

Supplementary Figures and Tables

Differential T Cell Signaling Pathway Activation by Tacrolimus and Belatacept after Kidney Transplantation: Post Hoc Analysis of a Randomised-Controlled Trial

NYNKE M. KANNEGIETER*; DENNIS A. HESSELINK; MARJOLEIN DIETERICH; GRETCHEN N. DE GRAAV; RENS KRAAIJEVELD; CARLA C. BAAN

*corresponding author

Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands

Address for Correspondence:

Nynke M. Kannegieter

Dept. of Internal Medicine, Division of Nephrology and Transplantation

Erasmus MC, University Medical Center Rotterdam

Room Na-522

Wytemaweg 80

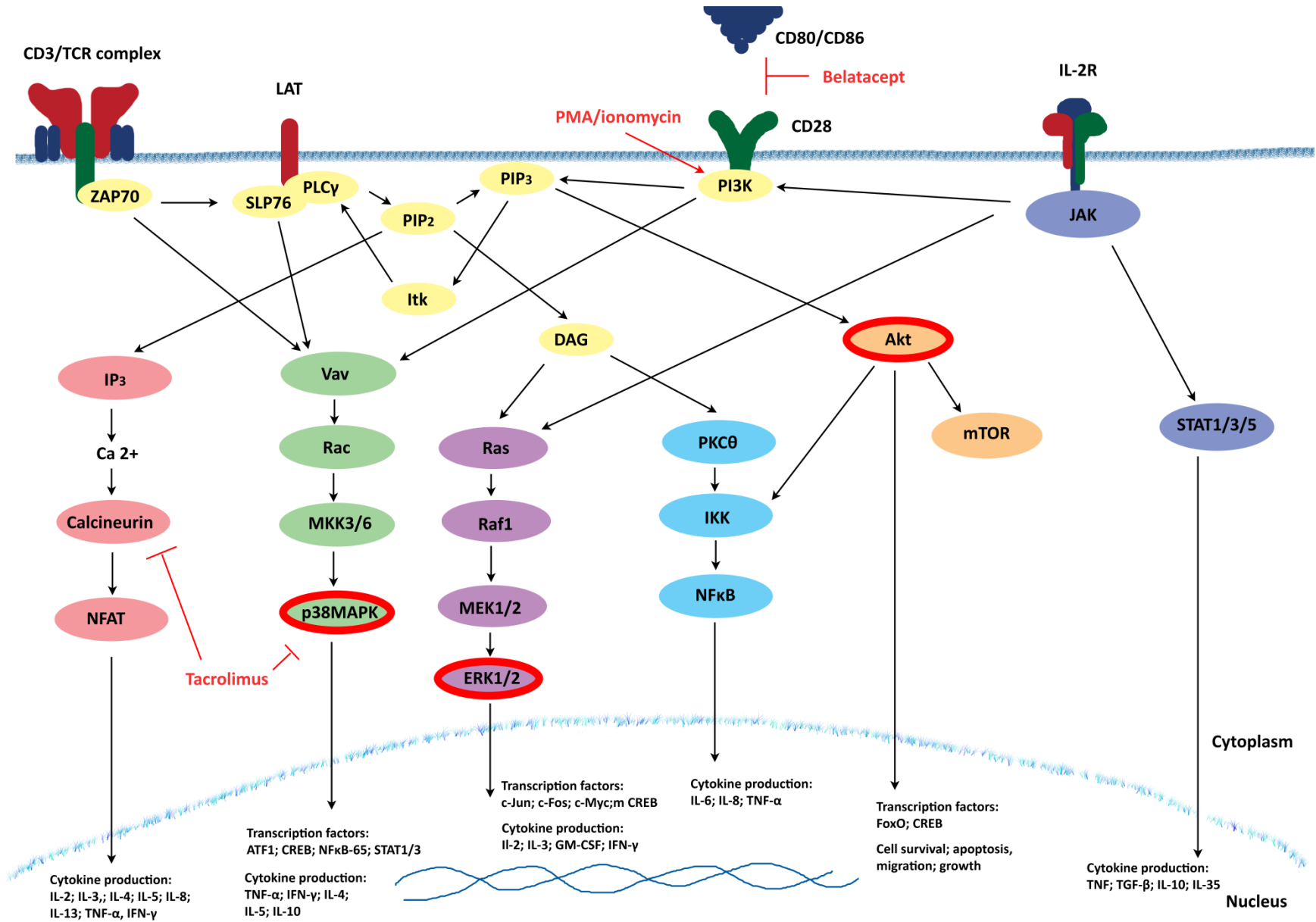
3015 CN, Rotterdam

The Netherlands

E-mail: n.kannegieter@erasmusmc.nl

Phone: +31-(0)10-7033867

Supplementary Figure S1:



Supplementary Figure S1. Schematic overview of T cell signaling pathways and their interaction downstream of the TCR. After antigen recognition by the TCR and after receiving a co-stimulatory signal via CD28, several intracellular signaling pathways become activated via phosphorylation. These pathways include the signaling molecules p38MAPK, ERK1/2 and Akt. Downstream of the signaling pathways, this activation will initiate gene-transcription and the control of T cell functions, such as cytokine production, cell survival, cell differentiation and cell apoptosis. Activation of the signaling pathways can be mimicked by the stimulation of the T cell with PMA/ionomycin. TAC is known to inhibit the calcineurin and p38MAPK pathway, while BELA blocks the co-stimulatory signal between the CD80/86 molecule on APC's and the CD28 molecule on T cells.

Supplementary Table S2: Baseline characteristics at time of transplantation*

	Belatacept group (n = 20)	Tacrolimus group (n = 20)	p
