A Randomized Trial of Bortezomib in Late Antibody-Mediated Rejection (BORTEJECT)

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Supplementary Material

Supplementary Table 1. Linear mixed models to assess inter-group differences in eGFR slope

Linear mixed model	eGFR slope (ml/min/1.73 m^2/year)	95% confidence interval	*p* Value
Model 1[a]			
Bortezomib	-4.68	-8.59 to -0.76	0.02
Placebo	-5.15	-8.87 to -1.42	0.008
Difference between study groups	0.47	-4.81 to 5.75	0.86
Model 2[b]			
Bortezomib	-5.87	-9.90 to -1.85	0.004
Placebo	-5.42	-9.34 to -1.50	0.008
Difference between study groups	-0.45	-5.98 to 5.07	0.87
Model 3[c]			
Bortezomib	-4.62	-8.65 to -0.58	0.025
Placebo	-5.87	-9.72 to -2.02	0.004
Difference between study groups	1.25	-4.23 to 6.73	0.65

[a]In model 1, eGFR values after graft loss were counted as missing.

[b]In model 2 (sensitivity analysis), eGFR values after graft loss were set to zero.

[b]In model 3 (sensitivity analysis), eGFR values obtained after 3 months were included.

Supplementary Table 2. Patients subjected to indication biopsies

Patient ID	Treatment allocation	Index biopsy		Indication biopsy				24-month biopsy	
		eGFR[a]	Diagnosis	Day	eGFR[a]	Diagnosis	Therapy	eGFR[a]	Diagosis
1	Bortezomib	21.1	c/a C4d+ ABMR	574	13.9	c/a C4d- ABMR	none	7.1[b]	c/a C4d- ABMR
2	Placebo	50.8	c/a C4d- ABMR	619	42.9	c/a C4d- ABMR; PVAN[c]	Tac/MPA reduction	31.1	c/a C4d- ABMR
4	Bortezomib	37.5	c/a C4d- ABMR	494	10.4	c/i C4d- ABMR	none	Dialysis (day 509)	no biopsy
6	Placebo	20.6	c/a C4d+ ABMR	506	10.1	c/a C4d- ABMR	none	Dialysis (day 619)	no biopsy
7	Placebo	27.3	c/a C4d- ABMR	519	18.0	c/a C4d+ ABMR	none	14.9	c/a C4d- ABMR
9	Bortezomib	48.6	c/a C4d+ ABMR	598	19.9	Nondiagnostic scarred tissue	none	Dialysis (day 612)	no biopsy
22	Placebo	46.2	a/a C4d+ ABMR	376[d]	15.5	c/a C4d+ ABMR BL lesion	IA, steroids	13.1	c/a C4d+ ABMR
37	Placebo	65.5	a/a C4d- ABMR	364	15.7	a/a C4d- ABMR	none	18.6	c/a C4d- ABMR

a/a, acute/active; ABMR, antibody-mediated rejection; c/a, chronic/active; c/i, chronic/inactive; eGFR, estimated glomerular filtration rate; IA, immunoadsorption; MPA, mycophenolic acid; PVAN, polyoma virus-associated nephropathy; SV40, simian virus 40; Tac, tacrolimus.

[a]eGFR according to the Mayo equation (ml/min/1.73 m^2)

[b]Patient No.1 returned to dialysis 729 days after study initiation, five days after the 24-month follow-up biopsy.

[c]Diagnosis of PVAN was based on immunohistochemical tubular staining for SV40 and BK viremia ($6x10^4$ copies/mL).

[d]Patient No. 22 discontinued her immunosuppression because of medication non-adherence at least four weeks before an indication biopsy that was performed for acute deterioration of graft function. Rejection treatment included high dose steroids (three days dexamethasone at 100 mg per day) and a 4-week course of IA with protein A (16 sessions).

Supplementary Table 3. Peripheral blood cell count in relation to treatment

Parameter	Bortezomib (n=21)	Placebo (n=23)	*p* Value
Hemoglobin levels (g/dL)			
Level at nadir, median (IQR)	8.9 (7.5-10.5)	10.0 (7.7-12.3)	0.20
Anemia grade at nadir[a], n (%)			
0	0 (0)	5 (21.7)	
I	8 (38.1)	7 (30.4)	
II	8 (38.1)	3 (13.0)	<0.001
IIII	1 (4.8)	4 (17.4)	
IV	4 (19.0)	4 (17.4)	
Thrombocyte count (x10^9/L)			
Level at nadir, median (IQR)	115 (67-163)	187 (143-261)	<0.001
Thrombocytopenia grade at nadir[a], n (%)			
0	7 (33.3)	15 (65.2)	
I	8 (38.1)	8 (34.8)	
II	2 (9.5)	0 (0)	0.002
IIII	3 (14.3)	0 (0)	
IV	1 (4.8)	0 (0)	
Leukocyte count (x10^9/L)			
Level at nadir, median (IQR)	3.7 (2.6-4.8)	5.6 (4.1-6.8)	0.002
Leukopenia grade at nadir[a], n (%)			
0	8 (38.1)	18 (78.3)	
I	10 (47.6)	5 (21.7)	
II	2 (9.5)	0 (0)	0.019
IIII	1 (4.8)	0 (0)	
IV	0 (0)	0 (0)	

IQR, interquartile range

[a]For grading of hematologic toxicities we used the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), which have also been applied in transplant candidates and recipients (see reference No. 19).

