Bortezomib: a new player in pre- and post-transplant desensitization?

Anne Lemy¹, Michel Toungouz² and Daniel Abramowicz¹

¹Renal Transplantation Clinic, Erasme Hospital, Brussels, Belgium and ²Immunobiology–Hemobiology–Transfusion, Erasme Hospital, Brussels, Belgium

Correspondence and offprint requests to: Anne Lemy; E-mail: Anne.Lemy@erasme.ulb.ac.be

Abstract

Several desensitization strategies have been investigated for the reversal of acute antibody-mediated rejection or for the removal of preformed anti-HLA antibodies, with the aim to promote access to renal transplantation. Today, their success appears limited or incomplete. Bortezomib, a selective inhibitor of the 26S proteasome, which is largely used in the treatment of multiple myeloma, could be a novel promising desensitizing agent. Its mechanism of action and preliminary clinical use in renal transplantation is reviewed here.

Keywords: bortezomib; desensitization; donor-specific antibodies; rejection

Introduction

The increasing number of, and cost of treating patients with end-stage renal disease is a major economic concern for health care systems. Compared with dialysis, kidney transplantation leads to improved patient survival and quality of life and is cost saving for the health payer.

Nevertheless, the access to transplantation is limited by the shortage of organs and, in some cases, by the presence of HLA antibodies (Abs) before transplantation. Therefore, efforts have been made to promote living kidney donation and several desensitization strategies have been investigated in order to remove anti-HLA Abs.

Nowadays, ~15% of all patients awaiting a deceased donor kidney transplant are sensitized to HLA because of pregnancy, blood transfusion or previous graft. Highly sensitized patients, defined by a PRA ≥85%, represent a minority of ~1% of patients on the waiting list. They have developed Abs against a large variety of HLA antigens and have the lowest chance to receive a cross-match (XM)-negative organ from standard allocation procedures. As a result, they often accumulate on the waiting list. At the present time, two strategies have been developed to overcome this problem.

Firstly, Eurotransplant has enrolled patients with a PRA ≥85% in the acceptable mismatch programme, which, by forbidding HLA antigens against which the patient has developed complement-fixing Abs, allows for an improved identification of XM-negative donors and gives the highest priority to allocation of these donors to highly sensitized patients [1,2].

Secondly, different desensitization protocols for the removal of anti-HLA Abs have appeared in order to increase the probability of a successful transplantation.

The presence of donor-specific anti-HLA antibodies (DSA), leading to a positive complement-dependent cytotoxicity (CDC) XM, has been associated for a long time with a high risk of graft loss due to hyperacute or early acute rejection (AR) and is, therefore, a contraindication for transplantation [3]. In recent years, HLA Abs screening assays, more sensitive than CDC, have become available. These assays fall into two categories, namely, ELISA-based methods and single HLA antigen-coated bead assays or single antigen-expressing cell lines used in a Luminex platform [4–6]. These new techniques are able to detect non-complement-fixing Abs but also Class I and Class II HLA Abs of lower titres. Up to now, DSA defined by such methods have been considered a risk factor, rather than as a contraindication for transplantation.

The presence of preformed anti-HLA Abs defined by ELISA has been clearly associated with a higher risk of antibody-mediated rejection (AMR), leading to poorer graft survival, especially when they are either donor-specific, strongly positive or related to a remote positive CDCXM [7].

The relevance of DSA identified by Luminex before transplantation has also been investigated in small retrospective studies [8–10]. All have shown that patients with DSA detectable by Luminex only had a higher frequency of biopsy-proven AR. However, the impact of preformed anti-HLA Abs on graft survival still remains unclear.

After transplantation, ~25% of kidney recipients develop de novo DSA, possibly consecutive to AR episodes. Since the last update of the Banff score for AR in 2007, AMR has become a distinct pathological entity whose diagnosis requires biopsy-proven histological features of glomerulitis and capillaritis, positive C4d staining of peritubular capillaries and the presence of DSA. Numerous studies have shown that post-transplant DSA but also non-DSA detected by the same screening approaches had
a deleterious impact on the incidence of acute and chronic rejection and graft survival [11–17].

