Supplementary Appendix

A Phase 2 Experimental Medicine Randomised Placebo Controlled Trial of Belimumab in Kidney Transplantation (BEL114424)

Gemma D. Banham*, Shaun M. Flint*, Nicholas Torpey, Paul A. Lyons, Don N. Shanahan, Adele Gibson, Christopher J.E. Watson, Ann-Marie O'Sullivan, Joseph A. Chadwick, Katie E. Foster, Rachel B. Jones, Luke R. Devey, Anna Richards, Lars-Peter Erwig, Caroline O. Savage, Kenneth G.C. Smith, Robert B. Henderson*, Menna R. Clatworthy* (*authors contributed equally to this work)

Contents:

BEL114424 eligibility criteria and study protocol

The BEL114424 study protocol, including full eligibility criteria is included as a separate file and will also be published at www.gsk-clinicalstudyregister.com.

The original protocol excluded donation after circulatory death (DCD) donors aged > 60 years old, or > 50 if they had died from a stroke, had a history of high blood pressure, or had a serum creatinine greater than 135µmol/L at the time of donation (i.e. extended criteria DCDs). An amendment to widen eligibility criteria was sought (protocol amendment 4, dated 16 October 2013), with the aim of increasing recruitment. The reasons for subjects not meeting inclusion criteria are listed in Table S1.

Table S1: Reasons for subjects not meeting inclusion criteria.

Reason not meeting inclusion criteria	Number
Donor characteristic	36
Alternative immunosuppression planned	21
0-0-0 mismatch	16
HLAi or ABOi transplant	15
Other disease/condition judged unsuitable by PI	12
Prior therapy	8
Transplant other than kidney	3
Recipient hepatitis	3
Donor hepatitis	2
Recipient unable to consent due to learning difficulties	2
Recipient HIV+	2
Drug sensitivity	1
Prior malignancy	1
Poor venous access	1
Total	**123**

Visit windows

Assessments were assigned to visit windows (slots) according to the date of assessment (for study schedule see study protocol).

For adverse events the on-treatment phase commenced from the start of the first infusion of belimumab/placebo and ended 28 days after the last dose. The post-treatment phase began the following day. For other primary and secondary endpoints, the on-treatment phase ended 35 days after the last dose to allow incorporation of the On-Trt Week 24 visit on day 168 +/- 7 days.

The visit slotting intervals for the Pre-Treatment and On-Treatment (On-Trt) phases for biomarker endpoints were as follows:

Week	Day relative to first infusion	Day	Visit Window
Baseline	≤0	≤0	≤0
On-Trt Week 4	28	28 - 27 Days or + 14 Days	Days 1 to 42
On-Trt Week 8	56	56 - 13 Days or + 14 Days	Days 43 to 70
On-Trt Week 12	84	84 - 13 Days + 42 Days	Days 71 to 126
On-Trt Week 24	168	168 - 41 Days +7 Days	Days 127 to 175

The visit slotting intervals for the Post-Treatment (Post-Trt) phase for biomarker endpoints were as follows:

Week	Day relative to (last infusion + 35 Days)	Day relative to last infusion	Day	Visit Window
Post-Trt Week 12	84	119	84 – 83 Days or + 56 Days	Days 1 to 140
Post-Trt Week 28	196	231	196 – 55 Days or + 154 Days	Days 141 to 350

If more than one assessment fell within the same visit window, the assessment nearest to the scheduled time point was used. If two assessments were equally close, the latter was used. Samples for transcriptomic and protoarray analysis were collected at baseline, at week 24 (+/- 7 days) and at week 52 (+/- 7 days) according to the study schedule, irrespective of the last dose of study drug given, so data for these endpoints are presented without window fitting. On-treatment samples for the B cell stimulation assay were limited so the last available on-treatment result is displayed ('Last On-Trt').

Study objectives and endpoints

Objectives	Endpoints
Co-primary	
Efficacy: To estimate the change in naive B cells following belimumab 10 mg/kg (or placebo) in addition to standard of care immunosuppressants in renal transplant patients from the time of transplantation up to 24 weeks	Change from baseline in naïve B cells from baseline to Week 24.
Safety: To assess the safety and tolerability of belimumab 10 mg/kg (or placebo) in renal transplant patients in addition to standard of care immunosuppressants.	AEs and SAEs, including AEs of special interest (opportunistic infections, malignancies, hypersensitivity and infusion reactions, all cause mortality and suicidality) Incidence and severity of infections Change from baseline and number of subjects outside the normal range for blood pressure, heart rate, and temperature Change from baseline and number of subjects outside the normal range for clinical chemistry and haematology parameters, with particular attention to white blood cell count and immunoglobulin levels
Secondary	
To further assess the pharmacodynamic effect of belimumab in addition to standard of care immunosuppressants in renal transplant patients at 24 weeks	Percent change from baseline in memory B cells, Activated memory B cells, Transitional B cells
To assess the phenotype and tolerogenic profile of the repopulating B cells after belimumab therapy at Week 52.	
To further assess the potential for efficacy of belimumab in addition to standard of care immunosuppressants in reducing the incidence of renal allograft rejection using biomarker and clinical outcomes.	Activated T cells, regulatory T cells, and ratio of activated: regulatory T cells All HLA-specific and donor HLA-specific antibody levels Proportion of subjects with episodes of acute rejection Serum creatinine Estimated glomerular filtration rate (eGFR) Immunosuppressant/corticosteroid use
Exploratory	
To further assess the potential for efficacy of belimumab in addition to standard of care immunosuppressants in reducing the incidence of renal allograft rejection using exploratory biomarkers.	Exploratory endpoints may include analyses of transcriptomic signatures, non-HLA antibody profiles, cytokine profiles and additional leukocyte subsets
Other	
To determine immunogenicity and pharmacokinetic profile of belimumab, and to characterize genetic variability that may affect efficacy or safety endpoints in renal transplant patients.	Incidence, titers, and specificity of anti-belimumab antibodies Serum concentrations of belimumab Characterize genetic variability (e.g., HLA typing)

Statistical methods

There were no formal statistical hypotheses tested in this study. An estimation approach was used to investigate the magnitude of the difference in the selected primary and secondary endpoints following treatment with belimumab relative to placebo. Because the study included no prospectively defined formal hypothesis testing, no adjustment for multiplicity was required for the primary and secondary endpoints.

The primary and, where specified, secondary analyses utilised a mixed model for repeated measures (MMRM) approach with fixed categorical effects of treatment, visit, donor organ status (coded as live donor, donated after brain death, donated after circulatory death) and treatment-by-visit interaction and fixed continuous covariates of baseline values and baseline values-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments.

All post hoc analyses are labelled as such. Analyses were performed using SAS version 9.3 except for transcriptomic and protein microarray analyses, which were performed using R version 3.3.1.

Enabling work in transplant patients (n=93 renal transplant recipients at the Cambridge Transplant Unit (unpublished data)) found that naive B cell counts at baseline in transplant patients were similar to SLE patients (Phase III BLISS-76 study[1]) after removing the top 5th percentile of SLE patients with highest baseline counts. By adjusting the SLE data in this way, and assuming transplant patients will vary in a similar way to SLE patients a standard deviation (SD) of 63.24 cells/mm^3 was observed. Assuming a sample size of 10 per group, and mean difference of -59 cells/mm^3 (observed in BLISS-76) we predicted a 95% confidence interval around the mean difference of (-114.43, -3.57).

Interim re-estimations of sample size were performed in November 2014 and February 2015 to take into account drop-outs and inform the potential benefit and requirement for further study enrolment. An unblinded statistician examined the variability in naive B-cells and compared to BLISS-76, looking for trends over time with subject profile plots. The observed treatment difference of the change from baseline in naïve B cells at Week 8 was used to make a predictive probability statement about the probability of achieving success at Week 24 for a particular total sample size. The predictive probability assessment suggested that "the benefit of recruiting an additional 10 evaluable subjects (30 evaluable overall) on the outcome of the study was negligible"

Microarray analysis

Leukocyte subsets were purified from whole blood then lysed as previously described [2]. Lysates (CD4+ T-cell) and PAXgene tubes (whole blood) were stored at -80°C until required. RNA was extracted from CD4+ T-cell lysates using an AllPrep Kit (Qiagen) and from whole blood using the PAXgene system (Qiagen). RNA was processed and hybridised to Human Gene ST 2.1 microarrays (Affymetrix) according to the manufacturer's instructions.

Affymetrix CEL files were imported into R/Bioconductor, normalised and summarised at the transcript cluster level using the oligo package (rma function, target = "core"). Transcript clusters were annotated using Ensembl biomaRt and manufacturer's own annotation and Affymetrix control probes were removed. Non-protein coding probes were also removed.

Weighted gene coexpression network analysis (WGCNA) was undertaken of the whole blood gene expression data using the WGCNA package in R/Bioconductor. One of the resulting whole blood gene expression modules was identified as representing a B-cell transcriptional programme on the basis of a unique overlap of module genes with the Gene Ontology class 'GO:0050853 BCR signalling pathway' (Supplementary Figure 1L). Module expression was determined as the value of the first principal component calculated from module gene expression, along standard analysis lines for WGCNA and using functions in the WGCNA package.

Differential gene expression analyses were performed using the limma package in R/Bioconductor. The model incorporated time-point and treatment group interaction terms and accounted for the repeated measures nature of the analysis (study participants contributed gene expression samples at three different timepoints) using the duplicateCorrelation function. Contrasts were defined for differential expression at baseline, 24 weeks and 52 weeks (Belimumab – Placebo at each timepoint). Estimates were adjusted using the eBayes function.

Where there was more than one transcript cluster per gene, differential expression was summarised into a single gene measure using the mean log-fold change. Immunoglobulin coding genes were identified from their Ensembl Biotype annotation. Cell cycle genes were identified using the Gene Ontology class 'GO:0007049 Cell cycle'.

