.70 (0.48-0.81)	0.44 (0.29-0.82)
TCMR score, median (IQR)	0.01 (0.01-0.18)	0.01 (0.01-0.01)	0.01 (0.01-0.02)
'all Rejection' score, median (IQR)	0.69 (0.43-0.83)	0.73 (0.44-0.84)	0.53 (0.42-0.80)
Atrophy/Fibrosis score, median (IQR)	0.68 (0.35-0.84)	0.39 (0.24-0.78)	0.79 (0.67-0.87)

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; IQR, interquartile range; MFI, mean fluorescence intensity; MLPTC, multilayering of peritubular capillary basement membranes; TCMR, T cell mediated rejection.

[a]For 1 patient (placebo group), biopsy material was not sufficient for g and cg scoring.

[b]For 1 patient (placebo group), biopsy material was not adequate for ptc scoring.

[c]Material for ultrastructural analysis of peritubular capillaries was available for 14 recipients (clazakizumab: n=8, placebo: n=6).

[d]cv score was not available for 2 recipients in the clazakizumab arm and 1 in the placebo arm (biopsy material inadequate for complete lesion scoring).

Supplementary Table 2. Baseline immunosuppression.

Parameter	Total (n=20)	Clazakizumab (n=10)	Placebo (n=10)
Initial immunosuppression			
Induction with antithymocyte globulin, n (%)	6 (30)	3 (30)	3 (30)
Induction with Interleukin-2 receptor antibody, n (%)	8 (40)	5 (50)	3 (30)
Tacrolimus, n (%)	9 (45)	4 (40)	5 (50)
Cyclosporine A, n (%)	10 (50)	5 (50)	5 (50)
Everolimus, n (%)	1 (5)	1 (10)	0
Azathioprine, n (%)	2 (10)	1 (10)	1 (10)
Mycophenolic acid derivatives, n (%)	17 (85)	8 (80)	9 (90)
Peri-transplant immunoadsorption, n (%)[a]	5 (25)	3 (30)	2 (20)
CDC crossmatch conversion before transplantation, n (%)	3 (15)	2 (20)	1 (10)
Immunsosuppression at the time of study inclusion			
Triple immunosuppression	18 (90)	9 (90)	9 (90)
Dual immunosuppression without steroids	2 (10)	1 (10)	1 (10)
Immunosuppressants			
Tacrolimus, n (%)	13 (65)	6 (60)	7(70)
Trough level (ng/mL), median (IQR)	6.0 (5.2-7.1)	5.6 (4.3-7.4)	6.0 (5.6-7.0)
Cyclosporine A, n (%)	6 (30)	4 (40)	2 (20)
Trough level (ng/mL), median (IQR)	123 (103-152)	138 (115-170)	85, 114

Everolimus, n (%)	1 (5)	0	1(10)
Trough level (mg/mL)	5.4	-	5.4
MMF, n (%)	10 (50)	6 (60)	4 (40)
Daily dose (mg), median (IQR)	1,250 (938-2,000)	1,250 (938-2,000)	1,250 (625-1,875)
EC-MPA, n (%)	10 (50)	4 (40)	6 (60)
Daily dose (mg), median (IQR)	720 (360-1,170)	1,260 (810-1,440)	450 (315-720)
Prednisolone, n (%)	15 (75)	8 (80)	7 (70)
Daily dose (mg), median (IQR)	5 (5-5)	5 (5-5)	5 (5-5)
Methylprednisolone, n (%)	3 (15)	1 (10)	2 (20)
Daily dose (mg), median (IQR)	4 (4-4)	4	4, 4

CDC, complement-dependent cytotoxicity; EC-MPA, enteric-coated mycophenolic acid; IQR, interquartile range; MMF, mycophenolate mofetil.

[a]Following our local standard, sensitized patients (until 2009: ≥40% CDC-PRA; since 2009: preformed DSA) were subjected to an earlier detailed protocol of peri-transplant immunoadsorption[1].

Supplementary Table 3. Adverse events by system organ class.

Adverse events	Part A[a]		Part B
	Clazakizumab (n=10)	Placebo (n=10)	Clazakizumab (n=19)[b]
Number of adverse events	50	44	129
Number of serious adverse events	3	1	9
Number (%) of patients with one or more adverse events	10 (100)	10 (100)	18 (90)
Number (%) of patients with one or more serious adverse events	3 (30)	1 (10)	7 (35)
Adverse events, number (%) of patients			
Infections and infestations	**5 (50)**	**8 (80)**	**13 (68.4)**
Upper respiratory tract infection	5 (50)	6 (60)	8 (42.1)
Urethritis and/or cystitis	1 (10)	2 (20)	3 (15.8)
Bronchitis	0	0	4 (21.1)
Herpes simplex	0	0	3 (15.8)
Pneumonia	0	0	2 (10.5)
Aseptic meningitis	0	0	1 (5.3)
Coxsackie viral infection	0	0	1 (5.3)
Ovarian abscess	0	0	1 (5.3)
Pyelonephritis	0	0	1 (5.3)
Skin papilloma	0	0	1 (5.3)
Gastrointestinal disorders	**5 (50)**	**7 (70)**	**11 (57.9)**

Diarrhoea	4 (40)	3 (30)	6 (31.6)
Abdominal pain	2 (20)	3 (30)	0
Gastroenenteritis	2 (20)	0	2 (10.5)
Nausea and/or vomiting	0	2 (20)	2 (10.5)
Diverticulitis	1 (10) [c]	0	1 (5.3) [d]
Dyspepsia	0	0	2 (10.5)
Aphtous ulcer	1 (10)	0	1 (5.3)
Dry mouth	0	0	1 (5.3)
Gastritis	0	1 (10)	0
Haemorrhoids	0	0	1 (5.3)
Noninfective gingivitis	0	0	1 (5.3)
Pancreatic enzyme abnormality	0	0	1 (5.3)
General disease and administration site conditions	**3 (30)**	**5 (50)**	**10 (52.6)**
Oedema	2 (20)	3 (30)	6 (31.6)
Injection site reactions	1 (10)	0	3 (15.8)
Fatigue	0	1 (10)	2 (10.5)
Malaise	1 (10)	0	1 (5.3)
Pyrexia	0	1 (10)	0
Skin and subcutaneous disorders	**1 (10)**	**2 (20)**	**8 (42.1)**
Erysipela	0	0	2 (10.5)
Alopecia	0	1 (10)	1 (5.3)

