Treatment of Antibody-Mediated Rejection in Kidney Transplant Recipients: A Single-Center Experience With a Bortezomib-Based Regimen

Janice G. Nigos, Swati Arora, Parineesha Nath, Sabiha M. Hussain, Richard J. Marcus, Tina Y. Ko, Kalathil K. Sureshkumar

Abstract

Objectives: **Antibody-mediated rejection after kidney transplant is less responsive to conventional antirejection therapies. The proteasome inhibitor bortezomib has activity against mature plasma cells that produce damaging donor-specific antibodies. We present our experience of using a bortezomib-based regimen in patients with severe antibody-mediated rejection.**

Materials and Methods: **A retrospective chart review was performed on patients with biopsy-proven antibody-mediated rejection after kidney transplant at our institution over 12 months. Diagnosis of antibody-mediated rejection was made on the basis of positive peritubular capillary C4d staining along with either histologic evidence of acute rejection or positive donor-specific antibody titers. Treatment for antibody-mediated rejection included plasmapheresis, intravenous immunoglobulin, steroids, single-dose rituximab (375 mg/m^2) along with bortezomib (1.3 mg/m^2) on days 1, 4, 8, and 11. Antibody-mediated rejection was diagnosed in 6 patients. Patients received induction with either alemtuzumab (n=4) or rabbit-antithymocyte globulin (n=2) and were maintained on a tacrolimus/mycophenolate mofetil/early steroid withdrawal protocol.**

Results: **Four of 6 patients responded to treatment. Patients had stable kidney function during follow-up (median 14 months) after bortezomib therapy.**

Conclusions: **In this series, we demonstrated the effectiveness of a bortezomib-based treatment regimen in achieving reduction of donor-specific antibody titers and stable renal function in patients experiencing severe antibody-mediated rejection.**

Key words: *Bortezomib, Donor-specific antibodies, Humoral rejection, Kidney transplant, Plasma cells*

From the Division of Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA 15212, USA

Corresponding author: *Kalathil K. Sureshkumar MD, FRCP (Glasg), FASN, 320 East North Avenue 4th Floor South Tower Pittsburgh, PA 15212, USA*

Phone: *+1 412 359 3319* ***Fax:*** *+1 412 359 4136* ***E-mail:*** *ksureshk@wpahs.org*

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Introduction

Antibody-mediated rejection (AMR) has gained importance because of the high risk of early graft loss that ranges from 27% to 40% in first year after an event.[1] Antibody-mediated rejection occurs in up to 20% to 30% of all acute rejection episodes, and 60% of the cases coexist with acute cellular rejection (ACR).[2] The increased recognition of AMR as a cause of early graft loss stimulated interest to find new therapeutic interventions. The mechanism of injury involves production of high levels of donor-specific antibodies (DSA) by plasma cells, and this knowledge has provided insights in developing therapeutic strategies. We present our experience with the use of bortezomib, a proteasome inhibitor with activity against mature plasma cells for the successful treatment of AMR in kidney transplant recipients along with a review of the literature.

Case Report

A retrospective chart review was performed on patients with biopsy-proven AMR after kidney transplant at our institution over 12 months (February 2010 to February 2011). Six patients were diagnosed with AMR based on positive peritubular capillary C4d staining along with either histologic

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evidence of acute rejection or positive DSA titers. Renal biopsies were analyzed by a single pathologist and graded using Banff updated 2005 criteria.[3] Donor-specific antibodies titers were measured using Luminexx platform (LABScreen, One Lambda, Inc, Canoga Park, CA, USA), were expressed as mean fluorescent intensity. Owing to the extreme sensitivity of this test and the uncertain clinical significance of lower values, a DSA level of 4000 mean fluorescent intensity was used as an arbitrary lower cutoff value for positivity. Mean age of the recipients was 43 ± 13 years, with panel reactive antibody range 0% to 80%, and 4 ± 1 human leukocyte antigen (HLA) mismatches. Of 6 patients, 5 were females, 5 had their first kidney transplant, 2 had live donors, 4 received alemtuzumab (30 mg IV intraoperatively) and 2 received rabbit-antithymocyte globulin (r-ATG) (6 mg/kg IV in 4 divided doses of 1.5 mg/kg/d). All patients were maintained on tacrolimus (trough level, 8-10 ng/mL), mycophenolate mofetil (500-1000 mg twice daily), and an early steroid withdrawal protocol (methylprednisolone 500 mg IV intraoperatively, 250 mg IV postoperative day [POD] No. 1, and 125 mg IV POD No. 2, prednisone 60 mg POD No. 3, and 30 mg POD No. 4). Patient demographics are shown in Table 1.

