**Response to reviewers’ comments**

**Manuscript ID:** EJH-2020-0055

**Title:** Nonmyeloablative matched stem cell transplantation with the optional reinforced stem cell infusion for hemoglobinopathies

Dear Editor,

We appreciate the editor and reviewers of “*European journal of Haematology*” for their effort and time 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.

Sincerely,

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**Reviewer #1**

Q1. The non-myeloablative conditioning of NIH consisted of 300 cGy total-body irradiation. Please, clarify that why did you employ a range of 300 or 400 cGy total body-irradiation? In addition, patients who received the optional reinforced stem cell infusion exposed to an additional 500 cGy total nodal irradiation. The cumulative toxicity of total-body or total-nodal irradiation, including hypothyroidism, hepatotoxicity, and cardiomyopathy, should be mentioned.

A1. Although the non-myeloablative conditioning of NIH consisted of 300 cGy total-body irradiation, we adopted 300 or 400 cGy in our study. Because 400 cGy total-body irradiation in most conditioning regimens for various hematologic disease was preferred in our transplantation center, we admixed 300 and 400 cGy total-body irradiation. On the other hand, currently available literatures of total body irradiation toxicity suggested the dose over 1200 cGy was associated with increased late sequels incidence, including neurologic complication, renal complication and secondary malignancies (Crit Rev Oncol Hematol. 2018 Mar;123:138-148). In addition, total-nodal irradiation was generally accepted as having less toxicity than total-body irradiation. Due to relatively short duration of follow-up, late complications followed by irradiation were not observed.

Q2. Table 1 should provide additional information: e.g. iron-chelating agents with serum ferritin level for β-thalassemia major patients and hydroxyurea for sick cell anemia patients.

A2. According to your comment, we have added corresponding information for β-thalassemia major and sickle cell anemia patients in Table 1.

Q3. Gene therapy is another curative therapy for β-thalassemia major and sickle disease. Therefore, the ‘only’ in sentence of ‘Allogeneic stem cell transplantation remains the only curative treatment option ~ hematologic disorders.’ (Line # ##) should be corrected.

A3. According to your comment, we have removed the ‘only’ in the corresponding sentence (Line # ##).

Q4. You should mention that graft was unmanipulated in patients receiving the optional reinforced stem cell infusion.

A4. We have specified that initial and the optional reinforced stem cells were infused without manipulation as follows: ‘, and then administered to patients without additional manipulation.’ (Line # ##) and ‘patients received the optional reinforced unmanipulated SC infusion from the same donor after total-nodal irradiation (TNI) at a single dose of 500 cGy.’ (Line # ##)

Q5. Please, clarify whether the same donor or another donor was employed in your report, when patients received the optional reinforced stem cell infusion.

A5. We employed the same donor of initial peripheral blood stem cell infusion, when patients received the optional reinforced stem cell infusion, which was added in manuscript as follows: ‘patients received the optional reinforced unmanipulated SC infusion from the same donor after total-nodal irradiation (TNI) at a single dose of 500 cGy.’ (Line # ##).

Q6. Would you like to describe the detailed parameters for packed red cell transfusion of patients in your cohort?

A6. According to your comment, we have added corresponding sentences as follows: ‘We have tried to adhere to the US and Thalassemia International Federation guidelines, Standards of care guidelines for thalassemia and Guidelines for the clinical management of thalassemia [4-6]. According to these guidelines, we have attempted to maintain target pre-transfusion hemoglobin level was between 9 and 10.5 g/dL through transfusing packed red cell every 2-5 weeks. Furthermore, if patients have cardiac dysfunction, they received packed red cell transfusion with a higher hemoglobin target level of 10-12 g/dL. We have attempted to maintain post-transfusion threshold for hemoglobin level of 14 g/dL or less with a mean level of 12 g/dL.’ (Line # ##)

Q7. Did receive sickle cell disease patients in your cohort routine penicillin prophylaxis?

A7. Sickle cell disease patients in our cohort did not receive routine penicillin prophylaxis because of they were more than 18 years old.

Q8. Peripheral blood donor T-cell chimerism at post-transplant day 30 may be not meaningful with your NMA conditioning. Would you consider removing corresponding sentences?

A8. According to your comment, we have removed the sentences regarding to peripheral blood donor T-cell chimerism at post-transplantation day 30.

**Reviewer #2**

Q1. Please, correct the discordant number of patients experiencing graft-versus-host disease in the ‘Results’ section and Figure 1, and Table 2.

A1. In the Table 2, we have corrected the mistake of GVHD column of UPN #04, who was hospitalized due not to graft-versus-host disease, but non-specific colitis.

Q2. The result that graft-versus-host disease occurred in three of 5 patients who received the optional reinforced infusion after post-transplant one year, whereas two patients who received the procedure before post-transplant 6 months did not should be emphasized.

A2. We entirely agree to your opinion, corresponding sentences with relevant references were added to the manuscript as follows: ‘Previous reports showed that incorporating alemtuzumab to the conditioning for depleting donor T cells contributed to reduce the GVHD incidence in the setting of alloSCT using NMA conditioning [22,23]. Three patients, in our cohort, who received the optional reinforced infusion due to impeding graft failure in early post-transplant period did not experience GVHD at all, whereas two of those who received the procedure due to declined PB donor T-cell chimerism after post-transplant one year experienced severe acute or chronic GVHD. It suggests that T-cell depletion by alemtuzumab could contribute to prevent GVHD in patients who received the optional reinforced infusion not after post-transplant one year, but in early post-transplant period, which might be resulted from gradually attenuated effect over time of alemtuzumab.’ (Line # ##)

Q3. The paradigm of which peripheral blood donor T cell chimerism > 50% should be maintained before tapering immunosuppressive agent to avoid graft rejection should be confirmed by larger studies. In fact, several adult patients, with sickle cell disease, of NIH study discontinued sirolimus earlier than required and did not experience graft rejection with sustained peripheral blood donor T-cell chimerism after post-transplant one year. It should be addressed.

A3. We have added corresponding sentences with relevant references as follows: ‘although the paradigm of which peripheral blood donor T-cell chimerism > 50% should be maintained before tapering immuno-suppressive agent to avoid graft rejection should be confirmed by larger studies.’ (Line # ##) and ‘In addition, several adult SCD patients of the NIH study discontinued sirolimus earlier than required and did not experience graft rejection with sustained PB donor T-cell chimerism after post-transplant one year [2].’ (Line # ##)

Q4. Authors should discuss that these data suggest that the optional reinforced stem cell infusion should be only performed for patients with impending graft failure during early post-transplant period because those who received the procedure after post-transplant one year had high risk of graft-versus-host disease, but low risk of graft rejection.

Q4. According to your opinion, we inserted corresponding sentences as follows: ‘Lastly, although our strategy was effective in preventing patients from receiving long-term immunosuppression, there was on recorded fatality by developing of acute GVHD after the optional reinforcedSC infusion. Since infused number of CD3+ cells appear to be associated with these complications, further studies are needed to determine the optimal cell dose of the optional reinforced SC infusion to minimize the risk of GVHD without sacrificing donor engraftment, especially in patients who received the procedure after post-transplant one year. In addition, considering risk/benefit of our strategy, the optional reinforced SC infusion should be also preferentially performed to patients with rapid decline of PB donor WB chimerism during the early post-transplant period.’ (Line # ##)