Table S2: Top 100 differentially expressed genes (by fold-change) in CD4+ T-cells comparing belimumab treated subjects and placebo at Week 24. P-values are unadjusted. Cell cycle column denotes whether a gene is annotated with GO:0007049 cell cycle in the Gene Ontology

		Week 0 (Baseline)		Week 24		Week 52		
Affymetrix ID	**Gene Symbol**	**log(2) FC**	**P-value**	**log(2) FC**	**P-value**	**log(2) FC**	**P-value**	**Cell cycle gene**
16996813	CD180	0.104	0.668	-1.081	0.018	0.615	0.125	
16844312	TOP2A	0.049	0.733	-1.032	0.028	-0.194	0.539	cell_cycle
17016403	HIST1H3G	-0.224	0.245	-0.973	0.110	-0.460	0.407	
16850477	TYMS	-0.014	0.915	-0.967	0.035	-0.215	0.503	cell_cycle
16976644	JCHAIN	0.106	0.651	-0.907	0.096	0.041	0.905	
16979515	CCNA2	-0.089	0.496	-0.895	0.073	-0.302	0.386	cell_cycle
16697471	B3GALT2	-0.332	0.283	-0.882	0.036	-0.445	0.225	
16977052	CXCL10	0.679	0.012	-0.882	0.225	1.433	0.034	
17019728	PLA2G7	0.044	0.912	-0.882	0.022	0.265	0.533	
16826230	NETO2	-0.037	0.730	-0.850	0.001	-0.252	0.333	
16877019	RRM2	0.136	0.274	-0.848	0.054	-0.277	0.358	cell_cycle
17075776	PBK	-0.011	0.918	-0.845	0.003	-0.159	0.416	cell_cycle
17101531	TLR7	0.264	0.271	-0.838	0.049	0.651	0.111	
16943181	GPR15	-0.595	0.074	-0.827	0.024	-0.629	0.086	
16912379	TPX2	0.033	0.827	-0.826	0.062	-0.427	0.106	cell_cycle
16965268	CD38	-0.129	0.204	-0.821	0.021	0.031	0.883	
16767335	CPM	0.045	0.796	-0.813	0.011	0.199	0.496	
16980096	TBC1D9	0.078	0.666	-0.804	0.018	0.413	0.301	
16986913	VCAN	0.082	0.844	-0.771	0.224	0.665	0.261	
16707468	KIF11	-0.003	0.985	-0.771	0.036	0.012	0.957	cell_cycle
16944618	CD86	0.221	0.356	-0.770	0.100	0.194	0.647	
16725227	MS4A14	-0.199	0.424	-0.770	0.017	0.340	0.376	
16968529	PTPN13	-0.257	0.188	-0.765	0.016	-0.339	0.133	
16851618	HRH4	-0.239	0.355	-0.764	0.043	-0.465	0.047	
16851397	RBBP8	0.064	0.713	-0.759	0.010	0.224	0.351	cell_cycle
16721835	WEE1	-0.049	0.768	-0.758	0.011	-0.447	0.062	cell_cycle
16801557	CCNB2	-0.116	0.379	-0.757	0.056	-0.341	0.311	cell_cycle
16677201	DTL	-0.081	0.319	-0.724	0.096	-0.194	0.483	cell_cycle
16843627	CCL3L3	-0.163	0.261	-0.714	0.093	0.200	0.460	
16719515	MKI67	-0.120	0.273	-0.706	0.043	-0.079	0.729	cell_cycle
16992744	FAM153B	-0.732	0.002	-0.704	0.012	-0.089	0.704	
16707551	CEP55	0.094	0.381	-0.700	0.033	-0.036	0.868	cell_cycle
16679349	KMO	0.290	0.203	-0.699	0.078	0.366	0.368	
17004518	LY86	-0.117	0.600	-0.696	0.054	0.351	0.345	
16696187	F5	-0.538	0.007	-0.693	0.009	-0.209	0.424	

16875997	ZNF154	-0.719	0.001	-0.693	0.003	-0.357	0.105	
16725041	FAM111B	-0.004	0.977	-0.692	0.100	-0.195	0.421	
16960577	P2RY12	0.168	0.318	-0.686	0.042	0.503	0.210	
16666509	IFI44	-0.065	0.809	-0.685	0.034	0.611	0.027	
16666326	ST6GALNAC3	0.157	0.225	-0.684	0.001	-0.099	0.723	
16852312	SKA1	-0.009	0.947	-0.683	0.048	-0.143	0.525	cell_cycle
16875467	LILRA4	-0.216	0.435	-0.681	0.270	-0.028	0.943	
16940172	CCR2	-0.315	0.127	-0.677	0.007	0.196	0.508	
16826160	SHCBP1	0.089	0.505	-0.669	0.015	0.041	0.850	
16840245	SCIMP	0.186	0.427	-0.664	0.090	0.197	0.617	
16665878	IL23R	-0.554	0.066	-0.652	0.066	-0.503	0.070	
17070229	ZC2HC1A	-0.096	0.638	-0.650	0.003	-0.059	0.718	
16928428	GRK3	0.063	0.801	-0.645	0.032	0.355	0.288	
16793225	DLGAP5	-0.121	0.191	-0.645	0.100	-0.297	0.230	cell_cycle
17094893	ALDH1A1	0.253	0.378	-0.644	0.168	0.686	0.228	
16747958	CLEC6A	0.468	0.221	-0.641	0.188	1.090	0.040	
17050350	LRRN3	-0.313	0.202	-0.640	0.089	-0.594	0.132	
16886105	HNMT	0.128	0.533	-0.636	0.091	0.540	0.161	
16663514	CDC20	-0.044	0.718	-0.635	0.037	-0.131	0.602	cell_cycle
16828886	GINS2	0.067	0.703	-0.624	0.094	-0.153	0.491	cell_cycle
16949625	CCDC50	-0.094	0.626	-0.621	0.051	-0.011	0.973	
16777685	FLT3	0.317	0.043	-0.620	0.096	0.503	0.263	
16789334	TDRD9	-0.292	0.288	-0.617	0.141	0.012	0.975	cell_cycle
16677425	CENPF	-0.147	0.200	-0.613	0.003	-0.148	0.331	cell_cycle
16671139	S100A9	0.340	0.334	0.609	0.331	0.705	0.165	
16813206	ANPEP	0.202	0.499	0.609	0.254	0.603	0.285	
16940444	CAMP	0.044	0.711	0.612	0.013	0.242	0.301	
16743816	PDGFD	0.386	0.145	0.619	0.015	0.002	0.996	
16872978	CD177	0.035	0.824	0.624	0.491	0.853	0.079	
16660360	CDA	0.348	0.198	0.636	0.267	0.473	0.371	
16983451	BASP1	0.144	0.473	0.636	0.314	0.646	0.205	
16672462	FCRL6	0.621	0.139	0.640	0.137	0.047	0.927	
16784760	DACT1	0.371	0.320	0.645	0.111	0.407	0.272	
16865540	FCAR	0.269	0.133	0.677	0.309	0.796	0.077	
17012582	ARG1	0.063	0.628	0.681	0.235	0.374	0.206	
16933766	OSM	0.271	0.086	0.689	0.014	0.150	0.450	
17052550	MGAM2	0.109	0.393	0.701	0.007	0.059	0.743	
16947173	MME	0.335	0.184	0.721	0.186	0.023	0.958	
16761617	MANSC1	0.076	0.576	0.723	0.056	0.275	0.448	
16819563	ADGRG3	0.314	0.093	0.728	0.265	0.442	0.358	
16686040	SVBP	0.697	0.004	0.740	0.009	0.663	0.011	
16695715	FCGR3B	0.386	0.083	0.743	0.105	0.613	0.185	
16747969	CLEC4D	0.378	0.055	0.744	0.324	1.162	0.052	
17101698	BMX	0.166	0.050	0.745	0.180	0.498	0.124	
16792859	PYGL	0.266	0.234	0.757	0.191	0.674	0.161	
16660436	ALPL	0.375	0.026	0.758	0.215	0.234	0.567	

16660059	PADI4	0.310	0.169	0.767	0.248	0.585	0.204
16820937	HP	-0.034	0.860	0.795	0.401	1.116	0.100
16681278	UTS2	0.729	0.062	0.805	0.097	0.668	0.146
16922759	KCNJ15	0.376	0.090	0.823	0.124	0.607	0.279
16998551	SLCO4C1	0.757	0.085	0.844	0.217	0.962	0.137
17052425	MGAM	0.549	0.044	0.850	0.275	0.633	0.328
16974529	FGFBP2	0.984	0.079	0.906	0.156	-0.129	0.847
16743686	MMP8	-0.086	0.809	0.914	0.398	1.323	0.068
16968213	ANXA3	0.129	0.530	0.932	0.235	0.853	0.130
16873501	PGLYRP1	0.187	0.181	0.947	0.140	0.649	0.173
16919547	SLPI	0.070	0.525	0.983	0.145	0.340	0.482
16956213	PROK2	0.376	0.254	0.984	0.109	0.330	0.553
17019877	CRISP3	0.174	0.421	0.999	0.352	0.896	0.120
16849400	SOCS3	0.314	0.320	1.022	0.005	0.485	0.173
17099463	ABO	0.680	0.225	1.030	0.101	0.410	0.454
16697370	PTGS2	0.384	0.332	1.041	0.153	0.361	0.590
16914395	MMP9	0.366	0.062	1.539	0.098	0.786	0.232
17017885	HLA-DRB5	1.863	0.125	1.548	0.181	1.218	0.320
17017900	HLA-DRB1	1.566	0.192	1.706	0.151	1.534	0.224

ProtoArray (Invitrogen) protein microarrays

Serum samples were stored at -80°C until required. ProtoArray Human Protein Microarray 5.1 slides were blocked in blocking buffer (50 mM HEPES, 200 mM NaCl, 0.01% Triton X-100, 25% glycerol, 20 mM reduced glutathione, 1.0 mM DTT, 1X Synthetic Block) at 4 °C for 1 hour. After blocking, arrays were rinsed once with freshly prepared PBST buffer (1X PBS, 0.1% Tween 20, and 1 X Synthetic Block). Arrays were then probed with a 1:500 dilution of each sample diluted in 5 mL of PBST buffer. Arrays were incubated for 90 minutes at 4°C in QuadriPERM 4-well trays (Greiner) with gentle agitation. After incubation, slides were washed five times (5 minutes per wash) in 5 ml PBST Buffer in 4-well trays. An Alexa Fluor®647-conjugated goat anti-human IgG antibody diluted in 5 ml PBST buffer to a 1.0 µg/ml final concentration was added to each array and allowed to incubate with gentle shaking at 4°C for 90 minutes. After incubation, the secondary antibody was removed, and arrays were washed as described above. Arrays were dried by spinning in a table top centrifuge equipped with a plate rotor at 200x gravity for 2 minutes. Arrays were then scanned using a Tecan PowerScanner™ fluorescent microarray scanner.

