Efficacy of Bortezomib as an Adjunctive Therapy for Refractory Chronic Active Antibody-Mediated Rejection in Kidney Transplant Patients: A Single-Center Experience

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ABSTRACT

Background. Chronic active antibody-mediated rejection (CAMR) has unsatisfactory prognosis in spite of intensive standard antihumoral treatment. Efficacy of additional bortezomib in CAMR remains uncertain.

Methods. A retrospective chart review was conducted among kidney transplant patients with biopsy-proven CAMR. Our standard CAMR protocol included plasma exchange, intravenous immunoglobulin, and rituximab. Repeated treatment was provided for refractory cases. Patients receiving at least 1 course of bortezomib were enrolled as the bortezomib group. Allograft outcome was compared among patients receiving repeated standard protocol alone and the bortezomib group.

Results. Thirteen and 15 patients were assigned to the bortezomib and control groups, respectively. Repeated bortezomib protocol was given for 1, 2, 3, and 4 courses in 6, 4, 1, and 2 patients, respectively. With a median follow-up time after treatment of 41.8 (18.3-47.4) months, the bortezomib group had a lower rate of glomerular filtration rate declination (-4.20 $\pm$ 4.89 mL/min/y vs -12.33 $\pm$ 10.44 mL/min/y; $P = .014$), a higher rate of disappearance of donor specific antibodies (69.2% vs 25%; $P = .03$), a lower rate of allograft loss (15.4% vs 66.7%; $P = .006$), and better allograft survival ($P = .006$).

Conclusion. In CAMR, additional bortezomib treatment was more effective in eliminating donor specific antibodies and improving allograft survival than standard protocol treatment.

ALLOGRAFT rejection is the principal obstacle of kidney transplantation. Post-transplant patients presenting for nonadherence or underimmunosuppression may develop de novo donor specific antibodies (DSA) and, subsequently, chronic active antibody-mediated rejection (CAMR) [1,2], which is the most common cause of late allograft failure [3]. Despite intensive antihumoral treatment—including plasma exchange (PE), intravenous immunoglobulin (IVIg), and rituximab infusion—outcomes of CAMR remain unfavorable in most cases [4]. Bortezomib, a proteasome inhibitor, appears to be an effective desensitizing agent [5] and can also salvage treatment in early acute antibody mediated rejection (AMR) [6,7]. However, the role of bortezomib in CAMR remains limited. The present study aimed to compare the efficacy between standard treatment alone and the addition of bortezomib in refractory CAMR.

PATIENTS AND METHODS

We reviewed all kidney transplant recipients with biopsy-proven CAMR according to the Banff 2013 classification at our center between January 1, 2005, and December 31, 2016. Patients presenting with DSA with persistent rejection on follow-up biopsy and

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receiving repeated courses of antihumoral treatment were enrolled in the study. Anti-HLA antibody was analyzed using the Luminex single antigen bead assay (One Lambda, Canoga Park, CA, United States) and reported as mean fluorescence intensity (MFI). C4d deposition was demonstrated using the immunofluorescence technique.

The patients were classified in 2 groups: standard treatment alone (control group) and bortezomib group. Our standard protocol for CAMR consisted of plasma exchange (PE) for 1 to 3 sessions followed by IVIg **1 g/kg/d** for 2 days. Rituximab **375 mg/m^2** was also administered in severe or refractory cases. Repeated treatment was provided for refractory CAMR. We also augmented the immunosuppressive regimen by replacing cyclosporine or mammalian target of rapamycin inhibitor by tacrolimus, meanwhile azathioprine is being replaced with mycophenolate. The daily dosage of mycophenolate was increased to **1.5 g to 2 g** of mycophenolate mofetil, as could be tolerated.

For the bortezomib group, bortezomib **1.75 mg** was administered subcutaneously on days 1, 4, 8, and 11, adjunctive to standard treatment. We also repeated bortezomib protocols in refractory cases. All treatments were provided based on the decision of the attending physician. Medical records were reviewed for baseline characteristics, histopathologic findings, DSA type and MFI, antihumoral treatment, and complications. DSA MFI, allograft outcomes, and patient outcomes at the end of July 31, 2017, were analyzed.

Baseline characteristics and demographic data were summarized as percentage (%) or mean ± standard deviation for normally distributed data and median with interquartile ranges for others. Categorical variables were compared using the χ^2 test. Continuous variables were compared using the Student *t* or Mann-Whitney *U* tests. The Kaplan-Meier method was used to compare allograft survival between both groups. All statistical analyses were executed using SPSS, Version 18.0 (Chicago, IL, United States).

