**Response to reviewers’ comments**

**Manuscript ID:** BMT-2019-900R

**Title:** Comparison of HLA-matched sibling and unrelated donor transplantation in adult patients with acquired severe aplastic anemia

Dear Editor,

We appreciate the editor and reviewers of “*Bone Marrow Transplantation*” for their time spent in reviewing our manuscript. We have made some corrections and clarifications regarding the following points in the revised manuscript according to the reviewers’ comments. We are pleased that you are interested in our paper and hope that the revised manuscript will be suitable for publication in your journal. Again, we thank you for your constructive comments.

\*The numbers in the parentheses indicate the column in the marked-up version of the revised manuscript.

Sincerely,

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**Reviewer #1**

Q1. Considering relatively high chronic GVHD and TRM incidences, I suspect that allogeneic SCT from WM-URD may be a reasonable option for not older (≥ 40 years old), but younger (< 40 years old) patients.

A1. When we performed subgroup analysis of the WM-URD group, no significant differences were observed in acute GVHD incidence (42.6% vs. 33.3% at day 100; *P* = 0.42), mild-to-moderate (42.6% vs. 47.2% at 6 years; *P* = 0.82), moderate-to-severe (31.5% vs. 27.8% at 6 years; P = 0.85), and severe chronic GVHD (13.0% vs. 11.1% at 6 years; *P* = 0.80) incidences; GF incidence (0% at 6 years in both); TRM incidence (9.9% vs. 11.1% at 6 years; *P* = 0.91); GFFS rate (59.3% vs. 61.1% at 6 years; *P* = 0.89); and OS rate (90.1% vs. 88.9% at 6 years; *P* = 0.91) between patients aged <40 years (n = 54) and those aged ≥40 years (n = 18). However, we agree with the reviewer’s opinion that the candidates of URD-SCT as the first-line treatment should be selected with caution. Hence, we added the following sentence in the Discussion section: “Until more clinical evidences are available, URD-SCT as the first-line treatment for SAA should be recommended in younger patients (<40 years old).” (# 363–364).

**Reviewer #2**

Q1. The authors should mention of a recently published study of “Vaht K et al. Biol Blood Marrow Transplant. 2019 Oct;25(10):1970-74”.

A1. In accordance with the reviewers’ comments, we mentioned two studies that provided a comparison of outcomes of adult SAA patients who received allogeneic MSD-SCT and URD-SCT in our manuscript:

a. Introduction section: “… except the two recently published retrospective studies [[14](#_ENREF_14), [15](#_ENREF_15)]” (#74)

b. Discussion section: “Only two recently published retrospective studies by Vaht et al. and Zhang et al. compared the OS rates of patients who received MSD-SCT and URD-SCT, which provided results similar to that of the current study (90.6% vs 83.3% at 5 years; *P* = 0.41 and 89.3% vs. 82.0% at 5 years; *P* = 0.40, respectively) [14, 15]. However, as these studies analyzed an extremely small sample of patients who received or did not receive heterogeneous transplant, they had substantial difficulty in drawing confirmatory results (#326–331).

Q2. The sentences in the ‘Abstract’ section could be improved. In addition, several bulky contents in manuscript should be shortened.

A2. We revised the Abstract and removed the unnecessary sentences to shorten the content of the manuscript.

a. The ‘Abstract’ was revised as follows: “The recent improvements in the outcomes of severe aplastic anemia (SAA) patients who received allogeneic stem cell transplantation (SCT) from unrelated donors (URD) suggest the possibility of its alternative first-line treatment. … Our study showed comparable OS between the MSD group and WM-URD group, which suggest that the URD-SCT can be used as a first-line treatment for adult SAA patients with WM-URD” (#29–42).

b. Results section:

‘~~The etiologies of the disease were categorized as follows: idiopathic in 234 (90.7%), pregnancy-associated in 9 (3.5%), hepatitis-related in 7 (2.7%), drug-induced in 5 (1.9%), and others in 3 (1.2%) patients.~~’ (#161–163)

‘~~Neutrophil engraftment incidences of the MSD, the WM-URD, and the PM-URD groups were 99.3% (95% confidence interval [CI], 95.4–99.9), 100%, and 97.0% (95% CI, 59.8–99.8) at day 28, respectively (~~*~~P~~* ~~= 0.79).’~~ (#181–183)

‘~~Platelet engraftment incidences in the MSD, the WM-URD, and the PM-URD groups were 88.0% (95% CI, 81.5–92.3), 81.9% (95% CI, 70.7–89.2), and 75.8% (95% CI, 56.4–87.4) at day 28, respectively (~~*~~P~~* ~~= 0.48).~~’ (#185–187)

c. Discussion section:

‘~~They also compared the outcomes of those who received IST and URD-SCT as a first-line treatment, which showed a significantly higher event-free survival rate of the latter group than that of the former group (40% vs. 92% at 2 years;~~ *~~P~~* ~~< 0.01).~~’ (#315–317)