GenePix 7 software was used to overlay the mapping of human proteins in the array list file to each array image with a fixed feature size of 130 µm (diameter). After aligning each of the 48 subarrays using spots from the AlexaFluor®-conjugated and murine antibodies printed in each subarray, the features were resized by the GenePix software to best fit the feature. Pixel intensities for each spot on the array were determined. Arrays were normalised and a signal for each spot on the array calculated by subtracting the local background signal. Microarray hybridisation and these initial processing steps were performed by ThermoFisher.

After removing data from control group spots and TNFSF13B (as bound by belimumab) the signal from duplicate spots was combined (mean) to give one value per unique antigen. This dataset was used to define a global threshold for 'significant' antibody binding (10,750) by maximising the signal-to-noise ratio for the comparison of number of antigen with a signal above threshold in samples at baseline (no immunosuppression) versus Week 24 (maximal immunosuppression). This approach avoided defining a threshold based on the parameter of interest (treatment group), relying on the biologically plausible hypothesis that there would be differences in circulating auto-/allo-antibody pre- and post-transplant.

This threshold was used to determine whether an individual antigen specificity had significant antibody binding (yes/no). New antigenic specificities post-transplant were defined as antigenic specificities with significant binding at Week 24 post-transplant not observed at baseline (Week 0). Kidney specific antigens were defined as those identified as such in [3] (Table S2).

Immunophenotyping

Immunophenotyping was performed by flow cytometry with absolute cell numbers calculated using TBNK counts.

Healthy control B cell stimulation assay

CD19+CD27+ memory B cells were enriched from PBMCs of 16 healthy donors using a human memory B cell isolation kit (Miltenyi Biotec) and cultured in vitro in the presence of CpG (100nM; Hycult Biotech) and CD40L (1µg/ml; R&D Systems) with 0 to 200ng/ml BLyS (GSK) for 48 hours. Phorbol 12-myristate 13-acetate (PMA) (50ng/ml; Sigma-Aldrich), ionomycin (500ng/ml; Sigma-Aldrich) and brefeldin A (5µg/ml; BioLegend) were added for the last 5 hours of culture and IL-6 and IL-10 quantified by flow cytometry. In 7 of these healthy donors, 15nM belimumab (GSK) was added in addition to 100ng/ml BLyS.

Subject with retrospective flow positive crossmatch

A single subject (subject 5) in the belimumab treatment group was found to have a positive flow cytometry cross-match with donor specific antibodies to DP1 and DP6 at baseline. This subject was receiving a second transplant and known to be sensitized against multiple HLA DP antigens. The HLA DP type of the donor was not known at the time of transplantation, and T and B lymphocyte CDC cross-match tests were negative. Both the donor DP type and flow cytometry cross-match result became available following transplantation. Had this information been available the subject would have been deemed ineligible for the study and given lymphocyte depleting induction therapy in view of his increased immunological risk. This subject experienced Banff IIA acute cellular rejection with features suspicious for early humoral rejection (day 6) associated with a rise in the titre of DP DSA and development of *de novo* DSA to HLA-A11 and HLA-B18. A further biopsy (day 34) demonstrated overt antibody mediated rejection. They were withdrawn from the study following a single dose of belimumab and the rejection responded to treatment with methlyprednisolone, plasma exchange and anti-thymocyte globulin.

Donor specific and anti-MICA antibodies

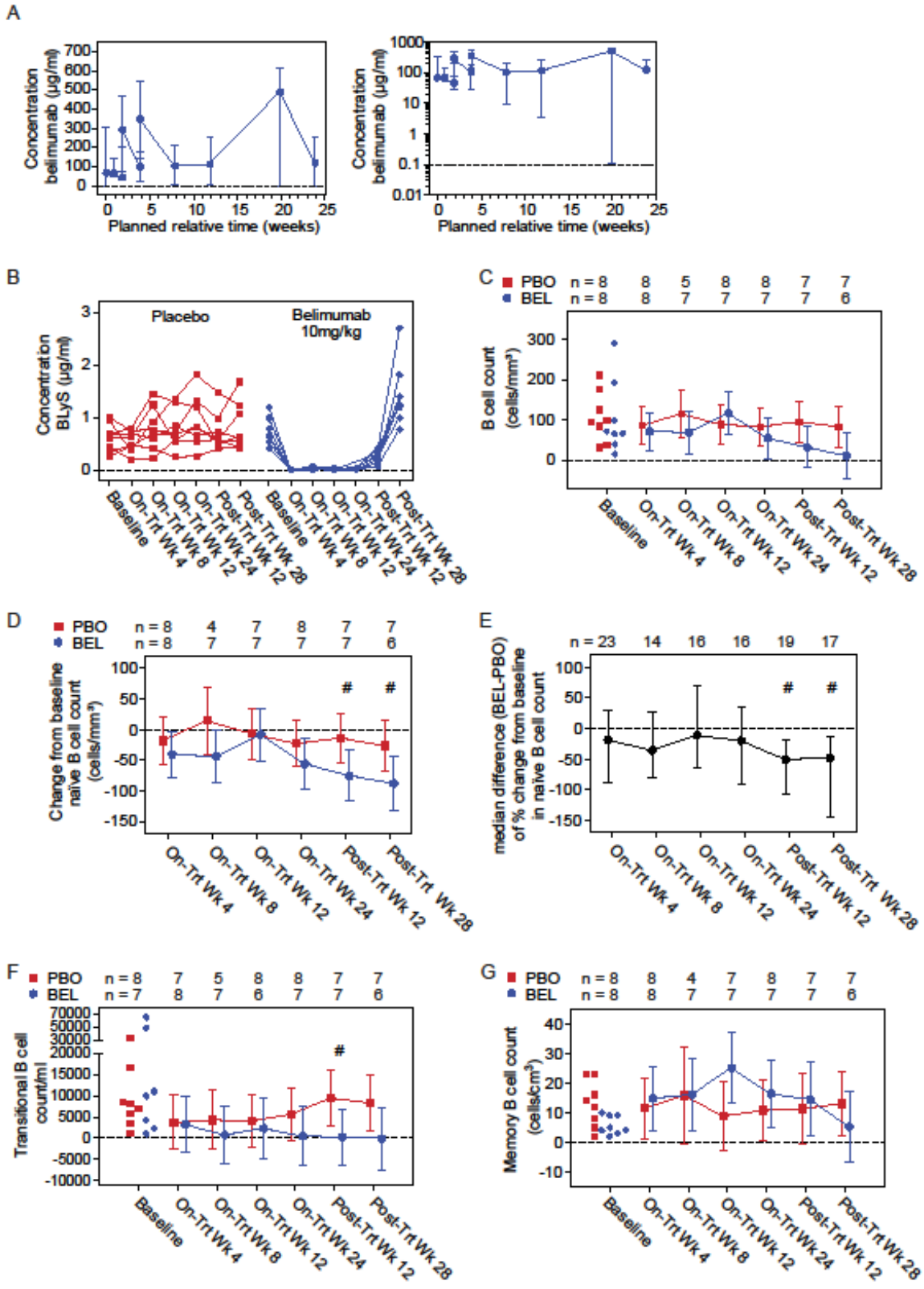
Two patients recruited to the study developed *de novo* DSA during follow-up; neither were part of the PP population having been withdrawn following a single dose of study drug (belimumab). Subject 5 was withdrawn due to a positive cross-match; subject 13 withdrew consent for study drug (Fig. S2C).

