Addition of bortezomib to high-dose cyclophosphamide therapy as a conditioning regimen for autologous peripheral blood stem cell harvest leads to an increased yield of hematopoietic stem cells

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Peripheral blood stem cell harvest (PBSCH) is a crucial procedure for autologous stem cell transplantation in patients with multiple myeloma. We herein report a retrospective study to verify the usefulness of bortezomib and high-dose cyclophosphamide therapy (Bor-HDCY) as a conditioning regimen for PBSCH. Thirty-three patients were evaluated. The median age at the first apheresis was 61 (interquartile range, 53–64) years old, and 18 (54.5%) patients were male. Bor-HDCY was performed in 15 patients, and HDCY was performed in 18. In the patients who underwent Bor-HDCY, the CD34+ cell count at the first apheresis was significantly higher than in the others (P<0.01), and the total CD34+ cell count also tended to be high (P=0.0933). In terms of apheresis days, two-thirds of the patients who underwent HDCY had two-day apheresis, whereas most who underwent Bor-HDCY had one-day apheresis. According to univariate analysis, Bor-HDCY (P<0.01), VRd (Bor, lenalidomide, and dexamethasone) as induction therapy (P=0.0529), and ≥VGPR before PBSCH (P=0.0767) were factors associated with a higher CD34+ cell count at first apheresis. Although multivariate analysis showed that there were no independently significant factors influencing the CD34+ cell count at the first apheresis, the stepwise selection method revealed that only the Bor-HDCY regimen remained in the final model (P<0.005). Bor-HDCY may be a useful conditioning regimen for increasing the CD34+ cell yield.

Keywords: bortezomib, high-dose cyclophosphamide therapy, conditioning regimen, autologous peripheral blood stem cell harvest, multiple myeloma
PATIENTS AND METHODS

Study design

This is a retrospective analysis to evaluate the number of CD34+ cells in PBSCH yields and adverse events in Bor-HDCY therapy compared to HDCY therapy, and we explored factors that influence the number of CD34+ cells. This study was approved by the Asahi General Hospital ethics committee and the in-hospital ethical review board prior to the initiation of the study.

Patients

All consecutive patients with MM and related diseases who underwent PBSCH at our hospital from October 2006 to December 2020 were included.

The conditioning regimen of PBSCH, CD34+ cell mobilization and harvesting

In the HDCY regimen, cyclophosphamide 2.0 g/m2 was administered on days 1 and 2; subcutaneous injection of lenograstim 10 µg/kg to mobilize peripheral blood stem cells was performed from days 13 to 16; and PBSCH was usually performed on day 17. In the Bor-HDCY regimen, Bor 1.3 mg/m2 was subcutaneously injected on days 1, 4, 8, and 11; cyclophosphamide 1.5 g/m2 was administered on days 8 and 9; subcutaneous injection of lenograstim 10 µg/kg was performed from days 21 to 24; and PBSCH was usually performed on day 25. Regarding the criterion of plerixafor usage, plerixafor was used in patients with a history of poor mobilization and who had received many regimens before PBSCH. In clinical practice, however, using plerixafor was left to the discretion of each physician. PBSCH was performed with a COBE Spectra® (Terumo BCT, Tokyo, Japan) from November 2006 to March 2013 and with a Spectra Optia® (Terumo BCT) from March 2013 until the study’s end. The total apheresis volume was set at either “3 times the total blood volume” or “200 ml per patient body weight”, whichever was smaller. Blood access was generally via a central venous catheter in most patients.

Statistics

To compare related factors of the HDCY and Bor-HDCY regimens, Fisher’s exact probability test was used for the nominal variables, and Welch’s test was used for the continuous variables. As a univariate analysis, Welch’s test was used to compare the differences between the CD34+ cell count at the first apheresis according to each factor. The following factors were included in the analysis: sex, male; age at PBSCH, >60 years old; depth of response before PBSCH, very good partial remission (VGPR) or better; plerixafor administration, yes; conditioning regimen, Bor-HDCY; ≥2 regimens until PBSCH; induction therapy, VRd (Bor, lenalidomide, and dexamethasone); and apheresis modality, Optia®. Factors with P <0.1 in the univariate analysis, plerixafor administration, and using Optia® were included in the multivariate regression analysis with the stepwise selection method using p-value.

