Peer Review File

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Reviewer A

This review summarizes changes which occur in the microenvirionment during AML.
Comments:
Comment 1. Be sure to define abbreviations when first used. There are many examplesof this; ? PDCD4, SCF, CTGF.
Reply 1. We thank the reviewer for bringing this to our notice. We have now made corresponding changes in the draft and have defined the abbreviations when used for the first time.
Changes in the text: Defined abbreviations when first used throughout the manuscript.

Comment 2. Line 176, 192, and some other places.; would not use etc. as the reader has no idea what else is meant to be included; would describe all the important components, all the various MSC subclasses, and it might even be good to incorporate tables which enumerate and describe these and how they change in AML.
Reply 2. We appreciate this great suggestion from the reviewer. We have now made corresponding changes in the mentioned sentences and wherever it seemed to be applicable. We have also incorporated the tables which enumerate and describe the various niche components (Table 1) and how they change in AML (Table 2)
Changes in the text: We added Table 1 and Table 2 and have cited it in the manuscript (see page 09, line 257 and page 09, line 268).

Comment 3. Lines 268-70, if DKK1 suppresses osteogenesis, how does that result in loss of osteoclasts?
Reply 3. We agree with the reviewer and clarify that induced expression of DKK1, a suppressor of osteogenesis, results in the loss of osteoblasts, and not osteoclasts.
Changes in the text: We have modified the text to Furthermore, AML-derived exosomes induced the expression of DKK1, a suppressor of osteogenesis, thereby resulting in the loss of osteoblasts (see page 10, line 292)

Comment 4. Line 329- reference 90 does not seem to be from the Scadden group.
Reply 4. We agree that all author's names are not included in the references. However, we clarified that reference 90 is from Scadden's group.
Changes in the text: We have added all the author's names for reference 90 in the references, including David T Scadden (see page 12, line 359).

Comment 5. The section on therapeutic options should be expanded; other CXCR4 inhibitors, E-selectin inhibitors, VLA4 inhibitors, and many more have been examined to modulate the AML microenvironment.

Reply 5. We thank the reviewer for the suggestion. Due to restrictions on word count, we have briefly discussed inhibitors as suggested that modulate the AML microenvironment and added everything in Table 3 with their clinical trial status.
Changes in the text: We added Table 3 and cited it in the manuscript on line 552 of page 18.

Minor;
Reply: We thank the reviewer for all the suggestions.

Line 50; majorly should be largely.
Changes in the text: We have now made the suggested change in the revised draft (see page 03, line 53).

Line 85 --? change "talking about"
Changes in the text: We have now modified this statement in the revised draft. (see page 04, line 90)

Line 197--? 'get differentiated" needs to be changed.
Changes in the text: We have changed the suggested wordings in the revised draft (see page 08, line 208).

Reviewer B

The review manuscript by Urs et al summarizes a very large body of work related to the cross-talk between AML blasts the microenvironment. It is generally well written and organized. It covers most of the important issues in the field. Important references are cited. A few suggestions are offered below.

Line 63: It would be helpful to specify how HSC is defined in ref 4 since there are various definitions leading to different frequency estimates.
Reply: We agree with the reviewer. We have not indicated details about the assay used to identify the HSCs in this reference article.
Changes in the text: The authors have used limiting dilution assay performed in nonobese diabetic severe combined immunodeficient (NOD/SCID) mice to identify the frequency of HSCs in human bone marrow. (see page 03, lines 65-67)

Lines 162-168: Regarding reference 43, is it correct to conclude that in this paper the effect of osteoclast disruption on HSC support was actually indirect, through a reduction in osteoblast activity? If yes, it would be helpful to spell that out for the reader.
Reply: Reviewer is correct! We appreciate the suggestion to spell out the results as suggested. We are happy to consider the suggestion and elaborate the observations in this study.

Changes in the text: This indirect regulation of HSC niche by the osteoclasts is dependent on their bone resorption activity and linked to their capacity to support osteoblast commitment. (see page 07, lines 174)

Line 170: Define SLAM.
Reply: We have defined signaling lymphocytic activation molecules expressing (SLAM) HSCs with context to SLAM HSCs as suggested. We have now specifically mentioned the SLAM markers used to identify these SLAM HSCs. We thank the reviewer for the same.
Changes in the text: We have added the definition for SLAM in the revised draft. (see page 07, lines 178-180)

Line 201: Explain the significance of CFU-F activity.
Reply: Significance of the CFU-F activity is mentioned as suggested by the reviewer.
Changes in the text: We have now mentioned that colony forming units – fibroblast (CFU-F) activity is the gold standard for measuring the MSC functionality (see page 08, lines 213-214).

To summarize the discussion of the endothelial and vascular niches, a table of the different subsets of stromal cells, what they secrete and how they influence HSCs would be useful.
Reply: We appreciate this great suggestion from the reviewer. We have now made corresponding changes to the manuscript. We have incorporated the table which enumerates and describes the various niche components and how they influence HSCs (Table 1).
Changes in the text: We added Table 1 to the manuscript and have cited it appropriately (see page 09, line 257).

Lines 268-270: One of the main points of ref 77 was that AML-derived exosomes alone were capable of impairing osteoblast maturation, due at least in part to DKK1. Suggest more emphasis on the role of exosomes here.
Reply: We thank the reviewer for their suggestions. We agree that in addition to the importance of DKK1 in AML niche, exosomes also play a role in AML niche modification and have added literature reports for the same in the manuscript.
Changes in the text: We have added the following to the text (see page 10, lines 293-299).
Several studies have highlighted the roles of AML-derived exosomes on modulation of BM niche (Pendse et al, May 2023). In the same study, the authors showed that AML exosomes induced downregulation of key HSC supporting factors CXCL12, SCF, and IGF1 in BM stromal cells, thereby decreasing their ability to support normal HSCs. Another study by Huan et al demonstrated that AML-derived exosomes induced downregulation of critical retention factors SCF and CXCL12 in stromal cells resulting in HSPC mobilization from the BM (Huan et al, Dec 2015).