RESULTS

Of 28 patients with refractory CAMR, 13 and 15 patients were assigned to the bortezomib and control group, respectively. All patients received transplantation and were regularly followed up at our center with a median follow-up time of 121.8 (55-218.6) months. For the control group, 10 patients were historical controls, 3 patients denied administration of bortezomib, 1 patient had DSA disappearance after repeated treatment, and 1 patient had not been offered bortezomib from the attending nephrologist. Baseline characteristics, pathologic findings, and treatment of patients in both groups are summarized in Table 1. Both groups had comparable time post-transplantation and demographic data. Patients in the bortezomib group tended to have lower serum creatinine and higher degree of proteinuria at diagnosis of CAMR but without reaching statistical significance.

In terms of the first allograft histopathology, peritubular capillaritis seemed to be more commonly noted in the bortezomib group (84.6% vs 53.3%, $P = .08$). Glomerulitis and positive C4d deposition also tended to be observed more often in the bortezomib group, without reaching significance ($P = .34$ and 0.19, respectively). DSA class II was detected among all patients in the bortezomib group and 77.8% of patients in the control group. Most were anti-HLA

Table 1. Baseline Characteristics, Pathologic Findings, and Treatment of Patients in Both Groups

Characteristics	Bortezomib (n = 13)	Control (n = 15)	*P* Value
Mean time after KT, mo	139.5 ± 45.3	110.2 ± 34.2	.06
Age at transplantation, y	33.1 ± 12.7	33.7 ± 6.9	.88
Female, %	30.8	20	.51
Living donor, %	76.9	53.3	.19
No. of HLA mismatch	3 (1-6)	3 (1-5)	.62
PRA pre-KT, %	0 (0-18.8)	0 (0-92)	.87
Serum creatinine at Dx, mg/dL	1.58 ± 0.32	1.82 ± 0.33	.07
eGFR at Dx, mL/min/1.73 m^2	51.2 ± 10.9	44.7 ± 10.0	.11
Urine protein, g/gCr	0.7 ± 0.6	1.2 ± 1.1	.11
Glomerulitis, %	100	93.3	.34
Peritubular capillaritis, %	84.6	53.3	.08
Concomitant acute cellular rejection, %	7.7	13.3	.63
IgA nephropathy, %	23.1	26.7	.83
Cg score	1 (0-3)	1 (0-3)	.98
Tubulointerstitial fibrosis, %	10 (0-30)	10 (0-40)	.58
Positive C4d deposit, %	76.9	53.3	.19
DSA class I, %	46.2	33.3	.49
DSA class II, %	100	80	.09
Maximum DSA MFI	11,207 (3892-21,166)	5386 (947-17,004)	.049
Number of PE session (sessions)	8.1 ± 5.5	5.5 ± 3.4	.16
Total IVIg dosage, g	533.9 ± 353.6	340.3 ± 355.4	.16
Total rituximab dosage, g	1353.9 ± 918.4	866 ± 464.7	.10

Data are summarized as percentage (%) or mean ± standard deviation for normally distributed data and median with interquartile ranges for others.

Abbreviations: Cg, chronic glomerulopathy; DSA, donor specific antibody; Dx, diagnosis; eGFR, estimated glomerular filtration rate; KT, kidney transplantation; IVIg, intravenous immunoglobulin; MFI, mean fluorescence intensity; PE, plasma exchange; PRA, panel reactive antibodies.

