Bortezomib-based antibody depletion for refractory autoimmune hematological diseases

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Key Points

- Proteasome inhibition has pleiotropic immunomodulatory properties and is cytotoxic to antibody-producing B lymphocytes and plasma cells.
- Bortezomib yields high response rates in antibody-mediated autoimmune hematological diseases refractory to conventional immunosuppression.

Certain patients with antibody-mediated autoimmune disease exhibit poor responses to conventional immunosuppression, including B-cell depletion with rituximab. Proteasome inhibitors such as bortezomib demonstrate pleiotropic immunomodulatory effects, including direct toxicity to antibody-producing cells. Here, we report preliminary evidence for the efficacy of bortezomib as salvage therapy for refractory autoimmune hematological disease. Thirteen treatment episodes in 10 patients with autoimmune hematological phenomena (autoimmune hemolytic anemia [AIHA; n = 8], acquired hemophilia (n = 1), immune thrombocytopenia (n = 1), and thrombotic thrombocytopenic purpura [TTP; n = 3]) and a median of 5 (range, 3-12) prior lines of therapy demonstrated an overall response rate of 77% (10 of 13) including 38% (5 of 13) complete remissions. The majority of clinical improvements were rapid, correlated with biomarkers of autoantibody reduction, and were associated with an acceptable safety profile. Responses appeared durable following treatment of TTP and acquired hemophilia; AIHA responses were more limited with a pattern of relapse following bortezomib cessation. These data provide proof of concept for the utility of proteasome inhibition as antibody depletion therapy in autoimmune disease.

Introduction

Antibody-mediated immune phenomena may occur de novo or in the context of immune dysregulation complicating lymphoid neoplasms and autoimmune diseases. Although the underlying mechanisms of autoreactivity are diverse, pathogenic antibody production represents a final common mechanism and potential targetable end point in these diseases. Where autoantibodies target cellular blood elements (eg, autoimmune hemolytic anemia [AIHA]) or plasma proteins (eg, acquired hemophilia, thrombotic thrombocytopenic purpura [TTP]), conventional immunosuppressive therapies are usually used to ameliorate both cell-mediated and humoral immune responses. In severe, relapsing, or refractory cases, selective B-cell depletion with rituximab may provide effective salvage and has been widely used in both AIHA1 and TTP.2 However, rituximab is unable to deplete CD20− plasma cells, including long-lived bone marrow and splenic forms responsible for chronic autoantibody production.3

The proteasome inhibitor bortezomib causes apoptosis in both clonal and reactive antibody-producing cells via an augmented unfolded protein response and the induction of endoplasmic reticulum stress.4 However, the effects of bortezomib on the immune system are much more diverse than simply depletions of antibody-producing cells. Immunomodulatory properties include downregulation of inflammatory...
signaling conveyed by NF-κB, depletion of autoreactive T-helper cells, and interference with antigen processing and presentation. On this basis, bortezomib has been prospectively evaluated as an adjunct to rituximab and plasma exchange (PEX) as a means to deplete alloantibodies in solid organ transplant recipients. More-over, reports of activity in autoimmune diseases including AIHA, immune thrombocytopenia, and TTP are emerging. Following our initial success utilizing bortezomib in a patient with refractory TTP, we now report our cumulative experience using this treatment approach in a series of patients with refractory antibody-mediated autoimmune hematological diseases.

**Study design**

All patients receiving “off-label” bortezomib for autoimmune hematological diseases at Monash University–affiliated hospitals (Victoria, Australia; patient catchment 1.5 million) and the Royal Hobart Hospital (Tasmania, Australia; patient catchment 300 000) were included in the study. Patients from Monash University–affiliated hospitals received bortezomib between 2012 and January 2016, and patients from Royal Hobart Hospital received bortezomib between 2015 and April 2016. All cases were considered to have exhausted reasonable conventional treatment options by their treating clinician, including rituximab. Consistent with local jurisdictional restrictions, off-label bortezomib use was approved on a case-by-case basis following independent hospital Drug and Therapeutic Committee review with Australian Therapeutic Goods Administration notification. As off-label high-cost drug use is neither privately funded in Australia nor reimbursed by Medicare, individual hospitals must approve and fund bortezomib on a case-by-case basis. By referring to such hospital “single patient use” requests, we were able to track all episodes of off-label (nonremunerated) bortezomib use at our respective state-funded institutions. The primary aim was to ascertain preliminary evidence for disease-specific treatment efficacy and potential therapy-related toxicities. Bortezomib was initiated at 1.3 mg/m² IV on days 1, 4, 8, and 11 on 21-day cycles; subsequent modification to weekly subcutaneous administration was performed where repeated cycles were required. Antiviral prophylaxis was administered according to institutional protocol. Where available, responses were classified utilizing previously defined criteria. In general, a complete response (CR) was indicated by sustained platelet recovery to >150 × 10⁹/L, as previously defined.

