Answering reviewers

Reviewer(s)' Comments:

Reviewer: 1 (01020409)
Comments to the Author

The review is well written, extensive and informative.

We thank the reviewer for these comments.

The authors describe the problems with PDX models, but they should discuss what the goals of a useful PDX model would be: in my view an MDS model needs to be transplantable in secondary mice to be able to evaluate genetic modifications or to test new compounds.

We agree and thank the reviewer for this valuable suggestion. We have added disease that is transplantable into secondary recipient mice to the list of characteristics that are desirable in mouse models of MDS (Page 11, paragraph 2, sentence 1).

The authors discuss the CSNK1A1 model but do not mention that CSKN1A1 is mutated in MDS patients with 5q-. Mutations and clinical impact should be discussed.

CSNK1A1 mutations in 5q- were described (Page 19, paragraph 2, sentence 4). However, as suggested we have expanded this section to include a discussion of the clinical implications of CSNK1A1 mutation (Page 22, paragraphs 2 and 3).

In the list of mouse models the authors should include MLL5.

We thank the reviewer for this suggestion. MLL5 has now been included in Table 2 (Page 40) and Table 3 (Page 42).

The authors state that splenomegaly in a cytopenic mouse may be considered equivalent to a hypercellular bone marrow. The authors should provide a reference if possible.

We have added two references: Perkins AS, PMID: 2659281 and Yang M et al, PMID: 23307598 (Page 11, paragraph 3, sentence 4).

Reviewer: 2 (01021691)
Comments to the Author

This is a very interesting manuscript covering an important research topic in the field of myeloid malignancies. Mouse models of MDS are very useful for our understandings of pathogenesis for MDS, but have been not available for many years. This review provides some novel insights into the biology of MDS.

We thank the reviewer for these comments.

However, there are several points that could complement and clarify some issues of this work:

Comments:

1. *The discussion/conclusion should be improved. The authors should discuss the advantages and disadvantages of models/techniques that have been used for generating mouse models for MDS.*

   We agree that this will strengthen the manuscript and thank the reviewer for the suggestion. We have added a section where the advantages and disadvantages of the techniques used to generate the models is discussed (Page 15, paragraph 4, and Page 16, paragraph 2).

2. *The authors should provide more precise information regarding murine MDS, such as:*
   *What are most common **dysplastic changes** of hematopoietic cells seen in mice? Which type of cytopenia? (pancytopenia? thrombocytopenia? etc).*

   We thank the reviewer for this suggestion. Cytopenias and dysplasia are now discussed (Page 13, paragraph 5).

3. *For diagnosis of myeloid malignancies including MDS in mice, it should be noted that the spleen is an essential organ for the diagnosis of hematological neoplasm. Unlike human leukemia, in murine models bone marrow is only variably involved but spleen in almost all cases (Perkins AS 1989; Curr Top Microbiol Immunol 149:3–21;*
   Yang M, et al., Annals of Hematology 2013; 92(5):595-604)

   We thank the reviewer for this suggestion. These papers have now been cited (Page 11, paragraph 3, sentence 4).

4. *What analyses should be done for diagnosis of MDS in mice? This information is very useful for scientists working in this field. Based on literatures, cytological (cytospins) and histological analyses must be performed for each mouse. For some reasons, megakaryocytes are underrepresented in cytospins, thus histology of spleen and bone marrow must be done for diagnosis of MDS. As cytology is irreplaceable for diagnosis of MDS, the following 2 manuscripts should be cited in the manuscript, which*

nicely described morphology and quantitative composition of hematopoietic cells in murine bone marrow and spleen of healthy subjects and diseased mice (Yang M, et al., Annals of Hematology 2013; 92(5):587-94; Yang M et al., Annals of Hematology 2013; 92(5):595-604).

We agree that this will strengthen the manuscript and thank the reviewer for drawing our attention to these very useful papers. We have added a section on the suggested work-up of MDS in mice and have cited the suggested publications (Page 33, paragraph 5).

5. *What significance does iron staining have for diagnosis of MDS in mice?*

This is a good question. We have incorporated a discussion of the utility of iron staining for recognition of MDS in mice to the section about the diagnostic work-up Page 34, paragraph 3, sentence 3.

6. *There are some mistakes in the manuscripts, that must be corrected:*
   a) *Table 2. It should be ASXL1 , not ASXL2*

   This has been corrected in Table 2 (Page 40).

   b) *Figure 1: 2 claims of the authors are not correct*
      - *<u>There is no cytogenetic abnormality in murine MDS.</u> So far, no detailed cytogenetic analyses have been performed in majority of reported murine MDS. Thus, it is unknown whether they have cytogenetic abnormality.*
      - *<u>There is no splenomegaly in patients with MDS.</u> It should be changed to "possible".*

   For cytogenetics, we have changed the figure to read: *"No recurrent abnormalities identified to date"* (Page 37).

   For splenomegaly, we have changed the figure to read: *"Possible"* (Page 37).

   c) *Some reference citations in Figure 2 are wrong. For example, the correct reference for MIR-145, MIR-146a is #140, not 96. The authors should check all references carefully once again.*

   We thank the reviewer for careful reading. The citations in Figure 2 have been corrected (Page 39). All tables and figure citations have been rechecked for accuracy.