P-values of <0.05 were considered to indicate statistical significance. All statistical analyses were performed using EZR (Saitama Medical Centre, Jichi Medical University, Japan; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html; Kanda, 2012), which is a graphical user interface for the R software program (The R Foundation for Statistical Computing, Vienna, Austria; version 2.13.0).7

RESULTS

Patients’ characteristics and apheresis results

A total of 33 patients underwent PBSCH. Two patients underwent a second course of PBSCH. The median age of the patients at PBSCH was 61 (interquartile range, 53–64) years old, and 18 (54.5%) patients were male. There were 31 patients with MM, 1 with AL amyloidosis, and 1 with POEMS syndrome. Sixteen patients were treated with Bd (Bor and dexamethasone) as the induction therapy, while nine were treated with VRd. Twenty-three patients underwent one regimen until PBSCH, five underwent two regimens, and five underwent three or more regimens. Sixteen patients achieved VGPR or better before PBSCH; seven achieved partial remission (PR), five had stable disease, three had progressive disease (including two with new mass lesions appearing, although the amount of M-protein was decreased), and the outcome was unknown in two patients. As the conditioning regimen for PBSCH, 18 patients underwent the HDCY regimen, and 15 underwent the Bor-HDCY regimen. Plerixafor was used in seven patients.

The median number of the CD34+ cell count at the first apheresis was 2.44 (interquartile range, 0.78–6.20) × 10^6/kg. The median number of the total CD34+ cell count was 4.55 (interquartile range, 2.36–6.91) × 10^6/kg. The number of apheresis required for a course of PBSCH was 1 day in 19 patients, 2 days in 13 patients, and 3 days in 1 patient.

Comparison between the HDCY and Bor-HDCY regimens

A comparison of the patients who underwent the Bor-HDCY and HDCY regimens is shown in Table 1. There were significant differences in the induction therapy, the timing of PBSCH, the apheresis modality, the use of Plerixafor, and the depth of response before PBSCH (P<0.0001, P<0.0001, P<0.01, P<0.05, and P<0.01, respectively). In the patients who underwent the Bor-HDCY regimen, the CD34+ cell count at the first apheresis was significantly higher than in the patients who underwent the HDCY regimen (P<0.01) (Fig. 1a), and the total CD34+ cell count also tended to be high (P=0.0933). In terms of apheresis days, two-thirds of the patients who underwent the HDCY regimen had one-day apheresis, whereas most patients who underwent the Bor-HDCY regimen had one-day apheresis. Among the 26 patients not using plerixafor, the 26 patients using Optia®, and the 16 patients who achieved VGPR or better before PBSCH, the CD34+ cell count at the first apheresis in those...
who received the Bor-HDCY regimen was significantly higher than in those who received the HDCY regimen (P<0.01, P<0.01, and P<0.05, respectively) (Fig. 1b-d), while among the 17 patients who did not achieve VGPR before PBSCH, the CD34+ cell count at the first apheresis was not significantly different between those who received the Bor-HDCY regimen and those who received the HDCY regimen (P=0.379) (Fig. 1e).

**Factors influencing the increase in the CD34+ cell count**

The results of the univariate analysis using Welch’s test are shown in Table 2. In the patients who were treated with VRd as induction therapy and those who achieved VGPR or better before PBSCH, the CD34+ cell count at the first apheresis tended to be higher than in the others (P=0.0529 and P=0.0767, respectively). In the patients who received the Bor-HDCY regimen, the CD34+ cell count at the first apheresis was significantly higher than in the others (P<0.01). Although multivariate regression analysis including these three factors, Optia® usage, and plerixafor administration showed that there were no independently significant factors influencing the CD34+ cell count at the first apheresis, the