### Table 1. Characteristics of patients and bortezomib responses

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, y</th>
<th>Sex</th>
<th>Prior therapies</th>
<th>Cycles to response</th>
<th>Concurrent therapies</th>
<th>Best response</th>
<th>Duration, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 AIHA, cold type</td>
<td>64</td>
<td>F</td>
<td>PDRL, AZA, HCG, R, IgIV</td>
<td>MR/PR</td>
<td>DM</td>
<td>PR</td>
<td>9+</td>
</tr>
<tr>
<td>#2</td>
<td>55</td>
<td>M</td>
<td>PDRL, PEX, R, RCD</td>
<td>2</td>
<td>DM</td>
<td>PR</td>
<td>5+</td>
</tr>
<tr>
<td>#3 AIHA, warm type</td>
<td>73</td>
<td>M</td>
<td>PDRL, AZA, R</td>
<td>–</td>
<td>1</td>
<td>CTX, DM</td>
<td>CR</td>
</tr>
<tr>
<td>#4</td>
<td>47</td>
<td>M</td>
<td>PDRL, AZA, CYA, CTX, R</td>
<td>1</td>
<td>PDRL</td>
<td>PR</td>
<td>4</td>
</tr>
<tr>
<td>#5</td>
<td>(at relapse)</td>
<td>PDRL</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>PDRL</td>
<td>NR</td>
</tr>
<tr>
<td>#6</td>
<td>49</td>
<td>F</td>
<td>PDRL, PEX, CYA, CTX, R, SP, ICE</td>
<td>1</td>
<td>PDRL, CTX</td>
<td>PR</td>
<td>5</td>
</tr>
<tr>
<td>#7 TTP</td>
<td>53</td>
<td>F</td>
<td>Me-PDRL, PEX, NAC, CTX, R</td>
<td>–</td>
<td>1</td>
<td>Me-PDRL, PEX, R</td>
<td>CR</td>
</tr>
<tr>
<td>#8</td>
<td>(at relapse)</td>
<td>PDRL, PEX, R</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>PDRL</td>
<td>CR</td>
</tr>
<tr>
<td>#9 ITP</td>
<td>48</td>
<td>F</td>
<td>PDRL, PEX, R</td>
<td>–</td>
<td>1</td>
<td>PDRL, PEX, R</td>
<td>CR</td>
</tr>
<tr>
<td>#10 Acquired hemophilia</td>
<td>45</td>
<td>M</td>
<td>PDRL, CTX, R</td>
<td>1</td>
<td>4</td>
<td>DM, DAN, ROMI</td>
<td>NR</td>
</tr>
</tbody>
</table>

* = not applicable; AZA, azathioprine; CTX, cyclophosphamide; CYA, cyclosporine A; DAN, danazol; DM, dexamethasone; ETP, eltrombopag; F, female; HCG, hydroxychloroquine; ICE, ifosfamide, carboplatin, etoposide; IgIV, IV immunoglobulin, LapS, laparoscopic splenunculus removal; ITP, immune thrombocytopenia; M, male; Me-PDRL, methylprednisolone; MMF, mycophenolate; MR, minor response; N, N-acetylcysteine; NR, nonresponder; OST, oseltamivir; PDRl, prednisolone; R, rituximab; R-CVP, rituximab, cyclophosphamide, vincristine, prednisolone; RCD, rituximab, cyclophosphamide, dexamethasone; ROMI, romiplostim; SP, splenectomy; VCR, vincristine.

*Response maintained by ongoing bortezomib treatment.
†Patient deceased.
‡Ongoing response at time of publication.
VIII (FVIII) >70% with inhibitor <0.4 Bethesda units; PR, FVIII >25% with a relative reduction in inhibitor of at least 50%; and minor remission, FVIII <25% with relative reduction in inhibitor of at least 50%.\(^\text{18}\) For AIHA, we defined CR as stable hemoglobin (Hb) >100 g/L without transfusions and immunosuppressive therapy and a PR as significantly reduced transfusion requirements or rise in Hb >20 g/L from baseline, associated with improved hemolytic markers (eg, bilirubin and lactate dehydrogenase levels) with stable or decreasing additional immunosuppressive therapy. Ninety-five-percent confidence intervals (CIs) for proportions were derived by Fisher exact test using OpenEpi (version 3).