**Pre- and post-transplant desensitizing strategies**

Because AMR responds only poorly to conventional anti-rejection treatment with steroid boluses, several desensitization strategies to prevent and to treat it have been developed. They include plasmapheresis, intravenous immune globulins (IVIG), rituximab and anti-thymocyte globulin (ATG). These agents either target B cells through different mechanisms of action or will decrease the titres and/or activity of anti-HLA Abs. Plasmapheresis removes anti-HLA Abs. Rituximab, a chimeric monoclonal Ab, binds to the CD20 receptor of B cells and depletes them through CDC, Ab-dependent cytotoxicity and stimulation of B-cell apoptosis. IVIG mediate their effect through inhibition of multiple pathways. The most obvious beneficial effect is attributed to their neutralizing effect on anti-HLA Abs. But IVIG are also able to neutralize cytokines involved in the allogeneic reaction as well as to block the effector mechanisms that mediate Ab-associated injury by inhibiting complement activation [18]. ATG, a polyclonal Ab preparation, depletes B and T cells by binding to multiple cell surface molecules.

Most renal transplantation centres advocated desensitization protocols for the removal of DSA before transplantation [19–28]. The therapies included plasmapheresis together with a low dose of IVIG, a high dose of IVIG and/or immunoabsorption, rituximab and a potent immunosuppressive regimen after transplantation. Often, desensitization was accompanied by induction therapy using either ATG, anti-IL2 Abs or alemtuzumab. However, highly sensitized patients have also been transplanted successfully after post-transplant prophylactic IVIG associated or not with plasmapheresis and rituximab.

Similar therapeutic approaches have been used for reversing acute AMR [26,28–34].

Today, two major issues related to the use of current desensitization therapies remain unsolved. Firstly, in many cases, low titres of DSA remain present and these approaches are, therefore, still complicated by high rates of early acute humoral rejection and late Ab-mediated injury, such as transplant glomerulopathy [35,36]. More and more, it appears that these depleting therapies incompletely prevent the histological lesions of acute humoral rejection, giving rise to a new entity named subclinical AMR, likely contributing to chronic allograft injury [37].

Secondly, a major pitfall with desensitization and the therapy of AMR is that most clinical trials are single-centre, non-randomized studies, with low number of patients, that eventually do not allow to clarify the hierarchical importance of plasmapheresis, IVIG rituximab or other agents.

To add confusion, it has been recently demonstrated that the classical desensitization strategies including rituximab, ATG and IVG did not target the terminally differentiated Ab-secreting plasma cells (PCs) responsible for the primary alloantibody production in the bone marrow and secondary lymphoid tissues [38]. A possible role of memory B cells in the persistent production of alloantibodies has also been suggested but remains unproven so far. Classically, memory B cells can become activated plasmablasts, which then convert to PCs within hours of exposure or re-exposure to an antigen [39].

**Immunomodulatory effects and clinical use of bortezomib**

Under physiological conditions, the NF-κB pathway is constitutively active only in a few types of cells, including neurons, B cells and thymocytes, and is always inactive in all other cell types [40]. However, dysregulation of NF-κB signalling has been associated with inflammatory disease and cancers. Indeed, constitutive activation of NF-κB signalling has been reported in cancer cells such as breast, colon, pancreatic, ovarian, lymphoma and melanoma. Hence, blockade of NF-κB signalling provided an attractive therapeutic strategy in autoimmune disease and cancer [41–45].