Eczema	0	1 (10)	1 (5.3)
Pruritus	1 (10)	1 (10)	0
Blister	0	0	1 (5.3)
Dry skin	0	0	1 (5.3)
Hirsutism	0	0	1 (5.3)
Impetigo	0	0	1 (5.3)
Intertrigo	0	0	1 (5.3)
Rash	1 (10)	0	0
Vascular disorders	**2 (20)**	**4 (40)**	**5 (26.3)**
Accelerated hypertension	2 (20)	3 (30)	3 (15.8)
Hypotension	0	1 (10)	1 (5.3)
Deep vein thrombosis	1 (10)	0	0
Thrombophlebitis	0	0	1 (5.3)
Musculoskeletal and connective tissue disorders	**4 (40)**	**2 (20)**	**4 (21.1)**
Musculoskeletal pain	2 (20)	1 (10)	3 (15.8)
Muscle cramps	3 (30)	1 (10)	1 (5.3)
Tenosynovitis	0	0	1 (5.3)
Nervous system disorders	**0**	**4 (40)**	**5 (26.3)**
Headache	0	3 (30)	4 (21.1)
Numbness	0	1 (10)	1 (5.3)
Respiratory, thoracic and mediastinal disorders	**2 (20)**	**1 (10)**	**4 (21.1)**

Dyspnea	1 (10)	1 (10)	2 (10.5)
Cough	0	0	3 (15.8)
Chest pain	0	0	1 (5.3)
Epistaxis	0	0	1 (5.3)
Nasal dryness	1 (10)	0	0
Pleural effusion	1 (10)	0	1 (5.3)
Small airway disease	1 (10)	0	0
Blood and lymphatic system disorders	**0**	**2 (20)**	**2 (10.5)**
Anemia	0	2 (20)	1 (5.3)
Leukopenia	0	1 (10)	1 (5.3)
Injury poisoning and procedural complications	**1 (10)**	**2 (20)**	**1 (5.3)**
Transplant biopsy complication[e]	1 (10)	2 (20)	0
Traumatic bone or joint injury	0	0	1 (5.3)
Cardiac disorders	**2 (20)**	**0**	**1 (5.3)**
Atrial fibrillation	1 (10)	0	1 (5.3)
Bradycardia	1 (10)	0	0
Palpitations	1 (10)	0	0
Psychiatric disorders	**0**	**0**	**3 (15.8)**
Sleep disturbance	0	0	3 (15.8)
Renal and urinary disorders	**1 (10)**	**1 (10)**	**1 (5.3)**
Acute renal injury	1 (10)	0	0

Aggravated proteinuria	0	1 (10)	0
Dysuria	0	0	1 (5.3)
Surgical and medical procedures	**1 (10)**	**0**	**2 (10.5)**
Mole excision	1 (10)	0	0
Nasal septum operation	0	0	1 (5.3)
Permanent thorax cavity drainage	0	0	1 (5.3)
Pleurodesis	0	0	1 (5.3)
Eye disorders	**0**	**0**	**2 (10.5)**
Ocular infection	0	0	2 (10.5)
Ear and labyrinth disorders	**0**	**1 (10)**	**0**
Ear pain	0	1 (10)	0
Otitis media	0	1 (10)	0
Metabolism and nutrition disorders	**0**	**0**	**1 (5.3)**
Folate deficiency	0	0	1 (5.3)
Hyperkalemia	0	0	1 (5.3)
Reproductive system and breast disorders	**0**	**0**	**1 (5.3)**
Pelvic pain	0	0	1 (5.3)

[a]Differences between groups (part A) were nonsignificant.

[b]One patient was withdrawn from the trial in part A was not included in the safety analysis of part B.

[c]Diverticulitis resolved after percutaneous abscess drainage and antibiotic therapy.

[d]Diverticulitis was complicated by colon perforation requiring open surgery (Hartmann's procedure).

[e]Transplant biopsy complications included skin hematoma, peritransplant hematoma, and arteriovenous fistula.

Supplementary Table 4. Diverticular disease and complications

Screening ID	Diverticulitis	Clazakizumab	Colon perforation	Diverticulosis	Assessment	Gender	Age	Renal disease	Yrs from Tx	Tx No.	Immunosuppression after inclusion
#001_Vienna	yes	2 doses	no	yes	CT	male	39	IgA nephropathy	9.9	1	CyA, MMF, Pred
#002_Vienna	no	-	-	no	CT	male	36	Postrenal cause	3.0	7	Tac, EC-MPA, Pred
#003_Vienna	no	-	-	no	MRI	female	41	Unknown	11.4	1	Tac, EC-MPA, Pred
#004_Vienna	no	-	-	no	Colonoscopy	female	37	Pyelonehritis	16.5	1	CyA, EC-MPA, Pred
#005_Vienna	no	-	-	no	CT	female	38	Goodpasture	11.3	1	Everolimus, EC- MPA, Pred
#008_Vienna	no	-	-	yes	CT	female	62	Unknown	18.9	1	CyA, EC-MPA, Pred
#009_Vienna	no	-	-	yes	CT	male	43	FSGN	4.8	2	Tac, MMF, Pred
#010_Vienna	no	-	-	yes	Colonoscopy	male	67	Unknown	17.4	4	CyA, EC-MPS, Pred
#011_Vienna	no	-	-	yes	CT	female	59	Chronic GN	3.1	2	Tac, MMF, Pred
#012_Vienna	no	-	-	yes	CT	female	60	Pyelonephritis	18.2	1	Tac, EC-MPA, Pred
#013_Vienna	no	-	-	no	CT	female	62	Unknown	4.2	4	Tac, MMF, Pred
#014_Vienna	no	-	-	yes	CT	male	60	Diabetic NP	1.6	1	Tac, MMF, Pred
#001_Berlin	no	-	-	-	no imaging	female	29	RPGN	11.4	1	CyA, MMF, Methypred
#015_Vienna	no	-	-	yes	CT	female	29	Postrenal cause	15.4	1	Tac, MMF, Pred
#016_Vienna	no	-	-	no	Colonoscopy	female	27	RPGN	24.1	1	Tac, EC-MPA,Pred
#002_Berlin	no	-	-	-	no imaging	male	41	IgA nephropathy	9.4	1	Tac, EC-MPA, Methypred
#017_Vienna	yes	4 doses	yes	yes	CT	male	62	PKD	3.9	2	Tac, MMF, Pred
#018_Vienna	no	-	-	no	Colonoscopy	male	42	IgA nephropathy	7.1	3	Tac, EC-MPA, Pred
#003_Berlin	no	-	-	-	no imaging	male	31	IgA nephropathy	6.8	1	Tac, MMF, Methypred
#004_Berlin	no	-	-	-	no imaging	male	52	Unknown	14.8	1	CyA, MMF

