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Bortezomib Against Refractory Antibody-Mediated Rejection After ABO-Incompatible Living-Donor Liver Transplantation: Dramatic Effect in Acute-Phase?

Tetsuya Tajima, MD,[1] Koichiro Hata, MD, PhD,[1] Hideaki Okajima, MD, PhD,[1] Momoko Nishikori, MD, PhD,[2] Kentaro Yasuchika, MD, PhD,[1] Jiro Kusakabe, MD,[1] Atsushi Yoshizawa, MD, PhD,[1] Ken Fukumitsu, MD, PhD,[1] Takayuki Anazawa, MD, PhD,[1] Hirokazu Tanaka, MD,[1] Seidai Wada, MD, PhD,[1] Junshi Doi, MD,[1] Akifumi Takaori-Kondo, MD, PhD,[2] and Shinji Uemoto, MD, PhD[1]

Abstract. Antibody-mediated rejection (AMR) is a refractory rejection after donor-specific antibody-positive or ABO blood-type incompatible (ABOi) organ transplantation. Rituximab dramatically improved the outcome of ABOi living-donor liver transplantation (LDLT); however, an effective treatment for posttransplant AMR, once occurred, is yet to be established. A 44-year-old woman with biliary cirrhosis underwent ABOi-LDLT from her sister (AB-to-A). Pretransplant rituximab diminished CD19/20-positive B lymphocytes to 0.6%/0.0%; however, AMR occurred on posttransplant day-6 with marked increase in both CD19/20 cells (17.1%/5.8%) and anti-B IgM/G-titers (1024/512). Despite rituximab readministration, steroid-pulse, intravenous immunoglobulin, and plasmapheresis, AMR was uncontrollable, with further increasing CD19/20 cells (23.0%/0.0%) and antibody-titers (2048/512). Bortezomib (1.0 mg/m^2) was thus administered on posttransplant day-9, immediately ameliorating CD19/20 cells (1.3%/0.0%) and antibody-titers (<256/128). Complete remission of refractory AMR was obtained by just 2 doses of bortezomib. Her liver function has been stable thereafter for over 3 years. This case highlighted the efficacy of bortezomib against refractory AMR after ABOi-LDLT. Unlike previous reports, the efficacy was very dramatic, presumably due to the administration timing near the peak of acute-phase AMR.

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Antibody-mediated rejection (AMR) is a refractory rejection after donor-specific antibody (DSA)-positive or ABO blood-type incompatible (ABOi) organ transplantation. AMR can cause irreversible damage not only to hepatic parenchyma but also to intrahepatic bile ducts, thereby predisposing to graft failure, unless appropriately treated.[1] Drug repositioning of rituximab, an anti-cluster of differentiation (CD)-20 monoclonal antibody, has dramatically altered the treatment paradigm of preoperative desensitization and outcome of ABOi living-donor liver transplantation (LDLT).[2] Once posttransplant AMR occurs, however, an effective treatment for this refractory rejection is yet to be established. Complete response is rarely achieved by conventional treatments, including steroid-pulse, plasmapheresis, intravenous immunoglobulin (IVIG), or even by rituximab readministration.

Bortezomib is a proteasome inhibitor that was originally developed for multiple myeloma.[3] Since the first report introducing bortezomib for AMR after kidney transplantation (KTx),[4] several reports have currently described its efficacy for

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[1] Department of Surgery, Division of Hepato-Biliary-Pancreatic Surgery and Transplantation, Kyoto University Graduate School of Medicine, Kyoto, Japan.

[2] Department of Hematology and Oncology, Kyoto University Graduate School of Medicine, Kyoto, Japan.

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Correspondence: Koichiro Hata, MD, PhD, Department of Surgery, Division of Hepato-Pancreato-Biliary Surgery and Transplantation, Kyoto University Graduate School of Medicine, Kawahara-cho 54, Shogoin, Sakyo-ku, Kyoto city, Kyoto 606–8507, Japan. (khata@kuhp.kyoto-u.ac.jp).

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AMR in other organ transplantation.[5-7] However, the use for AMR after ABOi-liver transplantation (LTx) is not yet fully elucidated. Here, we report a refractory case of AMR after ABOi-LDLT that was successfully treated with bortezomib, and its dramatic effect in acute phase.