Two of 6 patients (patients A and B) were initially treated with conventional therapy for AMR including plasmapheresis, intravenous immunoglobulin (IVIg), and steroids. Owing to the continued rise in serum creatinine and DSA titers, therapy with single-dose rituximab and 4 doses of bortezomib was initiated. In the other 4 patients, bortezomib was started on day 1 of diagnosis of AMR along with a single dose of rituximab (375 mg/m^2) and the rest of the conventional therapy for AMR. Peak serum creatinine and DSA titers at the time of diagnosis of AMR and end of treatment are shown in Table 2. Individual treatments of AMR in each patient are detailed in Tables 3 through 8.

Patients A and B remained to have excellent graft function 27 months after transplant. Patient C had 2 more episodes of biopsy-proven ACR after 4 and 5 months after AMR secondary to noncompliance that were treated with steroid boluses. Her last biopsy 6 months after AMR was negative for rejection. Patient D developed biopsy-proven ACR 7 months after AMR that was treated with steroid boluses. She had multiple episodes of acute kidney injury secondary to recurrent urinary tract infections and pyelonephritis. Her last transplant kidney biopsy was done 8 months after AMR, and it did not show any rejection. Patient E had refractory AMR and became dialysis-dependent. Patient F had chronic AMR. Her DSA titers did not improve with treatment. In summary, 2 of 6 patients did not respond to treatment but responders had stable

Table 1. Demographic Features of Study Subjects

Characteristic	Patient A	B	C	D	E	F
Age (y)	54	19	44	41	50	51
Sex	F	M	F	F	F	F
Race	White	White	White	African-American	White	White
ESRD cause	T2DM	FSGS	FSGS	Hypertension	Viral GN	T1DM
Previous transplants (No.)	0	1	0	0	0	0
Transplant type	LUKT	DDKT (ECD)	DDKT (ECD)	DDKT	LUKT	DDKT
CIT (h)	1.00	28.00	16.63	17.17	1.0	33.88
Donor CMV status	Negative	Positive	Negative	Negative	Positive	Negative
Recipient CMV status	Negative	Positive	Positive	Positive	Positive	Negative
DGF	No	Yes	No	No	No	No
PRA (%)	0	0	24	80	55	0
CDC crossmatch	Negative	Negative	Negative	Negative	Negative	Negative
No. of HLA mismatches	4	3	5	5	4	4
Induction	Alemtuzumab	Alemtuzumab	r-ATG	Alemtuzumab	Alemtuzumab	r-ATG
Maintenance	Tacrolimus, MMF	Tacrolimus, MMF	Tacrolimus, MMF	Tacrolimus, MMF	Tacrolimus, MMF	Tacrolimus, MMF
Time to AMR (d)	9	13	11	29	8	1357
Banff score	AMR II	AMR I	AMR II, ACR IB	AMR II	C4d positive	Chronic AMR, C4d negative

Abbreviations: ACR, acute cellular rejection; AMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; CIT, cold ischemia time; CMV, *Cytomegalovirus*; DDKT, deceased-donor kidney transplant; DGF, delayed graft function; ECD, expanded criteria donor; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; HLA, human leukocyte antigen; LUKT, living-unrelated kidney transplant; MMF, mycophenolate mofetil; PRA, panel reactive antibody; r-ATG, rabbit antithymocyte globulin; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus

Table 2. Donor-Specific Antibody and Serum Creatinine Values of Patients Who Received Kidney Transplant, Before and After Treatment for Antibody-Mediated Rejection