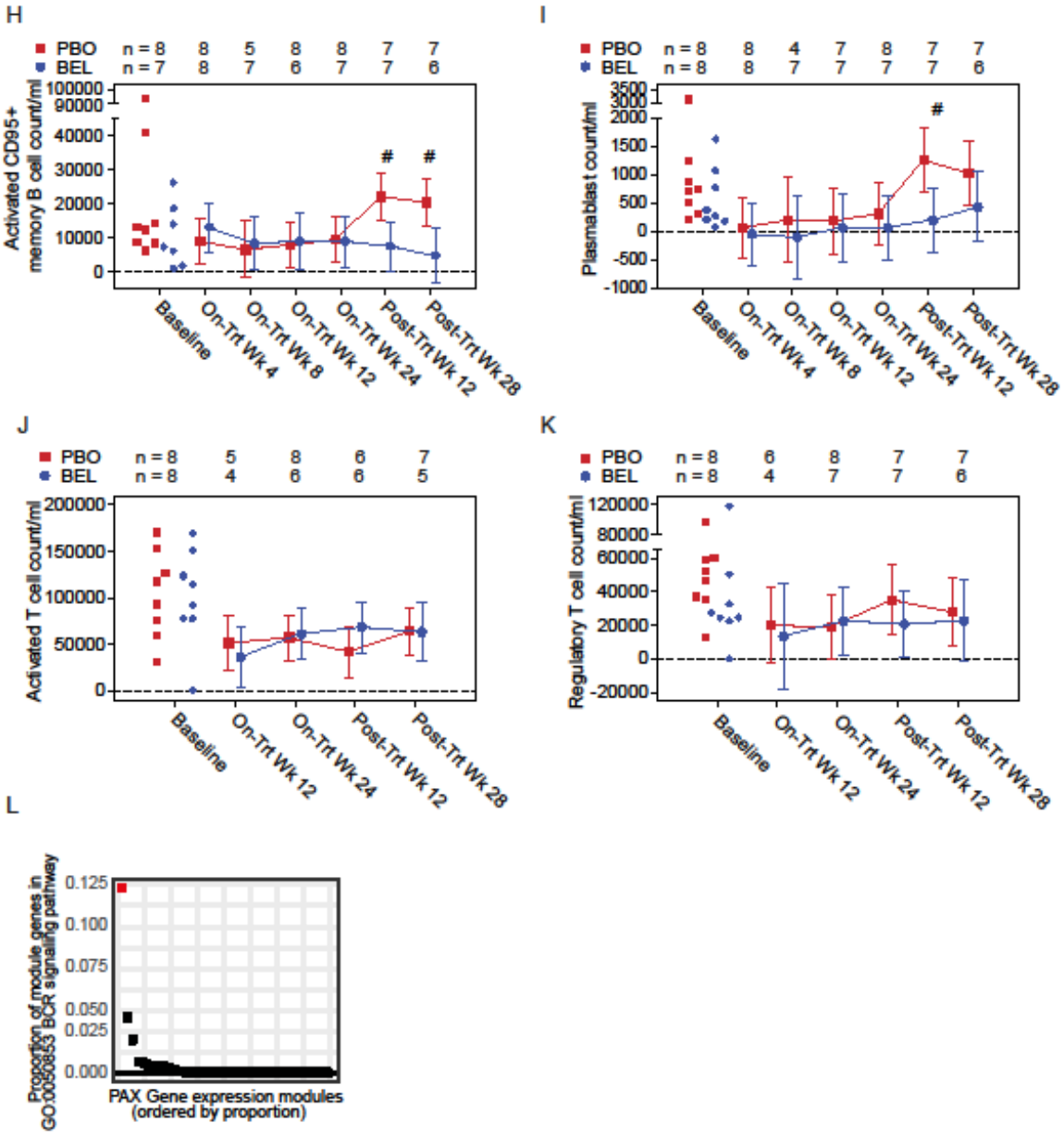
One patient in each group had positive anti-MICA antibodies at baseline; subject 19 received one dose of placebo and had positive levels throughout the first year post transplant; subject 27 received 5 doses of belimumab and antibody levels fell below the positive threshold. Subject 13 received a single dose of belimumab and developed *de novo* anti-MIC antibodies in addition to low level *de novo* DSA.

Subject with adoptive transfer of donor HLA specific allosensitization

Subject 21 had no HLA antibodies detectable at baseline but received a kidney from a sensitised donor. Post-transplant, high levels de novo IgG HLA class I- and class II-specific antibodies were measurable with no antibodies to donor HLA. A similar antibody profile was found in the recipient of the paired deceased donor kidney (not part of this study) and in stored donor serum[4].

Figure S1: Belimumab Pharmacokinetics and Impact on Peripheral B and T Cell Subsets





Panel A shows the median serum concentration of belimumab over time (left panel linear scale; right panel logarithmic scale). The lower limit of detection of 100ng/ml is marked with a dotted line. Error bars indicate range. Pharmacokinetics (PK) population that consisted of all subjects randomised to belimumab treatment that were treated with at least one dose and had a PK sample that was analysed (N=12).

Panel B shows serial measurements of serum BLyS concentration (µg/ml) in individual subjects.

Panel C shows B cell count (cells/mm^3).

Panel D shows the adjusted mean (95% CI) change from baseline in naïve (CD20+CD27-) B cell count, by visit and treatment in the PP population.

Panel E shows the Hodges Lehman estimates and 95% CI for median difference (belimumab 10mg/kg (BEL) – placebo (PBO)) of percent change from baseline in naïve B-cell (CD20+CD27-) count by visit in the MITT population (sensitivity analysis of primary endpoint).

Panel F shows transitional ($CD24^{br}CD38^{br}IgD^{+}$) B cell count/ml.

Panel G shows memory ($CD20^{+}CD27^{+}$) B cell count (cells/cm^3).

Panel H shows activated memory ($CD95^{+}CD27^{+}$) B cell count (cells/ml) by visit and treatment group.

Panel I shows plasmablast ($CD19^{+}CD27^{+}CD38^{+}$) count/ml.

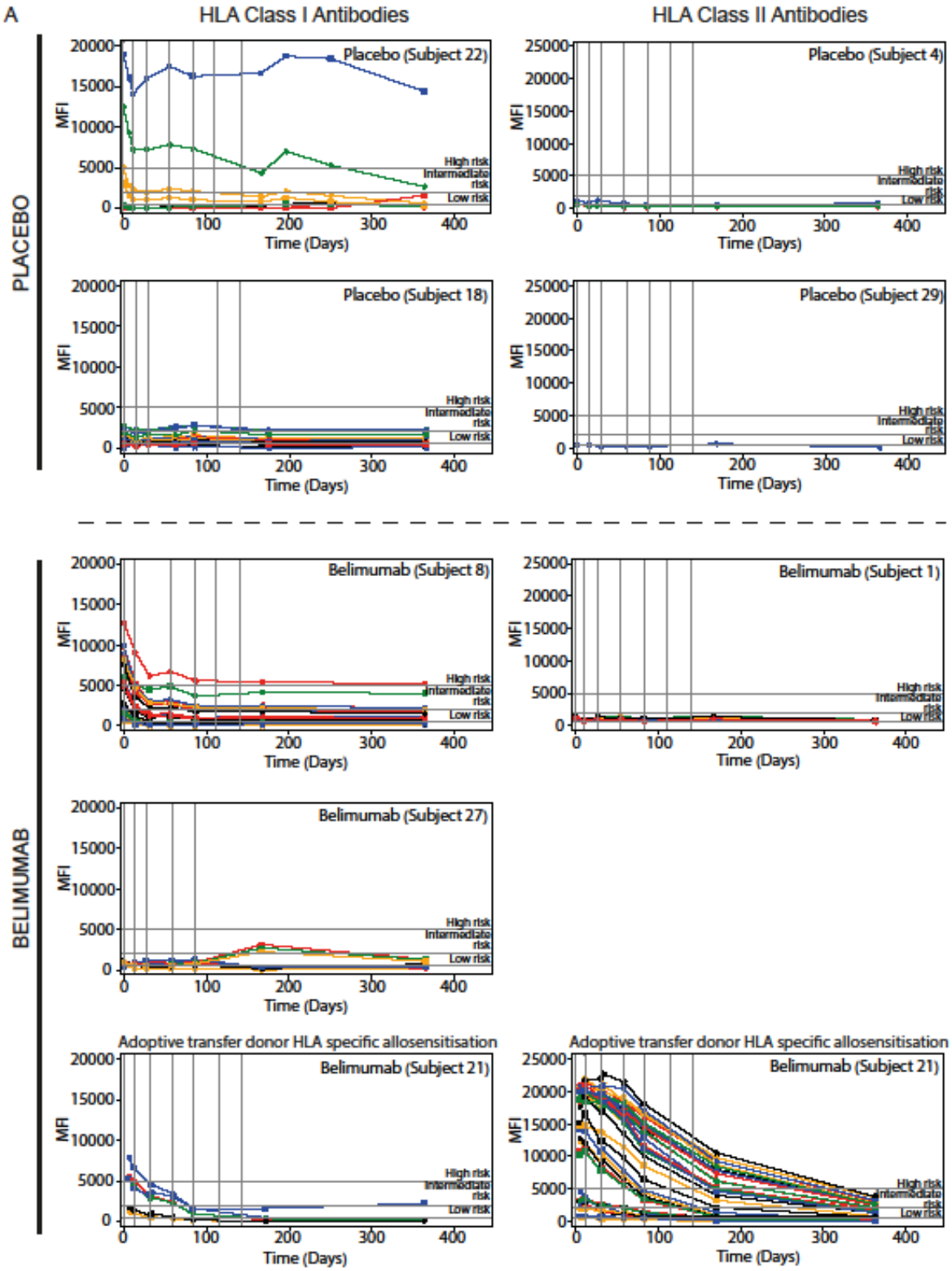
Panel J shows activated T cell ($CD4^{+}CD25^{hi}CD45RA^{-}$IL $7R^{hi}$) count/ml