CASE DESCRIPTION

A 44-year-old Japanese woman with end-stage biliary cirrhosis was referred to our hospital for LTx. The patient underwent surgical resection of a teratoma at the hepatoduodenal ligament and concomitant choledocho-choledochostomy at 9 months old. Thereafter, she had bile duct stricture and resultant repeated cholangitis, resulting in complete portal vein obstruction. Esophageal transection and splenectomy were performed in her childhood due to esophageal varices rupture. She also underwent cholecystectomy and choledocolithotomy at 19 years, and endoscopic sphincterotomy at 32 years for recurrent choledocholithiasis; however, refractory cholangitis did not subside. Finally, she was referred to our hospital for LTx. Child-Pugh-Turcotte and Model for End-stage Liver Disease scores on admission were 11 and 21, respectively (Table 1). Her sister volunteered as a living-donor, though their blood-type combination was incompatible (AB-to-A). Her left-lobe satisfied our donor criteria[8] with graft/recipient body weight ratio of 0.91%.

As a preoperative desensitization, 300 mg/body (200 mg/m^2) of rituximab was administered 3 weeks before the elective LDLT, and CD19/20-positive (CD19+/20+, respectively) B lymphocytes decreased to 0.6% (7.8 cells/μL) and 0.0%, respectively. In addition, oral mycophenolate-mofetil (0.5 g/day) for 14 days, and tacrolimus (trough, 5 ng/mL) for 7 days were given before LDLT. Postoperatively, tacrolimus trough ranged from 10 to 15 ng/mL, and mycophenolate-mofetil was raised from 0.5 to 1.5 g/day. Methylprednisolone (10 mg/kg body weight) was intraoperatively administered before reperfusion, and gradually tapered postoperatively.[9]

The operation was difficult because of severe adhesions from 4 previous laparotomies, frequent cholangitis, and complete portal vein obstruction. Although re-laparotomy was necessary for postoperative hemorrhage on posttransplant day (PTD)-1, the patient's condition stabilized thereafter. She started oral intake and walking on PTD-3. On PTD-6, however, serum aspartate aminotransferase and alanine aminotransferase suddenly increased from 68/67 on PTD-5 to 229/169 U/L, and total bilirubin rose from 8.8 to 19.3 mg/dL. Anti-B IgM/G titers markedly increased from 256/64 (PTD-5) to 1024/512 (PTD-6), with significant increase of CD19$^+$, CD20$^+$, and CD19$^+$/20$^-$ B lymphocytes from preoperative 0.6%, 0.0%, and 0.6% (7.8, 0.0, and 7.8 cells/μL, respectively) to 17.1%, 5.8%, and 11.3% (126.3, 42.8, and 83.4 cells/μL, respectively) on PTD-6 (Figure 1 and Table 1). She was clinically and serologically diagnosed as acute and severe AMR. Rituximab (300 mg/m^2) was readministered on PTD-6, followed by IVIG, steroid-pulse, and repeated plasma exchange. Despite such intensive cares, AMR was uncontrollable, accompanied by systemic inflammatory response syndrome (SIRS) with high fever, tachycardia, and diminished bile and urine (Figure 1). Though CD20$^+$ cells were depleted by rituximab readministration, CD19$^+$/20$^-$ cells remained highly activated (23.0% [81.5 cells/μL]), and anti-B IgM/G titers further increased up to 2048/512, representing emerging CD20-negative B cells with plasmacytic differentiation.[10] Thus, bortezomib (1.0 mg/m^2) was administered on PTD-9 with no other therapeutic options. CD19$^+$/20$^-$ cells and anti-B IgM/G titers both dramatically decreased to 1.3% (3.5 cells/μL) and <256/128, respectively, and bile and urine volume rapidly recovered within half a day (Figure 1). Though refractory AMR became completely quiescent by just 1 dose of bortezomib, an additional dose (1.0 mg/m^2) was given on PTD-12 as a consolidative treatment.[3] Complete remission was thus obtained with just 2 doses of bortezomib (Figure 1), and she was discharged on PTD-70 (Table 1). Her liver function has been stable thereafter for >3 years, without any intrahepatic biliary lesions.