Age (years)	57 (25-76)	55 (21-76)	0.88
Male / female	14 (70%) / 6 (30%)	16 (80%) / 4 (20%)	0.72
Ethnicity			1.00
• Caucasian	17 (85%)	16 (80%)	
• African	2 (10%)	2 (10%)	
• Asian	1 (5%)	2 (10%)	
Body weight (kg)	79.0 (56.6-111.4)	93.6 (51.4-120.0)	0.06
HLA A mismatch (mean ± SD)	1.1 (± 0.7)	1.4 (± 0.5)	0.13
HLA B mismatch (mean ± SD)	1.3 (± 0.5)	1.5 (± 0.5)	0.51
HLA DR mismatch (mean ± SD)	1.1 (± 0.4)	1.3 (± 0.4)	0.70
Current PRA (%)	0 (0-5)	0 (0-17)	0.30
Peak PRA (%)	4 (0-6)	4 (0-21)	0.78
CMV status			0.80
• Donor + / Recipient -	3 (15%)	2 (10%)	
• Donor + / Recipient +	4 (20%)	7 (35%)	
• Donor - / Recipient -	7 (35%)	6 (30%)	
• Donor - / Recipient +	6 (30%)	5 (25%)	
Donor age (years)	59 (24-71)	51 (22-80)	0.18
Related / unrelated donor	6 (30%) / 14 (70%)	5 (25%) / 15 (75%)	1.00
Cause of end-stage renal disease			0.09
• Diabetes mellitus	3 (15%)	7 (35%)	
• Hypertension	2 (10%)	5 (25%)	
• IgA nephropathy	1 (5%)	3 (15%)	
• Polycystic kidney disease	3 (15%)	3 (15%)	
• Obstructive nephropathy	3 (15%)	1 (5%)	
• Unknown	5 (25%)	0 (0%)	
• Other	3 (15%)	1 (5%)	
Renal replacement therapy			0.91
• None (pre-emptive)	10 (50%)	12 (60%)	
• Hemodialysis	7 (35%)	6 (30%)	
• Peritoneal dialysis	3 (15%)	2 (10%)	
Time on dialysis (days)	425 (123-2782)	605 (465-1519)	0.41
Number of kidney transplantation			1.00
• First	19 (95%)	20 (100%)	1.00
• Second	1 (5%)	-	

Continuous variables are presented as medians (plus ranges) and categorical variables as numbers (plus percentages), unless otherwise specified

BPAR, biopsy-proven acute rejection; CMV, cytomegalovirus; HLA, human leukocyte antigen; PRA, panel reactive antibodies (current = PRA at time of transplantation, peak = historically highest measured PRA); SD, standard deviation.