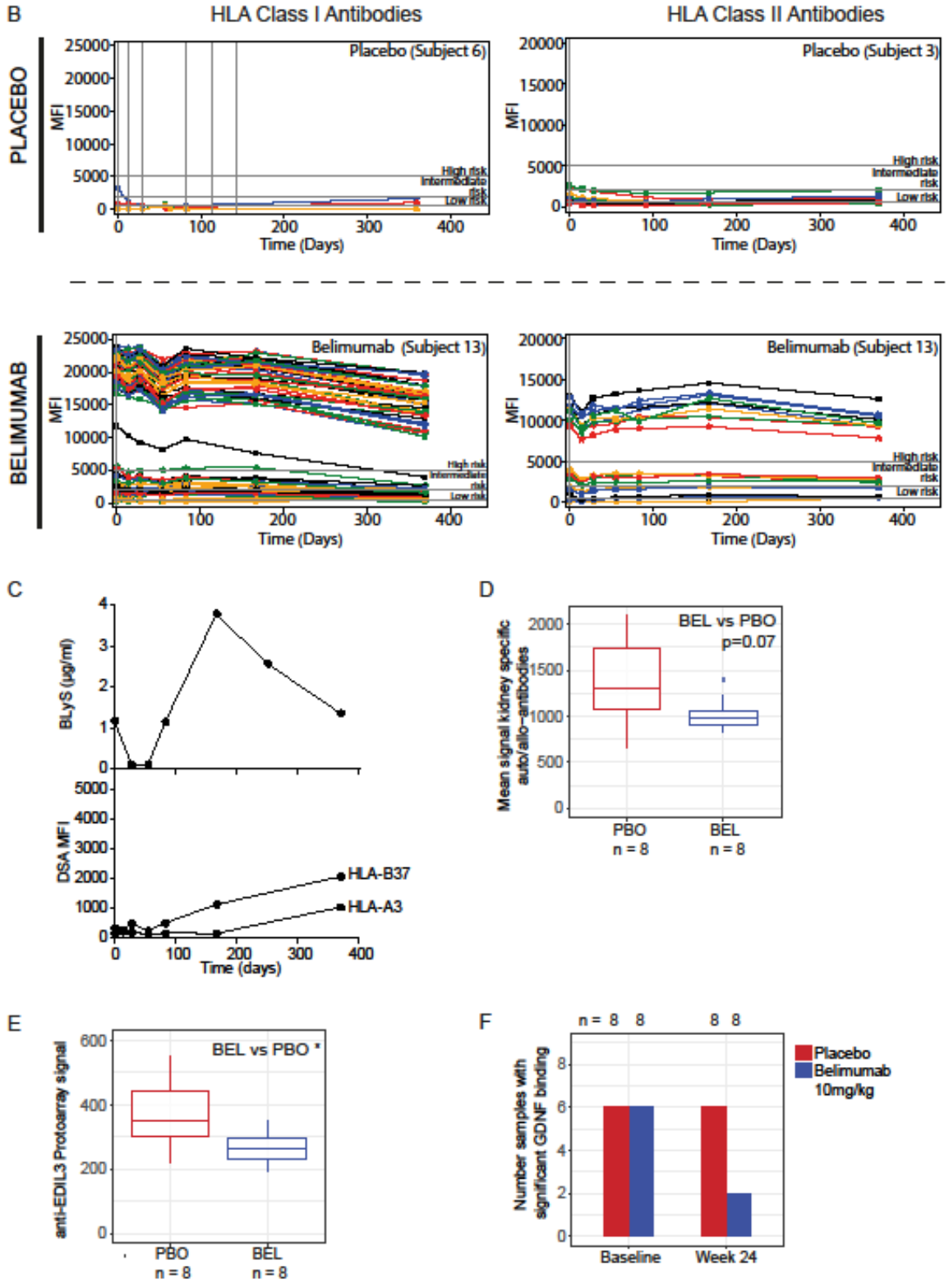
Panel K shows regulatory T cell ($CD4^{+}CD25^{hi}$IL-$7R^{lo}$) count/ml

B-D and F-K performed on PP population. E performed on MITT population. C and F-K show raw values at baseline for comparison and adjusted mean estimate with 95% confidence intervals, obtained from the same MMRM model as used for the primary endpoint, at subsequent timepoints. # indicates that the 95% confidence interval of the treatment difference does not include zero. In J and K weeks 4 and 8 excluded due to known effects of basiliximab on CD25 surface expression. C, G and I represent analyses performed *post hoc.* Where n numbers are displayed, these represent the number of subjects assayed at each timepoint.

Panel L shows the proportion of module genes within each module identified during weighted correlation network analysis of whole blood RNA in the GO:0050853 BCR signalling pathway. A single module (coloured red) was enriched for BCR signalling and analysed further for the effects of belimumab treatment. Analysis performed on MITT population at baseline and PP thereafter.

Figure S2: HLA and Non-HLA Antibodies





Serum samples before and after treatment were screened for HLA antibodies using Luminex HLA class I and class II antibody detection beads (LABScreen™ Mixed, One Lambda) and HLA antibody specificities were determined using Luminex single antigen HLA-specific antibody detection beads (LABScreen Single Antigen HLA Class I and Class II, One Lambda). The normalized mean fluorescence intensity (MFI) for each single antigen bead with an MFI above the detection limit of 500 at any timepoint is shown in individuals from the PP population (A) and those not in the PP population (B). Vertical lines show when doses of study drug were received. Subject 6 received plasma exchange for FSGS between days 31 and 64. Subjects 3 and 13 withdrew

consent for study drug following a single dose. Adoptive transfer donor HLA specific allosensitization as described in [4].

Panel C shows the relationship between serum BLyS and *de novo* donor-specific HLA antibody over time for subject 13 who received a single dose of belimumab on day 0 prior to withdrawal of consent for study drug. Serum was sampled for BLyS levels at Day 0, week (W) 4, W8, W16, W24, W36 and W52. The first increase in serum BLyS was detected at W24, with a fall in levels thereafter to baseline by W52. Antibodies were measured using Luminex single antigen HLA-specific antibody detection beads (LABScreen Single Antigen HLA Class I and Class II, One Lambda); donor specificity was determined manually by comparison of donor and recipient HLA-types. A low MFI MHC class I B37 antibody became detectable at W24, and a further A3 antibody at W52, the latter in the context of a falling serum BLyS level.

Panel D shows the mean protoarray signal from kidney specific antigens (defined in [3]) at week 24. Horizontal lines on boxplot correspond to median and interquartile ranges. Patients are coloured by treatment group. The Wilcoxon rank-sum test was used to compare the belimumab treatment group (BEL) and placebo treatment group (PBO); exact p value displayed.

Panel E shows the distribution of the mean protoarray signal from two antigens on the array annotated to EGF-like repeats and discoidin I-like domains 3 (EDIL3) (BC053656.1; BC030828.1) by treatment group at week 24. Horizontal lines on boxplot correspond to median and interquartile ranges. PP population. The Wilcoxon rank-sum test was used to compare the belimumab and placebo treatment groups; * denotes $p<0.05$.

Panel F shows the number of subjects in each treatment group with above threshold protoarray binding to glial cell-derived neurotrophic factor (GDNF) at baseline and week 24. PP population; Eight subjects were tested at each timepoint for both treatment groups.

D, E and F represent *post hoc* analyses. Where n numbers are displayed, these represent the number of subjects assayed at each timepoint.

Table S3: Concomitant maintenance immunosuppression (*post hoc* analysis).
1. In addition, for treatment of rejection (week 2), one subject in the placebo group received 500mg x 3 pulsed iv methylprednisolone and one subject in the belimumab group received 1000mg x 3 pulsed iv methylprednisolone.
2. One subject in the placebo arm switched to mycophenolate sodium (Myfortic) at week 52; one subject in the belimumab group switched at week 4. mycophenolate sodium 720mg approximately equivalent to mycophenolate mofetil 1g. One subject in the belimumab group was on azathioprine 50mg on day 231 through to week 52, having stopped MMF on day 120.

	Placebo (N=8)			Belimumab 10mg/kg (N=8)		
	n	Mean (SD)	Median(Range)	n	Mean (SD)	Median (Range)
Daily prednisolone dose (mg)[1]						
week 1	8	20.0 (0.0)	20.0 (20.0-20.0)	8	20.0 (0.0)	20.0 (20.0-20.0)
week 4	8	8.8 (3.3)	8.8 (5.0 -15.0)	8	8.4 (2.3)	10.0 (5.0-10.0)
week 12	8	5.0 (1.3)	5.0 (2.5-7.5)	8	4.7 (0.9)	5.0 (2.5-5.0)
week 24	8	5.3 (2.1)	5.0 (2.5-10.0)	8	6.9 (5.8)	5.0 (2.5-20.0)
week 52	8	4.3 (1.8)	5.0 (0.0-5.0)	8	4.7 (1.6)	5.0 (2.5-7.5)
Tacrolimus daily dose (mg)						
week 1	8	10.3 (3.1)	8.5 (8-15)	7	7.8 (4.6)	6.5 (0-13)
week 4	8	12.1 (5.8)	11.5 (5-24)	8	9.6 (3.7)	11.5 (4-14)
week 12	8	9.6 (6.3)	9.0 (4-24)	8	8.3 (4.0)	8.0 (3-15)
week 24	8	7.5 (3.9)	6.5 (4-16)	8	7.1 (4.1)	7.0 (3-15)
week 52	8	6.1 (1.5)	6.0 (4-8)	8	6.1 (2.9)	7.0 (2.5-10)
Tacrolimus level (µg/L)						
week 1	8	5.6 (2.8)	4.4 (2.8-10.5)	8	7.3 (1.8)	6.9 (4.9-10.8)
week 4	7	10.7 (5.8)	9.3 (4.5 - 22.8)	7	8.3 (2.4)	8.7 (3.3-10.3)

week 12	8	7.9 (2.1)	8.5 (3.7 - 10.5)	7	8.6 (2.1)	7.4 (6.2-11.6)
week 24	7	6.6 (1.0)	7.0 (4.5 - 7.6)	6	8.4 (3.5)	8.5 (3.5 - 13.0)
week 52	5	4.8 (3.0)	6.0 (0.0 - 7.4)	8	6.1 (1.8)	5.9 (3.7 - 9.4)
MMF equivalent dose (mg)[2]						
week 1	8	937.5 (176.8)	1000 (500-1000)	8	1062.5 (176.8)	1000 (1000-1500)
week 4	8	1062.5 (176.8)	1000 (1000-1500)	8	1062.5 (267.3)	1000 (500-1500)
week 12	8	812.5 (530.3)	1000 (0-1500)	8	875 (517.5)	1000 (0-1500)
week 24	8	750 (534.5)	1000 (0-1500)	8	687.5 (530.3)	750 (0-1500)
week 52	8	625 (443.2)	750 (0-1000)	8	687.5 (530.3)	750 (0-1000)

Table S4: Analysis for change from baseline in naïve B cell count by visit (MITT and PP populations).