Bortezomib, a selective inhibitor of the 26S proteasome is largely used in the treatment of multiple myeloma, a clonal B-cell malignancy characterized by aberrant expansion of PCs within the bone marrow and extramedullary sites. This agent’s anti-PCs activity derives from several mechanisms including inhibition of NF-κB pathway, induction of caspase 8/9-mediated apoptosis, cleavage of DNA repair enzymes and blockade of IL-6 production [46]. Therefore, bortezomib, through an inhibitory effect on PCs, could be an additional therapeutic option for desensitization in the field of transplantation, either to increase the chance of receiving a XM-negative donor for sensitized patients awaiting renal transplantation or for the treatment of AMR. Perry et al. demonstrated in a recent paper that the classical desensitization strategy including rituximab, ATG and IVIG were ineffective against Ab production. They isolated and cultured PCs from bone marrow aspirates of kidney transplant recipients before transplantation. Apoptotic cells were counted and compared in control, bortezomib-, rituximab-, ATG- and IVG-treated groups. Unlike current desensitization agents, they showed in vitro that proteasome inhibition with bortezomib triggered apoptosis of CD138+CD20+ bone marrow-derived PCs and blocked anti-tetanus toxoid and anti-HLA IgG secretion in marrow supernatant [38]. However, the impact of proteasome inhibition on memory B cells or plasmablasts is still unknown so far. It was recently reported in primates that the concomitant low-dose administration of rapamycin and bortezomib could suppress the proliferation of memory B cells without affecting the survival of regulatory T cells and limit the production of pro-inflammatory cytokines, such as IL6 and IFN-γ, to a greater extent than that achieved by any single agent [47] (Figure 1).

Bortezomib also mediates apoptosis of activated T cells by preventing degradation of IκB and blocking NF-κB nuclear translocation and subsequent transcription of IL-1, IL-6 and TNF-α [48,49]. The mechanisms of
apoptosis in T cells were recently explored by Berges and colleagues. They showed that, in CD4+ T cells activated by allogeneic dendritic cells (DC), bortezomib induced the loss of mitochondrial membrane potential, leading to the translocation of pro-apoptotic proteins from mitochondria to the cytoplasm, thereby enhancing caspase 3 and 9 activities [50]. Treg cells seem to be spared by proteasome inhibition. In vitro assays showed that long-term culture of CD4+ T cells in the presence of anti-CD3, anti-CD28 monoclonal Abs, IL-2 and bortezomib did not affect naïve Treg cell viability but promoted the emergence of a regulatory T-cell population that inhibits proliferation, IFN-γ production and CD40 ligand expression among stimulated effector T cells [51].

Bortezomib has also inhibitory effects on the maturation of DC, whose main role is to present antigens, including HLA antigens, to T cells. It was shown in vitro that the treatment of DC generated from peripheral blood monocytes of healthy volunteers with bortezomib prevented their TLR4-induced maturation in response to stimuli of bacterial (lipopolysaccharide) or endogenous sources (TNF-α and CD40 ligand). Namely, DC pretreatment with the proteasome inhibitor inhibited the up-regulation of co-stimulatory molecules CD80, CD86 and CD40 which mediate T-cell adhesion and activation and prevents the processing of antigen-presenting cells by shutting down peptide generation from proteasome. Bortezomib also reduced the phagocytic capacities and cytokines production from DC, shut down peptide generation from proteasome and inhibited the processing of antigen-presenting cells [52].

Finally, bortezomib also showed in vitro a dose-dependent inhibition of angiogenesis through indirect mechanisms like a reduction of VEGF and IL-6 release by tumour cells and prevention of tumour adaptation to hypoxia [53,54]. Therefore, this molecule could be attractive to treat or at least to slow down chronic allograft injury or chronic rejection, events in which a lymphangiogenesis process was incriminated. Along this line, B cells present within inflammatory infiltrates strongly express the angiogenic cytokine VEGF [55,56].

Bortezomib has proved to be effective and safe in the treatment of untreated or refractory/relapsed multiple myeloma and in relapsed mantle cell lymphoma [57]. Of note, bortezomib does not benefit all patients. Some authors have recently identified a novel mechanism of
bortezomib resistance in myeloma patients mediated by REDD1 gene overexpression inducing inhibition of mammalian target of rapamycin kinase complex 1 (mTORC1) activity, suggesting that the use of mTOR inhibitors in myeloma patients could be deleterious [58]. We still do not know yet if the heterogeneity of response in multiple myeloma due to genomic variations in malignant PCs also takes place in to the transplantation setting.