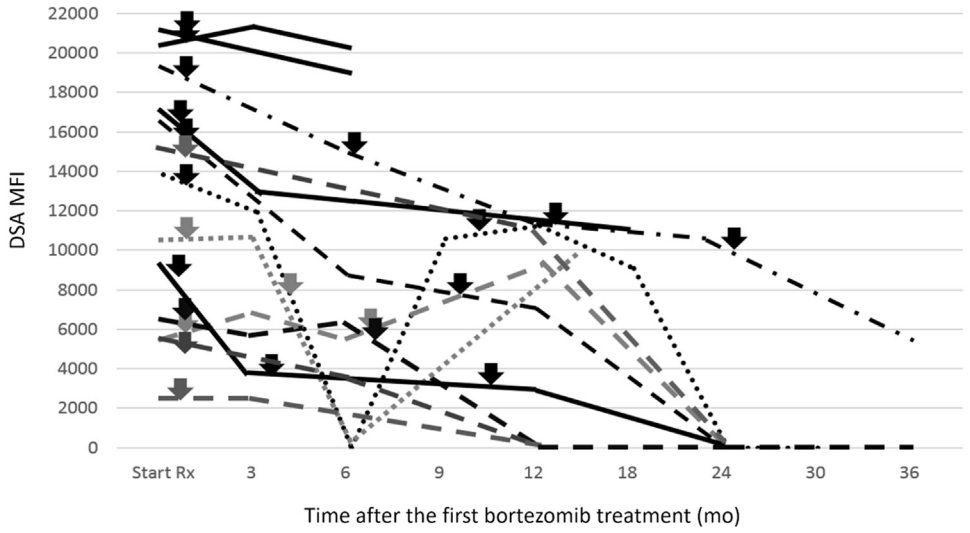

Fig 1. Maximum DSA MFI of patients in the bortezomib group at the beginning and during follow-up. Arrows indicate the standard treatment followed by bortezomib. Three patients, represented as a solid line, had persistently high DSA MFI. One patient, represented as a dotted and dashed line, had gradually decreased DSA MFI after repeated bortezomib protocol. Seven patients, represented as dashed line, experienced the disappearance of DSA. Two patients, represented as a dotted line, experienced the reappearance of DSA. DSA, donor specific antibody; MFI, mean fluorescence intensity; Rx, treatment.

DQ antibodies, and maximum DSA MFI was significantly higher in the bortezomib group (11,206 vs 5386, $P = .049$).

To treat CAMR, all patients in both groups received repeated courses of PE followed by IVIg and at least 1 dose of rituximab. No significant difference was found regarding the number of PE sessions and accumulated dosages of IVIg and rituximab between both groups. Six patients in the bortezomib group received bortezomib for 1 cycle while 4, 1, and 2 patients got 2, 3, and 4 cycles, respectively.

DSA disappearance after treatment was significantly higher in the bortezomib group (69.2% vs 25%; $P = .03$). The maximum DSA MFI of patients in the bortezomib group at the beginning and during follow-up is shown in Fig 1. Median follow-up time after the first bortezomib dose was 41.8 (18.3-47.4) months. Nine patients in the bortezomib group experienced loss of DSA; however, 2 patients had reoccurrence of DSA, which disappeared after an additional course of treatment in 1 patient. DSA MFI started to decrease after the bortezomib treatment for 3 months. Median time to complete remission of DSA was 12 (6-12) months. Three patients had persistently high DSA MFI following treatment, 2 patients presented noncompliance, while 1 patient developed a serious infection and did not receive further treatment. The DSA MFI of 1 patient gradually reduced following 4 cycles of bortezomib.

Mean follow-up time after CAMR was 3.8 ± 2.4 years. Graft loss was more prevalent in the control group (66.7% vs 15.4%; $P = .006$). Mean rate of glomerular filtration rate (GFR) declination annually in the control group was significantly higher than that of the bortezomib group (-12.33 ± 10.44 mL/min/y vs -4.20 ± 4.89 mL/min/y; $P = .014$). Survival analysis revealed superior graft survival in the bortezomib group ($P = .006$) as shown in Fig 2.

Regarding complications, cytomegalovirus viremia and disease was found among 3 patients in the bortezomib group and 2 patients in the standard treatment group. BK viruria and viremia was observed in 3 patients in the bortezomib group and 4 patients in the standard treatment group. Three patients in each group suffered from urinary tract infection with sepsis. One patient in the bortezomib group developed

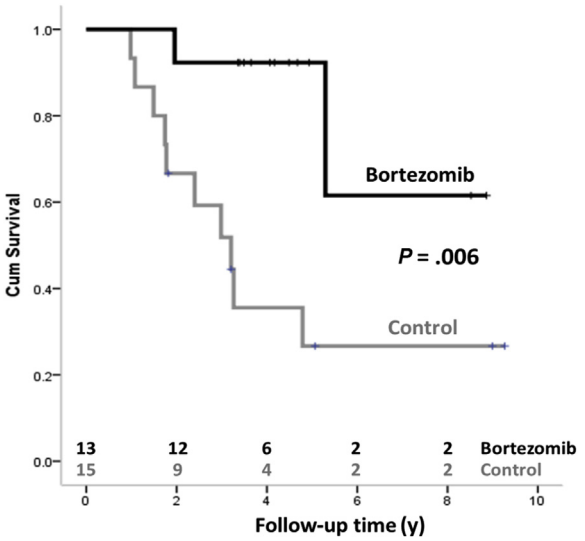

Fig 2. Survival analysis comparing allograft survival between the bortezomib and control groups.

mild reversible peripheral neuropathy after 2 treatment cycles.

DISCUSSION

Advances in immunosuppression and medical care have notably improved early post-transplant outcomes, but strategies for successful long-term graft survival remain elusive. CAMR is one of the most challenging conditions in the late period after kidney transplantation. Despite similar pathologic findings, late or CAMR had worse allograft survival rates than acute AMR [3,8]. The natural course of CAMR resembles "chronic glomerulonephritis," which gradually destroys an allograft. No promising treatment is available for CAMR in most cases [9]. From our experience, efficacy of standard treatment for acute AMR, including PE, IVIg, and rituximab, is unsatisfactory for CAMR [3].