1. Adjusted Mean Differences(treatment-placebo) and 95% confidence intervals for differences are obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. Naïve B cell count = CD20+CD27- cells/mm^3. This data is represented visually in Figure 2A (MITT population) and Figure S1D (PP population)

MITT Population: Unadjusted baseline values naïve B cell count (cells/mm^3)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	13	13	97.5	58.22	84	25	196
Belimumab 10mg/kg	Baseline	12	12	119.2	104.87	78.5	11	357

MITT Population: Change from baseline in naïve B cell count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk4	13	12	29.0 (23.65)	(-19.2, 77.2)		
Belimumab 10mg/kg		12	11	-11.7 (24.24)	(-61.0, 37.7)	-40.7 (33.79)	(-109.5, 28.1)
Placebo	On-Trt Wk8	13	6	35.0 (29.14)	(-23.4, 93.4)		
Belimumab 10mg/kg		12	8	-16.5(27.35)	(-71.5, 38.6)	-51.5 (40.58)	(-132.9, 29.9)
Placebo	On-Trt Wk12	13	8	19.4 (26.54)	(-34.1, 72.8)		
Belimumab 10mg/kg		12	8	3.1 (27.31)	(-51.9, 58.0)	-16.3 (38.45)	(-93.6, 61.0)
Placebo	On-Trt Wk24	13	9	4.0 (25.55)	(-47.7, 55.6)		
Belimumab 10mg/kg		12	7	-30.4 (27.50)	(-85.8, 24.9)	-34.4 (37.24)	(-109.5, 40.7)
Placebo	Post-Trt Wk12	13	11	0.8 (24.11)	(-48.3, 49.8)		
Belimumab 10mg/kg		12	8	-58.0 (26.52)	(-111.5, -4.5)	-58.7 (35.72)	(-131.0, 13.6)
Placebo	Post-Trt Wk28	13	10	5.4 (24.67)	(-44.6, 55.5)		
Belimumab 10mg/kg		12	7	-53.8 (27.83)	(-109.7, 2.1)	-59.3 (37.12)	(-134.1, 15.6)

PP Population: Unadjusted baseline values naïve B cell count (cells/mm^3)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	91.9	56.26	79.5	25	184
Belimumab 10mg/kg	Baseline	8	8	96.1	87.55	61.0	11	274

PP Population: Change from baseline in naïve B cell count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk4	8	8	-18.4 (19.11)	(-56.6, 19.9)		
Belimumab 10mg/kg		8	8	-40.5 (19.02)	(-78.6, -2.5)	-22.2 (26.75)	(-75.7, 31.4)
Placebo	On-Trt Wk8	8	4	14.2 (27.38)	(-40.5, 68.9)		
Belimumab 10mg/kg		8	7	-44.3 (21.66)	(-87.6, -0.9)	-58.5 (35.75)	(-129.9, 13.0)
Placebo	On-Trt Wk12	8	7	-7.1 (20.44)	(-48.0, 33.8)	-0.9 (29.93)	(-60.7, 59.0)

Belimumab 10mg/kg		8	7	-8.0 (21.53)	(-51.1, 35.1)		
Placebo	On-Trt Wk24	8	8	-22.9 (19.11)	(-61.1, 15.4)		
Belimumab 10mg/kg		8	7	-56.2 (20.47)	(-97.1, -15.2)	-33.3 (27.64)	(-88.6, 22.0)
Placebo	Post-Trt Wk12	8	7	-14.0 (20.44)	(-55.0, 26.9)		
Belimumab 10mg/kg		8	7	-75.3 (20.44)	(-116.2, -34.4)	-61.2 (29.08)	(-119.4, -3.0)
Placebo	Post-Trt Wk28	8	7	-26.7 (20.37)	(-67.4, 14.1)		
Belimumab 10mg/kg		8	6	-88.3 (22.82)	(-133.9, -42.7)	-61.6 (30.49)	(-122.6, -0.7)

Table S5: Non-parametric analysis of percent change from baseline in naïve B cell count.

1. Median difference and 95% CI of difference obtained using the Hodges-Lehman method. Naïve B cell count = CD20+CD27- cells/mm^3. This data is represented visually in Figure S1E.

MITT population

Comparison	N	Visit	n	Median difference % change from baseline naïve B cell count[1]	95% CI of median difference
Belimumab 10mg/kg vs placebo	25	On-Trt Wk4	23	-18.7	(-88.8, 30.8)
		On-Trt Wk8	14	-36.1	(-81.4, 27.2)
		On-Trt Wk12	16	-11.5	(-65.5, 70.1)
		On-Trt Wk24	16	-20.1	(-90.9, 34.5)
		Post-Trt Wk12	19	-50.4	(-107.0, -18.0)
		Post-Trt Wk28	17	-48.3	(-144.7, -13.0)

Table S6: B cell populations at week 24 expressed as percentage of total B-cells (*post hoc* analysis). Median difference from baseline to week 24 (percentage at week 24 – percentage at week 0) in the labelled B cell populations by treatment group with each population expressed as a percentage of B cells. This data is represented visually in Figure 2B.

	Placebo (N=8)				Belimumab 10mg/kg (N=8)			
Subset	n	Median difference (%)	IQR	Range	n	Median difference (%)	IQR	Range
Naïve	8	9.277	(4.036, 10.503)	(-65.407, 12.67)	8	-36.231	(-50.492, -29.662)	(-69.66, 52.963)
Memory	8	-9.451	(-12.14, -6.752)	(-12.912, -2.497)	8	26.284	(12.117, 40.853)	(-58.19, 48.231)
Non switched memory	8	1.081	(-0.53, 2.602)	(-1.709, 72.622)	8	10.953	(8.632, 17.545)	(5.227, 21.429)
Switched memory	8	-5.518	(-7.392, -4.074)	(-9.417, -2.113)	8	20.653	(13.785, 34.642)	(-57.145, 49.29)
DN memory	8	-3.598	(-5.195, -0.386)	(-8.947, 2.285)	8	0.883	(-1.082, 6.538)	(-2.566, 9.761)

Table S7: Analysis of memory B cell (%CD19) by visit.

1. Adjusted mean differences (treatment-placebo) and 95% confidence intervals for differences obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. This data is represented visually in Figure 2C.

PP Population: Unadjusted baseline values

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	12.1	4.32	12.5	6	17
Belimumab 10mg/kg	Baseline	8	8	8.3	6.84	4.5	3	22

PP Population: Change from baseline in memory B cell count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk4	8	8	10.3 (3.13)	(3.8, 16.9)		
Belimumab 10mg/kg		8	8	31.0 (3.25)	(24.2, 37.8)	20.7 (4.62)	(11.0, 30.3)
Placebo	On-Trt Wk8	8	4	12.2 (3.68)	(4.8, 19.7)		
Belimumab 10mg/kg		8	8	30.4 (3.25)	(23.5, 37.2)	18.1 (5.00)	(7.8, 28.4)
Placebo	On-Trt Wk12	8	7	8.6 (3.20)	(2.0, 15.2)		
Belimumab 10mg/kg		8	7	28.9 (3.31)	(22.0, 35.8)	20.3 (4.67)	(10.6, 30.0)
Placebo	On-Trt Wk24	8	8	9.6 (3.13)	(3.1, 16.1)		
Belimumab 10mg/kg		8	7	32.6 (3.36)	(25.6, 39.6)	23.0 (4.70)	(13.2, 32.8)
Placebo	Post-Trt Wk12	8	7	12.3 (3.20)	(5.6, 18.9)		
Belimumab 10mg/kg		8	7	38.0 (3.31)	(31.1, 44.9)	25.7 (4.69)	(16.0, 35.5)
Placebo	Post-Trt Wk28	8	7	13.5 (3.23)	(6.8, 20.1)		
Belimumab 10mg/kg		8	6	29.0 (3.68)	(21.5, 36.5)	15.5 (5.10)	(5.1, 26.0)

Table S8: Analysis of non-HLA antibodies via ProtoArray (*post hoc* analysis)

Patient serum from baseline and week 24 was hybridised to ProtoArray (Invitrogen) protein microarrays. P values relate to Wilcoxon rank-sum tests used to compare the belimumab and placebo treatment groups. Kidney specific auto/allo-antibodies were defined in (Li 2009). IQR interquartile range; EDIL3 - EGF-like repeats and discoidin I-like domains 3 (EDIL3) (mean signal of two probes annotated to EDIL3 on array (BC053656.1; BC030828.1)); GDNF - Glial glial cell-derived neurotrophic factor (GDNF). This data is represented visually in Figure 2G-H; S2D-F.

	Placebo (n=8)	Belimumab 10mg/kg (n=8)	P-value
Number new auto/allo-antibodies above threshold (median (IQR)(range))	55.5 (27.5-91) (3-130)	15.5 (8.75-20) (1-44)	0.0474
Number new kidney specific auto/allo-antibodies above threshold (median (IQR)(range))	12.5 (10.25-14.75) (3-20)	8.0 (5.75 - 10.0) (5-12)	0.0524
Mean signal of kidney specific auto/allo-antibodies (median (IQR)(range))	1307.3 (1076-1743) (662-2096)	983.5 (914-1064) (823-1396)	0.0650
EDIL3 protoarray signal at week 24 (median (IQR)(range))	353.6 (305-442) (221-550)	261.9 (232-296) (191 - 350)	0.0379
Number of samples with above threshold GDNF binding			
- baseline	6	6	
- week 24	6	2	

Table S9: Analysis of B cell intracellular cytokine production following *ex-vivo* stimulation (*post hoc* analysis). PBMC were stimulated *ex vivo* for 5 hours and intracellular cytokine production quantified by flow cytometry**.** IL-6 and IL-10 secreting B cells are expressed as a percentage of CD19+ cells. Wilcoxon rank-sum tests were performed to compare samples by treatment and visit. This data is represented visually in Figure 3A-C.