DISCUSSION

Generally, AMR is comprehensively diagnosed based on clinical, serological, and histological findings.[11,12] In this case, AMR was clinically and serologically diagnosed without liver biopsy because of moderate ascites on liver surface, severe thrombocytopenia (1.4 × 10^4/μL), and insufficient recovery of coagulability, all of which were potential risks for bleeding complication. However, drastic increase in both anti-B antibody-titers and CD19$^+$, CD20$^+$, and CD19$^+$/20$^-$ cells definitely manifested acute/severe AMR. The sudden deterioration of liver function with tachycardia, high fever, and hypouresis indicated concomitant SIRS from acute/severe AMR. Conventionally, AMR is treated with steroid-pulse, plasmapheresis, IVIG, or rituximab; however, these approaches cannot deplete antibody-producing cells, that is, activated plasma cells and antibody-secreting B cells.[13] Since the patient became rapidly exacerbated despite all such treatments, bortezomib was administered with no other promising options.

In the first report introducing bortezomib against AMR after KTx,[4] 6 patients were treated with bortezomib, rituximab, IVIG, and plasmapheresis, showing >50% reduction of DSA levels. In contrast, posttransplant desensitization with bortezomib failed to decrease DSA in chronic AMR.[14] Another report described that bortezomib with steroids, plasmapheresis, and IVIG was effective in 20 AMR cases after KTx, but the recovery rate of renal function was just 25%.[15] More recently, a randomized, placebo-controlled trial using bortezomib against late AMR (2–14 after KTx) reported that bortezomib neither exhibited any protective effects to renal allografts nor reduced DSA, despite significant toxicities.[16]

By contrast, just one dosing of bortezomib dramatically diminished severely increased CD19$^+$/20$^-$ cells, followed by significant reduction of anti-B IgM/G titers and very quick recovery from SIRS in this case. Thus far, there are a few reports describing the efficacy of bortezomib against AMR after DSA-positive deceased-donor LTx[17] and ABOi-LDLT;[18] however, no such dramatic effect, observed in this case, was reported. To our knowledge, there is little evidence demonstrating different efficacies of bortezomib by administration timing or the phase of AMR. Bortezomib selectively inhibits 26S proteasome that is necessary for plasmacytic proliferation/differentiation. Proteasome inhibition results in protein overload and subsequent endoplasmic-reticulum stress, finally leading to myeloma cell death.[19] Therefore, efficacy of bortezomib is likely enhanced by proliferating phase of plasma cells. Theoretically, whole B cell lineage including plasma cells should be diminished as soon as possible to control AMR before sufficient sensitization by donor antigens. Therefore,

TABLE 1.

Summary of laboratory data

	Before rituximab administration	Before transplant	PTD-2	PTD-5	PTD-6	PTD-7	PTD-9	PTD-10	PTD-12	PTD-14	PTM-1	Before discharge (PTM-2)	PTM-3	PTM-6
AST (unit/L)	150	118	179	68	229	47	28	20	27	19	40	46	48	64
ALT (unit/L)	34	27	65	67	169	44	24	17	25	26	40	24	34	40
LDH (unit/L)	165	155	212	465	1173	327	251	226	180	188	242	269	231	159
T-Bil (mg/dL)	20.4	23.5	3.4	8.8	19.3	10.7	9.7	6.0	3.9	2.5	1.4	0.6	0.7	0.9
PT-INR	1.25	1.29	1.59	1.35	1.44	1.17	1.19	1.18	1.20	1.24	1.15	1.15	1.09	0.93
sCre (mg/dL)	0.66	0.67	1.35	1.18	1.12	1.24	1.40	1.3	1.07	1.00	1.05	1.16	0.93	0.94
Platelet ($\times 10^4$/μL)	29.6	31.8	10.3	4.2	1.4	3.6	5.0	7.7	13.0	18.5	43.0	44.2	38.6	49.1
Anti-B IgM (titer)	256	256	–	256	1024/512[a]	2048/1024[a]	2048/1024[a]	1024/256[a]	256	512	128	16	128	64
Anti-B IgG (titer)	4	4	–	64	512/128[a]	256/256[a]	512/256[a]	256/128[a]	128	128	32	64	64	32
CD19+ (% [cells/μL])	–	0.6 (7.8)	–	–	17.1 (126.3)	23.0 (81.5)	9.4 (73.8)	1.9 (6.1)	1.3 (3.5)	0.7 (4.0)	0.0	0.0	25.7 (291.3)	20.2 (382.5)
CD20+ (% [cells/μL])	–	0.0	–	–	5.8 (42.8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.7 (291.3)	20.2 (382.5)
CD19+/CD20- (% [cells/μL])	–	0.6 (7.8)	–	–	11.3 (83.4)	23.0 (81.5)	9.4 (73.8)	1.9 (6.1)	1.3 (3.5)	0.7 (4.0)	0.0	0.0	0.0	0.2 (3.8)
IgG (mg/dL)	3218	–	–	–	–	–	–	–	–	–	950	–	1373	–
IgM (mg/dL)	148	–	–	–	–	–	–	–	–	–	47	–	75	–
Anti-HLA antibody: Class I	Negative	Negative	–	–	–	–	–	–	–	–	–	–	–	Negative
Anti-HLA antibody: Class II	Negative	Negative	–	–	–	–	–	–	–	–	–	–	–	Negative
Tacrolimus trough level (ng/mL)			11.8	19.0	16.4	14.7	23.0	17.3	11.9	12.4	9.5	6.7	8.1	5.6