CT, computer tomography; CyA, cyclosporine A; EC-MPA, enteric-coated mycopehnoloc acid; GN, glomerulonephritis; IgA, immunoglobulin A; Methylpred, methylprednisolone; MMF, mycophenolate mofetil; MRI, magnetic resonance imaging; NP, nephropathy; PKD, polycystic kidney disease; Pred, prednisolone; RPGN, rapid progressive glomerulonephritis; Tac, tacrolimus.

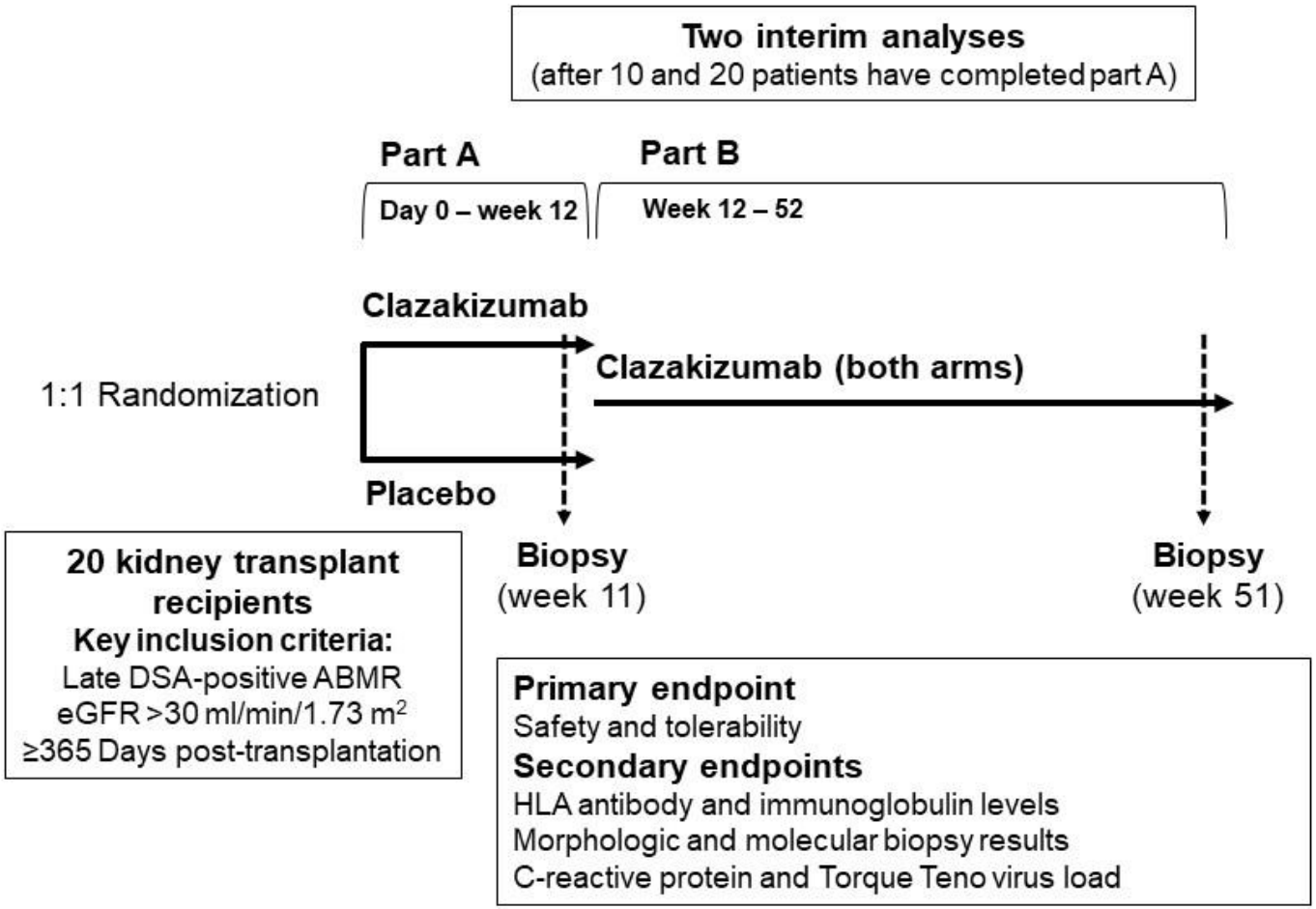
Supplementary Table 5. Resolution of ABMR activity in four recipients – Serial biopsy results

Screening ID (Randomization - Part A)	**Biopsy**	**ABMR category (Banff 2017)**	**Banff single lesion scores**				**ABMR score (MMDx)**
			g	**ptc**	**cg**	**c4d**	
#004_Vienna (Clazakizumab)	Index	Chronic active	2	0	3	2	0.35
	Week 11	Chronic active	2	0	3	0	0.21
	Week 51	Chronic[a]	1	0	3	0	0.17
#008_Vienna (Placebo)	Index	Chronic active	-[b]	3	-[b]	0	0.29
	Week 11	Chronic active	2	1	3	0	0.25
	Week 51	Chronic[a]	1	0	3	0	0.14
#0018_Vienna (Placebo)	Index	Chronic active	1	1	3	0	0.41
	Week 11	Chronic active	0	2	2	0	0.34
	Week 51	Chronic[a]	1	0	2	0	0.08
#004_Berlin (Clazakizumab)	Index	Chronic active	2	0	3	0	0.73
	Week 11	Chronic active	1	1	2	0	0.86
	Week 51	Chronic[a]	1	0	3	0	0.23