Treatment	Visit	N	n	Endpoint	Median	p value
Placebo	Baseline	8	6	IL-6 secreting B cells	29.833	
Belimumab 10mg/kg		8	2	IL-6 secreting B cells	35.833	0.8571
Placebo	Last On-Trt	8	4	IL-6 secreting B cells	32.183	
Belimumab 10mg/kg		8	6	IL-6 secreting B cells	20.250	0.0667
Placebo	Post-Trt Wk12	8	8	IL-6 secreting B cells	42.617	
Belimumab 10mg/kg		8	8	IL-6 secreting B cells	29.175	0.0379
Placebo	Post-Trt Wk28	8	3	IL-6 secreting B cells	33.067	
Belimumab 10mg/kg		8	4	IL-6 secreting B cells	32.183	0.8571
Placebo	Baseline	8	6	IL-10 secreting B cells	1.352	
Belimumab 10mg/kg		8	2	IL-10 secreting B cells	2.702	0.2857
Placebo	Last On-Trt	8	4	IL-10 secreting B cells	1.575	
Belimumab 10mg/kg		8	6	IL-10 secreting B cells	4.307	0.0667
Placebo	Post-Trt Wk12	8	8	IL-10 secreting B cells	2.458	
Belimumab 10mg/kg		8	8	IL-10 secreting B cells	4.555	0.0499
Placebo	Post-Trt Wk28	8	3	IL-10 secreting B cells	2.960	
Belimumab 10mg/kg		8	4	IL-10 secreting B cells	3.750	0.8571
Placebo	Baseline	8	6	Ratio of IL-10/IL-6	0.043	
Belimumab 10mg/kg		8	2	Ratio of IL-10/IL-6	0.076	0.2857
Placebo	Last On-Trt	8	4	Ratio of IL-10/IL-6	0.049	
Belimumab 10mg/kg		8	6	Ratio of IL-10/IL-6	0.180	0.0095
Placebo	Post-Trt Wk12	8	8	Ratio of IL-10/IL-6	0.059	
Belimumab 10mg/kg		8	8	Ratio of IL-10/IL-6	0.127	0.0070
Placebo	Post-Trt Wk28	8	3	Ratio of IL-10/IL-6	0.093	
Belimumab 10mg/kg		8	4	Ratio of IL-10/IL-6	0.117	0.8571

Table S10: Analysis of B cell subset intracellular cytokine production following *ex-vivo* stimulation (*post hoc* analysis). Peripheral blood mononuclear cells were stimulated *ex vivo* for 5 hours and intracellular cytokine production quantified by flow cytometry**.** The IL-10/IL-6 ratio for individual subsets of naïve (CD27-), transitional (CD24hiCD38hiIgD+), CD24+CD27+ memory, switched memory (CD27+IgD-), and non switched memory (CD27+IgD+) B cells at post treatment Wk 12. Wilcoxon rank-sum tests were performed to compare samples by treatment. This data is represented visually in Figure 3D.

	Median IL-10/IL-6 at post Trt Week 12		
Subset	Placebo (n=8)	Belimumab 10mg/kg (n=8)	P-value
Naïve	0.035	0.060	0.1327
TrB	0.100	0.225	0.0109
CD34+CD27+ memory	0.150	0.230	0.0780
Switched memory	0.225	0.275	0.4880
Non-switched memory	0.120	0.180	0.0185

Table S11: Summary of percent change from baseline of IL10:IL6 ratio (relative to 0ng/mL BLyS) in PBMC from healthy volunteers enriched for CD27+ memory B cells and stimulated for 48 hours with increasing quantities of BLyS (*post hoc* analysis). In the presence of increasing quantities of BLyS a more inflammatory cytokine milieu was observed. This change was blocked by the addition of belimumab. N, number of healthy volunteers tested for each experimental condition. T-tests were performed to determine

whether the mean percentage changes from baseline differed significantly from 0 for each experimental condition. This data is represented visually in Figure 3E.

BLyS (ng/ml)	Belimumab (nM)	n	Mean change from baseline (%)	SD	95% CI of mean	p value
25	0	8	-18.47	13.850	-30.05, -6.89	0.0070
50	0	6	-20.47	19.447	-40.88, -0.06	0.0495
100	0	15	-22.90	24.595	-36.52, -9.28	0.0029
200	0	7	-22.64	15.218	-36.72, -8.57	0.0077
100	15	7	0.63	20.448	-18.28, 19.54	0.9377

Table S12: Median serum concentration of belimumab over time. The lower limit of detection of 100ng. Pharmacokinetics (PK) population that consisted of all subjects randomised to belimumab treatment that were treated with at least one dose and had a PK sample that was analysed. Values at day 0 pre IV dose were imputed. This data is represented visually in Figure S1A-B.

Time	N	n	median serum concentration of belimumab (µg/ml)	minimum serum concentration of belimumab (µg/ml)	maximum serum concentration of belimumab (µg/ml)
Day 0 (pre IV dose)	12	12	0.00	0.00	0.00
Day 0 (post IV dose)	12	12	212.63	134.40	309.60
Week 1	12	12	67.06	49.98	140.98
Week 2 (pre IV dose)	12	12	45.76	28.56	73.10
Week 2 (post IV dose)	12	9	291.94	207.19	468.23
Week 4 (pre IV dose)	12	10	100.08	26.23	177.61
Week 4 (post IV dose)	12	8	349.29	141.54	550.07
Week 8 (pre IV dose)	12	10	103.19	9.55	210.42
Week 12 (pre IV dose)	12	10	113.79	3.34	257.04
Week 20 (post IV dose)	12	8	489.89	0.11	616.84
Week 24	12	11	119.37	0.00	253.97

Table S13: Analysis of B cell count by visit (*post hoc* analysis).

1. Adjusted Mean Difference (treatment-placebo) and 95% confidence intervals for differences are obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. B cell count = CD19+ cells/mm^3. This data is represented visually in Figure S1C.

PP Population: Unadjusted baseline values B cell count (cells/mm^3)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	107.6	62.33	96	32	211
Belimumab 10mg/kg	Baseline	8	8	105.8	91.41	70	16	291

PP Population: Change from baseline in B cell count by visit

Treatment	Visit	N	n	Adjusted mean	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk4	8	8	86.4	(39.4, 133.5)		
Belimumab 10mg/kg		8	8	71.2	(24.4, 118.0)	-15.2 (33.01)	(-81.2, 50.7)
Placebo	On-Trt Wk8	8	5	115.8	(56.0, 175.7)		
Belimumab 10mg/kg		8	7	70.3	(17.4, 123.2)	-45.5 (39.90)	(-125.3, 34.2)
Placebo	On-Trt Wk12	8	8	89.2	(42.1, 136.2)		
Belimumab 10mg/kg		8	7	117.1	(64.3, 169.9)	27.9 (35.19)	(-42.4, 98.2)
Placebo	On-Trt Wk24	8	8	83.5	(36.4, 130.6)		
Belimumab 10mg/kg		8	7	55.6	(5.2, 105.9)	-27.9 (34.02)	(-95.9, 40.0)

Placebo	Post-Trt Wk12	8	7	96.2	(45.4, 147.0)		
Belimumab 10mg/kg		8	7	32.6	(-18.0, 83.2)	-63.6 (36.28)	(-136.1, 8.9)
Placebo	Post-Trt Wk28	8	7	84	(33.7, 134.2)		
Belimumab 10mg/kg		8	6	12.1	(-44.2, 68.4)	-71.9 (37.58)	(-147.0, 3.2)

Table S14: Analysis of transitional B cell count by visit.

1. Adjusted mean difference (treatment-placebo) and 95% confidence intervals for differences are obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. Transitional B cell count = CD19+CD24b+CD38b+IgD+ cells/ml. This data is represented visually in Figure S1F.

PP Population: Unadjusted baseline values transitional B cell count (cells/ml)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	10437.19	10071.917	7592.85	1233.2	32697.0
Belimumab 10mg/kg	Baseline	8	7	20380.89	25680.973	9931.33	1005.2	64790.5

PP Population: Change from baseline in transitional B cell count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk4	8	7	3738 (3123.2)	(-2720, 10197)		
Belimumab 10mg/kg		8	8	3240 (3178.6)	(-3350, 9831)	-498 (4433.1)	(-9658, 8663)
Placebo	On-Trt Wk8	8	5	4309 (3429.8)	(-2692, 11311)		
Belimumab 10mg/kg		8	7	779 (3283.7)	(-5993, 7550)	-3531 (4677.9)	(-13120, 6059)
Placebo	On-Trt Wk12	8	8	4092 (3055.0)	(-2251, 10434)		
Belimumab 10mg/kg		8	6	2315 (3446.5)	(-4743, 9373)	-1777 (4544.2)	(-11129, 7576)
Placebo	On-Trt Wk24	8	8	5586 (3031.6)	(-717, 11890)		
Belimumab 10mg/kg		8	7	452 (3371.0)	(-6469, 7374)	-5134 (4519.6)	(-14442, 4174)
Placebo	Post-Trt Wk12	8	7	9464 (3119.9)	(3012, 15917)		
Belimumab 10mg/kg		8	7	308 (3178.8)	(-6283, 6899)	-9156 (4427.7)	(-18307, -6)
Placebo	Post-Trt Wk28	8	7	8348 (3141.0)			
Belimumab 10mg/kg		8	6	-33 (3614.9)	(-7396, 7329)	-8382 (4847.8)	(-18276, 1513)

Table S15: Analysis of memory B cell count by visit (*post hoc* analysis).

1. Adjusted mean difference (treatment-placebo) and 95% confidence intervals for differences are obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. Memory B cell count = CD20+CD27+ cells/mm^3. This data is represented visually in Figure S1G.

PP Population: Unadjusted baseline values memory B cell count (cells/mm³)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	12.9	7.75	13.0	2	23
Belimumab 10mg/kg	Baseline	8	8	5.8	3.11	4.5	2	10

PP Population: Change from baseline in memory B cell count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk4	8	8	11.5 (5.12)	(1.2, 21.7)		
Belimumab 10mg/kg		8	8	14.9 (5.40)	(4.1, 25.7)	3.4 (8.16)	(-12.9, 19.7)
Placebo	On-Trt Wk8	8	4	15.8 (8.20)	(-0.6, 32.2)		
Belimumab 10mg/kg		8	7	16.1 (6.06)	(4.0, 28.2)	0.3 (11.71)	(-23.1, 23.7)
Placebo	On-Trt Wk12	8	7	8.9 (5.92)	(-2.9, 20.8)		
Belimumab 10mg/kg		8	7	25.1 (5.97)	(13.2, 37.1)	16.2 (9.57)	(-2.9, 35.3)
Placebo	On-Trt Wk24	8	8	10.7 (5.12)	(0.5, 20.9)		
Belimumab 10mg/kg		8	7	16.4 (5.60)	(5.2, 27.6)	5.7 (8.22)	(-10.7, 22.2)
Placebo	Post-Trt Wk12	8	7	11.4 (5.98)	(-0.6, 23.3)		
Belimumab 10mg/kg		8	7	14.5 (6.16)	(2.2, 26.9)	3.2 (9.91)	(-16.7, 23.0)
Placebo	Post-Trt Wk28	8	7	13.0 (5.31)	(2.4, 23.7)		
Belimumab 10mg/kg		8	6	5.3 (6.04)	(-6.8, 17.4)	-7.8 (8.59)	(-25.0, 9.4)

Table S16: Analysis of activated memory B cell (CD95%+) count by visit.