### Table 1. Comparison of the HDCY and Bor-HDCY regimens

|                      | HDCY (n=18)          | Bor-HDCY (n=15)         | p-value |
|----------------------|----------------------|-------------------------|---------|
| Age at PBSCH (years) | 60.5 (IQR, 49.3–63.5)| 62.0 (IQR, 56.5–66.0)  | 0.151   |
| Male/female          | 9/9                  | 9/6                     | 0.729   |
| Induction therapy    |                      |                         |         |
| Bd                   | 12                   | 4                       | <0.0001 |
| VRd                  | 0                    | 9                       |         |
| KrD                  | 0                    | 1                       |         |
| VAD                  | 4                    | 0                       |         |
| MP                   | 1                    | 1                       |         |
| HD-Dex               | 1                    | 0                       |         |
| Timing of PBSCH      |                      |                         |         |
| 2006-2010            | 5                    | 0                       | <0.0001 |
| 2011-2015            | 10                   | 0                       |         |
| 2016-2020            | 3                    | 15                      |         |
| Apheresis system     |                      |                         |         |
| COBE®                | 7                    | 0                       | <0.01   |
| Optia®               | 11                   | 15                      |         |
| Regimens until PBSCH |                      |                         |         |
| 1                    | 14                   | 9                       | 0.310   |
| 2                    | 3                    | 2                       |         |
| 3 or more            | 1                    | 4                       |         |
| Plerixafor           |                      |                         |         |
| Yes                  | 1                    | 6                       | <0.05   |
| No                   | 17                   | 9                       |         |
| Depth of response before PBSCH |            |                         |         |
| VGPR or better       | 5                    | 11                      | <0.05   |
| Other                | 13                   | 4                       |         |
| CD34+ cell count at the first apheresis (×10⁶/kg) | 1.37 (IQR, 0.63–3.18) | 5.80 (IQR, 2.85–8.10) | <0.01  |
| Total CD34+ cell count (×10⁶/kg) | 3.37 (IQR, 1.62–54.70) | 5.80 (IQR, 2.88–8.10) | 0.0933 |
| Apheresis days       |                      |                         |         |
| 1                    | 6                    | 13                      | <0.001  |
| 2                    | 12                   | 1                       |         |
| 3                    | 0                    | 1                       |         |

HDCY, high-dose cyclophosphamide; Bor, bortezomib; PBSCH, peripheral blood stem cell harvest; IQR, interquartile range; Bd, bortezomib and dexamethasone; VRd, bortezomib, lenalidomide and dexamethasone; KrD, carfilzomib, lenalidomide and dexamethasone; VAD, vincristine, doxorubicin and dexamethasone; MP, melphalan and prednisolone; HD-Dex, high-dose dexamethasone; VGPR, very good partial response
Fig. 1. Comparison of the CD34+ cell count at the first apheresis between patients who received the Bor-HDCY and HDCY regimens. (a) Among all the patients (n=33). (b) Among the patients who were not using plerixafor (n=26). (c) Among the patients who were using Optia® (n=26). (d) Among the patients who achieved VGPR or better before PBSCH (n=16). (e) Among the patients who did not achieve VGPR before PBSCH (n=17).

Table 2. Results of univariate analysis of the factors influencing the number of CD34+ cells

| Factor                                | CD34+ cells (×10^6/kg) | Standard deviation | p-value |
|---------------------------------------|------------------------|--------------------|---------|
| Sex                                    |                        |                    |         |
| Male                                  | 4.26                   | 4.44               | 0.893   |
| Female                                | 4.05                   | 4.29               |         |
| Age at PBSCH                           |                        |                    |         |
| ≤60 years                             | 5.43                   | 4.51               | 0.160   |
| >60 years                             | 3.24                   | 4.01               |         |
| Induction therapy                     |                        |                    |         |
| VRd                                   | 7.42                   | 5.81               | 0.0529  |
| Other                                 | 2.95                   | 2.88               |         |
| Previous regimens until PBSCH         |                        |                    |         |
| 1 regimen                             | 4.72                   | 4.63               | 0.209   |
| ≥2 regimens                           | 2.89                   | 3.28               |         |
| Depth of response before PBSCH        |                        |                    |         |
| ≥VGPR                                 | 5.58                   | 5.32               | 0.0767  |
| <VGPR                                 | 2.84                   | 2.59               |         |
| Plerixafor                             |                        |                    |         |
| Yes                                   | 6.75                   | 7.43               | 0.293   |
| No                                    | 3.47                   | 2.84               |         |
| Conditioning regimen                  |                        |                    |         |
| Bor-HDCY                              | 6.40                   | 5.04               | <0.01   |
| HDCY                                  | 2.30                   | 2.43               |         |
| Apheresis modality                    |                        |                    |         |
| COBE®                                 | 2.71                   | 2.36               | 0.162   |
| Optia®                                | 4.56                   | 4.69               |         |