Bortezomib, a proteasome inhibitor, depletes plasma cells that generate antibodies. Therefore, bortezomib has been combined with PE, IVIg, and rituximab as a rescue therapy for acute AMR with some supporting evidence [6,7]. Because of the benefits of inhibiting class I major histocompatibility complex expression [6], bortezomib can reduce anti-HLA class I antibodies more effectively than class II antibodies in a desensitization protocol [5]. However, the role of adjunctive bortezomib in CAMR, a more troublesome condition mostly triggered by anti-HLA class II antibodies, requires further elucidation.

Our study showed the advantages of adjunctive bortezomib in abolishing DSA, both class I and II, and delaying the progression of CAMR. We monitored DSA after treating and provided additional treatment in the case of persistently high DSA without complications. Optimum time to follow-up DSA after bortezomib was 3 to 6 months. Based on an in vitro study, bortezomib may have limited efficacy to reduce DSA in serum because of compensated B cell proliferation generating new antibodies quickly [10]. However, our multitarget protocol might solve this underlying pathophysiology.

The BORTEJECT study, a randomized controlled trial of bortezomib in late AMR, was recently reported [11]. Eskandary et al [11] investigated whether 2 cycles of bortezomib were able to slow the change in estimated GFR slope compared with placebo. Despite a trend to reduce DSA levels, bortezomib affected neither the rate of GFR declination nor allograft survival after 2 years of follow-up. Therefore, it seemed that proteasome inhibitors had no benefit in late AMR. This result was dissimilar to our study; however, the treatment strategy differed from this study. We added bortezomib to standard antihumoral treatment and did not provide bortezomib alone. Both BORTEJECT and our study found no significant increase in infectious complications, but neuropathy was more evidenced in the BORTEJECT trial. Novel irreversible proteasome inhibitor with less neurotoxicity, carfilzomib, may be beneficial.

Our study had some limitations. First, it was a single-center study. Second, it was a retrospective study in nature, which might have affected the results; however, all refractory CAMR patients were included to reduce bias. Third, we had limited DSA data during follow-up for the control group, of which most patients were historical controls that lacked DSA results. Fourth, we did not repeat the biopsy when the DSA turned negative without clinical deterioration. Lastly, the treatment had a relatively high cost. Even though we proposed an optional treatment for a desperate condition, a randomized controlled trial regarding this strategy should be conducted to confirm our result.

In conclusion, adjunctive bortezomib was more effective than standard treatment alone in eliminating DSA, stabilizing allograft function, and improving allograft survival. DSA monitoring after treatment should be accomplished for consideration of further management.

REFERENCES

[1] Loupy A, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. Nat Rev Nephrol 2012;8:348–57.

[2] Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. Am J Transplant 2012;12:1157–67.

[3] Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. Am J Transplant 2012;12:388–99.

[4] Larpparisuth N, Premasathian N, Vareesangthip K, Cheunsuchon B, Parichatikanon P, Vongwiwatana A. Clinicopathologic characteristics and outcomes of late acute antibody-mediated rejection in Thai kidney transplant recipients: a single-center experience. Transplant Proc 2014;46:477–80.

[5] Woodle ES, Shields AR, Ejaz NS, Sadaka B, Gimita A, Walsh RC, et al. Prospective iterative trial of proteasome inhibitor-based desensitization. Am J Transplant 2015;15:101–18.

[6] Walsh RC, Alloway R, Girnita A, Woodle ES. Proteasome inhibitor-based therapy for antibody-mediated rejection. Kidney Int 2012;81:1067–74.

[7] Cicora F, Paz M, Mos F, Roberti J. Use of bortezomib to treat anti-HLA antibodies in renal transplant patients: a single-center experience. Transpl Immunol 2013;29:7–10.

[8] Walsh RC, Brailey P, Girnita A, Alloway RR, Shields AR, Wall GE, et al. Early and late acute antibody-mediated rejection differ immunologically and in response to proteasome inhibition. Transplantation 2011;91:1218–26.

[9] Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 2009;9: S1–155.

[10] Kwun J, Burghuber C, Manook M, Iwakoshi N, Gibby A, Hong JJ, et al. Humoral compensation after bortezomib treatment of allosensitized recipients. J Am Soc Nephrol 2017;28:1991–6.

[11] Eskandary F, Regele H, Baumann L, Bond G, Kozakowski N, Wahrmann M, et al. A randomized trial of bortezomib in late antibody-mediated kidney transplant rejection. J Am Soc Nephrol 2018;29:591–605.