Reviewer’s report

Title: Infliximab therapy together with tyrosine kinase inhibition targets leukemic stem cells in chronic myeloid leukemia

Version: 0 Date: 18 Jan 2019

Reviewer: Paolo Gallipoli

Reviewer’s report:

In this manuscript Herrmann and colleagues study the role of microenvironmental cues in survival of CML LSC using mostly a very elegant and well characterised mouse model. They specifically focus, based on previous literature in the field and their gene expression data, on the role of TNF-alpha and demonstrate a subtle but consistent in vitro and in vivo effect of targeting TNF-alpha signalling in supporting LSC survival and/or function above all in combination with standard BCR-ABL TKI treatment.

The manuscript is well written, the data are clearly presented and overall the conclusions appropriate. My main comments are as follow:

1) Is TNF-alpha production in BCR-ABL expressing cells kinase dependent? It would be good if the authors could test this as it might help explaining the effects of combined treatment

2) The authors state that the effects of IFX in vitro are possibly not due to direct binding to TNF-alpha and one wonder if they could possibly be off target. This might explain why the effects of the murine TNF-alpha antibody MP6 are less obvious and only seen in the presence of TNF-alpha. The alternative explanation however is that MP6 is not as good therapeutic and ideally the authors should try the TNF-alpha rescue also in the IFX treated cells. This would allow one to see if indeed TNF-alpha plays a role in the effects seen with IFX regardless whether this is via direct binding or indirect effects.

3) In vivo experiments in figure 3 show only a mild phenotype with the Nilotinib + IFX combination which is surprising compared to the in vitro data in figure 2. Is this because TNF-alpha plays a bigger role in vivo than in vitro and IFX is not targeting it? Could the authors speculate as to why this is?

4) Why in the in vivo experiments the authors do not have a IFX only control arm ? They had it in vitro and showed efficacy on its own so ideally it should be shown for the in vivo experiments too?

5) Why LSK % engraftment from the donor is so low in WT transplanted mice (figure 3g) given that the recipient were lethally irradiated and the total CD45.1 (donor) % is around 75% (figure3b)?
6) In the secondary transplant experiments of figure 4 the authors do not see any effects on survival with combination therapy. They speculate this is due to the high number of cells transplanted. Have they tried to lower the number of cells transplanted or a limiting dilution analysis to bring out this phenotype? Ideally this should be done as it would strengthen or disprove their conclusions. This is even more important as most of the effects the authors see in the secondary transplantation in the blood composition of the combined treatment animals is due to a reduction of the B220 population (figure 4e) which one has to assume is not the malignant CML population in this model of chronic phase disease. The effects on the actual blasts (figure 4c) with the combined treatment are only marginal compared to the nilotinib only arm. If the authors cannot perform these experiments, they should explain their findings taking into account all the above and discuss their current data more cautiously in terms of the effects on the leukemia initiating cell population. Also could the authors speculate as to why the B220 appears to be preferentially targeted by the combined treatment?

7) In the discussion, the authors provide some possible explanations on the mechanism behind the efficacy of IFX in combination with nilotinib and particularly its putative role in reducing IFN-gamma production in the spleen of the CML mice. Can the authors expand on that a bit more? Is this due to the effects of IFX on T-cell population/subsets? Given the effects seen in the secondary transplant on the B220 population, it might be nice to show also any effects on the T-cell population which is the likely source of IFN-gamma and other cytokines? Also they discuss the known effects of TNF-alpha on NFKB signalling and this prompts the obvious question if IFX has any effect on NFKB signalling itself. Have the authors looked at this as this might also explain the effects on cytokine production they already show with combination treatment?

Minor comments:

1) Page 7, line 158. Nilotinib concentration is stated as being 100mM which is far too high. I suspect this was 100nM as per figure legend and should be corrected. Similarly, page 15 line 361, und should be changed to and.

2) The authors state that the 2 mice treated with the combination therapy who died during the delivery of therapy did not show splenomegaly. Could they however explain the likely cause of death. I suspect this might have been infection or radiation effect but if it was due to the therapy is worth mentioning.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.
Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report
including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal