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SUPPLEMENTAL DIGITAL CONTENT

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A Randomized Controlled Clinical Trial Comparing Belatacept With Tacrolimus After De Novo Kidney Transplantation

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Detailed additionalMaterials and Methods

Additional (immunosuppressive) treatment – detailed information

The additional immunosuppressive therapy was identical in both groups and consisted of basiliximab (Simulect; Novartis Pharma B.V., Arnhem, the Netherlands) in a dose of 20 mg administered intravenously on day 0 (immediately before kidney transplant reperfusion) and day 4 after transplantation. Patients also received a starting dose of 1000 mg mycophenolate mofetil (MMF; CellCept; Roche Pharmaceuticals, Woerden, the Netherlands) twice daily aiming for plasma mycophenolic acid (MPA) predose concentrations between 1.5 and 3.0 mg/L. In addition, all patients received prednisolone in a dose of 50 mg twice daily intravenously on days 0–3, followed by 20 mg orally once daily (on days 4–14), after which the dose was tapered to 5 mg at month 3 after transplantation. Patients continued to receive 5 mg of prednisolone for the rest of the first posttransplant year.

All patients received trimethoprim/sulfamethoxazole prophylaxis for Pneumocystis Jirovecii pneumonia for at least 3 months. Patients receiving a kidney from a cytomegalovirus (CMV)-positive donor and patients who were seropositive for CMV received prophylaxis with valganciclovir for a duration of 6 months.

Additional antirejection therapy consisted of 3 doses of 1000 mg methylprednisolone intravenously for 3 consecutive days. In case of glucocorticoid-resistant rejection, lymphocyte-depleting therapy with 1 dose of 30 mg of alemtuzumab was administered subcutaneously.[1]

Primary end points – BPAR scoring methods

BPAR was scored as part of routine clinical care by a renal pathologist (M.C.C.) per the Banff '15 classification using 2 μm paraffin sections stained for HE, PAS, Jones and immunohistochemistry for C4d on 4 μm sections. After the completion of the study, all biopsies were reviewed again in a blinded fashion by 2 pathologists (M.C.C. and J.v.d.T.) per the Banff '15 classification.[2] In case of discrepancy, biopsies were reviewed and consensus was reached.

Safety

Data on clinical outcomes and (serious) adverse events [(S)AEs] were collected for safety and included patient- and graft survival, estimated GFR (eGFR), proteinuria, and development of donor-specific anti-HLA antibodies (DSA). DSA were retrospectively measured in patient sera 1 day before transplantation, and 1, 6 and 12 months after transplantation. In addition, we monitored delayed graft function, malignancies, (opportunistic) infections, posttransplant diabetes mellitus (PTDM), neurologic events, and acute tacrolimus-induced nephrotoxicity. PTDM was defined as the need for glucose-lowering medical therapy that persisted after month 3 posttransplantation in a patient not needing such treatment pretransplantation. Acute tacrolimus nephrotoxicity was defined as any ≥15% increase of serum creatinine with a return to baseline after tacrolimus dose reduction and after exclusion of other causes of renal transplant function deterioration.

Routine laboratory investigations included blood glucose, glycated hemoglobin (HbA1c), thrombocytes, leucocytes, hemoglobin (Hb), mean corpuscular volume (MCV), low-density lipoproteins (LDL), high-density lipoproteins (HDL) and triglycerides. Blood pressure and body weight were measured at every visit to the outpatient clinic.

Laboratory Studies – detailed information

Blood samples were collected on days 0 (pretransplant), 4, 30, 90, and months 6 and 12. Serum was collected on days 0, 15, 30, and months 6 and 12. Blood and sera were also collected during clinically suspected rejection, before additional antirejection therapy was given. In addition, blood and urine samples were collected on a routine basis as part of routine clinical care. Proportions of $CD8^{+}CD28^{-}$, $CD4^{+}CD57^{+}PD1^{-}$ and $CD8^{+}CD28^{++}$ EMRA T cells were determined pretransplantation (1 day before transplantation) and posttransplant (3 months after transplantation or during rejection) on thawed isolated peripheral blood mononuclear cells.

Absolute numbers of cells in blood

The Becton & Dickinson (BD Biosciences, San José, CA) multi-test 6-color, CD14 FITC (Serotec, Kidlington, United Kingdom) and TruCount Tubes were used to measure absolute numbers of $CD3^{+}$ T cells, $CD4^{+}$ T-helper cells, $CD8^{+}$ cytotoxic T cells, and $CD14^{+}$ monocytes. Absolute numbers were measured in 50 µL blood in the presence of 0.5 mL BD Pharm Lyse. All proportions of subsets measured in PBMCs (see below) were calculated back to these absolute numbers.

Flow cytometry of cytotoxic T cells in peripheral blood mononuclear cells (PBMCs)

Using the Ficoll density method, PBMCs were isolated and stored at -190°C before further characterization. T cells were identified by CD3 (AF700, BD), CD4 (V450, BD) and CD8a (APC-eF780, eBioscience). The immuno-regulatory receptor PD-1 (PE, BioLegend), the cytotoxic marker CD57 (FITC, BD), and the co-stimulatory molecule CD28 (APC, BD) were

determined on $CD4^+$ and $CD8^+$ T cells. EMRA $CD8^+$ T cells were defined by $CD8^+$ $CCR7^-CD45$ RO^-, using CCR7 (PE, BD) and CD45RO (PE-Cy7, BD). Intracellular expression of GrB (PE-CF594, BD) was also assessed.

Saturation of CD86 on monocytes and B cells in blood

The surface expression of free CD86 on $CD14^+$ monocytes was assessed using the Lyse-Wash method per the manufacturer's instruction. Cells were surface-stained in 100 µL blood and erythrocytes were subsequently lysed in 2 mL BD FACS Lysing solution, and washed away before measurement. Monoclonal antibodies used were the leukocyte marker CD45 PerCP (BD); CD19 PE-Cy7 (BioLegend); CD14 FITC (Serotec); and the for belatacept competitive binder of the co-stimulatory molecules of the CD28-pathway, CD86 PE (clone HA5.2B7 Beckman Coulter, Brea, CA).[3] Numbers of CD86 molecules per monocyte were calculated by using QuantiBrite beads per manufacturer's manual (BD).

Detection of serum DSA

Using the Luminex single antigen bead assay (Thermo Fisher Scientific, Waltham, MA) as previously described,[4] the development of DSA was determined by measuring the presence of DSA against HLA class I and II before and at different set time points after transplantation in serum. The MFI cut-off for positivity was 1000.

Panel reactive antibodies

Sera were tested for HLA-antibody specificities by standard National Institutes of Health (NIH) complement-dependent microlymphocytotoxicity test (LCT) using a panel of 54 donors yielding a measurement of the PRA (Panel Reactive Antibody). Ifsamples tested positive using a Human Linker for Activation of T cell ELISA (LAT) or a Complement-Dependent Cytotoxicity Crossmatch(CDC), HLA antibodies were specified with Luminex single antigen test (LABScreen SA, One Lambda Inc., Canoga Park, CA, USA).

Statistical analyses – additional information

Patient, graft and biopsy-proven acute rejection (BPAR)-free survival were defined as 1) time from transplantation to mortality, 2) time from transplantation to transplant nephrectomy, reinitiation of dialysis or (preemptive) retransplantation, and 3) time from transplantation to the diagnosis of BPAR, respectively, or as the end of the 12-month follow-up period, whichever came earlier.

In addition to intention-to-treat analyses, on-therapy analyses were conducted and included evaluable patients who were still on their assigned regimen 12 months after transplantation.

Categorical variables (+ reference groups) in the univariable Cox regression analyses included treatment arm (belatacept *vs.* tacrolimus), gender (female *vs.* male), ethnicity (noncaucasian*vs.* Caucasian), HLA mismatches (4 or more *vs.* less than 4), HLA-DR mismatches (2 *vs.* 1), highest PRA, and CMV serostatus (positive *vs.* negative).

SUPPLEMENTAL RESULTS

On-therapy analysis

The on-therapy analysis at month 12 revealed that eGFR and protein/creatinine ratios were similar between nonrejecting tacrolimus and belatacept-treated patients: median eGFR 57 (45-89) and 58 (37-84) mL/min per 1.73m^2, respectively (SDC, Table 3). Graft-loss censored median eGFR in belatacept-treated patients that suffered from rejection (n = 7) was 36 (28-76) mL/min per 1.73m^2 at month 12, which was lower than in the nonrejectingbelatacept group, p = 0.001.

Graft function in time in belatacept-treated rejectors

The graft function before, during and after BPAR (after additional antirejection therapy) is displayed in SDC, Table 4, for the belatacept-group. Before and after BPAR the highest eGFR is depicted for each patient. It should be noted 6 patients had a decrease in eGFR after BPAR was diagnosed (including 3 graft losses), 2 patients had a similar eGFR after treatment for BPAR, and 3 patients had an improved eGFR.

Donor-specific and nondonor-specific anti-HLA antibodies (DSA and non-DSA)

None of the patients had DSA pretransplantation. During the first posttransplant year, 2 patients developed DSA, both in the belatacept group (Figure 3 and SDC, Table 5). One month after transplantation, patient no. 2 in the belatacept group developed DSA against HLA-DQ2 (Median Fluorescence Intensity [MFI] 3787; most likely C1q-negative[5], but these disappeared hereafter without additional therapy and no AR occurred. Patient no. 20 in the belatacept group developed DSA during her 4th rejection episode (right before losing her graft), which were also detectable in the cross match-dependent cytotoxicity test, against HLA-A1 (MFI 18000), B8

(MFI 22700), DR3 (MFI 11000), DR52 (MFI 5500) and DQ2 (MFI 16500) (SDC, Table 5). At this time, she had already been switched to a tacrolimus-based regimen and had been treated with methylprednisolone and alemtuzumab (Figure 3).

Two and 3 patients, in the belatacept and tacrolimus group, respectively, had nondonor specific anti-HLA antibodies (non-DSA) pretransplantation. In both the belatacept and the tacrolimus group 2 patients developed non-DSA after transplantation (SDC, Table 5).

Pharmacokinetic drug monitoring

SDC, Table 2 depicts belatacept doses, tacrolimus doses and predose concentrations (C_0), MMF doses and mycophenolic acid (MPA) C_0, and prednisolone doses. MPA C_0 were not different between the belatacept and tacrolimus groups after 12 months: 2.30 (0.99–3.54) and 1.83 (0.57–3.67) mg/mL, respectively; $p = 0.25$. Also, prednisolone doses were similar between the belatacept and tacrolimus group in month 12; $p = 0.59$.

Pharmacodynamic drug monitoring

The number of belatacept-free CD86 molecules on monocytes was calculated by measuring the MFI of bound anti-CD86-PE antibodies. These antibodies bind to CD86 molecules to the same epitope but with lower affinity than belatacept, which allows for measurement of free CD86 molecules.[3] A typical example is depicted for the MFIs of CD86-PE on monocytes for a patient treated with belatacept and a patient treated with tacrolimus (SDC, Figure 2A). As evidenced by a linear mixed model, belatacept significantly decreased free CD86 molecules on monocytes at different time points after transplantation compared to tacrolimus (SDC, Figure 2B). Free CD86 molecules/monocyte were 5.9-fold (95%-CI 5.4 to 7.7-fold) higher on day 4 and 5.3-fold (95%-CI 4.0 to 7.0-fold) higher 1 month after transplantation in

tacrolimus-treated patients compared to belatacept-treated patients, p <0.0001. Hereafter the difference in free CD86 molecules/monocyte between the belatacept- and tacrolimus-treated patients reduced, because almost half of the belatacept-treated patients had been converted to tacrolimus-based therapy. In these patients (n = 8), free CD86 expression returned to baseline 3–5 months after conversion (SDC, Figure 2C). Pretransplant values for (future) rejectors and nonrejectors in the belatacept group were significantly different: 753 (428 – 928) free CD86 molecules/monocyte *versus* 882 (528 – 1528) cells/monocyte, respectively, p = 0.04 (SDC, Figure 2D). However, the pretransplant values showed a great overlap between rejectors and nonrejectors, and the numbers of pretransplant CD86 molecules on monocytes were not associated with acute rejection risk (Table 5). No significant differences between (future) rejectors and nonrejectors were observed in posttransplant dynamics of free CD86 molecules/monocyte (SDC, Figure 2E).

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SUPPLEMENTAL TABLES

SDC, Table 1: Adverse events, intention-to-treat analysis*

	Belatacept group (n = 20)	**Tacrolimus group (n= 20)**	**p**
Blood or lymphatic system	0.75 (0.97)	1.00 (0.92)	0.22
• Leucopenia	7	7	
• Anemia	6	10	
• Thrombocytopenia	1	1	
• Other	1	2	
Bleeding and thrombotic events	0.30 (0.57)	0.40 (0.60)	0.52
• Major bleeding	0	2	
• Minor bleeding	4	2	
• Thrombosis	2	4	
Cancer	0	0	-
Cardiovascular	0.95 (0.83)	1.20 (0.83)	0.33
• Acute coronary syndrome / myocardial ischemia	1	1	
• Cardiac decompensation / volume overload	2	3	
• Hypertension	12	17	
• Other	4	3	
Gastrointestinal	0.65 (0.67)	0.60 (1.00)	0.40
• Diarrhea	2	4	
• Other	11	8	
Infection	2.25 (1.86)	1.90 (1.83)	0.46
• Opportunistic infection	0.45 (0.69)	1.90 (1.83)	0.57
▪ BKV	2	1	
▪ CMV	1	2	
▪ EBV	1	0	
▪ HSV	0	1	
▪ VZV	0	0	
▪ Fungal	5	2	
• Other infection	1.80 (1.70)	1.60 (1.64)	0.61
▪ Urinary tract infection	20	14	
▪ Upper respiratory tract infection	8	4	
▪ Pneumonia	2	0	
▪ Gastrointestinal infection	1	2	
▪ Other	5	12	
Locomotor system disorder	0.25 (0.55)	0.20 (0.52)	0.70
Metabolism or nutrition	1.75 (1.16)	2.00 (1.56)	0.84
• Posttransplant diabetes mellitus	1	7	
• Hypo- / hyperglycemic	4	9	

dysregulation			
• Calcium disorder (hypo- / hypercalcemia)	6	3	
• Potassium disorder (hypo- / hyperkalemia)	6	9	
• Hypophosphatemia	6	6	
• Dyslipidemia	8	4	
• Liver enzyme abnormality	3	1	
• Other	1	1	
Nervous system	0.50 (1.00)	0.65 (0.88)	0.36
• CVA/TIA	1	0	
• Tremor	2	8	
• Headache	1	1	
• Other	6	4	
Skin-related disorders	0.15 (0.37)	0.30 (0.47)	0.26
Surgical or procedural complication	0.10 (0.31)	0.20 (0.52)	
• Acute tubular necrosis	1	2	
• Delayed graft function	1	1	
• Renal infarction	0	1	
• Other	0	0	
Tacrolimus-induced nephrotoxicity	0.05 (0.22)	0.40 (0.60)	-
Urological complication	0.55 (0.76)	0.60 (0.88)	0.96
• Hydronephrosis	1	4	
• Urinary leakage	2	1	
• Other	8	7	
Wound-related problem	0.15 (0.37)	0.25 (0.44)	0.44
• Wound infection	2	3	
• Other	1	2	
Other	1.80 (1.44)	2.20 (2.04)	0.63
Total	10.25 (4.18)	11.90 (5.43)	0.41

* Meannumber of adverse events (+standard deviation) per patient are depicted for both treatment groups for the different categories of adverse events. Numbers of adverse events per subcategory are depicted per treatment group.

BKV, BK virus; CMV, cytomegalovirus; CVA, cerebrovascular accident; EBV, Epstein-Barr virus; HSV, herpes simplex virus; N/A, not applicable; VZV, varicella zoster virus; TIA, transient ischemic attack.

SDC, Table 2: Clinical outcomes, intention-to-treat analysis*

	Belatacept group (n = 20)						Tacrolimus group (n = 20)						
	n	**M3**	**n**	**M6**	**n**	**M12**	**n**	**M3**	**n**	**M6**	**n**	**M12**	**p**[†]
Blood pressure													
• Systolic / diastolic (mmHg)	18	137 (98 - 167) / 83 (40 - 94)	17	138 (93 - 181) / 80 (50 - 109)	17	147 (106 - 165) / 81 (50 - 85)	20	144 (108 – 178) / 85 (59 – 98)	20	138 (96 – 184) / 84 (55 – 95)	19	145 (110 – 170) / 85 (45 – 97)	0.64 / 0.42
Kidney function													
• Creatinine (µmol/L)	18	127 (73 – 276)	17	114 (74 – 219)	17	128 (71 – 207)	20	122 (64 – 242)	20	126 (61 – 179)	19	126 (79 – 179)	0.80
• eGFR (mL/min)	18	52 (18 – 72)	17	62 (26 – 88)	17	54 (28 – 89)	20	50 (23 – 80)	20	53 (33 – 85)	19	50 (33 – 84)	0.57
• Protein/Creatinine ratio (mg/mmoL)	18	19.3 (5.2 – 443.2)	17	18.2 (5.8 – 87.7)	17	13.2 (5.7 – 343.8)	20	15.3 (7.3 – 115.0)	20	12.1 (4.2 – 209.6)	19	9.0 (5.3 – 43.5)	0.44
Glucose metabolism													
• Glucose (mmol/L)	18	5.6 (4.7 – 9.4)	17	5.5 (2.9 – 13.7)	17	5.6 (2.9 – 13.7)	20	6.2 (3.7 – 10.7)	20	6.6 (4.7 – 13.5)	19	6.1 (4.3 – 26.7)	0.06
• HbA1c (mmol/mol)	6	36 (29 – 74)	3	37 (33 – 50)	5	41 (33 – 49)	6	48 (37 – 67)	5	42 (30 – 73)	10	46 (33 – 75)	0.31
Lipids													
• Cholesterol total (mmol/L)	18	4.6 (2.9 – 7.5)	16	4.7 (3.0 – 6.9)	16	4.7 (3.4 – 7.2)	20	4.5 (2.9 – 6.5)	20	4.5 (3.2 – 5.9)	19	4.7 (3.1 – 6.9)	0.55
• Triglycerides (mmol/L)	18	2.1 (1.1 – 4.1)	16	1.9 (1.1 – 4.0)	16	2.2 (1.2 – 3.2)	20	2.0 (0.8 – 5.3)	20	1.8 (0.7 – 4.2)	19	1.6 (0.9 – 5.9)	0.13
• HDL-cholesterol (mmol/L)	18	1.1 (0.7 – 3.0)	16	1.2 (0.9 – 3.1)	16	1.2 (0.8 – 3.5)	20	1.3 (0.8 – 2.7)	20	1.2 (0.6 – 2.8)	19	1.4 (0.8 – 3.4)	0.66
• LDL-cholesterol (mmol/L)	18	3.0 (1.2 – 5.3)	16	2.8 (1.0 – 4.9)	16	2.8 (1.3 – 5.3)	20	2.4 (1.2 – 4.4)	20	2.6 (1.2 – 4.3)	19	2.7 (1.2 – 4.3)	0.30
Hematology													

• Hemoglobin (mmol/L)	18	7.2 (5.0 – 9.5)	17	7.6 (6.3 – 9.6)	17	8.2 (7.0 – 9.9)	20	7.5 (6.5 – 9.4)	20	7.7 (6.2 – 10.5)	19	8.4 (6.5 – 10.5)	0.85
• MCV (fL)	18	96 (89 – 100)	17	93 (88 – 98)	17	92 (83 – 97)	20	94 (69 – 106)	20	90 (68 – 102)	19	88 (72 – 108)	0.20
• Thrombocytes (×10^9/L)	17	222 (162 – 401)	17	232 (119 – 477)	17	214 (138 – 394)	20	231 (148 – 495)	20	235 (131 – 457)	19	245 (163 – 380)	0.21
• Leucocytes (×10^9/L)	18	6.3 (1.0 – 15.5)	17	6.9 (1.9 – 11.1)	17	6.4 (2.2 – 17.4)	20	5.9 (1.3 – 11.8)	20	7.4 (1.7 – 14.2)	19	8.4 (4.0 – 12.0)	0.12
Pharmacokinetics													
• Belatacept dose (mg)	16	800 (575 – 938)	11	400 (300 – 45)	10	381 (300 – 450)	-	N/A	-	N/A	-	N/A	N/A
• Tacrolimus dose (mg)	2	10.0 (10.0 – 10.0)	6	5.5 (3.5 – 10.0)	7	5.0 (3.0 – 8.0)	20	4.0 (2.0 – 8.0)	20	4.0 (2.0 – 6.0)	19	4.0 (2.5 – 7.0)	0.19
• Tacrolimus concentration (ug/L)	2	2.2 (1.5 – 5.5)	6	5.8 (4.2 – 8.3)	7	7.2 (4.5 – 8.6)	20	7.0 (4.1 – 10.7)	20	6.3 (2.6 – 9.9)	19	6.8 (4.4 – 13.3)	0.53
• Mycophenolate mofetil dose (mg)	18	1000 (500 – 2000)	17	1000 (500 – 2000)	17	1000 (500 – 2000)	20	1000 (500 – 2000)	19	1000 (0 – 2000)	18	1000 (0 – 2000)	0.47
• Mycophenolate acid concentration (mg/mL)	17	3.04 (0.52-10.00)	16	2.45 (0.98 – 5.21)	17	2.30 (0.99 – 3.54)	20	2.53 (1.03 – 10.00)	19	1.69 (0.96 – 4.24)	18	1.83 (0.57 – 3.67)	0.25
• Prednisone dose (mg)	18	5.0 (5.0 – 10.0)	17	5.0 (5.0 – 10.0)	17	5.0 (5.0 – 10.0)	20	5.0 (5.0 – 10.0)	20	5.0 (5.0 – 10.0)	19	5.0 (2.5 – 10.0)	0.59

* Censored for graft loss and death; † Comparison between patients from the belatacept group and the tacrolimus group 12 months after transplantation

Target tacrolimus C0 of 5 – 10 ng/mL were achieved in 75%, 85% and 95% of patients in the tacrolimus group 3, 6 and 12 months after transplantation, respectively. Target MPA C0 of 1.5 – 3.0 mg/mL were achieved in 45%, 40% and 40% of patients in the tacrolimus group respectively 3, 6 and 12 months after transplantation, and in 30%, 40% and 60% of patients in the belatacept group respectively 3, 6 and 12 months after transplantation.

Data present medians (plus ranges).

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high density lipoproteins; LDL, low density lipoproteins; M3, 3 months after transplantation; M6, 6 months after transplantation, M12, 12 months after transplantation; MCV, mean corpuscular volume

SDC, Table 3: Graft function 12 months after transplantation

	Belataceptnonrejectors (n=9)	**Belatacept rejectors, censored for graft loss (n=8)**	**Belatacept rejectors, including graft loss (n=11)**	**Tacrolimus (n=19)**	**Tacrolimus nonrejectors (n=17)**
Creatinine (µmol/L)	106 (71-143)	163 (93-207)	-	126 (79-179)	119 (79-178)
eGFR (mL/min)	57 (45-89)	36 (28-76)	34 (0-76)	50 (33-84)	58 (37-84)
Protein/Creatinine ratio	11.4 (7.9-25.0)	12.2 (5.7-343.8)	-	9.0 (5.3-43.5)	9.0 (5.3-43.5)

Data are medians (plus ranges). Graft function was compared between 1) the belatacept-treatedrejectors and belatacept-treatednonrejectors and 2) the tacrolimus-treated and belatacept-treated nonrejectors, using the Mann-Whitney U test. Creatinine concentrationat month 12 was significantly higher and eGFR at month 12 was consequently significantly lower in belatacept-treated rejectors than in belatacept-treated nonrejectors, both p=0.001. These parameters did not differ between nonrejectingbelatacept-treated and tacrolimus-treated patients at month 12.

In the group of "Belatacept rejectors, including graft loss" the 3 patients that lost their grafts were set to an eGFR of zero on month 12. Creatinine and Protein/Creatinine ratio were not calculated for this group, since these could not be determined for the 3 patients after graft loss.

eGFR, estimated glomerular filtration rate

SDC, Table 4: Response to antirejection therapy in belatacept-treated rejectors

No. (Patient)	Creatinine			eGFR		
	Best before	BPAR	Best after	Best before	BPAR	Best after
3	84	132	93	59	35	52
5	89	698	N/A	56	5	0
6	155	211	136	39	27	45
7	148	188	164	45	34	40
13	89	107	93	80	65	76
14	109	148	110	72	50	71
15	227	279	145	25	19	41
16	106	210	152	62	28	41
17	305	807	N/A	14	5	0
19	325	367	161	18	16	41
20	162	175	N/A	33	30	0

Patient numbers are the same depicted as in Figure 3. For detailed clinical course per patient, please refer to this figure. Creatinine and estimated glomerular filtration rates (eGFR) are given for the 10 belatacept-treated rejectors before, during and after rejection (when applicable, before second rejection episodes). Both before and after rejection the highestmeasuredeGFRs are depicted. Patients no. 6, 16 and 19 were switched to tacrolimus (almost) immediately after rejection occurred. Patients no. 5 lost her graft immediately after rejection, and patients no. 17 and 20 were switched to tacrolimus, but still lost their grafts thereafter (eGFRs after rejection were set to zero). Patients no.7, 14 and 15 were switched to tacrolimus after a second episode of acute rejection. Patient no. 13 was diagnosed with BPAR after revision of the biopsy, and was therefore not treated with additional antirejection therapy. This patient had an isolated v-lesion which may explain the excellent outcome despite no treatment. Finally, patient no. 3 was switched after his third rejection episode.

N/A, not applicable

SDC, Table 5: Anti-HLA antibodies in serum

		Belatacept group (n = 20)	Tacrolimusgroup (n = 20)	p
Donor-specific	*Preexistent*	-	-	-
	De novo	2 (10%) *[Patients no. 2 and 20]*	-	0.49
Nondonor-specific	*Preexistent*	2 (10%) *[Patients no. 2 and 12]*	3 (15%) *[Patients no. 3, 11 and 12]*	1.00
	De novo	2 (10%) *[Patients no. 7 and 20]*	2 (10%) *[Patients no. 6 and 20]*	1.00

Patient numbers are the same as in Figure 3. None of the patients had donor-specific antihuman leukocyte antigen (HLA) antibodies (DSA) pretransplantation. During the first posttransplant year, 2 patients developed DSA, both in the belatacept group. Patient no. 2 in the belatacept group developed DSA against HLA-DQ2 (MFI 3787) 1 month after transplantation, but this disappeared hereafter without additional therapy and no acute rejection occurred. Patient no. 20 in the belatacept group had DSA, which were also detectable in the cross match-dependent cytotoxicity test, against HLA-A1 (MFI 18,000), -B8 (MFI 22700), -DR3 (MFI 11000), -DR52 (MFI 5500) and -DQ2 (MFI 16500) during her 4th rejection episode right before losing her graft. Now, she was already switched to a tacrolimus-based regimen and had been treated with multiple methylprednisolone and alemtuzumab gifts (Figure 3).

Two and 3 patients, in the belatacept and tacrolimus group, respectively, had nondonor-specific anti-HLA antibodies (non-DSA) pretransplantation. Patient no. 2 in the belatacept group had non-DSA against DR1 that remained present after transplantation, without clinical consequences. Patient no. 12 in the belatacept group had non-DSA against HLA-Dp11 which disappeared after transplantation. No rejection occurred. Patients no. 3, 11 and 12 in the tacrolimus group had non-DSA pretransplantation against HLA-B76, -DP1, and -DP; -DR4; and -B15; respectively. Only patient no. 3 suffered from an acute rejection Banff type 2B. No serum was available from this patient at the time of rejection, but in sera from month 1 to 12 no non-

DSA were detected. Also in the other 3 patients,preexistent anti-HLA antibodies disappeared after transplantation.

Two patients in the belatacept group (no. 7 and no. 20) developed non-DSA. Patient no. 7 in the belatacept group developed non-DSA against HLA-DQ3 (measured on day 30) before he was diagnosed with an acute Banff type 2B rejection 44 days after transplantation. These non-DSA were also positive during rejection. After treatment with methylprednisolone they were no longer detectable and remained so throughout follow-up. Patient no. 20 in the belatacept group developed non-DSA against HLA-A24, -A68, and -DQ3 simultaneously with DSA. Two patients in the tacrolimus group (no. 6 and no. 20) developed non-DSA, without clinical consequences in the first year after transplantation against HLA-DP14 and HLA-A24, respectively.

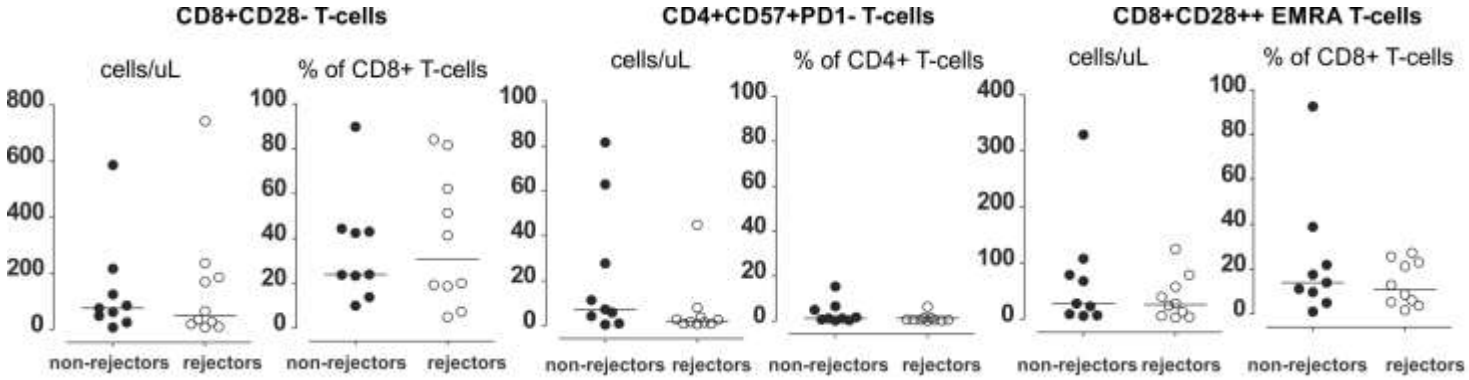
SDC, Table 6: Baseline characteristics of (future) rejectors and nonrejectors in the belatacept group

	Belatacept (n= 20)		
	rejectors (n= 11)	**nonrejectors (n= 9)**	**p**
Age at transplantation (years)	47 (25-76)	60 (40-74)	0.41
Male / female	7 (64%) / 4 (36%)	7 (78%) / 2 (22%)	0.49
Ethnicity			0.07
• Caucasian	11 (100%)	6 (67%)	
• African	-	2 (22%)	
• Asian	-	1 (11%)	
Body weight (kg)	83.3 (63.5 - 111.4)	76.0 (56.6 - 98.6)	0.26
HLA A mismatch (mean ± SD)	1.0 (± 0.6)	1.1 (± 0.8)	0.84
HLA B mismatch (mean ± SD)	1.4 (± 0.5)	1.2 (± 0.4)	0.63
HLA DR mismatch (mean ± SD)	1.2 (± 0.4)	1.0 (± 0.5)	1.00
Current PRA (%)	0 (0 - 4)	0 (0 - 5)	0.55
Peak PRA (%)	4 (0 - 6)	4 (0 - 5)	0.37
CMV status at transplantation			0.37
• Donor + / Recipient -	1 (9%)	2 (22%)	
• Donor + / Recipient +	2 (18%)	2 (22%)	
• Donor - / Recipient -	6 (55%)	1 (11%)	
• Donor - / Recipient +	2 (18%)	4 (44%)	
Donor age at transplantation (years)	60 (43 - 69)	53 (24 – 71)	0.33
Related / unrelated donor	4 (36%) / 7 (64%)	2 (22%) / 7 (78%)	0.64
Cause of end-stage renal disease			0.90
• Diabetes mellitus	1 (9%)	2 (22%)	
• Hypertension	-	2 (22%)	
• IgA nephropathy	-	1 (11%)	
• Polycystic kidney disease	2 (18%)	1 (11%)	
• Obstructive nephropathy	2 (18%)	1 (11%)	
• Unknown	3 (27%)	2 (22%)	
• Other	3 (27%)	0 (0%)	
Renal replacement therapy			0.37
• None (preemptive)	7 (64%)	3 (33%)	
• Hemodialysis	3 (27%)	4 (44%)	
• Peritoneal dialysis	1 (9%)	2 (22%)	
Time on dialysis therapy (days)	560 (147-2633)	425 (123-2782)	1.00
Number of kidney transplantation			1.00
• First	10 (91%)	9 (100%)	
• Second	1 (9%)	-	

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

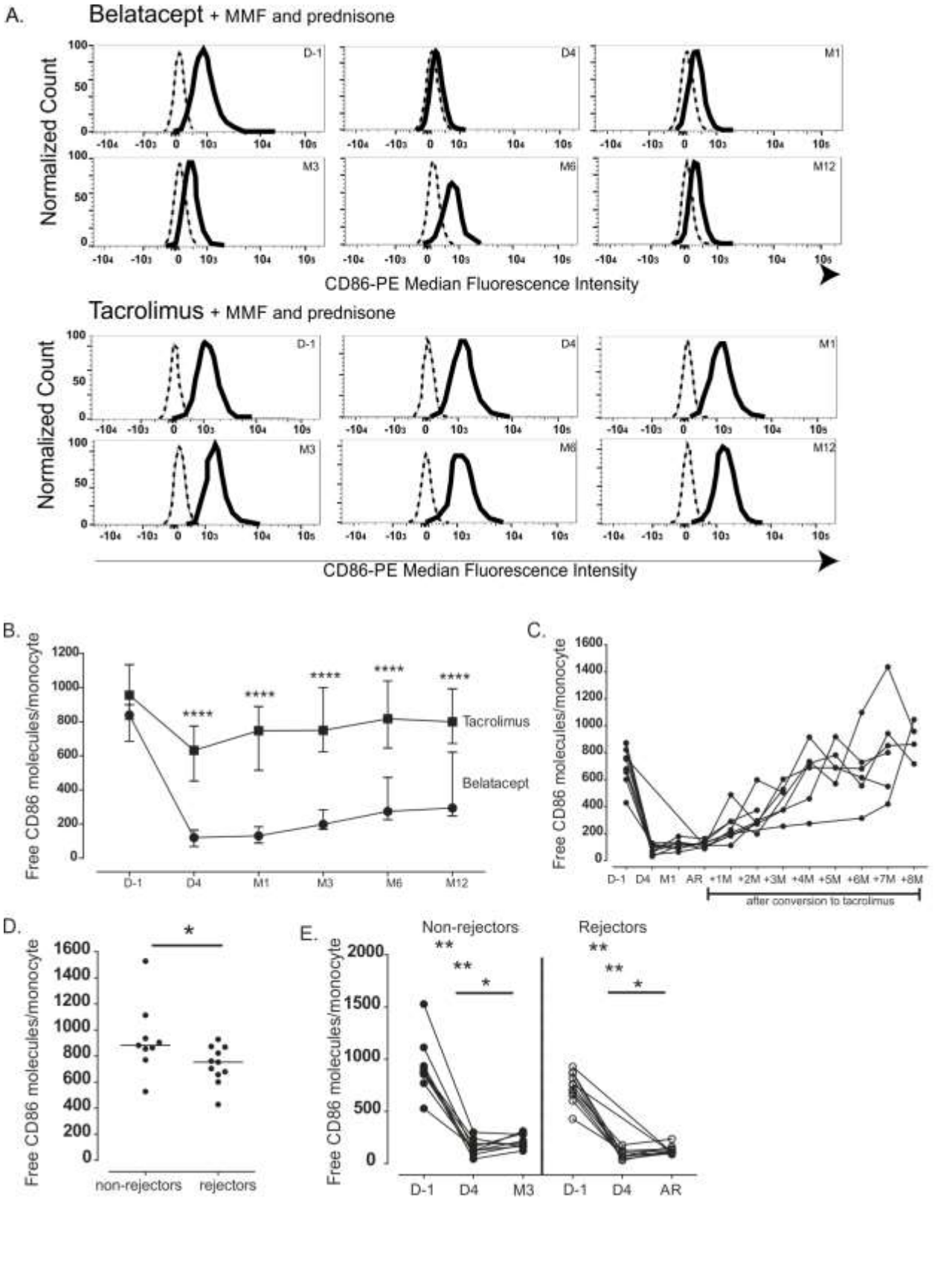
BPAR, biopsy-proven acute rejection; CMV, cytomegalovirus; HLA, human leukocyte antigen; PRA, panel reactive antibodies; SD, standard deviation.

