RE: **Manuscript No:** 67832 (WJCO); "***Hematopoietic stem cell mobilization strategies to support high-dose chemotherapy: a focus in relapsed/refractory germ-cell tumors***", by Porfyriou *et al*.

Dear Science Director,

We greatly appreciated the review comments by Reviewer #1 regarding the above manuscript submitted to the *World Journal of Clinical Oncology*. We are glad to let you know that we have made the appropriate amendments according to reviewer's suggestions and resubmit a revised manuscript for consideration in the journal.

We look forward to hearing from you in the near future.

Sincerely,

Eleni Porfyriou, M.D.

Reviewer #1:

General comments: - the paper is mostly focused on a general dissertation on mobilization strategies before autologous stem cell transplantation and only very shortly on this particular topic in the setting of germ-cell tumors. It seems that the main topic is a review of the possible mobilization strategies rather than a specific focus on ASCT mobilization techniques for GCT. I would recommend either expanding the section devoted to GCT or modify the title of the manuscript.

Reply*:** We thank the Reviewer for his constructive and fair criticism. Since our review was proposed to be focused on HSC mobilization strategies regarding relapsed/refractory GCTs, and as pointed-out by the Reviewer, the manuscript is more a general overview of HSC mobilization mechanisms and strategies in general with a relatively short part devoted to GCTs, we have modified the title to reflect this issue as follows: "Hematopoietic stem cell mobilization strategies to support high-dose chemotherapy: a focus in relapsed/refractory germ-cell tumors***".
Moreover, we had already explained in the originally submitted manuscript, in Introduction-page 3; 1st paragraph, that "*Indications, as far as strategies appropriate for achieving adequate CD34+ cell numbers for these patients are limited due to the lack of data and are generally based on standard approaches for HSC mobilization that have been applied in other disease settings. Hence, the establishment of standard mobilization and remobilization techniques for patients with GCTs who failed the initial mobilization protocols should become a high priority.*"

Other comments: The manuscript is a very good review of the present literature, however to some extent lacks novelty. I would recommend better underlying critical issues of the topic, future directions and areas that mostly need further investigations. - the manuscript would benefit from one (or more) summarizing figure(s). I would also recommend adding a table with the recruiting clinical trials addressing new mobilization strategies for GCT or for any indications.
***Reply*:** We agree and added a figure (named Fig. 1) outlining current approaches in use regarding HSC mobilization in GCTs. As at *Clinicaltrials.gov* the studies concerning HSC mobilization agents and strategies do not particularly focus in GCTs and most studies are published and few still recruiting in other disease settings, we preferred to discuss these under *(d) Future novel approaches*, on page 17, we have

added 3 paragraphs concerning novel CXCR4 antagonists applied for HSCs mobilization, currently in NHL & MM, as follows: "*Most novel HSC mobilizing agents developed are initially tested in MM and NHL patients*
candidates for ASCT and their successful application in this setting will allow their further testing in patients with relapsed/refractory GCTs and other solid tumors where HDCT and autografting is an indication at some point during disease course.
As CXCR4 antagonists, like plerixafor, emerged as potent agents to rescue "hard-to-mobilize" patients with MM, NHL, GCTs and some rare solid tumors, research in this area is expanding with the development of novel CXCR4 inhibitors, such as motixafortide [BL-8040; BKT140 (4F-benzoyl-TN14003)] a 14-residue bio-stable synthetic peptide, which binds CXCR4 with much greater affinity compared to plerixafor; i.e. (84 vs. 4 nmol/L). An interim analysis of the phase 3 GENESIS trial of motixafortide vs. placebo, both with G-CSF, for HSC mobilization in MM demonstrated an almost 4.9-fold efficacy in obtaining the primary endpoint of a target of 6.0x10 6 CD34+ cells/kg with up to 2 apheresis sessions and that 5.6-fold more patients achieved that target with one apheresis. Moreover, the motixafortide arm allowed 88.3% of patients to proceed to transplant, as opposed to 10.8% in the placebo arm [(133)]. Another peptide CXCR4 antagonist, clinical stage compound balixafortide (POL6326) was applied in healthy volunteers and proved to be safe, well tolerated, and induced efficient mobilization of HCs at doses ≥1500 μg/kg and predicted to yield an adequate collection of 4x10 6 CD34+ cells/kg in a single apheresis [(134)] .
Another area of interest in HSC mobilization examines the role of the sphingosine-1-phosphate/S1P receptor 1 (S1P/S1P1) axis and studies in mice demonstrated an additional PB HSC mobilization benefit for S1P1 agonist (SEW2871) co-treatment in combination with a CXCR4 antagonist but not human G-CSF [(135)]. However, this approach still remains at an experimental level with no apparent clinical testing so far.", and further down on last paragraph, page 18-top of page 19, we have added: "*A novel mobilization strategy was developed and tested in mice through combined targeting of the chemokine receptor CXCR2 on granulocytes and VLA4 in HSCs demonstrating rapid and synergistic mobilization along with an enhanced recruitment of long-term re-populating HSCs. This was achieved when a CXCR2 agonist (a truncated form of GRO-β; tGRO-β) was administered in conjunction with a VLA4 inhibitor leading to rapid and potent HSC mobilization and represents an exciting potential future strategy for clinical development [(147)]. A G-CSF–free mobilization regimen using a tGRO-β compound, MGTA-145; a CXCR2 agonist, in combination with plerixafor was developed in the context of in vivo HSC transduction as a gene therapy approach in a mouse model of β-thalassemia [(148)]. The MGTA-145+plerixafor combination resulted in robust mobilization of HSCs. Importantly, compared with G-CSF+plerixafor, MGTA-*

145+plerixafor led to significantly less leukocytosis and no elevation of serum interleukin-6 levels and was thus likely to be less toxic [(148)]. However, the above regimen has not been tested for HSCs mobilization in neoplastic diseases so far."

Moreover, under *Conclusion* we have included a new paragraph 2 stating: "*Algorithms to improve the efficiency of HSCs mobilization ("just-in-time" and pre-emptive) by minimizing the number of failures, obtain the desired CD34+ HSCs dose for one or more transplants with the least apheresis sessions, and therefore reduce overall healthcare costs, are urgently required. As novel HSC mobilizing agents are initially tested in pre-clinical experimental models and hematologic malignancies, such as NHL and MM, their application in solid tumors, candidates for ASCT and in particular GCTs, is lagging behind. Moreover, the two axes responsible for HSC retention in the BM stroma explored are the CXCR4-CXCL12 (SDF-1) and the VLA4 (α4/β1)-VCAM1 pathways, and novel inhibitors to these interactions are explored, either alone or in combination with G-CSF, or with GRO-β/CXCR2 axis co-stimulation.*".

Minor comment:- in the Abstract the abbreviation at line 7 HDC should be corrected in HDCT.

Reply: We agree and corrected at that point.