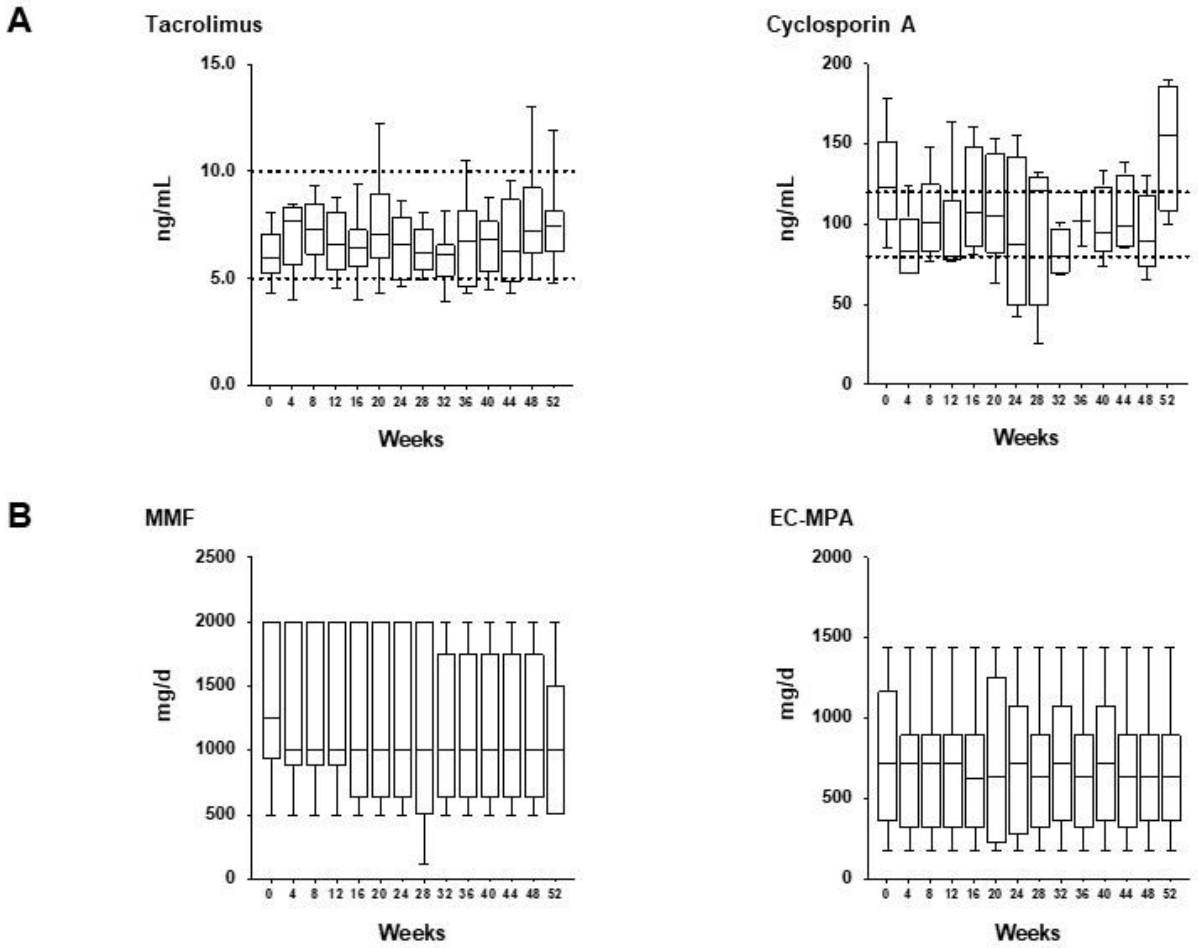
ABMR, antibody-mediated rejection; cg, transplant glomerulopathy; g, glomerulitis; MMDx, molecular microscope diagnostic system; ptc, peritubular capillaritis.

[a]According to the Banff 2017 update[2], the term “chronic ABMR” was applied for cg without evidence of current/recent antibody interaction with the endothelium, but with a prior documented diagnosis of chronic active ABMR.