Preliminary results in the prophylaxis of graft vs host disease after allogeneic stem cell transplantation also seem to be promising [59]. Bortezomib-containing regimens are being evaluated but not yet approved in patients with various other cancers including prostate cancer, non-small cell lung cancer, Waldenstrom’s macroglobulinaemia, chronic lymphocytic leukaemia and non-Hodgkin’s lymphoma. Pre-clinical data also suggest that proteasome inhibition could be attractive as a therapeutic agent in autoimmune diseases, such as lupus and rheumatoid polyarthritis [43,44].

### Administration modality and tolerability profile

The recommended dose of bortezomib is 1.3 mg/m² to be administered as an intravenous bolus injection over 3–5 s. In patients with relapsed/refractory myeloma, bortezomib 1.3 mg/m² should be administered on Days 1, 4, 8 and 11 of a 21-day cycle for up to eight cycles. There should be a break of at least 72 h between consecutive doses [60]. Bortezomib is widely distributed to peripheral tissues and extensively bound to human plasma proteins. It is primarily metabolized by the cytochrome P450 enzymes CYP3A4, CYP2C19 and CYP1A2. Bortezomib is eliminated more rapidly after a first dose than after subsequent doses (half-life time, 12 vs 76–108 h) [60]. The bortezomib tolerance profile is largely derived from the APEX Phase III trial, which compared the efficacy of bortezomib vs high-dose dexamethasone in refractory or relapsed multiple myeloma and from the VISTA trial which compared the use of melphalan and prednisone with or without bortezomib in previously untreated patients with multiple myeloma who were ineligible for high-dose therapy [61,62]. Main drug-related adverse events that were reported in more than 15% of patients enrolled in these two studies consisted of asthenic conditions (fatigue, weakness and malaise), gastrointestinal disorders, pyrexia, thrombocytopenia, neutropenia and peripheral neuropathy, psychiatric disorders and anorexia. They could generally be managed by dosage modifications and supportive therapy.

Antiviral prophylaxis should be considered in patients treated with bortezomib as HZV infections are common, especially in cancer patients. Vaccination against HZV before bortezomib use should be contemplated in HZV-naïve patients. Weekly monitoring of CMV antigenaemia should be performed in seropositive patients at risk of reactivation or disease [61,63]. Bortezomib should be withheld at the onset of at least Grade 3 non-haematologic or Grade 4 haematologic adverse events and reintroduced once the symptoms have resolved. Dosage adjustments of bortezomib are not necessary in patients with renal failure [60]. Bortezomib should be administered preferably after dialysis, as the procedure may reduce its concentration. Reduction in the dose of bortezomib is also recommended for patients with treatment-related neuropathic pain and/or peripheral neuropathy [60]. The pharmacokinetics of bortezomib has not been investigated in patients with hepatic impairment.

### Clinical use of bortezomib in transplantation: initial experience

Until now, there are eight case series reporting on the clinical use of bortezomib in the reversal of AR (Table 1) or the decrease of anti-HLA Abs (Table 2). Firstly, Perry’s group successfully treated two positive XM kidney recipients for early acute AMR with four courses of bortezomib 1.3 mg/m² in addition to daily plasmapheresis and IVIG. They demonstrated in vivo a transient decrease of bone marrow-derived PCs in bone marrow aspirates. They also showed that serum HLA alloantibody titres and number of specific