[a]Represent anti-B IgM/G titers just after plasma exchange each day.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; $CD19^+$, CD19-positive B lymphocytes; $CD19^+/CD20^-$, CD19-positive/CD20-negative cells; $CD20^+$, CD20-positive B lymphocytes; LDH, lactate-dehydrogenase; PT-INR, prothrombin time-international normalized ratio; PTD, posttransplant day; PTM, posttransplant mo; sCre, serum creatinine; T-Bil, total bilirubin; –, no data.

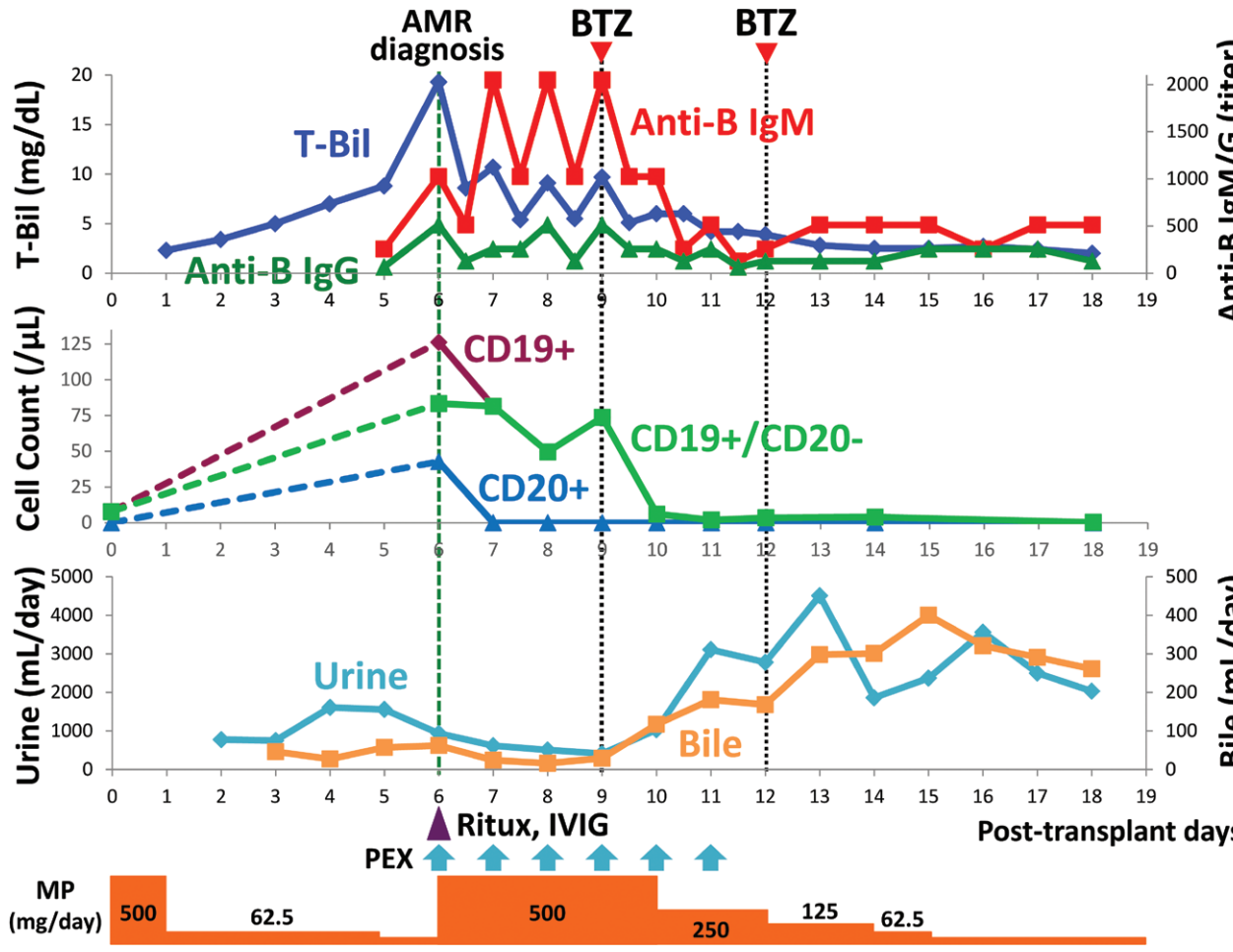

FIGURE 1. Postoperative course. On PTD-6, the patient's liver function tests suddenly worsened, with marked increase of $CD19^+/20^+$ cell counts and anti-B IgM/G titers. Despite intensive cares including rituximab readministration (300 mg/m^2), IVIG, steroid-pulse (10 mg/kg of MP for 3 days, and tapered thereafter by half every 2 days), and repeated PEX, AMR was uncontrollable with remaining $CD19^+/CD20^-$ cells. Although PEX provided transient reduction in the antibody-titers just after PEX; however, the titers rapidly rebounded up to the pre-PEX level or higher by the next morning. Just one dosing of bortezomib on Day 9, however, remarkably suppressed the rebound of both anti-B IgM and IgG antibody-titers. Moreover, the patient developed systemic inflammatory response syndrome with high fever, tachycardia, and diminished bile, and urine. Bortezomib was thus administered on PTD-9 with no other promising options. Bile and urine volume rapidly recovered within half a day, and both $CD19^+/CD20^-$ cell counts and anti-B IgM/G titers dramatically decreased. Note that $CD19^+/CD20^-$ cells were rapidly diminished after the bortezomib administration on PTD-9, which correspond to CD20-negative antibody-secreting B cells, such as short-lived plasma cells and/or plasmablasts[10] by flow cytometry (FACSCalibur HG; BD Biosciences, San Jose, CA) using phycoerythrin (PE)-conjugated anti-CD19 antibody (clone SJ25C1, BD Biosciences) and fluorescein isothiocyanate (FITC)-conjugated anti-CD20 antibody (clone L27, BD Biosciences). The absolute cell numbers were calculated from the percentage of the