Supplementary Table 4. Immunosuppressive therapy: trough levels and daily doses

Parameter	**Bortezomib (n=21)**	n assessed	**Placebo (n=23)**	n assessed
Tacrolimus-based therapy at baseline, n (%)	10 (47.6)		14 (60.9)	
Trough levels (ng/ml), median (IQR)				
Day 0	5.5 (4.6-6.7)	10	5.6 (4.5-6.0)	13
3 months	7.9 (6.5-9.9)	9	6.8 (6.0-8.2)	14
6 months	7.2 (5.5-8.6)	10	8.0 (6.5-8.8)	14
12 months	7.3 (6.3-9.5)	10	8.6 (6.7-9.7)	14
18 months	7.8 (6.7-11.0)	10	7.8 (6.9-9.6)	14
24 months	7.6 (6.3-8.2)	8	8.5 (5.6-10.6)	14
Cyclosporin A-based therapy at baseline, n (%)	10 (47.6)		6 (26.1)	
Trough levels (ng/ml), median (IQR)				
Day 0	53 (38-77)	10	60 (51-96)	5
3 months	74 (66-122)	8	94 (77-102)	6
6 months	74 (60-122)	10	100 (79-181)	6
12 months	80 (66-101)	10	83 (69-138)	6
18 months	73 (67-85)	8	80 (57-153)	6
24 months	56 (50-80)	7	61 (51-69)	5
Sirolimus-based therapy at baseline, n (%)	0 (0)		1 (4.3)	
Trough levels (ng/ml)				
Day 0	-	0	5.3	1
3 months	-	0	6.1	1
6 months	-	0	7.6	1
12 months	-	0	5.1	1
18 months	-	0	7.2	1
24 months	-	0	5.7	1
Everolimus-based therapy at baseline, n (%)	1 (4.8)		1 (4.3)	
Trough levels (ng/ml)				
Day 0	3.4	1	6.8	1

3 months	3.6	1	8.9	1
6 months	4.0	1	7.1	1
12 months	8.7	1	9.6	1
18 months	5.2	1	5.9	1
24 months	5.3	1	5.8	1
MMF-based therapy at baseline, n (%)	9 (42.9)		10 (43.5)	
Daily dose (g), median (IQR)				
Day 0	1.0 (0.75-1.75)	9	1.25 (1.0-2.0)	10
3 months	1.0 (1.0-1.5)	13	1.5 (1.0-2.0)	11
6 months	1.0 (1.0-1.5)	13	1.5 (1.0-2.0)	11
12 months	1.0 (1.0-1.5)	13	1.75 (1.38-2.0)	10
18 months	1.0 (1.0-1.5)	13	2.0 (1.38-2.0)	10
24 months	1.0 (1.0-1.5)	11	1.75 (1.0-2.0)	10
EC-MPA-based therapy at baseline, n (%)	7 (33.3)		10 (43.5)	
Daily dose (g), median (IQR)				
Day 0	0.54 (0.36-1.08)	7	0.72 (0.36-0.81)	10
3 months	0.72 (0.36-1.08)	7	0.72 (0.36-0.72)	11
6 months	0.72 (0.36-1.44)	7	0.72 (0.36-1.08)	11
12 months	0.72 (0.41-1.26)	8	0.72 (0.36-1.08)	11
18 months	0.72 (0.41-1.26)	8	0.72 (0.36-1.08)	11
24 months	0.72 (0.54-1.44)	7	0.54 (0.36-0.81)	10
Azathioprine-based therapy at baseline, n (%)	0 (0)		1 (4.3)	
Daily dose (mg)				
Day 0	-	0	12.5	1
3 months	-	0	12.5	1
6 months	-	0	12.5	1
12 months	-	0	12.5; 25	2
18 months	-	0	12.5; 25	2
24 months	-	0	12.5; 25	2
Prednisolone-based therapy at baseline, n (%)	18 (85.7)		20 (87.0)	

Daily dose (mg), median (IQR)				
Day 0	5.0 (5.0-5.0)	18	5.0 (5.0-5.0)	20
3 months	5.0 (5.0-5.0)	21	5.0 (5.0-5.0)	23
6 months	5.0 (5.0-5.0)	21	5.0 (5.0-5.0)	23
12 months	5.0 (5.0-5.0)	21	5.0 (5.0-5.0)	23
18 months	5.0 (5.0-5.0)	21	5.0 (5.0-5.0)	23
24 months	5.0 (5.0-5.0)	18	5.0 (5.0-5.0)	22

IQR, interquartile range; MMF, mycophenolate mofetil; EC-MPA, enteric-coated mycophenolic acid.
[a]With the exception of a higher MMF dose after 12 (p=0.036) and 18 months (p=0.026) in the placebo arm, differences between the two study groups were nonsignificant (p>0.05).