### Table 1. Clinical use of bortezomib in control of AR

<table>
<thead>
<tr>
<th>Studies</th>
<th>No. of treated AR episodes</th>
<th>Histological findings</th>
<th>C4d</th>
<th>Post-Tx days at initiation of bortezomib</th>
<th>Schema of administration of bortezomib</th>
<th>Adjuvant therapy</th>
<th>Reversibility of AR</th>
<th>Decrease of HLA MFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every &amp; al.</td>
<td>3</td>
<td>Banff Ia</td>
<td>1/3</td>
<td>147–239</td>
<td>1.3 mg/m² × 4</td>
<td>PP (1) rituximab (1)</td>
<td>Complete (3)</td>
<td>Significant (3)</td>
</tr>
<tr>
<td>1</td>
<td>Banff Ib</td>
<td>1</td>
<td>95</td>
<td></td>
<td>1.3 mg/m² × 4</td>
<td>PP</td>
<td>Complete (1)</td>
<td>Significant (3)</td>
</tr>
<tr>
<td>1</td>
<td>Banff Iia</td>
<td>0</td>
<td>1766</td>
<td></td>
<td>1.3 mg/m² × 4</td>
<td>ATG</td>
<td>Complete (1), no reversibility (1)</td>
<td>Significant (1)</td>
</tr>
<tr>
<td>2</td>
<td>Borderline</td>
<td>0/2</td>
<td>265–2825</td>
<td></td>
<td>1.3 mg/m² × 4</td>
<td>PP (1)</td>
<td>Complete (2)</td>
<td>Significant (2)</td>
</tr>
<tr>
<td>Perry &amp; al.</td>
<td>2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.3 mg/m² × 4</td>
<td>Daily PP + IVIg</td>
<td>Complete (2)</td>
<td>Significant (2)</td>
</tr>
<tr>
<td>Walsh &amp; al.</td>
<td>2</td>
<td>Acute tabular injury (1/2), glomerulitis (2)</td>
<td>2/2</td>
<td>14–15</td>
<td>1.3 mg/m² × 4 (2) + 1.3 mg/m² × 4 (1)</td>
<td>PP × 7, rituximab (3.75 mg/m²) mPDS</td>
<td>Complete (2)</td>
<td>Significant (2)</td>
</tr>
</tbody>
</table>

PP, plasmapheresis; ATG, antithymocyte globulin; NA, non available.
Table 2. Clinical use of bortezomib in removal of post-transplant anti-HLA Abs