Measurement	Patient A	B	C	D	E	F
DSA*						
Pretreatment peak	13,494 (HLA A2)	16,552 (HLA DR4)	14,700 (HLA DR15)	9597 (HLA B13)	11,800 (HLA DR11)	7500 (HLA DQ1)
At treatment end	3000	6000	2100	250	520	6000
At last follow-up	Not detected	Not detected	Not detected	Not detected	NA	7311 (HLA DQ5) 5742 (HLA DQ6)
Serum creatinine (μmol/L)†						
Pretreatment peak	609.96	1051.96	362.44	300.56	654.16	221.0
At treatment end	123.76	114.92	194.48	150.28	654.16	194.48
At last follow-up	114.92	114.92	433.16	353.6	Hemodialysis	247.52

Abbreviation: HLA, human leukocyte antigen
*Normal range < 4000. DSA titers are expressed as mean fluorescent intensity (MFI)
†Normal range, 53-106 μmol/L

Table 3. Treatment for Antibody-Mediated Rejection: Patient A

Postoperative Day	Serum Creatinine Level (μmol/L)*	Treatment
Pretreatment	556.92	NA
4	88.40	NA
6	309.40	NA
9	415.48	MP 500 mg IV, PP 1.5 vol, IVIg 20 g
10	459.68	MP 500 mg IV
11	530.40	MP 500 mg IV, PP 1.5 vol, IVIg 20 g
12	556.92	MP 500 mg IV
13	609.96	PP 1.5 vol, IVIg 20 g, rituximab 375 mg/m^2, bortezomib 1.3 mg/m^2, prednisone 20 mg daily with slow taper
16	503.88	PP 1.5 vol, bortezomib 1.3 mg/m^2, IVIg 20 g
20	353.60	PP 1.5 vol, bortezomib 1.3 mg/m^2, IVIg 20 g
23	274.04	PP 1.5 vol, bortezomib 1.3 mg/m^2, IVIg 20 g
25	123.76	NA

Abbreviations: IV, intravenous; IVIg, intravenous immunoglobulin; MP, methylprednisolone; PP, plasmapheresis
*Normal range, 53-106 μmol/L

Table 4. Treatment for Antibody-Mediated Rejection: Patient B

Postoperative Day	Serum Creatinine Level (μmol/L)*	Treatment
Pretreatment	1759.16	NA
7	1166.88	NA
11	1228.76	NA
12	1211.08	MP 250 mg IV
13	1131.52	MP 250 mg IV
14	1051.96	MP 250 mg IV, PP 1.5 vol, IVIg 20 g
16	627.64	PP 1.5 vol, IVIg 20 g
18	300.56	PP 1.5 vol, IVIg 20 g
20	185.64	PP 1.5 vol, IVIg 20 g
22	176.80	PP 1.5 vol, IVIg 20 g
25	167.96	PP 1.5 vol, IVIg 20 g
35	203.32	Rituximab 375 mg/m2
44	335.92	PP 1.5 vol, bortezomib 1.3 mg/m^2, IVIg 20 g
51	159.12	PP 1.5 vol, bortezomib 1.3 mg/m^2, IVIg 20 g
57	141.44	PP 1.5 vol, bortezomib 1.3 mg/m^2, IVIg 20 g
90	114.92	PP 1.5 vol, bortezomib 1.3 mg/m^2, IVIg 20 g

Abbreviations: IV, intravenous; IVIg, intravenous immunoglobulin; MP, methylprednisolone; PP, plasmapheresis
*Normal range, 53-106 μmol/L

kidney function during median follow-up of 14 months (range, 3-27 mo) after bortezomib therapy.

Discussion

We present our experience with the successful use of bortezomib in 4 out of 6 patients who presented with AMR after kidney transplant. The repeat kidney biopsies after completion of treatment were consistent with resolution of AMR although the clinical outcomes varied over the long-term follow-up.

Use of currently available therapies for AMR (plasmapheresis, IVIg, rATG, rituximab) have shown inconsistent and suboptimal results. This could be due to their inability to suppress mature plasma cell activity, which is the major source of antibody production in AMR. The beneficial effects of bortezomib (Millennium: The Takeda Oncology Company, Cambridge, MA, USA), an anti-plasma cell agent used to treat AMR, was first reported by Everly and associates in 2008 in patients with refractory AMR and ACR.[4] Bortezomib was originally synthesized in 1995 and approved by the US Food and Drug Administration in 2003 for treating multiple myeloma.