1. Adjusted mean difference (treatment-placebo) and 95% confidence intervals for differences are obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. Activated memory B cell count = CD19+ CD27+ CD95+ cells/ml. This data is represented visually in Figure S1H.

PP Population: Unadjusted baseline values activated memory B cell count (cells/ml)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	24516.5	29798.59	12610.3	6091	93078
Belimumab 10mg/kg	Baseline	8	7	10535.3	9295.42	7131.4	876	25999

PP Population: Change from baseline in activated memory B cell count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk4	8	8	8839.1 (3293.27)	(2151.0, 15527.1)		
Belimumab 10mg/kg		8	8	12900.5 (3556.85)	(5677.0, 20124.0)	4061.48 (4940.18)	(-5965.7, 14088.6)
Placebo	On-Trt Wk8	8	5	6396.7 (4196.14)	(-2029.2, 14822.7)		
Belimumab 10mg/kg		8	7	8189.6 (3850.17)	(411.1, 15968.1)	1792.88 (5983.11)	(-10234.5, 13820.2)
Placebo	On-Trt Wk12	8	8	7696.3 (3293.38)	(1008.1, 14384.5)		
Belimumab 10mg/kg		8	6	8833.6 (4112.07)	(550.4, 17116.8)	1137.35 (5391.68)	(-9747.1, 12021.8)
Placebo		8	8	9266.8 (3295.62)	(2574.4, 15959.2)		

Belimumab 10mg/kg	On-Trt Wk24	8	7	8735.3 (3720.62)	(1203.8, 16266.8)	-531.55 (5029.91)	(-10727.4, 9664.3)
Placebo	Post-Trt Wk12	8	7	21892.8 (3479.10)	(14854.1, 28931.5)	- 14538.52 (5104.63)	(-24875.4, -4201.6)
Belimumab 10mg/kg		8	7	7354.3 (3560.25)	(124.4, 14584.1)		
Placebo	Post-Trt Wk28	8	7	20274.1 (3420.31)	(13347.0, 27201.3)	- 15749.48 (5372.70)	(-26595.5, -4903.5)
Belimumab 10mg/kg		8	6	4524.7 (4053.36)	(-3642.2, 12691.5)		

Table S17: Analysis of plasmablast count by visit (*post hoc* analysis).

1. Adjusted mean difference (treatment-placebo) and 95% confidence intervals for differences are obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. Plasmablast count = CD27+CD38+CD19+ (EVENTS). Values for this rare subset have been normalised and converted to count/mL using the formula: normalised count/mL = [(event count) / (CD19+ event count)] * (CD19+ count per mm^3) * 1000 cells/ml. This data is represented visually in Figure S1I.

PP Population: Unadjusted baseline values plasmablast count (cells/mm^3)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	973.2	935.50	726.9	218	3143
Belimumab 10mg/kg	Baseline	8	8	575.7	542.24	324.6	80	1629

PP Population: Change from baseline in plasmablast count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk4	8	8	63.5 (270.58)	(-479.6, 606.7)		
Belimumab 10mg/kg		8	8	-50.2 (269.52)	(-591.4, 491.1)	-113.7 (386.43)	(-889.3, 661.9)
Placebo	On-Trt Wk8	8	4	206.4 (376.37)	(-546.1, 958.9)		
Belimumab 10mg/kg		8	7	-100.8 (368.77)	(-838.3, 636.7)	-307.2 (479.12)	(-1265.9, 651.4)
Placebo	On-Trt Wk12	8	7	187.0 (289.06)	(-392.5, 766.5)		
Belimumab 10mg/kg		8	7	70.3 (297.79)	(-526.5, 667.1)	-116.7 (427.98)	(-974.1, 740.7)
Placebo	On-Trt Wk24	8	8	307.7 (270.90)	(-236.0, 851.5)		
Belimumab 10mg/kg		8	7	74.7 (283.62)	(-494.1, 643.6)	-233.0 (393.20)	(-1021.8, 555.8)
Placebo	Post-Trt Wk12	8	7	1259.1 (288.55)	(680.6, 1837.7)		
Belimumab 10mg/kg		8	7	203.7 (286.98)	(-371.8, 779.1)	-1055.5 (416.23)	(-1889.7, -221.2)
Placebo	Post-Trt Wk28	8	7	1032.8 (287.02)	(457.3, 1608.2)		
Belimumab 10mg/kg		8	6	432.2 (308.99)	(-186.5, 1051.0)	-600.6 (425.67)	(-1453.2, 252.1)

Table S18: Analysis of activated T Cell count by visit.

1. Adjusted mean difference (treatment-placebo) and 95% confidence intervals for differences are obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. Activated T-cell count= CD4+ CD25hi CD45RA- IL7Rhi cells/ml. This data is represented visually in Figure S1J.

PP Population: Unadjusted baseline values activated T cell count (cells/ml)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	103344.1	47314.16	105357.3	31488	170276
Belimumab 10mg/kg	Baseline	8	8	100793.4	52470.57	103341.5	542	169844

PP Population: Activated T cell count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk12	8	5	51203.4 (14618.11)	(21342.6, 81064.2)	-15043.8 (21896.18)	(-59669.7, 29582.0)
Belimumab 10mg/kg		8	4	36159.5 (16350.26)	(2849.4, 69469.7)		
Placebo	On-Trt Wk24	8	8	57278.7 (11872.54)	(32803.8, 81753.5)	3881.2 (17477.72)	(-31954.2, 39716.7)
Belimumab 10mg/kg		8	6	61159.9 (13288.02)	(33935.5, 88384.3)		
Placebo	Post-Trt Wk12	8	6	41835.9 (13483.91)	(14211.7, 69460.1)	26387.0 (18975.60)	(-12451.7, 65225.7)
Belimumab 10mg/kg		8	6	68222.9 (13529.41)	(40540.1, 95905.8)		
Placebo	Post-Trt Wk28	8	7	63901.1 (12475.65)	(38257.5, 89544.7)	-650.8 (19371.60)	(-40233.6, 38932.0)
Belimumab 10mg/kg		8	5	63250.3 15250.65)	(32152.3, 94348.4)		

Table S19: Analysis of regulatory T cell count by visit.

1. Adjusted mean difference (treatment-placebo) and 95% confidence intervals for differences are obtained from MMRM model, with fixed categorical effects of treatment, visit, donor type and treatment-by-visit interaction and fixed continuous covariates of baseline and baseline-by-visit interaction. A compound symmetry variance structure was used to model the within-patient errors, shared across treatments. Regulatory T-cell count = CD4+ CD25hi IL-7Rlo cells/ml. This data is represented visually in Figure S1K

PP Population: Unadjusted baseline values regulatory T cell count (cells/ml)

Treatment	Visit	N	n	Mean	SD	Median	Min	Max
Placebo	Baseline	8	8	50119.4	24537.03	49473.7	12884	97360
Belimumab 10mg/kg	Baseline	8	8	37496.1	35027.59	26074.9	192	117226

PP Population: Regulatory T cell count by visit

Treatment	Visit	N	n	Adjusted mean (SE)	95% CI of adjusted mean	Adjusted mean difference (SE)[1]	95% CI of difference
Placebo	On-Trt Wk12	8	6	19855.8 (11089.98)	(-2594.7, 42306.3)	-6357.8 (20260.67)	(-47373.3, 34657.8)
Belimumab 10mg/kg		8	4	13498.0 (15530.96)	(-17942.7, 44938.8)		
Placebo	On-Trt Wk24	8	8	19196.7 (9437.89)	(90.7, 38302.7)	3486.5 (13654.12)	(-24154.8, 31127.8)
Belimumab 10mg/kg		8	7	22683.3 (9933.17)	(2574.6, 42791.9)		
Placebo	Post-Trt Wk12	8	7	35047.0 (10367.51)	(14059.1, 56034.9)	-14278.4 (14198.12)	(-43021.0, 14464.2)
Belimumab 10mg/kg		8	7	20768.6 (9794.01)	(941.6, 40595.5)		
Placebo	Post-Trt Wk28	8	7	27998.4 (10183.27)	(7383.4, 48613.3)	-5143.3 (16598.08)	(-38744.3, 28457.8)
Belimumab 10mg/kg		8	6	22855.1 (12004.05)	(-1445.8, 47156.0)		

References

1. Stohl W, Hiepe F, Latinis KM, et al. Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. *Arthritis Rheum* 2012; **64**(7): 2328-37.

2. Lyons PA, Koukoulaki M, Hatton A, et al. Microarray analysis of human leucocyte subsets: the advantages of positive selection and rapid purification. *BMC Genomics* 2007; **8**: 64.

3. Li L, Wadia P, Chen R, et al. Identifying compartment-specific non-HLA targets after renal transplantation by integrating transcriptome and "antibodyome" measures. *Proc Natl Acad Sci U S A* 2009; **106**(11): 4148-53.

4. Maxfield SJ, Taylor CJ, Kosmoliaptsis V, et al. Transfer of HLA-Specific Allosensitization From a Highly Sensitized Deceased Organ Donor to the Recipients of Each Kidney. *Am J Transplant* 2015; **15**(9): 2501-6.