cells in total lymphocytes. AMR, antibody-mediated rejection; BTZ, bortezomib; $CD19^+$, CD19-positive B lymphocytes; $CD19^+/CD20^-$, CD19-positive and CD20-negative cells; $CD20^+$, CD20-positive B lymphocytes; IVIG, intravenous immunoglobulin; MP, methylprednisolone; PEX, plasma exchange; PTD, posttrasnplant day; Ritux, rituximab; T-Bil, total bilirubin.

combined administration of rituximab and bortezomib immediately after AMR diagnosis may be recommended to suppress refractory AMR at present. Daratumumab, anti-CD38 antibody, may also be a promising candidate to diminish activated plasma cells and to minimize AMR instantly.[20]

The standard regimen of bortezomib for multiple myeloma, consisting of four 1.3 mg/m^2 doses on Day 1, 4, 7, and 11 in the first 2 weeks, followed by 1-week interval, is usually repeated until remission or severe adverse events.[3] However, single cycle of the same regimen is often administered for AMR.[4,14-16] In this case, we reduced the dose from 1.3 to 1.0 mg/m^2, and from 4 to 2 doses, because (1) all serological/clinical parameters improved just after one dosing, and the patient condition recovered quickly; and (2) the patient already received other intensive immunosuppression. Such dose control enabled no adverse effects of bortezomib in this case, for example, viral infections, thrombocytopenia, or peripheral neuropathy.[14,17] Although combined use of rituximab and bortezomib has a potential risk of persistent B cell depletion and hypogammaglobulinemia, she was not affected by any of those (Table 1).

In conclusion, this case highlighted the significant potential of bortezomib against refractory, acute-phase AMR after ABOi-LDLT. The effect was substantial, perhaps due at least partially to the administration timing on highly proliferated phase of plasma cells near the peak of acute AMR. Further studies are required to confirm the efficacy and safety of bortezomib for AMR, and to evaluate the phase-dependent difference of its efficacy in both acute and late/chronic rejections.

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