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of patients</th>
<th>Post-Tx days at use of bortezomib</th>
<th>DSA MFI before bortezomib</th>
<th>Serum creatinine before bortezomib</th>
<th>Schema of administration of bortezomib</th>
<th>Adjuvant therapy</th>
<th>DSA MFI after initiation treatment with bortezomib</th>
<th>Serum creatinine after bortezomib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idica et al.</td>
<td>1</td>
<td>82</td>
<td>2500–6600 (ClI + ClII)</td>
<td>1.4 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS/ATG</td>
<td>&lt;1000 (86 days)</td>
<td>1.6 mg/dL</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>69</td>
<td>1200–2500 (ClI), 10 400 (ClII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4 + 1.3 mg/m² × 1</td>
<td>PP/mPDS</td>
<td>&lt;1000 (20 days), &lt;1000 (147 days)</td>
<td>1 mg/dL</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>65</td>
<td>2800–3700 (ClII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>IVIg/ATG/CP, PP/mPDS</td>
<td>&lt;1000 (87 days), 2300 (179 days)</td>
<td>1.5 mg/dL</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>71</td>
<td>6600–13 200 (ClII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 3</td>
<td>IVIg/ATG/CP, PP/mPDS</td>
<td>&lt;1000 (144 days)</td>
<td>1.3 mg/dL</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>69</td>
<td>1100–2000 (ClI)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>IVIg/ATG/CP, PP/mPDS</td>
<td>&lt;1000 (18 days), 1.3 mg/dL</td>
<td>1.3 mg/dL</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>62</td>
<td>1000–11 000 (ClII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4 + 1.3 mg/m² × 3</td>
<td>IVIg/ATG/CP, PP/mPDS, rituximab</td>
<td>9200 (126 days)</td>
<td>1.2 mg/dL</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>105</td>
<td>1300–2000 (ClIII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>CP/ATG/PP/mPDS, rituximab</td>
<td>&lt;1000 (14 days), &lt;1000 (81 days)</td>
<td>1.6 mg/dL</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>68</td>
<td>1400–4600 (ClI + ClII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>CP/ATG/PP/mPDS, rituximab</td>
<td>&lt;1000 (19 days), &lt;1000 (96 days)</td>
<td>1.4 mg/dL</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>123</td>
<td>2800–10 000 (ClIII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4 + 1.3 mg/m² × 4</td>
<td>CP/ATG, PP/mPDS</td>
<td>7500 (55 days)</td>
<td>1.6 mg/dL</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>85</td>
<td>1000–14 000 (ClII)</td>
<td>0.9 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>IVIg, PP/mPDS, rituximab</td>
<td>&lt;1000–8000 (118 days), 9000 (74 days)</td>
<td>1.5 mg/dL</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>7</td>
<td>1100–11 300 (ClI)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>IVIg/ATG/CP, PP/mPDS, rituximab</td>
<td>1300 (29 days)</td>
<td>1.2 mg/dL</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>122</td>
<td>9600 (ClIII)</td>
<td>1.4 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>IVIg/ATG/CP, PP/mPDS, rituximab</td>
<td>&lt;1000 (45 days), &lt;1000 (91 days)</td>
<td>1.3 mg/dL</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>81</td>
<td>1400–5200 (ClI)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 3</td>
<td>IVIg/ATG/CP, PP/mPDS</td>
<td>&lt;1000 (45 days), 1.3 mg/dL</td>
<td>1.3 mg/dL</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Author</th>
<th>No. of patients</th>
<th>Post-Tx days at use of bortezomib</th>
<th>DSA MFI before bortezomib</th>
<th>Serum creatinine before bortezomib</th>
<th>Schema of administration of bortezomib</th>
<th>Adjuvant therapy</th>
<th>DSA MFI after initiation treatment with bortezomib</th>
<th>Serum creatinine after bortezomib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trivedi et al.</td>
<td>1</td>
<td>71</td>
<td>2500–6800 (ClI + ClIII)</td>
<td>1.4 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (30 days), &lt;1000 (80 days)</td>
<td>1.4 mg/dL</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>68</td>
<td>3500 (ClI + ClII)</td>
<td>0.9 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (20 days), &lt;1000 (154 days)</td>
<td>1.0 mg/dL</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>54</td>
<td>2800–3700 (ClI)</td>
<td>1.6 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (87 days), &lt;1000 (172 days)</td>
<td>1.5 mg/dL</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>59</td>
<td>2200–2500 (ClIII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (18 days), &lt;1000 (173 days)</td>
<td>1.2 mg/dL</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>76</td>
<td>2800–11 000 (ClI + ClIII)</td>
<td>1.2 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>9000 (84 days)</td>
<td>1.3 mg/dL</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>95</td>
<td>1300–2000 (ClI + ClIII)</td>
<td>1.2 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (14 days), &lt;1000 (78 days)</td>
<td>1.5 mg/dL</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>25</td>
<td>2900 (ClI)</td>
<td>1.8 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (24 days), &lt;1000 (158 days)</td>
<td>1.4 mg/dL</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6</td>
<td>4000–11 300 (ClI + ClIII)</td>
<td>1.1 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>9400 (168 days)</td>
<td>1.1 mg/dL</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>33</td>
<td>2000–4400 (ClI + ClIII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (25 days), &lt;1000 (89 days)</td>
<td>1.4 mg/dL</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>77</td>
<td>2300–5200 (ClI + ClIII)</td>
<td>1.4 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (67 days), &lt;1000 (81 days)</td>
<td>1.4 mg/dL</td>
</tr>
<tr>
<td>Sberro-Soussan et al.</td>
<td>10</td>
<td>66</td>
<td>2000–2300 (ClI + ClII)</td>
<td>1.3 mg/dL</td>
<td>1.3 mg/m² × 4</td>
<td>PP/mPDS</td>
<td>&lt;1000 (84 days)</td>
<td>1.4 mg/dL</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>880</td>
<td>3159 (ClIII)</td>
<td>±350 μmol/L</td>
<td>1.3 mg/m² × 4</td>
<td>None</td>
<td>±3500 (150 days)</td>
<td>±350 μmol/L</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>425</td>
<td>4590 (ClI)</td>
<td>173 μmol/L</td>
<td>1.3 mg/m² × 4</td>
<td>None</td>
<td>±2700 (150 days)</td>
<td>±190 μmol/L</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1125</td>
<td>5096 (ClI)</td>
<td>165 μmol/L</td>
<td>1.3 mg/m² × 4</td>
<td>None</td>
<td>±4000 (150 days)</td>
<td>±180 μmol/L</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>700</td>
<td>±1500 (ClI + ClII)</td>
<td>±150 μmol/L</td>
<td>1.3 mg/m² × 4</td>
<td>None</td>
<td>±2000 (150 days)</td>
<td>±150 μmol/L</td>
</tr>
</tbody>
</table>