Our approach to AMR followed the protocol described by Everly and associates.[4] The first step is plasmapheresis, which helps not only in removing previously secreted antibodies but also, in increasing the metabolic demands on memory B cells and plasma cells and thereby, enhancing their

Table 5. Treatment for Antibody-Mediated Rejection: Patient C

Postoperative Day	Serum Creatinine Level (μmol/L)*	Treatment
Pretreatment	784.99	NA
3	654.16	NA
6	177.68	NA
10	364.21	MP 500 mg IV
11	330.62	MP 500 mg IV
12	266.08	MP 500 mg IV, PP 1 vol, IVIg 15 g, rituximab 375 mg/m^2, bortezomib 1.3 mg/m^2
13	218.35	MP 500 mg IV
14	160.89	Prednisone 30 mg, PP 1 vol, IVIg 15 g
16	140.56	Prednisone 30 mg, PP 1 vol, bortezomib 1.3 mg/m^2
18	NA	Patient missed treatment
20	156.47	Prednisone 30 mg, PP 1 vol, bortezomib 1.3 mg/m^2
23	166.19	Prednisone 30 mg, PP 1 vol, bortezomib 1.3 mg/m^2
31	194.48	NA

Abbreviations: IV, intravenous; IVIg, intravenous immunoglobulin; MP, methylprednisolone; PP, plasmapheresis
*Normal range, 53-106 μmol/L

Table 6. Treatment for Antibody-Mediated Rejection: Patient D

Postoperative Day	Serum Creatinine Level (μmol/L)*	Treatment
Pretreatment	577.25	NA
1	638.25	NA
5	422.55	NA
15	329.73	NA
22	228.07	NA
28	350.06	MP 250 mg IV, hemodialysis for volume overload
29	200.67	MP 250 mg IV
30	264.32	MP 250 mg IV, hemodialysis for volume overload
32	NA	Hemodialysis for volume overload
33	190.06	Prednisone 30 mg, PP 1.5 vol, IVIg 20 g, rituximab 375 mg/m^2, bortezomib 1.3 mg/m^2
36	276.69	Prednisone 30 mg, PP 1.5 vol, IVIg 20 g, bortezomib 1.3 mg/m^2, hemodialysis for volume overload
40	304.98	Prednisone 30 mg, PP 1 vol, IVIg 20 g (unable to give third dose of bortezomib because of thrombocytopenia)
42	259.90	Prednisone 30 mg, PP 1 vol, IVIg 20 g (unable to give fourth dose of bortezomib because of thrombocytopenia)
44	205.09	Prednisone 30 mg, PP 1 vol, IVIg 20 g
47	149.40	Prednisone 30 mg, PP 1 vol, IVIg 20 g

Abbreviations: IV, intravenous; IVIg, intravenous immunoglobulin; MP, methylprednisolone; PP, plasmapheresis
*Normal range, 53-106 μmol/L

susceptibility to proteasome inhibition.[5,6] The usual prescription includes 1.0 to 1.5 volume exchange using albumin solution daily or on alternate days, continued until serum creatinine falls within 30% of previous baseline values. This modality does not suppress antibody production and in fact, can cause a rebound in DSA levels after plasmapheresis. Pulse corticosteroids, hypothesized to treat AMR by potentially down-regulating B-cell activity, were given concomitantly.

The second step is the administration of IVIg products derived from pooled human plasma. The exact mechanism of action of IVIg is unclear,

Table 7. Treatment for Antibody-Mediated Rejection: Patient E

Postoperative Day	Serum Creatinine Level (μmol/L)*	Treatment
Pretreatment	577.25	NA
4	126.41	NA
7	257.24	NA
8	250.17	MP 250 mg IV
9	232.49	PP 1.5 vol
10	234.26	MP 500 mg IV, rituximab 375 mg/m^2, bortezomib 1.3 mg/m^2
11	213.04	PP 1.5 vol, MP 500 mg IV
13	218.35	PP 1.5 vol, prednisone 30 mg, bortezomib 1.3 mg/m^2
14	211.28	Prednisone 30 mg
17	222.77	Prednisone 30 mg, bortezomib 1.3 mg/m^2
20	265.20	Bortezomib 1.3 mg/m^2
23	303.21	PP 1.5 vol, IVIg 10 g
25	307.63	IVIg 10 g
27	333.27	IVIg 10 g, rituximab 375/m^2, bortezomib 1.3 mg/m2
29	376.58	PP 1.5 vol, IVIg 10 g
31	529.52	IVIg 10 g, bortezomib 1.3 mg/m^2
39	656.81	Started on hemodialysis

