Format for ANSWERING REVIEWERS

June 10, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 19435-review.doc).

Title: Cytomegalovirus reactivation after autologous stem cell transplantation in myeloma and lymphoma patients: a single-center study

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The manuscript has been improved according to the suggestions of reviewers:

Answers to reviewer 00504674 (RED BOLD in the revision version of the MS)

In their manuscript entitled "CYTOMEGALOVIRUS REACTIVATION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MYELOMA AND LYMPHOMA PATIENTS: A SINGLE-CENTER STUDY" Marchesi and his colleagues present a retrospective cohort study of CMV infection in HSCT patients. Congretulations! The authors conducted the outstanding care for CMV infection in HSCT patients. Also, the findings are also interesting. Many statements regarding referenced literature are correct. The manuscript is well-written and clear.

Major issues 1. The important issue is the lack of clarity regarding methodology. The author does not describe specific strategies of prophylaxis and preemptive treatment for CMV infection. Although current internal guidelines do not suggests detailed algorhithms stil due to lack of evidences, each center has its own strategy for those prophylaxis and preemptive strategies. Didn't all patient have antiviral CMV prophylaxis? If so, how long did subjects have? According to your data (median onset of CMV reactivation= 33days), it seems that your center does not use prophylaxis. In addition, preemptive strategy must be clear in the manuscript. Do you start iv ganciclovir or just oral valganciclovir? Or do you just decrease immunsuppressants? How about the cut-off value of CMV viremia to triger antiviral treatment? As far as we know, the cut-off value of triggering CMV preemptive treatment has been established yet. Occasionally it is not practical to start antiviral treatment, when the subject has extremely minimal valude of CMV viremia. If you decrease immunosuppressants to control mild CMV viremia, the likelihood of another complications such as GVHD may be increased and also affect the patient's outcomes.

Answer: all patients were treated with Valaciclovir prophylaxis starting to day of

transplant for 6 months after, as described in the methods section. No specific CMV prophylaxis was adopted. Moreover, we have a well structured algorithm for start anti-CMV treatment (see *Criteria for diagnosis of CMV symptomatic infection and end-organ disease* section of the manuscript), but a pre-emptive strategy (e.g. treatment only based on CMV PCR test) was not adopted. The choose of antiviral agent to use for symptomatic reactivation treatment was based on clinical features of the patients (e.g. Foscarnet for patients with a graft failure). We have clarified this aspect in the revised version of the MS (page 7, line 7 from the bottom). As for the immunosuppressants, this aspect is not applicable to our study, considering that all patients underwent an autologous but not allogeneic hematopoietic stem cell transplantation.

2. When you monitored study subjects, was risk stratification (CMV mismatch between donor and recipients) included in the study? According to literatures about HSCT and SOT, CMV IgG matching play an important role in predicting CMV reactivation (eg. donor CMV IgG positivity is protective). If your study does not include this, at least you must mention this as a limiation.

Answer: this aspect is not applicable to our study, considering that all patients underwent an autologous but not allogeneic hematopoietic stem cell transplantation.

3. The next flaw of the study may be about statistics. First, the study period is controversial. your last follow-up day is January 2015, which means the shortes follow-up period of your cohort is just 3-4 months. This is so-called selection bias. Therefore, substantial cases of late-onset CMV infection seems to be excluded. To include late-onset CMV reactivation, the minimum fu period should be at least 1-2 years. In addition, the result of Table 3 is confusing. The ORs are < 1.0, which seems that HBCcIgG and T cell NHL are protective role in predicting CMV reactivation. According to your data, ORs of HBCcIgG and T cell NHL may be 6.9 and 4.2, respectively. Please, clarify this. In summary, please describe prophylaxis and preemptive strategy of your own center. Second, please check if the risk stratification based on CMV IgG matching is needed. Finally, check the possibility of the selection bias and ORs.

Answer: As for the first point, the follow-up period in our study has been established on the basis of previously studies, suggesting that CMV reactivation is an early event in autologous setting and late reactivations are very rare, in contrast to allogeneic transplant. In this point of view, our follow-up is limited at 100 days from autograft (time in which we observe a complete immune reconstitution). As for the second point, I believe that this is a good observation. We have revised the ORs, as suggested (In Table 3 and, as consequence, in the revised version of the manuscript).

- Answers to reviewer 00504828 (BLUE BOLD in the revision version of the MS)

The authors analyzed cytomegalovirus infection-related complications after autologous stem cell transplantation in multiple myeloma and several types of lymphoma patients.

The manuscript is well-written, and will be even greater by adding a little more background (see "minor comments"). I have no major concerns on this manuscript. Major comments None. Minor comments 1. Page 4, introduction. Because this manuscript has direct clinical impact, it would be nice to include some numbers related to CMV infection-related mortality. For example, "". 2. Page 4, lines 17~19 "…because of the low likelihood of progression…treatment with Fludarabine, Cladribine or Alemtuzumab.". If the authors briefly described why the combination of CD34-selected grafts and these treatment could make patients more susceptible to progression from CMV infection to disease, it would greatly help non-experts. 3. Page 12, line 18 "…a CMV co-infection TROUGH direct interaction…". Isn't it a type of "THROUGH"? Please check. 4. Acknowledgment: Did not authors receive any funding to conduct this research? If yes, they should acknowledge the funding agencies.

Answer: 1. Data about the mortality rate of CMV infection in autologous setting are very contrasting. The CMV-related mortality reported in the published studies ranged notably between 0 and 100%. We added this consideration in the Introduction section of the revised MS version, as suggested (page 5, line 14 from the top). 2. A briefly description of the mechanism with which prior therapy with Fludarabine or Alentuzumab and an ASCT with CD34+ selected cells are considered risk factors for CMV end-organ disease was provided in the revised version of the MS (page 5, line 12 from the bottom). 3. TROUGH was corrected as THROUGH in the revised version of the MS, as indicated. 4. We have not funding to conduct this study.

- Answers to reviewer 00504545

In my opinion this is a very well designed and performed study on this subject. It contains also a very useful clinical information. I have no suggestions to include on Congratulations to the authors for your interesting study!

Answer: none.

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