PP, plasmapheresis; ATG, antithymocyte globulin; mPDS, methylprednisolone; CP, cyclophosphamide.
cities identified by Luminex were much lower at 1-year follow-up than at the time of transplant before bortezomib treatment, while serum IgG levels remained within normal range, thereby reducing the potential risk of infections. Other results have also been reported regarding the successful use of bortezomib in the prompt reversal of acute AMR after renal and lung transplantation together with a long-lasting reduction in DSA levels [64,65]. In Everly’s study, six kidney recipients with biopsy-proven features of mixed AMR and T-cell-mediated rejection (TCMR) refractory to classical antihumoral agents, such as rituximab, IVIg, ATG or plasmapheresis, were treated with four courses of bortezomib 1.3 mg/m². Bortezomib was administered at a median time of 743 days after transplantation. Median follow-up was 7 months. In all patients except one, bortezomib lead to a prompt reversal of AMR and TCMR. Five patients out of six experienced a 50% reduction in DSA, independent of HLA Class I or II specificity, within 2–4 weeks after the first bortezomib dose regardless of the magnitude of DSA levels. Bortezomib therapy was relatively well tolerated. Opportunistic infections were not observed.

Walsh reported on a bortezomib-based therapy combining plasmapheresis, methylprednisolone and a single dose of rituximab for the treatment of AMR occurring within the first 2 weeks after transplantation. Patients experienced prompt AMR reversal and elimination of detectable DSA within 14 days of bortezomib-based therapy. Renal function was excellent with urinary protein excretion in the normal range until 6 months after AMR episode. However, one patient presented a repeated elevation of DSA 2 months after proteasome inhibition therapy without C4d deposition or histological evidence of AMR. Retreatment with another cycle of bortezomib provided for durable lowering of DSA levels [66].

Up to now, proofs are still lacking that the decrease in DSA is really due to bortezomib therapy or is part of the natural history of AMR. Indeed, Burns et al. examined the course of DSA levels after renal transplantation of positive XM kidney recipients and their relationship with AMR. They found that the increase in DSA levels after acute humoral rejection in patients keeping a well-functioning graft was transient, returning to pre-transplant levels after a few weeks. A possible explanation could be a competition between newly generated PCs and existing PCs for survival niches in order to become long-lived PCs [67].

Two studies related to the use of bortezomib as a desensitization agent have been performed in the post-transplant setting, in the absence of AR [68,69]. The first concerned the treatment of 13 renal transplant patients, with one or two cycles of bortezomib, in whom DSA were detected within 2 months after transplantation. Serum creatinine was stable. DSA titres before bortezomib ranged from 1000 to 14 000 MFI. Patients were given plasmapheresis at each dose of bortezomib. Additional therapies as clinically needed included rituximab, cyclophosphamide, ATG, IVIg and corticosteroids. Ten patients out of 13 had a decrease in DSA titres to <1000 MFI within 14–144 days after the initiation of bortezomib therapy. In five patients, DSA MFI remained undetectable up to nearly 6 months after the initial dose [68].

Trivedi’s study showed in the early post-transplant period that bortezomib therapy (1.3 mg/m² at Days 1, 4, 8 and 11) with adjuvant methylprednisolone (250 mg) followed by plasmapheresis also resulted in a substantial reduction of both HLA DSA and non-DSA levels in 9 out of 11 kidney recipients with MFI <10 000. This occurred within a median time of 24 days from treatment initiation, in the absence of AR [69]. In addition to plasmapheresis and bortezomib, 6 out of 11 patients also received one dose of rituximab to provide depletion of precursor and early naïve B lymphocytes. Three of the nine patients experienced recurrent DSA. In two patients, DSA remained refractory. In these two studies, patients with MFI >10 000 partially responded, suggesting maybe the need for additional cycles of bortezomib.