‘~~A large European Group for Blood and Marrow Transplantation registry-based study by Bacigalupo et al. showed insignificantly different OS rates between the MSD and the URD groups (HR 1.20; 95% CI 0.93–1.55;~~ *~~P~~* ~~= 0.16) [~~[~~23~~](#_ENREF_23)~~]. However, this study has difficulty in representing the comparable OS rates of adult SAA patients who received MSD-SCT and URD-SCT, considering the proportion of patients aged of ≤ 20 years approaching to approximately 50%.~~’ (#321–326)

**Reviewer #3**

Q1. It is noticeable that this issue had been provided by one study published recently, which compared the outcomes of adult SAA patients who underwent upfront URD-SCT or MSD-SCT and concluded that URD-SCT may be an effective and feasible option for first-line treatment in adult SAA patients without an appropriate MSD. (Zhang Y, et al. Biol Blood Marrow Transplant. 2019 Aug; 25(8):1567-75.). The authors should take into account and compare the previous investigation and the current study in the ‘Discussion’ section.

A1. In accordance with the reviewers’ comments, we mentioned two studies that provided a comparison of the outcomes of adult SAA patients who received allogeneic MSD-SCT and URD-SCT in our manuscripts:

a. Introduction section: “…except two recently published retrospective studies [[14](#_ENREF_14), [15](#_ENREF_15)] (# 74)

b. Discussion section: “Only two recently published retrospective studies by Vaht et al. and Zhang et al. compared the OS rates of patients who received MSD-SCT and URD-SCT, which provided results similar to that of the current study (90.6% vs 83.3% at 5 years; *P* = 0.41 and 89.3% vs. 82.0% at 5 years; *P* = 0.40, respectively) [14, 15]. However, because these studies analyzed an extremely small sample of patients who received or who did not received heterogeneous transplant, they had substantial difficulty in drawing confirmatory results” (#326–331).

Q2. The authors mentioned that low-dose rabbit ATG (1.25 mg/kg intravenously for 2 days) has been administered to patients who URD-SCT since August 2009. The GVHD incidences of the URD groups were relatively higher compared with those of published similar studies, which may be relevant to the inadequate rabbit ATG dose. The authors should discuss the available literatures on the association between ATG dose and high GVHD incidences.

A2. Because we did not know the optimal dose of rabbit ATG for SAA patients with Korean ethnicity, who received URD-SCT. The conditioning protocols of our institute have recommended the gradual increase in the ATG dose, considering the risk-benefit ratio. Bryant et al. reported significantly lower chronic GVHD incidence with significant GFFS improvement in patients who received URD-SCT with low-dose rabbit ATG (2.5 mg/kg), which was the same dose used in our study, compared with those who received MSD-SCT without rabbit ATG (Biol Blood Marrow Transplant. 2017; 23(12): 2096-101). Several studies conducted in Asian groups reported that low-dose ATG (1.5–2.5 mg/kg) could significantly decrease GVHD incidence, suggesting that the optimal ATG dose for Asian patients might be different from that for Caucasian patients (Park et al, Biol Blood Marrow Transplant 2017; 23: 1498-1508, and Shishido et al, Bone Marrow Transplant 2018; 53: 634-639).

Since Aug 2009, we commenced the administration of low-dose rabbit ATG (1.25 mg/kg intravenously for 2 days) to SAA patients receiving URD-SCT from HLA-mismatched donor and/or PB stem cells; results showed that the incidence rates of mild-to-severe, moderate-to-severe, and severe chronic GVHD in patients treated with low-dose rabbit ATG significantly decreased from 53.8% to 28.2% (*P* = 0.01), 44.4% to 13.7% (*P* < 0.01), and 20.4% to 3.9% (*P* = 0.01), respectively, compared with those who received transplantation without rabbit ATG before July 2009. These findings were already described in the “Discussion” section: “Our recently published report for adult SAA patients who received URD-SCT using PM-URD or PB stem cells may provide a possible solution for this issue [[16](#_ENREF_16)]. It showed significantly lower acute grades (II–IV) and chronic GVHD incidences (31.2% vs. 61.5% at day 100; *P* < 0.01 and 21.9% vs. 65.4% at 3 years; *P* < 0.01, respectively) in patients who received low-dose ATG (2.5 mg/kg) compared to patients who did not receive low-dose ATG” (#344-348). Based on these results, we amended the conditioning protocols in December 2016, then a relatively high-dose rabbit ATG (2.5 mg/kg for 2 days) has been administered to SAA patients who received URD-SCT. It was also described in the “Patients and Methods” section as follows: ‘Thereafter, the protocol was amended so that all patients, who have received URD-SCT since December 2016, received rabbit ATG (2.5 mg/kg for 2 days).’ (#109–110).

Q3. The significance of two time-periods of Mar 2002 to July 2009 and Aug 2009 to May 2018, needs to be mentioned either here or in the ‘Discussion’ section, if it is relevant.