Lines 317-319: This sentence is just an introduction so its own subheading is not needed.
Reply: We agree with the reviewer and have deleted the subheading.
Changes in the text: We have modified the text as follows (see page 12, lines 345-351).
The altered BM niche in AML reciprocally interacts with the AML blasts and influences their proliferation, survival, and apoptosis through various mechanisms. The following sections will focus on how these altered BM components affect leukemogenesis.

Line 342: Please clarify that treatment with a b2 adrenergic agonist (not the receptor itself) limited LSC expansion.
Reply: We agree with the reviewer and clarify that it was an error on our end. We have reworded the line to clarify that the treatment with a b2 adrenergic agonist limited LSC expansion.
Changes in the text: We have modified the text to- Furthermore, they showed that inhibition of the β2 adrenergic receptor by antagonists resulted in extended leukemic cell proliferation while treatment with β2 adrenergic agonist limited leukemic stem cell expansion by rescuing the healthy BM niche (see page 12, lines 374-375).

Section starting at line 380: Three of these 4 paragraphs of this section are more about immune evasion than chemotherapy resistance. Consider separating into a specific section on the topic of immune evasion. Then a section about stroma-mediated chemotherapy resistance could be expanded. There is a large body of literature about contact-dependent resistance and soluble factor-dependent resistance that is not well covered.
Reply: We thank the reviewer for the suggestion which improved our manuscript. As per the suggestion we have separated the first 3 paragraphs into a separate section on 'mechanisms of immune evasion'. Further, we have expanded the stroma-mediated therapy resistance section by adding contact-dependent and soluble factor-dependent resistance.
Changes in the text: We have included the mechanisms of immune evasion section (see page 12, lines 414-450). A paragraph about contact-dependent and soluble factor-dependent resistance is added (see page 19-20, lines 489-500).

Section starting at line 446: Suggest mentioning the E selectin antagonist from Glycomimetics that is in clinical trials.
Reply: We agree with the reviewer and as per the suggestion we have mentioned the E-selectin inhibitor from Glycomimetics GMI-1271 (Uproleselan) in the text and Table 3.
Changes in the text: We have modified the text to - A potent E-selectin inhibitor GMI-1271 may reduce the adhesion of AML cells to the stroma and enhance chemotherapy efficiency (Table 3) (see page 18, lines 549-552).

Reviewer C

This is a well written overview that takes on a very large problem (the marrow microenvironment) and breaks it down into smaller pieces while also providing a good overview of how the marrow microenvironment is hijacked by AML.

I have two major comments.
1. More in depth consideration for some marrow effects could be done. For example the role of cholesterol in AML development has been well established- trials have even been done using statins in treatment. This could be expanded upon.
https://pubmed.ncbi.nlm.nih.gov/15161671/
https://pubmed.ncbi.nlm.nih.gov/29407182/
https://pubmed.ncbi.nlm.nih.gov/34731885/
Reply 1: We thank the reviewer for the suggestion. As per suggestion, we have added information on elevated cholesterol levels leading to chemoresistance.
Changes in the Text: We have modified the text as follows:
There are many reports suggesting cholesterol homeostasis as one of the mechanisms of AML chemoresistance. Cholesterol levels are significantly increased in AML samples exposed in vitro to chemotherapy. Blocking these elevated acute cholesterol levels may sensitize AML cells for therapy (see page 19, lines 569-572).

2. The only figure is excellent. Additional figures though would be beneficial that describe mechanisms by which the microenvironment is changed. Tables also would be useful- 1-2 tables that discuss the components the bone marrow niches (Table 1) and the mechanism by which AML cells co-opt these components (Table 2). A table with clinical trials that have targeted the marrow would also be useful as this has been done in some ways already.
Reply 2: We agree with the reviewers. In response to their comment, we have added a figure that describes BM niche-conferred mechanisms of therapeutic resistance in AML. Furthermore, we have added 3 tables to discuss bone marrow niches, their modifications in AML, and clinical trials targeting the marrow.
Changes in the text: We added 3 tables and a figure at appropriate sections and cited them in the manuscript.

Minor
Page 5, Line 119
Suggest to use definition rather than defining
Changes in the text: We have changed the suggested wordings in the revised draft. (see page 05, line 125)

Page 12 Line 327 should be either Scadden's group or The Scadden group. be replicated through the manuscript.
Reply: We agree with the reviewer and have modified all instances of “Scadden group”

to Scadden's group in the manuscript.
Changes in the text: We have modified the text as follows:
Scadden's group has shown a decreased number of mature osteo-lineage cells in the BM niche of mice transplanted with MLL-AF9 AML cells. (see page 10, lines 284)
Scadden's group has shown that activation of TGFb1 signaling through parathyroid hormone receptors on osteoblasts enhances engraftment in the MLL-AF9 mouse model. (see page 12, lines 359)

Reviewer D

The authors summarize the current knowledge on normal bone marrow niche and describe the changes in leukemia. They proved a punctual, exhaustive description of the impact of the different niche components on leukemogenesis process, and on the development of resistance to therapy. Moreover they mention the possible future therapeutic option to reverse the permissive bone marrow microenvironment and increase leukemia response.
The paper is clear, concise but exhaustive, references updated and the figure well described.
Reply: We thank the reviewer for investing their valuable time for going through the manuscript and appreciating the work done.