Since bortezomib was administered as part of a multi-drug regimen, its intrinsic properties could not be firmly established from these previous studies. Indeed, it is reasonable to think that other desensitizing therapies might have potentiated bortezomib by different mechanisms. Rituximab, inefficient against PCs, probably reduced PC generation from the memory B-cell population. We can also hypothesize that the removal of circulating Abs by plasmapheresis resulted in a rebound of Ab production, thereby enhancing the sensitivity to proteasome. Synergy between bortezomib and dexamethasone has also been clearly demonstrated in multiple myeloma. Dexamethasone leads to the down-regulation of MAPKs and p70S6K involved in proliferative/anti-apoptotic signalling pathways activated in multiple myeloma cells [70,71].

A recent article described four cases in which bortezomib was used as the sole desensitization therapy in patients exhibiting late subclinical AMR accompanied by persistent DSA [72]. All patients received bortezomib (1.3 mg/m²) on Days 1, 4, 8 and 11 without any modification of their maintenance immunosuppressive regimen. HSV and HZV prophylaxis was given for 6 months. Sera were tested at Days 0, 20 and 40 for DSA by Luminex and for the presence of anti-HSV and anti-HbS IgG. Total IgG were also monitored. This protocol failed to significantly decrease HLA Abs within 40 days after the infusion of a single cycle of bortezomib, as well as at 5 months post-treatment. Neither viral serology titres nor total serum levels were affected by bortezomib. Unlike other case series, bortezomib was not well tolerated. Three patients experienced bilateral conjunctivitis, which was severe and long-lasting in one case.

Another potential interest in the use of bortezomib could be the lowering of Abs in sensitized patients before transplantation in order to increase their chance to receive a XM-negative donor organ and reduce the waiting time on dialysis. However, a recent report on the first clinical experience of bortezomib in two pre-sensitized renal transplant candidates has been inconclusive [73].

Finally, very recent animal data have also suggested a potential benefit of bortezomib for the control of chronic rejection in a rat cardiac allograft model. In that study, bortezomib treatment of recipients was associated with a significant reduction of the levels of anti-HLA IgG and a trend to a reduction of intra-graft C4d deposition. Bortezomib also tended to reduce fibrosis and histological lesions of transplant vasculopathy [74].
Conclusion and perspectives

Considering both its mechanisms of action and initial clinical experience, bortezomib appears as a novel and promising therapeutic agent for the reversal of primary or refractory AMR, as well as for anti-HLA desensitization. However, we still lack a definitive proof of efficiency of this agent as well as a clear definition of the treatment scheme including the number of cycles required and the potential need for a combination with another agent, such as steroids boluses, rituximab, plasmapheresis or IVIG. These questions can only be answered in multi-centric, prospective, randomized and controlled studies combining the evaluation of the efficacy and safety of bortezomib as a potential desensitization agent with the analysis of its impact on graft outcome.

Conflict of interest statement. None declared.

References

2. Claas FH, Rahmel A, Doxiadis II. Enhanced kidney allocation to highly sensitized patients by the acceptable mismatch program. Transplantation 2009; 88: 447–452
15. Lee PC, Zhu L, Terasaki PI et al. HLA-specific antibodies developed in the first year posttransplant are predictive of chronic rejection and renal graft loss. Transplantation 2009; 88: 568–574
18. Toungouz M, Denys C, Dupont E. Blockade of proliferation and TNF-α production occurring during the mixed lymphocyte reaction by IFN-γ specific natural antibodies contained in intravenous immunoglobulins (IVIg). Transplantation 1996; 62: 1292–1296
26. Montgomery RA, Zachary AA, Racusen LC et al. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. Transplantation 2000; 70: 887–895

Received for publication: 16.4.10; Accepted in revised form: 22.7.10