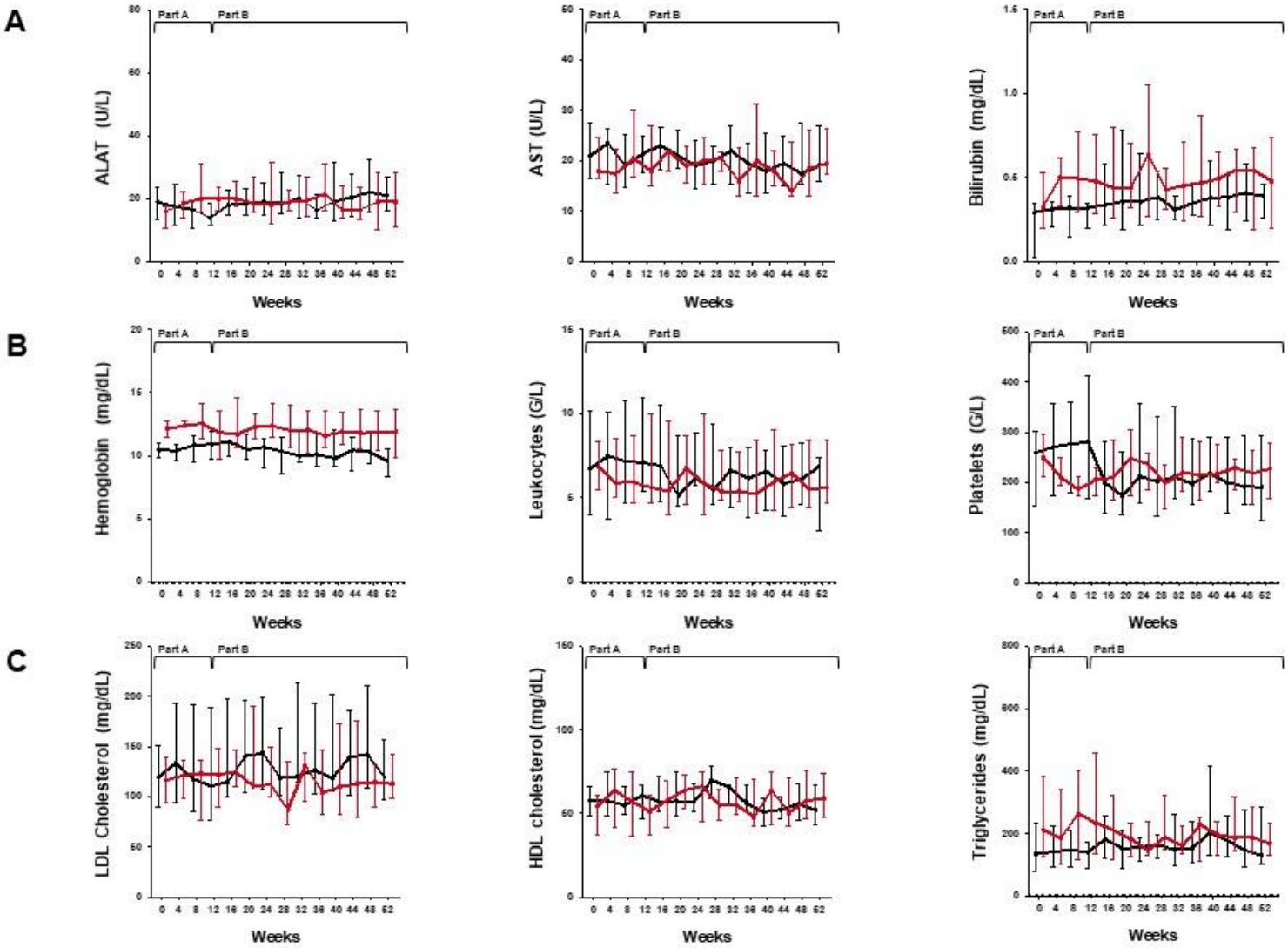
[b]For one patient (#008_Vienna), index biopsy material was not sufficient for g and cg scoring.



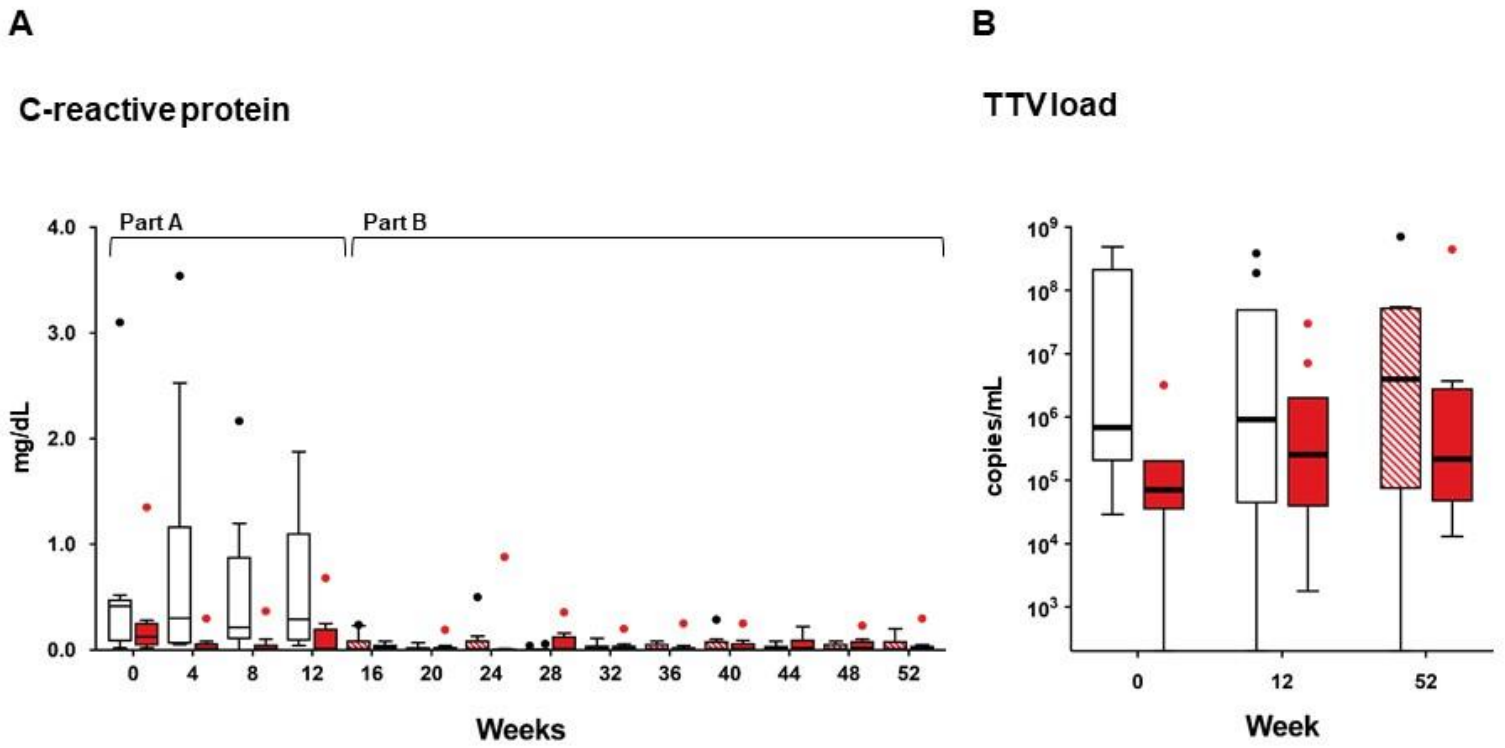
Supplemental Figure 1. Summary of trial protocol. ABMR=antibody-mediated rejection; DSA=donor-specific antibody, eGFR=estimated glomerular filtration rate; HLA=human leukocyte antigen.