PBSCH, peripheral blood stem cell harvest; VRd, bortezomib, lenalidomide and dexamethasone; VGPR, very good partial remission; HDCY, high-dose cyclophosphamide; Bor, bortezomib
stepwise selection method revealed that only the Bor-HDCY regimen remained in the final model (P<0.005) (Table 3).

Adverse events

During the period from the conditioning regimen to PBSCH, 21 patients developed febrile neutropenia and 5 developed other infections, including sepsis in 2 patients, catheter infection in 2 patients, and urinary tract infection in 1 patient. Seventeen patients presented with nausea of grade 3, diarrhea, or loss of appetite. Other adverse events included a drug fever, drug eruption, hemorrhagic cystitis, stomatitis, subclinical tumor lysis syndrome, and redness at the bortezomib injection site. No cases developed peripheral neuropathy (Table 4).

DISCUSSION

Although our study is a retrospective study, this is the first study to statistically show that the Bor-HDCY regimen can yield more CD34+ cells without increasing adverse events than HDCY. In the patients who underwent the Bor-HDCY regimen, the CD34+ cell count at the first apheresis was significantly higher than the patients who underwent the HDCY regimen, and the total CD34+ cell count also tended to be high. Of note, most patients who underwent the Bor-HDCY regimen had one-day apheresis. Because the Bor-HDCY regimen was newly started in September 2016, it cannot be denied that there were differences in the patient background compared to those receiving the HDCY regimen such as the rate of plerixafor usage, the induction therapy, the depth of response before PBSCH, and the apheresis modality. Therefore, we performed some analyses with limited target patients, such as patients who were not using plerixafor, patients who were using Optia®, patients who achieved VGPR or better, and patients who did not achieve VGPR. These analyses showed that among even the patients who were not using plerixafor, who were using Optia®, or who achieved VGPR or better, the Bor-HDCY regimen achieved a significantly higher CD34+ cell count than the HDCY regimen. Although the multivariate regression analysis including five factors (VRd as induction therapy, achievement of VGPR or better, the Bor-HDCY regimen, using plerixafor, and using Optia®) showed that there were no independently significant factors influencing the CD34+ cell count at the first apheresis, the stepwise selection method revealed that only the Bor-HDCY regimen remained in the final model.

We explored factors that influence the CD34+ cell number at the first apheresis, not the total CD34+ cell number. In the 2000s, the target CD34+ cell count was an amount sufficient to perform ASCT twice, as some reports showed the usefulness of tandem transplantation for MM.8,9 However, the superiority of tandem transplantation disappeared with the emergence of effective novel agents for MM,10 and twice the necessary amount for ASCT was no longer needed. Because the target total CD34+ cell count was determined by each attending physician, the analysis of the total CD34+ cell count was considered to be biased.

There are several hypotheses regarding the increase in CD34+ cell yields with the combination of bortezomib based on gene expression and pathway analyses. One is that the

| Table 3. Results of multivariate analysis of the factors influencing the number of CD34+ cells |
|---------------------------------------------------------------|
| **Regression coefficient estimate** | Standard deviation | T-statistic | p-value |
| Bor-HDCY | 2.41 | 2.06 | 1.17 | 0.252 |
| ≥VGPR | 0.73 | 1.69 | 0.44 | 0.661 |
| VRd | 2.12 | 2.18 | 0.97 | 0.339 |
| Plerixafor | 1.28 | 1.87 | 0.68 | 0.499 |
| Using Optia® | -0.93 | 1.95 | -0.48 | 0.635 |
| Final model of the stepwise selection method using p-value |
| Bor-HDCY | 4.10 | 1.34 | 3.06 | <0.005 |

