Peer Review File
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Review Comments

The paper titled "FLT3 mutation-related immune checkpoint molecule absent in melanoma 2 (AIM2) contributes to immune infiltration in pediatric and adult acute myeloid leukemia" is interesting. study identified AMI2 as an ICM linked to FLT3 mutations. AMI2 may be involved in the activation of suppressive immune cell populations, such as macrophages, neutrophils, and monocytes. AIM2 could serve as a promising immunotherapeutic target for combination therapy with FLT3 inhibitors in AML. However, there are several minor issues that if addressed would significantly improve the manuscript.

1)What is the correlation between the immunophenotype and prognosis of patients with AML? It is recommended to add relevant content.

Reply: Thank you for your constructive suggestion. Our analysis utilizing the ssGSEA algorithm to determine immune cell enrichment scores revealed no significant correlation between OS and the enrichment scores of neutrophils (*Supplementary figure 1A*), macrophages (*Supplementary figure 1B*), Tem cells (*Supplementary figure 1C*), or Th17 cells (*Supplementary figure 1D*) in AML patients.

2)Please combine literature analysis on how to establish predictive genetic features and explore the determinants of AML prognosis.

Reply: Thank you for your constructive suggestion. Adverse prognostic factors associated with AML include an age greater than 60 years, a prior history of myelodysplastic syndromes (MDS) or myeloproliferative neoplasms (MPN), treatment-related or secondary AML, hyperleukocytosis, central nervous system involvement, chromosomal abnormalities or molecular genetic markers indicative of poor prognosis, and the failure to achieve complete remission after two courses of induction chemotherapy (1-3). Our findings indicate that the immune checkpoint molecule AIM2, which is related to FLT3 mutations, is associated with poor prognosis. Conversely, our analysis revealed that the enrichment scores of four major immune cell types do not correlate with the prognosis of AML. Nevertheless, immunoinvasive AML has been associated with poor prognosis (4), and various immune cell enrichment scoring algorithms may yield disparate prognostic outcomes. While our study did not identify a statistically significant correlation between macrophage enrichment and poor prognosis in AML, there was a tendency for lower macrophage enrichment to be linked with better prognostic outcomes. It is well-documented that macrophage enrichment is correlated with poor prognosis in various tumor types (5,6).

Consequently, the relationship between immune cell enrichment and AML prognosis warrants further investigation with a larger sample size to achieve more definitive conclusions. (lines 426-443)

3)It is recommended to add AIM2 and FLT3 molecular cytology experiments for verification and functional research.

Reply: Thank you for your insightful comments. In alignment with the objectives of our study, our intention was to employ bioinformatics analysis for the preliminary screening of the involvement of FLT3 mutation-related immune checkpoint molecules in tumor immunity within AML. It appears that the original title may not have sufficiently conveyed this specific focus, potentially leading to misunderstandings. A cursory review of the title might suggest that our study encompasses cell and animal experiments. Consequently, we have decided to revise the title of the article as follows: FLT3 mutation-related immune checkpoint molecule absent in melanoma 2 (AIM2) contributes to immune infiltration in pediatric and adult acute myeloid leukemia: evidence from bioinformatics analysis. Additionally, we assure that the functions of AIM2 and FLT3 will be further validated in vivo and in vitro in subsequent research.

4)How to understand the tumor immune microenvironment of AML? What impact will the tumor microenvironment have on the immune checkpoint inhibitor response? It is recommended to add related content.

Reply: Thank you for your insightful comments. The TME in AML frequently demonstrates immunosuppressive characteristics, including the accumulation of TAMs, regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs) (7-9). These elements can impede effector T cell function and diminish the effectiveness of immune checkpoint inhibitors (8-10). Furthermore, AML cells may directly suppress T cell activity and attenuate the response to immune checkpoint inhibitors through the expression of PD-L1 and other immune checkpoint molecules (10). Considering the intricate interactions between AML immune cells and various environmental components, it is plausible to hypothesize that the future of AML immunotherapy resides in the strategic integration of complementary immunotherapy approaches with chemotherapeutic agents or other inhibitors targeting carcinogenic pathways. (lines 413-424)

5)All figures are not clear enough, please upload again.

Reply: Thank you for your comments. All the figures have met the requirements of the Journal and have been uploaded in the submission system again.

6)This article is best to supplement clinical experimental research. This is more helpful to support the conclusion of this article.

Reply: Thank you for your constructive comments. As you are aware, we have revised the title to reflect the research outcomes based on bioinformatics. Incorporating additional clinical trials would undoubtedly enhance the robustness of our current conclusions. Given that our present findings represent preliminary investigations into the FLT3 mutation-related immune checkpoint molecule AIM2, future studies will focus on a more detailed analysis of the expression levels of AIM2 and FLT3 in clinical samples.

References

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