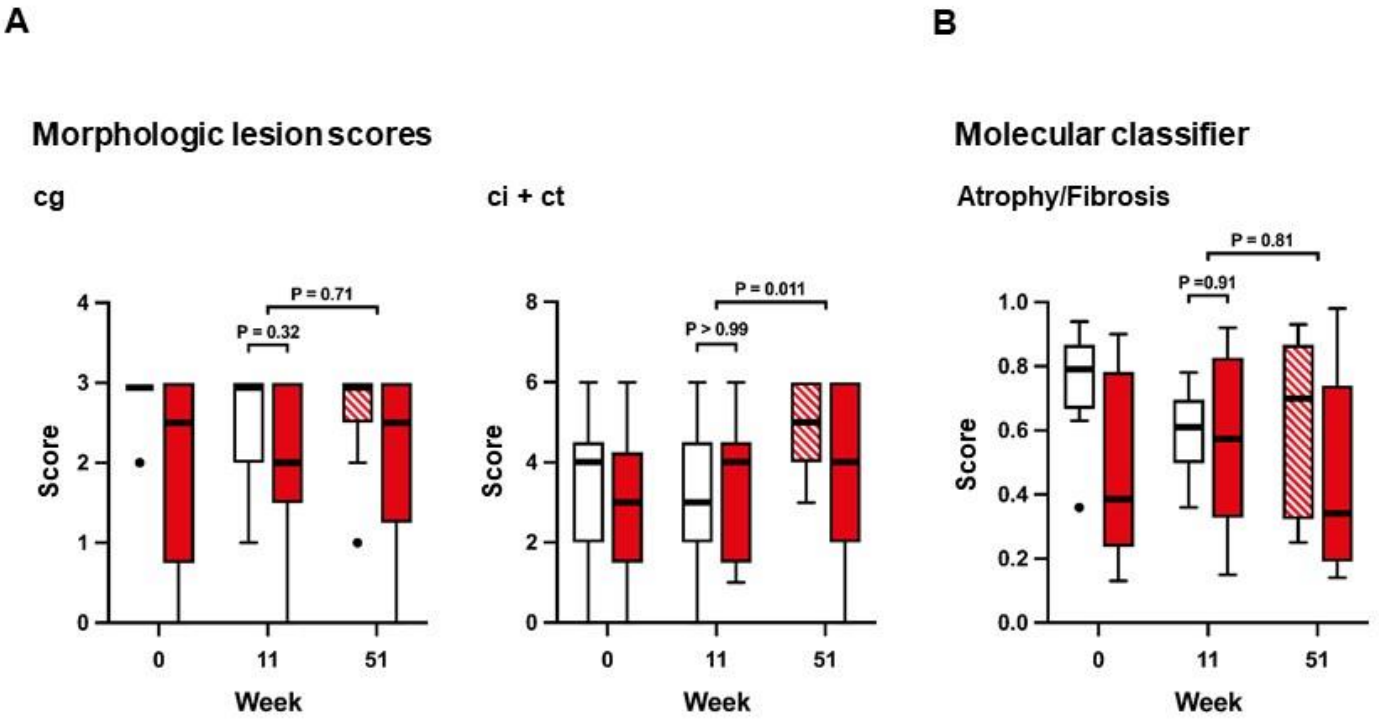
Supplemental Figure 2. Immunosuppression levels and dosage. Shown are (A) calcineurin inhibitor trough levels and (B) dosages of mycophenolate mofetil (MMF) and enteric-coated mycophenolic acid (EC-MPA). Horizontal dashed lines indicate the target range of trough levels.



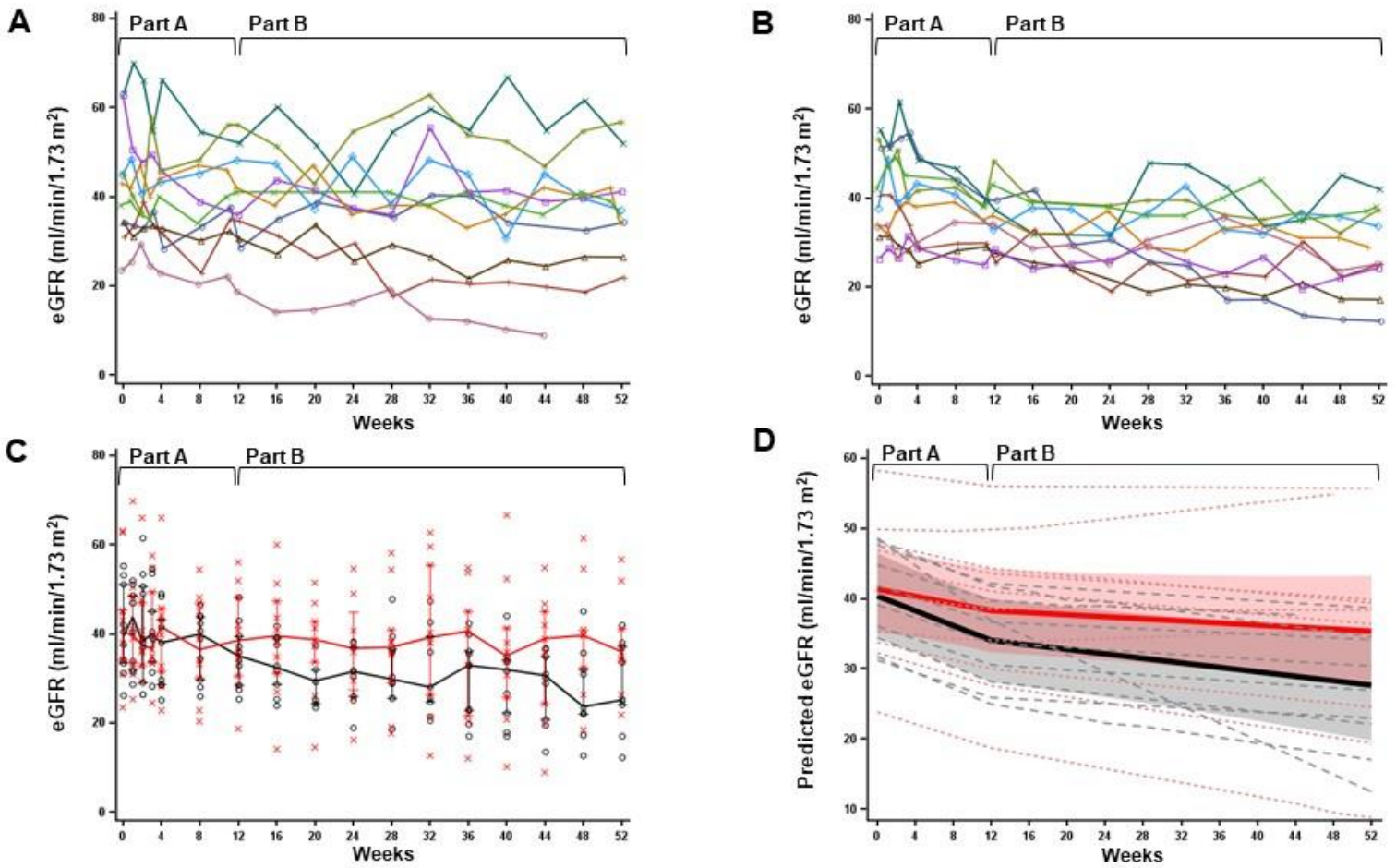
Supplemental Figure 3. Safety lab. Shown are median (interquartile range) levels of (A) liver parameters, (B) hematologic variables and (C) lipids (randomization in part A: placebo: black lines and bars; clazakizumab: red lines and bars). ALT = alanine aminotransferase; AST = aspartate aminotransferase; HDL = high-density lipoprotein; LDL = low-density lipoprotein.