Supplementary Table S3: Incidence of rejection according to the treatment group*

	Belatacept group (n = 20)	Tacrolimus group (n = 20)	p
Borderline	0 (0%)	0 (0%)	-
Type 1			1.00
• 1A	0 (0%)	0 (0%)	
• 1B	1 (5%)	1 (5%)	
Type 2			0.004
• 2A	2 (10%)	1 (5%)	
• 2B	6 (30%)	0 (0%)	
Type 3	1 (5%)	0 (0%)	1.00
Mixed	1 (5%)	0 (0%)	1.00
Total BPAR	11 (55%)	2 (10%)	0.006

The incidence of the first rejection episodes is given. The highest Banff score is depicted if sequential biopsies were performed.

BPAR, Biopsy-proven acute rejection

Supplementary Table S4: Immunosuppressive drugs dose and pre-dose concentrations

	<i>TAC-treated patients</i>					<i>BELA-treated patients</i>			
	TAC dose (mg)	TAC C₀ (ng/ml)	MMF dose (mg)	MPA C₀ (µg/ml)	PRED dose (mg)	BELA dose (mg)	MMF dose (mg)	MPA C₀ (µg/ml)	PRED dose (mg)
Day 4	16.7 (± 2.5)	16.8 (± 6.2)	2000 (± 0)	3.35 (± 1.59)	20.0 (± 0)	792 (± 153)	2000 (± 0)	3.85 (± 1.55)	20.0 (± 0)
Day 30	8.9 (± 4.6)	9.8 (± 3.3)	1750 (± 546)	2.69 (± 1.57)	12.5 (± 3.3)	767 (± 106)	1682 (± 603)	3.39 (± 1.74)	10.5 (± 2.7)
Day 90	4.7 (± 1.4)	6.8 (± 1.7)	1342 (± 473)	2.83 (± 1.62)	5.4 (± 1.3)	774 (± 130)	1364 (± 393)	3.27 (± 1.57)	5.0 (± 0)
Day 180	4.3 (± 1.1)	6.3 (± 1.5)	1181 (± 499)	2.17 (± 0.98)	4.7 (± 1.2)	383 (± 63)	1222 (± 441)	2.15 (± 1.34)	5.0 (± 0)
Day 360	4.2 (± 1.3)	7.0 (± 2.3)	1103 (± 493)	2.02 (± 0.92)	4.3 (± 1.5)	382 (± 58)	1139 (± 377)	1.99 (± 0.63)	5.0 (± 0)

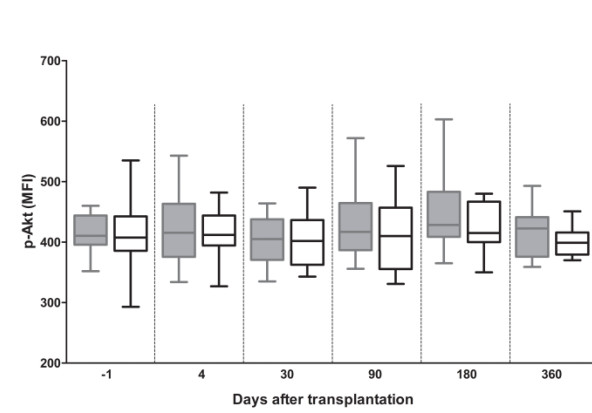
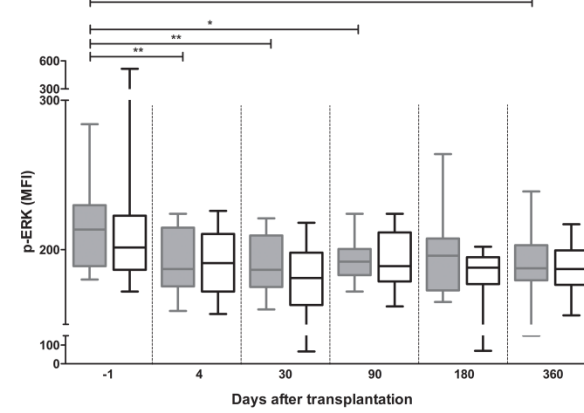
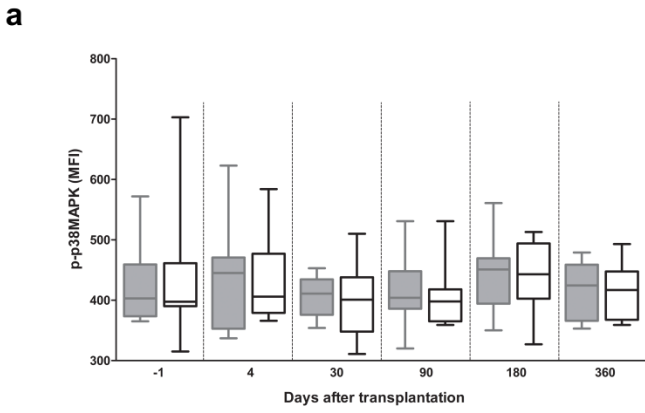
mean (±SD)