Bor-HDCY, bortezomib and high-dose cyclophosphamide; VGPR, very good partial remission; VRd, bortezomib, lenalidomide and dexamethasone

| Table 4. Adverse events of the HDCY and Bor-HDCY regimens |
|---------------------------------------------------------------|
| **Infections** | **Febrile neutropenia** | **Other infections** | **Gastrointestinal symptoms** | **Other adverse events** |
| HDCY | 13 | 11 | 2 | 8 | 3 |
| Bor-HDCY | 13 | 10 | 3 | 9 | 3 |

HDCY, high-dose cyclophosphamide; Bor, bortezomib
Bor-modulated expression of the ephrin receptor and its ligands has potential importance in CD34+ cell migration from bone marrow. Another is that Bor decreases the expression of VLA-4, an adhesion factor of hematopoietic stem cells, and increases the induction of hematopoietic stem cells into peripheral blood when used in combination with G-CSF preparations.11

VGPR or better before PBSCH and VRd as induction therapy were other factors which tended to influence an increased CD34+ cell count in the univariate analysis. One of the factors related to the risk of mobilization failure is bone marrow infiltration by primary disease at mobilization.12 It is understandable that deeper remission of MM would lead to an increase in CD34+ cells. VRd is one of the most effective induction therapies, leading to deeper remission than other induction therapies. In our study, plerixafor administration was not a significant influential factor, even in the univariate analysis. This is because the number of patients who were administered plerixafor was small, and plerixafor was used only in patients where mobilization difficulty was predicted. In contrast, the Bor-HDCY regimen was introduced in September 2016 and has been used in all patients since June 2017.

Common adverse events in our study were infectious diseases, such as febrile neutropenia, and gastrointestinal symptoms such as nausea and diarrhea. Regarding the frequency of adverse events, there was no significant difference between the Bor-HDCY and the HDCY regimens. No adverse events of peripheral neuropathy were observed. This may be because Bor was administered via subcutaneous injection in all patients and had already been administered as induction therapy in most patients. The total CY dose in the Bor-HDCY regimen was reduced from 4 g/m² in the original HDCY regimen to 3 g/m². Regarding the CY dose in the conditioning regimen for PBSCH, it has been reported that a low dose (2 g/m²) proved to be as effective as an intermediate-high dose (≥3 g/m²) for stem cell mobilization, suggesting that de-escalation of the CY dose is reasonable, given that CY is used solely for stem cell mobilization and not for its antimielyoma activity.13 In recent years, there have been many patients who achieved sufficiently deep remission before PBSCH because of advances in myeloma treatment. However, given that adverse events were still observed in our study, the cyclophosphamide dose of the Bor-HDCY regimen may be able to be further reduced.

The limitations of our study are that it is a retrospective study with a limited number of patients. In our hospital, the Bor-HDCY regimen was introduced in September 2016 and has been used in all patients since June 2017. Because our study was retrospective, the existence of “chronological effects” due to advances in myeloma treatment and stem cell collection cannot be denied. Because of the use of VRd as induction therapy (approved from December 2015 in Japan) and carfilzomib (approved from August 2016 in Japan), the number of patients who achieved VGPR or better before PBSCH was increased. New procedures to increase CD34+ cell yields have become available such as Optia (from March 2013 in our hospital) and plerixafor (approved from February 2017 in Japan). We performed this analysis as carefully as possible, excluding these factors, but there are limits. Large-scale prospective studies in a uniform population are desired. In addition, we did not analyze the therapeutic effects of the HDCY and Bor-HDCY regimens. This is because there was a large difference in the treatment effect before PBSCH, and in most patients who have undergone PBSCH in recent years, ASCT has been performed without a treatment evaluation for the conditioning regimen for PBSCH, as VGPR or better has been achieved before PBSCH.

In conclusion, our study suggests that Bor-HDCY therapy is a useful conditioning regimen for increasing the number of CD34+ cells collected in clinical practice. Large-scale prospective studies in a uniform population are desired.

FUNDING

This study received no funding.

CONFLICT OF INTEREST

The authors declare no conflicts of interest in association with the present study.

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