**Results and discussion**

Thirteen treatment episodes were documented in 10 patients (median age, 54 years [range, 45-81 years]; 4 females) with the following disorders: AIHA, cold type \((n = 2)\); AIHA, warm type \((n = 4)\); TTP \((n = 2)\); ITP \((n = 1)\); and acquired hemophilia \((n = 1)\) (Table 1). Four patients had coexisting immune-related disorders or indolent hematological malignancies: immunoglobulin M monoclonal gammopathy of undetermined significance \((n = 1)\), splenic marginal zone lymphoma \((n = 1)\), systemic lupus erythematosus \((n = 1)\), and psoriatic arthritis \((n = 1)\). All were rituximab exposed upon receiving initial bortezomib therapy, with a median of 5 prior lines of therapy (range, 3-12). The overall response rate (PR + CR) was 77\% (CI, 46\%-95\%), including 38\% (CI, 14\%-68\%) CRs. The majority of patients \((n = 6)\) responded maximally within 1 cycle (range, 1-4 cycles). With a median follow-up of 12 months (range, 6 days to 40 months), 8 patients remain alive, including 4 in durable remission following cessation of bortezomib and reduction or cessation of concomitant immunosuppressive therapy. Definitive comments regarding disease-specific responses are precluded by the small number of patients. However, responses appeared abrupt.
and durable in patients treated for TTP and correlated with laboratory parameters of autoantibody depletion, specifically restoration of ADAMTS13 activity and reductions in anti-ADAMTS13 inhibitor titer (Figure 1A). Notably, the response in case #8 occurred in the context of "inhibitor boosting" following initiation of plasma exchange and initiation of rituximab, suggesting rapid amelioration of aberrant antibody production with bortezomib and continued rituximab administration (Table 1; Figure 1A). Durable remission was also observed in a patient with acquired hemophilia, as we recently reported.20

In contrast to the sustained remissions observed in TTP patients, AIHA responses appeared less durable. For example, case #1 experienced a significant improvement in hemoglobin with initiation of bortezomib (Table 1; Figure 1B). However, as hemolytic markers appeared to trend upwards in the bortezomib-free interval between day 11 and day 21, dosing was changed to continuous weekly subcutaneous therapy from the second month of treatment (Figure 1B). Unfortunately, subsequent attempts to wean bortezomib therapy resulted in abrupt increases in hemolysis necessitating continued therapy. In addition to the temporal relationship between commencing bortezomib and apparent clinical responses, this case also illustrates that cessation of bortezomib may lead to abrupt worsening of disease activity in certain patients. A temporal relationship between drug withdrawal and disease recrudescence further strengthens the assertion of causality. In contrast, case #4 experienced an immediate improvement in hemolysis within the first cycle of bortezomib, allowing cessation of cyclosporine, cyclophosphamide, and a 75% reduction in prednisolone (Table 1). Bortezomib was ceased in the context of possible hepatotoxicity during cycle 2, but subsequently reinitiated at relapse 4 months later. No objective response was observed at rechallenge despite 8 further doses of bortezomib. Despite case reports of efficacy in ITP,11 no objective response was observed in an isolated case of severe multirefractory disease, however, this patient only received 2 bortezomib doses.

Bortezomib appeared well tolerated with only 2 patients manifesting adverse events potentially attributable to proteasome inhibition: self-limiting low-grade diarrhea and an episode of grade 3 hepatotoxicity in the context of high alcohol intake which necessitated bortezomib cessation (although it did not recur with subsequent rechallenge at later relapse [case #4]). Two patients died during the follow-up period, 1 due to uncontrollable bleeding secondary to ITP (case #9) and another due to metastatic carcinoma (with AIHA in PR [case #1]). No peripheral neuropathy or infective complications were experienced.

Together with isolated case reports, these findings provide proof of concept for proteasome inhibitor–based therapy in refractory autoantibody-mediated hematological disease. All bortezomib episodes occurred in the context of concurrent immunomodulatory therapies that are likely to have contributed to depletion of antibody-producing cells. In many cases, we had documented effective B-cell depletion in the peripheral blood using flow cytometry prior to bortezomib administration. Indeed, the combined effects of rituximab-mediated B-cell depletion and bortezomib-mediated plasma cell depletion may explain apparent treatment efficacy. Although the contribution of antecedent therapy cannot be discounted, the temporal relationship between bortezomib administration and clinical improvement (almost universally within 1 cycle) argues in favor of a bortezomib-specific treatment effect.

In the absence of prospective data, disease-specific response rates are uncertain, due to possible positive reporting bias. However, although this is a case series, we have reported consecutive cases treated at our institutions representing an unbiased experience. Although absolute numbers are low, the apparent high response rate in TTP is consistent with a recent series of 6 consecutive cases from UK referral centers where 5 patients achieved remission.16 Although formal prospective evaluation of proteasome inhibition in ultra-rare diseases like TTP is unlikely to be feasible, more common presentations such as refractory AIHA should be amenable to trial development. Registry data may also prove invaluable for capturing disease-specific response rates and safety end points.

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Authorship
Contribution: S.R., J.S., and S.S.O. analyzed the data and wrote the manuscript; and P.A.W., Huy Tran, Z.S.K., J.D.M., Huyen Tran, T.-C.T., S.F., S.D.C., J.V.C., and A.J. provided patient information and critically evaluated the manuscript.

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References