Supplementary Figure S5

p38MAPK in CD4+CD28+ cells

ERK in CD4+CD28+ cells

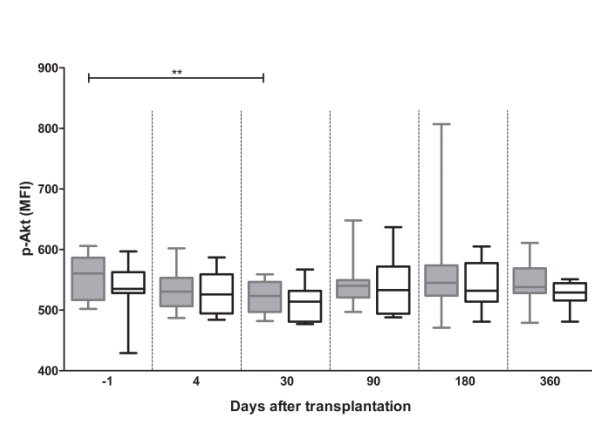
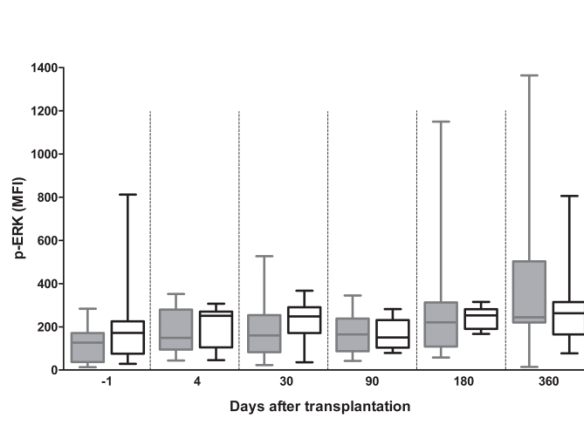
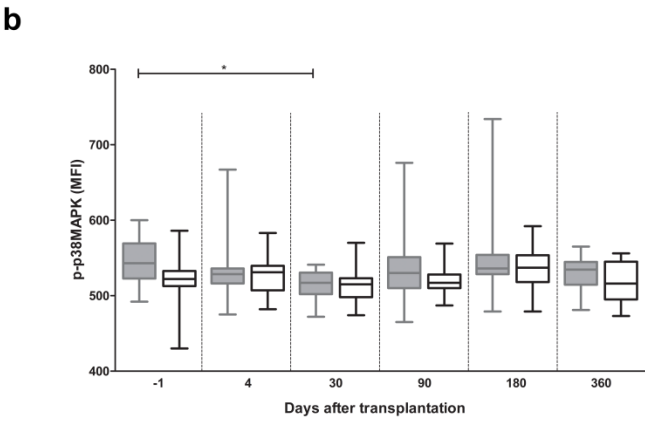
Akt in CD4+CD28+ cells