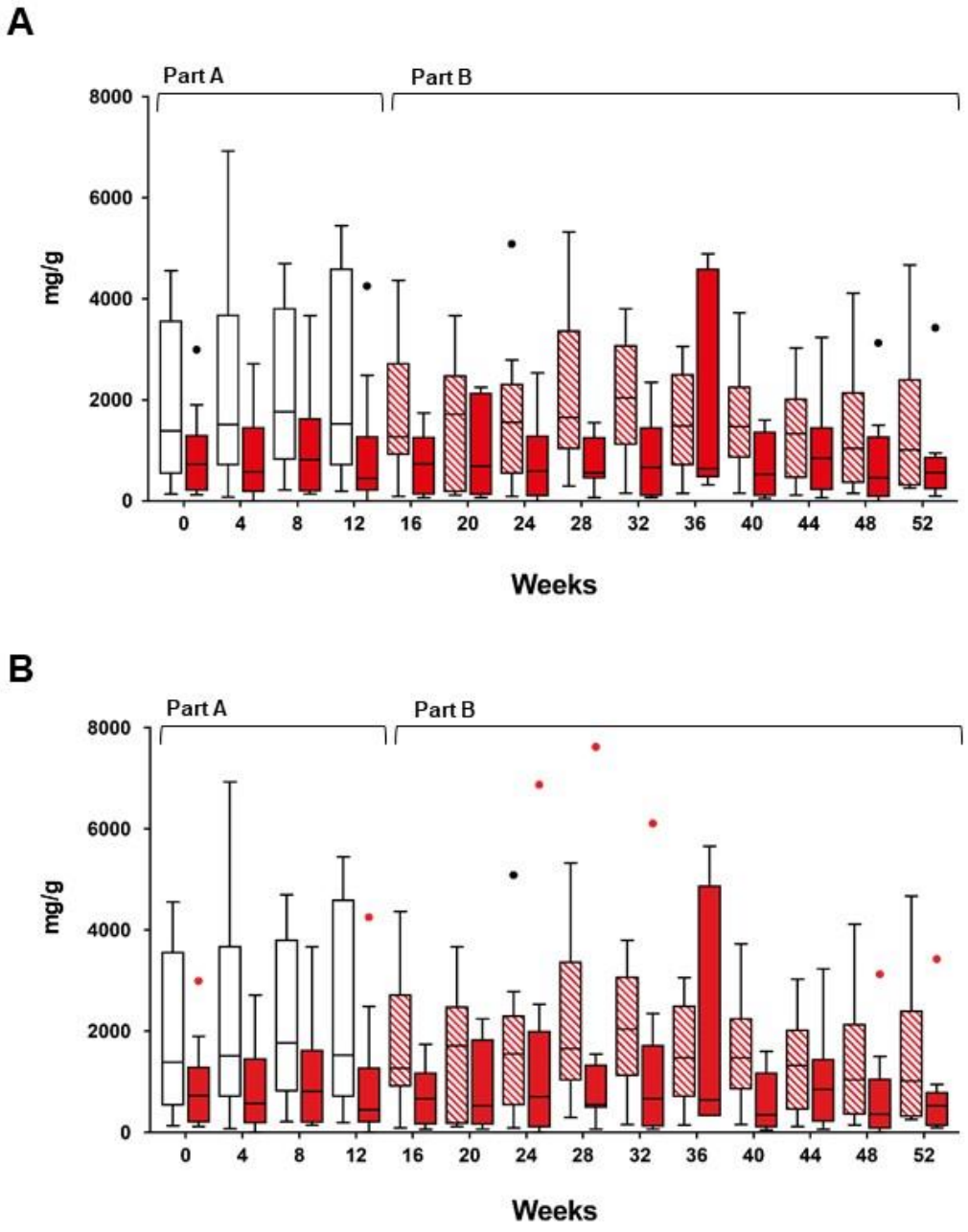
Supplemental Figure 4. C-reactive protein and Torque Teno Virus (TTV) levels. Shown are the levels of (A) C-reactive protein and (B) TTV viral load in patients randomized to clazakizumab (red closed boxplots) versus placebo (part A: open boxplots; part B: red hatched boxplots).



