**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 why you employed a range of 300 or 400 cGy total body-irradiation and why patients who received the optional reinforced stem cell infusion were 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. Currently available literature on total body irradiation toxicity suggested that doses over 1200 cGy were associated with increased late sequelae incidence, including neurologic complications, renal complications and secondary malignancies (Crit Rev Oncol Hematol. 2018 Mar;123:138-148). In addition, total-nodal irradiation is generally accepted as having less toxicity than total-body irradiation. Due to the relatively short duration of follow-up, late complications from by irradiation were not observed in the patients of our cohort.

Q2. Table 1 should provide additional information (e.g. iron-chelating agents with serum ferritin levels for β-thalassemia major patients and hydroxyurea for sickle cell anemia patients).

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

Q3. Gene therapy is another curative therapy for β-thalassemia major and sickle disease. Therefore, “only” in sentence of “Allogeneic stem cell transplantation remains the only curative treatment option … hematologic disorders.’ should be corrected.

A3. According to your comment, we removed “only” in the corresponding sentence (Line # 48).

Q4. You should mention that the grafts were unmanipulated in patients receiving optional reinforced stem cell infusions.

A4. We specified that the initial and optional reinforced stem cells were infused without manipulation as follows: “Subsequently, peripheral blood (PB) stem cells (target CD34+ cell dose of 10 × 106/kg) from the MSD were mobilized … and administered to patients without additional manipulation.” (Line # 88 – 90) and “the 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 # 96 – 97).

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

A5. We employed the same donor for an optional reinforced stem cell infusion as for the initial peripheral blood stem cell infusion, which was added in to the manuscript as follows: “the 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 # 96 – 97).

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

A6. According to your comment, we added the corresponding sentences as follows: ‘We 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]. … We attempted to maintain post-transfusion hemoglobin threshold level of 14 g/dL or less with a mean of 12 g/dL.’ (Line # 111 – 117)

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

A7. The sickle cell disease patients in our cohort did not receive routine penicillin prophylaxis because 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 the corresponding sentences?

A8. According to your comment, we removed the sentences regarding 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, Figure 1, and Table 2.

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

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

A2. We entirely agree with your opinion and the corresponding sentences with relevant references were added to the manuscript as follows: ‘Previous reports showed that incorporating alemtuzumab into the conditioning regimen for depleting donor T cells contributed to reducing the GVHD incidence in the setting of alloSCT using NMA conditioning [22,23]. … These results suggest that future optional reinforced SC infusion should be preferentially performed to patients with impending graft failure during the early post-transplant period.” (Line # 337–347)

Q3. The paradigm of which peripheral blood donor T cell chimerism > 50% should be maintained before tapering immunosuppressive agents to avoid graft rejection should be confirmed by larger studies. In fact, several adult patients with sickle cell disease 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. This should be addressed.

A3. We added the corresponding sentences with relevant references as follows: ‘although the paradigm of which PB donor T-cell chimerism > 50% should be maintained before tapering immunosuppressive agent to avoid graft rejection should be confirmed by larger studies.’ (Line # 305 - 307) and ‘Several adult SCD patients in 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 # 328 - 330)

Q4. The authors should discuss that these data suggest that the optional reinforced stem cell infusion should only be performed for patients with impending graft failure during the 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 the corresponding sentences as follows: ‘Lastly, although our strategy was effective in preventing patients from receiving long-term immunosuppression, there was on recorded fatality from the development of acute GVHD after an optional reinforcedSC infusion. Since the number of CD3+ cells appears to be associated with these complications, further studies are needed to determine the optimal cell dose in 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.’ (Line # 370 - 375)