A3. In accordance with the reviewer’s comment, we analyzed the outcomes of patients who received allogeneic SCT between March 2002 to July 2009 and Aug 2009 to May 2018. However, no significant differences were observed between the two groups, including GF incidence (6.8% vs. 9.9% at 6 years; *P* = 0.29), TRM incidence (11.7% vs. 7.5% at 6 years; *P* = 0.46), GFFS rate (61.4% vs. 73.8% at 6 years; *P* = 0.09), and OS rate (88.3% vs. 89.1% at 6 years; *P* = 0.45).

Q4. Currently recommended TBI dose for SAA patients who receive URD-SCT is 200–300 cGy. In this study, the conditioning regimen for the URD group consists of 400–800 cGy TBI, which might raise the secondary malignancies incidence in the long run. Besides, the authors mentioned "secondary malignancies in 4 (1.6%)" (#219). Whether is the secondary malignancies of those patients associated with relatively high TBI dose should be commented on.

A4. Of four (1.6%) patients who died of secondary malignancies, two (50.0%) (acute myeloid leukemia and myelodysplastic syndrome, respectively) belonged to the MSD group who did not receive TBI-based conditioning. Following URD-SCT, two (50.0%) patients (esophageal cancer and cervical cancer, respectively) died of secondary malignancies following URD-SCT. A large retrospective analysis (Curtis et al. N Engl J Med. 1997; 336: 897-904) reported that the incidence rates of secondary malignancies increased only if patients received ≥ 1,400 cGy TBI. Therefore, these results suggest that the administration of 400–800 cGy TBI may not increase the risk of secondary malignancy. However, as secondary malignancies associated with high TBI dose still occur, we modified the conditioning protocols by reducing the TBI dose from 800 cGy to 600 cGy, and then from 600 cGy to 400 cGy.

Q5. There are published literatures of GVHD prophylactic approaches. This needs to be a stronger point.

A5. In accordance with the reviewer’s comment, we added the following phrase in the “Discussion” section and cited the relevant reference: “…emerging prophylactic approaches of various action mechanisms, including T-cell depletion, functional inhibition of donor T-cell activation, inhibition of signals mediated by extracellular mediators, and B-cell depletion [35]” (#349–351).

Q6. In Table S1, 18 patients received URD-SCT as first-line treatment without preceding ATG-based IST. However, disease courses were longer than 12 months in 15 out of 18 (83.3%) patients. Whether did these 15 patients received CsA monotherapy or supportive cares before transplantation? If they previously received CsA monotherapy, URD-SCT also should be recognized as second-line treatment. Therefore, the authors should define the standard of the first-line URD transplantation in ‘Methods’ section.

A6. We added the following sentences in the “Methods” section accordance with the reviewer’s comment: “In addition, allogeneic SCT as a first-line treatment is only indicated in patients who did not previously receive IST, including horse or rabbit ATG plus CsA or CsA monotherapy” (#126–128). The long interval from diagnosis to transplantation was mainly observed in patients who were categorized as having moderate severity at diagnosis and then experiencing progression to severe disease. In our institution, CsA monotherapy has been only used in patients with transfusion-dependent disease of moderate severity or older age and/or comorbidities who are not indicated or ineligible for ATG-based IST and allogeneic SCT.

Q7. I do not understand how the GF incidences of 15.9% vs. 0% (*P* = 0.06) between the MSD and the URD groups were not significantly different. The statistics for this should be reviewed. Besides, I think that the secondary GF incidence in the MSD group of 15.9% is too high.

A7. The insignificance of the GF incidence of the MSD and the URD groups was observed in a limited number of patients from both groups. Our statistician reviewed the statistic results with raw data and confirmed our results were correct. In addition, we already mentioned the following limitation in the “Discussion” section: “only a limited number of patients received allogeneic SCT as a first-line treatment, especially in the URD groups.” (#360–361). In addition, a relatively high secondary GF incidence was also mentioned in the “Discussion” section: “A substantially high secondary GF incidence only in the MSD group may be an additional limitation in our study. The GF incidence rates reported in previously published studies using Flu-based conditioning were low (from 0% to 13.9%) [[27-29](#_ENREF_27)], supporting the hypothesis that the high secondary GF incidence in the MSD group was not only due to the low intensity of the conditioning regimen. … Consequently, it suggests that a Flu-based conditioning might be insufficient to overcome the possible occurrence of GF in these high-risk patients of the MSD-SCT group.” (#294–302).

Q8. Retrospective nature should be more emphasized.

A8. We edited the following relevant sentences in the “Discussion’ section”: “However, this is a retrospective study with an unbalanced distribution of clinical and transplant-related characteristics of the donor-type groups, although it is an unavoidable feature of a real-world description. In addition, only a limited number of patients received allogeneic SCT as a first-line treatment, especially in the URD groups. Hence, our results should be interpreted with caution. Until more clinical evidences are available, URD-SCT as the first-line treatment for SAA should be recommended in younger patients (<40 years old). Therefore, future well-designed prospective studies are warranted to confirm our results (#359-365).