p38MAPK in CD8+CD28+ cells

ERK in CD8+CD28+ cells

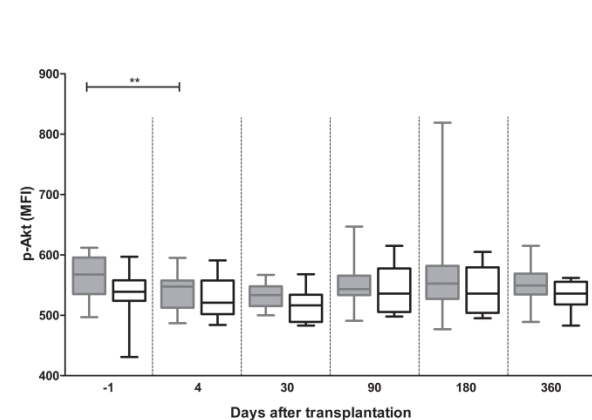
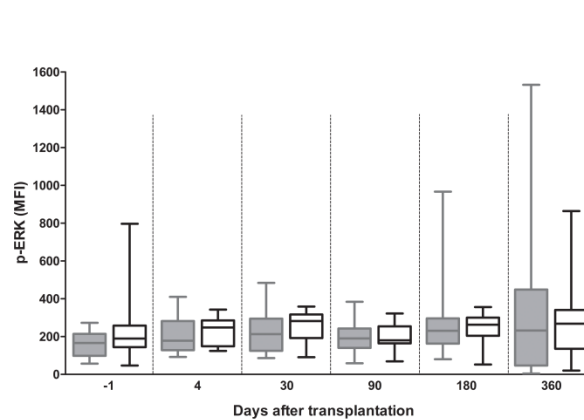
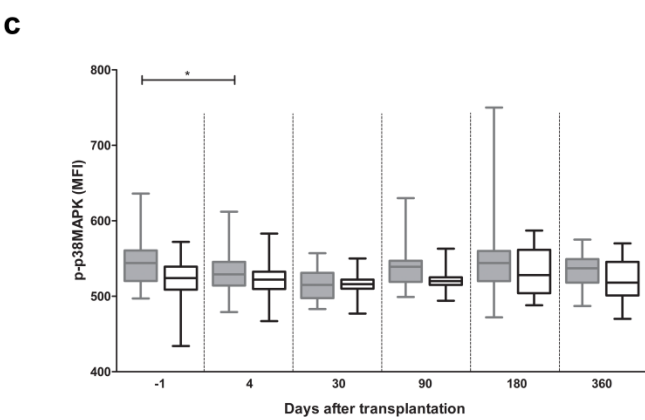
Akt in CD8+CD28+ cells



p38MAPK in CD8+CD28- cells

ERK in CD8+CD28- cells

Akt in CD8+CD28- cells



Supplementary Figure S5. P-p38MAPK, p-ERK and p-Akt in unstimulated T cell subsets of TAC (*grey*)- and BELA (*white*)-treated patients before and after transplantation. **a)** p-p38MAPK (left), p-ERK (middle) and p-Akt (right) within CD4⁺CD28⁺ T cells. P-ERK was significantly decreased in TAC-treated patients, but not in BELA-treated patients. **b)** p-p38MAPK (left), p-ERK (middle) and p-Akt (right) within CD8⁺CD28⁺ T cells. P-p38MAPK and p-AKT were only inhibited at day 30 after transplantation when TAC was given. **c)** p-p38MAPK (left), p-ERK (middle) and p-Akt (right) within CD8⁺CD28⁻ T cells. Phosphorylation inhibition is comparable with CD8⁺CD28⁺ T cells. (Data are plotted as box and whiskers indicating total range; n=20 TAC-treated patients and n=20 BELA-treated patients) *) p < 0.05, **) p < 0.01, ***) p < 0.001

Supplementary Table S6. Univariate regression analysis between patient demographic characteristics and signaling protein phosphorylation