SUPPLEMENTAL FIGURES



SDC, Figure 1. CD8+CD28-, CD4+CD57+PD1- and CD8+CD28++ EMRA T cells during rejection or 3 months after transplantation. CD4+ and CD8+ T cells were gated from 7-AAD negative CD3+ lymphocytes (based on forward and sideward scatter) and EMRA T cells were gated as CCR7- and CD45RO- T cells (See Figure 4). The absolute numbers and percentages of CD8+CD28-, CD4+CD57+PD1- andCD8+CD28++ EMRA T cells are presented for nonrejectors 3 months after transplantation and for rejectors during acute rejection beforeadditional antirejection therapy was given.

N.B.: From 1 rejector no materials were obtained during rejection, because biopsy-proven acute rejection was diagnosed in retrospect after revision by a second pathologist.


A.
Belatacept + MMF and prednisone
Normalized Count
D-1
D4
M1
M3
M6
M12
CD86-PE Median Fluorescence Intensity
Tacrolimus + MMF and prednisone
Normalized Count
D-1
D4
M1
M3
M6
M12
CD86-PE Median Fluorescence Intensity
B.
Free CD86 molecules/monocyte
Tacrolimus
Belatacept
D-1
D4
M1
M3
M6
M12
C.
Free CD86 molecules/monocyte
D-1 D4 M1 AR +1M +2M +3M +4M +5M +6M +7M +8M
after conversion to tacrolimus
D.
Free CD86 molecules/monocyte
non-rejectors
rejectors
E.
Non-rejectors
Rejectors
Free CD86 molecules/monocyte
D-1 D4 M3 D-1 D4 AR

SDC, Figure 2. Pharmacodynamic drug monitoring of belatacept.
The median fluorescence intensity (MFI) of CD86 was assessed on circulating monocytes in belatacept and tacrolimus-treated patients using a competitive monoclonal antibody (clone HA5.2B7, solid line) with an IgG control (dotted line) (A). Free CD86 molecules per monocyte were calculated from MFIs (medians + interquartile ranges) and compared between the belatacept (triangles) and tacrolimus (squares) group on different time points in an intention-to-treat analysis using a linear mixed model (B). Free CD86 molecules/monocyte in tacrolimus-treated patients compared to belatacept-treated patients were 5.9-fold (95% CI 4.5 to 7.7-fold) higher on day 4; 5.3-fold (95% CI 4.0 to 7.0-fold) higher on month 1; 3.7-fold (95% CI 2.8 to 4.8-fold) higher on month 3; 2.6-fold (95% CI 2.0 to 3.4-fold) higher on month 6; and 2.1-fold (95% CI 1.6 to 2.8-fold) on month 12. Free CD86 molecules/monocytes were measured in n = 8 patients which were converted to a tacrolimus-based therapy after acute belatacept-resistant rejection (C). Numbers of free CD86 molecules/monocytes pretransplantation were compared between nonrejectors(n = 9) and rejectors (n = 11) in the belatacept group (D), as well as CD86 molecules/monocyte on day 4 and month 3 or during rejection after transplantation (E).
AR, acute rejection; D-1, 1 day pretransplantation; D4, 4 days after transplantation; M1, 1 month after transplantation; M3, 3 months after transplantation; M6, 6,
months after transplantation; M12, twelve months after transplantation; MMF, mycophenolate mofetil
* $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$
N.B.: In (D) black lines represent the medians; the upper and lower border of the boxes represent the 25th and 75^{th} percentiles; the error lines represent 10th and 90th percentiles.