**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.

\*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. The non-myeloablative conditioning of NIH consisted of 300 cGy total-body irradiation. Please, clarify that why did you employ a range of 300–400 cGy total body irradiation? In addition, patients who received the optional reinforced stem cell infusion exposed to an additional dose of 500 cGy. The cumulative toxicities 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 employed 300–400 cGy. Because 400 cGy total body irradiation in many conditioning regimens of 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 toxicities suggested the dose over 1200 cGy was associated with increased incidence of late sequeles, 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 toxicities than total body irradiation. Considering these findings, only small proportion of patients in our cohort experienced late complications followed by total body and total nodal irradiation. We have added corresponding sentences as follows:

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

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

Q3. Gene therapy is another curative therapy for thalassemia and sickle disease. Therefore, the ‘only’ in sentence of ‘Allogeneic stem cell transplantation remains the only curative treatment option ~ hematologic disorders.’ (Page 3, Line 49) should be corrected.

A3. According to your comment, we have removed the ‘only’ in the corresponding sentence (Page 3; Line # 46).

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

A4. We have specified that initial and reinforced stem cells were infused without manipulation as follows: ‘, and then administered to patients without manipulation.’ (Page 4; Line # 92–93) 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.’ (Page 5; Line #99-100)

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 as follows: ‘patients received the optional reinforced unmanipulated SC from the same donors after total-nodal irradiation (TNI) at a single dose of 500 cGy.’ (Page 5; Line # 99).

Q6. Please, describe the detailed parameters for packed red cell transfusional support of patients in your cohort.

A6. According to your comment, we have added following sentences as follows: ‘We have tried to adhere to the US and Thalassemia International Federation guidelines and Guidelines for the clinical management of thalassemia. Briefly, 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 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.’ (Page ; 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 of peripheral blood donor T-cell chimerism at post-transplant 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 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 incidence of acute and chronic GVHD in the setting of alloSCT using NMA conditioning [19,20]. Three patients, in our cohort, who received the optional reinforced infusion due to impeding graft failure in early post-transplant period did not experience acute and chronic 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.’ (Page 13; Line # 323-331).

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.’ (Page 12; Line #295-297) and ‘In addition, several adult SCD patients of the 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 [2]’ (Page 13; Line # 317-319).

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

Q4. According to your opinion, we inserted corresponding sentences as follows: ‘The above-mentioned results make us to consider that the optional reinforced SC infusion should be preferentially performed to patients with rapid decline of PB donor WB chimerism during the early post-transplant period.’ (Page 14; Line #337-339) and ‘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 restrictively performed to patients with rapid decline of PB donor WB chimerism during the early post-transplant period.’ (Page 15; Line #368-371).