	Predictor	p-p38MAPK		p-ERK		p-Akt	
		β	<i>p value</i>	β	<i>p value</i>	β	<i>p value</i>
Before transplantation	Age (years)	0.381	0.045	-0.175	0.374	0.126	0.523
	Gender (male vs female)	-0.267	0.169	0.267	0.169	-0.273	0.160
	Ethnicity (non caucasian vs caucasian)	-0.370	0.053	-0.016	0.934	-0.324	0.092
	Bodyweight before transplantation (kg)	0.222	0.257	0.321	0.096	0.210	0.284
	Highest PRA (%)	0.068	0.730	0.049	0.805	0.069	0.727
	HLA total mismatches (4 or more vs 3 or less)	-0.046	0.815	0.102	0.607	-0.075	0.704
	HLA DR mismatches (2 vs 1)	-0.228	0.243	0.197	0.315	0.147	0.457
	CMV (negative vs positive)	0.234	0.230	-0.363	0.058	0.142	0.470
	Renal replacement therapy (no vs yes)	-0.067	0.735	-0.023	0.906	-0.099	0.616
	CD86 molecules/monocyte pre-transplantation	-0.065	0.741	-0.038	0.847	-0.101	0.607
	Donor age (years)	0.217	0.267	-0.100	0.613	0.044	0.823
	Donor gender (male vs female)	-0.032	0.873	-0.072	0.715	-0.029	0.884
Day 4	Age (years)	0.423	0.031	0.049	0.806	-0.252	0.205
	Gender (m vs f)	-0.346	0.084	0.021	0.915	-0.033	0.872
	Ethnicity (non caucasian vs caucasian)	-0.285	0.159	-0.006	0.975	-0.152	0.448
	treatment group (tacrolimus vs belatacept)	0.118	0.566	-0.134	0.505	0.242	0.225
	Bodyweight before transplantation (kg)	-0.026	0.900	0.253	0.203	0.087	0.667
	Highest PRA (%)	0.029	0.890	-0.203	0.310	0.231	0.246
	HLA total mismatches (4 or more vs 3 or less)	-0.084	0.689	-0.261	0.188	-0.247	0.214
	HLA DR mismatches (2 vs 1)	-0.349	0.087	-0.054	0.789	-0.430	0.025
	CMV (negative vs positive)	0.270	0.191	0.112	0.577	0.054	0.788
	Renal replacement therapy (no vs yes)	0.087	0.681	-0.081	0.686	0.057	0.779
	CD86 molecules/monocyte pre-transplantation	-0.061	0.771	-0.027	0.895	-0.144	0.473
	Donor age (years)	0.442	0.027	-0.239	0.230	0.133	0.510
Donor gender (male vs female)	-0.047	0.823	-0.092	0.649	0.112	0.577	
Day 360	Age (years)	0.388	0.055	0.213	0.307	0.049	0.816
	Gender (m vs f)	-0.213	0.307	-0.090	0.669	-0.115	0.586
	Ethnicity (non caucasian vs caucasian)	-0.007	0.974	-0.211	0.312	-0.054	0.797
	treatment group (tacrolimus vs belatacept)	0.180	0.389	-0.099	0.639	-0.092	0.662
	Bodyweight before transplantation (kg)	-0.162	0.438	0.161	0.442	-0.045	0.829
	Highest PRA (%)	-0.131	0.533	-0.157	0.453	-0.148	0.481
	HLA total mismatches (4 or more vs 3 or less)	-0.148	0.481	-0.136	0.518	-0.281	0.173
	HLA DR mismatches (2 vs 1)	0.020	0.923	-0.106	0.613	0.154	0.462
	CMV (negative vs positive)	0.285	0.167	0.316	0.124	0.346	0.090
	Renal replacement therapy (no vs yes)	0.080	0.705	0.198	0.344	-0.046	0.828
	CD86 molecules/monocyte pre-transplantation	0.279	0.176	0.171	0.414	0.303	0.141
	Donor age (years)	0.424	0.035	0.369	0.070	0.155	0.458
Donor gender (male vs female)	-0.330	0.107	0.060	0.774	-0.275	0.184	