Abbreviations: IV, intravenous; IVIg, intravenous immunoglobulin; MP, methylprednisolone; PP, plasmapheresis
*Normal range, 53-106 μmol/L

Table 8. Treatment for Antibody-Mediated Rejection: Patient F

Postoperative Day	Serum Creatinine Level (μmol/L)*	Treatment
Pretreatment	583.44	NA
1	459.68	NA
350	97.24	NA
1357	167.96	High DSA titer, biopsy was C4d negative
1358	178.57	PP 1.0 vol, MP 250 mg IV, rituximab 375 mg/m^2, bortezomib 1.3 mg/m^2
1365	194.48	PP 1.0 vol, bortezomib 1.3 mg/m^2
1370	185.64	PP 1.0 vol, bortezomib 1.3 mg/m^2
1373	221.0	PP 1.0 vol, bortezomib 1.3 mg/m^2
1470	194.48	NA

Abbreviations: IV, intravenous; IVIg, intravenous immunoglobulin; MP, methylprednisolone; PP, plasmapheresis
*Normal range, 53-106 μmol/L

however, it is hypothesized that they suppress immunoglobulin synthesis, has anti-idiotypic activity against DSA, blocks Fc receptor, inhibits complement activation, and possesses anticytokine activity. When given with plasmapheresis, IVIg helps replenish lost gammaglobulin and decreases the risk of infection. The recommended dose is 100 mg/kg after each session and 300 to 400 mg/kg for 1 to 2 days after the last session of plasmapheresis with a cumulative dose of 1000 mg/kg.[7] The third step is to give rituximab, a chimeric anti-CD20 antibody that directly inhibits B-cell proliferation by antibody-, cell-, and complement-mediated cytotoxicity and induces cellular apoptosis. It has been demonstrated that the high density of CD20+ B cells is found in the biopsies of patients with steroid-resistant rejection episodes. The main limitation of rituximab is the inability to remove CD20-negative plasma cells that continue to produce HLA antibodies.[8] The final step in treatment is to administer bortezomib, a boronic acid dipeptide that specifically inhibits 26S proteasome preventing activation of the transcriptional activator nuclear factor kappa B. This leads to disruption of normal cell homeostasis and plasma cell apoptosis.[9] Targeting the plasma cells directly destroys the source of damaging DSA. The recommended dose is 1.3 mg/m²/dose × 4 doses.

Experience with the use of bortezomib in treating AMR thus far is limited to case reports and series. Perry and associates report successful use of bortezomib in 2 patients after transplant AMR with reduction in the number of bone marrow plasma cells and normal renal function at 1 year after transplant.[10] Walsh and associates showed undetectable DSA levels within 14 days of bortezomib-based therapy in 2 living-donor transplant recipients who developed AMR within first 2 weeks of renal transplant.[4] Everly and associates reported 2 different case series of patients with AMR and coexisting ACR with prompt reversal of rejection with use of bortezomib.[4,11] They did not observe any opportunistic infections or serious adverse events except transient gastrointestinal adverse effects and thrombocytopenia with bortezomib therapy. Sberro and associates did not show any significant reduction in DSA titers in 4 renal transplant recipients with subacute AMR at 5 months after transplant with the use of 4 doses of bortezomib alone.[12] These findings are consistent with 1 of our patients (Patient F), who had chronic AMR with elevated DSA titers that did not improve with bortezomib therapy. Trivedi and associates demonstrated reduction in DSA titers with bortezomib therapy in 9 out of 11 patients with stable graft function at 4 months after transplant.[13] Our study had a longer follow-up after AMR therapy. We have followed these patients closely with monthly serum creatinine, DSA titers, and periodic renal allograft biopsies.

In conclusion, our experience in treating AMR after kidney transplant demonstrates the effectiveness of a bortezomib-based treatment regimen in achieving reduction of DSA titers and stable renal function over a relatively longer follow-up. Patients tolerated treatment without significant adverse effects, but larger studies with longer follow-ups are required for a more-definitive evaluation of this approach.

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