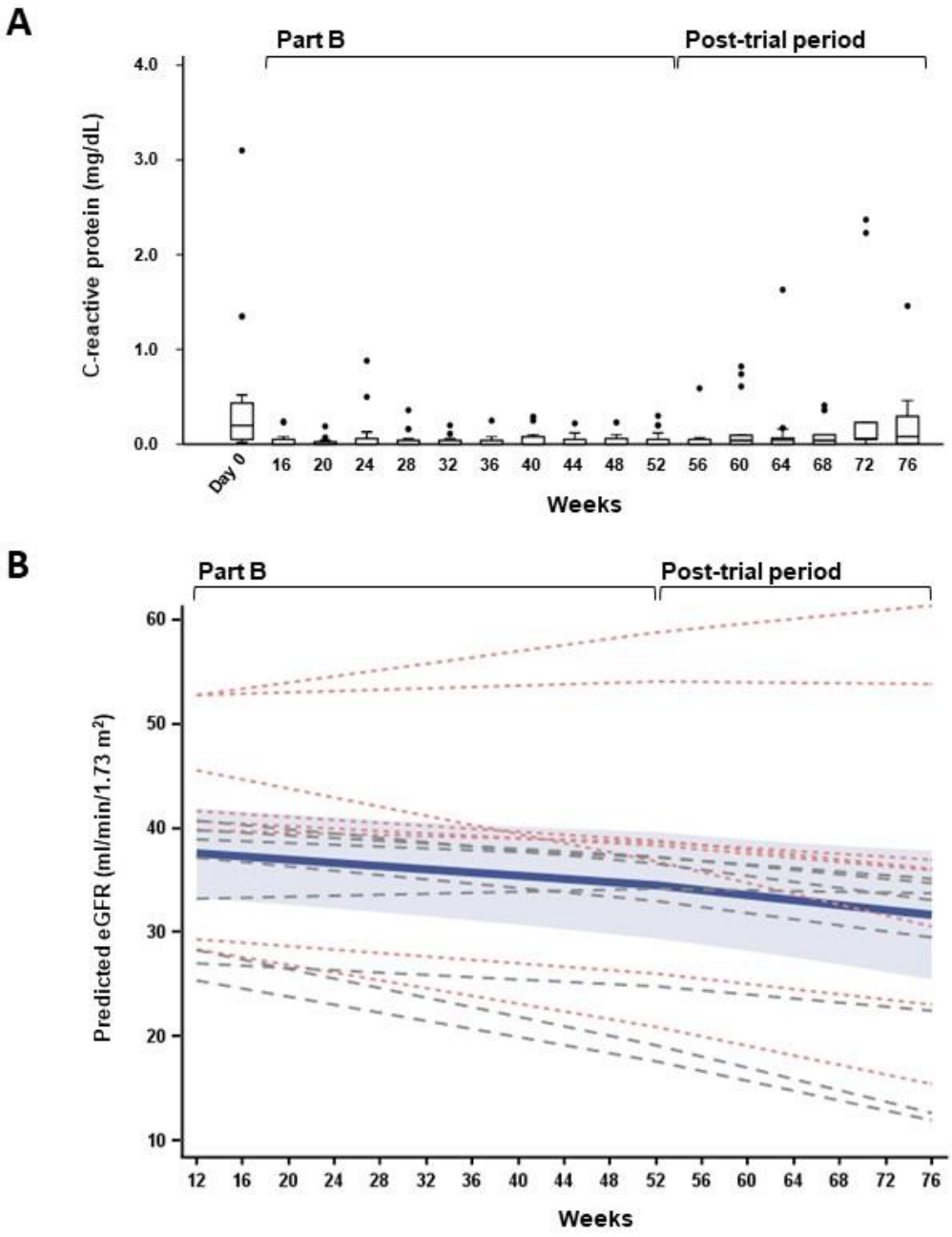
Supplemental Figure 5. Morphologic and molecular features of chronic injury. Morphologic (cg, ci+ct) and molecular (atrophy/fibrosis) scores are shown for patients randomized to clazakizumab (red closed boxplots) versus placebo (part A: open boxplots; part B: red hatched plots). We applied unpaired Mann Whitney U test for group comparisons (clazakizumab vs. placebo) at the end of part A and paired Wilcoxon test to evaluate changes under clazakizumab (overall cohort) in open-label extension (part B).



Supplemental Figure 6. Analysis of renal function including two patients withdrawn from the trial. The individual course of estimated glomerular filtration rate (eGFR) is shown for patients allocated to receive clazakizumab (A) versus placebo (B). Panel C shows median, interquartile range and individual levels of eGFR (clazakizumab: red line, bars and asterisks; placebo: black lines, bars and circles). Panel D shows individual (dashed lines) and mean eGFR slopes (solid lines; shaded areas represent 95% confidence intervals) in relation to treatment in part A (clazakizumab: red lines; placebo: black lines). In part A, the mean slope of eGFR differed significantly between groups (P=0.028). In part B, patients switched from placebo to clazakizumab showed a significant improvement in the eGFR slope (P<0.001), while inter-group differences became non-significant (P=0.40).



Supplemental Figure 7. Spot urine protein/creatinine ratio. Shown are the median levels of spot urine protein/creatinine ratio for patients randomized to clazakizumab (red closed boxplots) versus placebo (part A: open boxplots; part B: red hatched plots). Results are shown for patients on active treatment (A) and for all randomized patients including the two patients who were withdrawn from the trial because of serious adverse events (B).



Supplemental Figure 8. C-reactive protein and renal function after the end of the trial. Boxplots in panel A show levels of C-reactive protein at day 0, during part B (clazakizumab in all patients), and over 6 months after the last trial visit. Panel B shows individual (dashed lines) and mean estimated glomerular filtration (eGFR) slopes (solid line; shaded areas represent 95% confidence intervals) calculated for part B and the 6-month post-trial period (the two patients withdrawn from the trial and another two recipients for whom only a single eGFR value was available for the post-trial period were excluded from the analysis).

References

1. Schwaiger, E, Eskandary, F, Kozakowski, N, Bond, G, Kikic, Z, Yoo, D, et al.: Deceased donor kidney transplantation across donor-specific antibody barriers: predictors of antibody-mediated rejection. *Nephrol Dial Transplant,* 31**:** 1342-1351, 2016

2. Haas, M, Loupy, A, Lefaucheur, C, Roufosse, C, Glotz, D, Seron, D, et al.: The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant,* 18**:** 293-307, 2018