Peer Review File

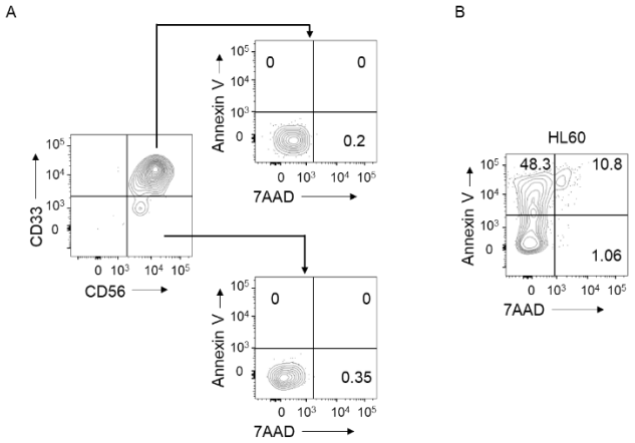
Article information: https://dx.doi.org/10.21037/tcr-21-733

Reviewer Comments

Major Comments:
My major concern is how do we know you are analyzing NK cells and not myeloid blasts. Unless I am misinterpreting the manuscript, it seems one of the main statements is that the CD3-CD56+ cells are NK cells although these cells are also CD33+. This would be consistent with expression of CD56 (Neural cell adhesion molecule-1, NCAM-1) on myeloid blasts which is a known poor prognostic marker and is present in 20% of AML cases.

Reference PMID: 30814062

I do not think this can be argued given the CD33 positivity unless there is additional immunophenotypic evidence for something lymphoid and not myeloid. Was this done? If instead the point is that these blasts that are CD33+ and CD56+ are resistant to killing then please clarify.
Reply: We appreciate your insightful comments and helpful suggestions. It is of great significance to verify whether $CD33^+$ $CD56^+$ cells are resistant to killing. In accordance with your important suggestion, we had purified $CD56^+$ cells through magnetic-activated cell sorting (Miltenyi Biotec) from PBMC of healthy donor, and this patient. The $CD56^+$ cells purified from the patient were firstly marked with CFSE in accordance with the manufacturer's instructions, then washed and used as target cells (effector to target ratio = 10:1). When co-cultured with $CD56^+$ cells purified from PBMC of healthy donor, we found the proportions of $7AAD^+$ or Annexin V^+ $CD33^+$ $CD56^+$ cells or $CD33^-$ $CD56^+$ cells were very low **(as shown below, A)**. What's more, at the same time, when the HL60 cells were co-cultured with $CD56^+$ cells purified from PBMC of healthy donor, we found the proportion of Annexin V^+ HL60 cells was high **(as shown below, B)**. Therefore, we demonstrated that the blast of CD33+ CD56+ cells in our study was resistant to killing.



(A) The expressions of 7AAD and Annexin V in CFSE$^+$ CD56$^+$ cells from the patient after co-cultured with CD56$^+$ cells purified from PBMC of healthy donor. (B) The expressions of 7AAD and Annexin V in HL60 cells after co-cultured with CD56$^+$ cells purified from PBMC of healthy donor.

Results

1. How was relapse confirmed? By morphology with >5% blasts or flow?
2. Was the secondary breast tumor a myeloid sarcoma? If not then was it an epithelial cancer?

Reply: Thanks for your insightful comments.

1. The relapse was first confirmed by morphology with >5% blasts.
2. The secondary breast tumor a myeloid sarcoma.

Minor Comments:

Abstract- Introduction

1. I would say instead "Based on genetic risk allogeneic stem cell transplant is the only curative treatment for some forms of AML"

Reply: We really appreciate your helpful suggestion. In the revised version of the manuscript we have corrected “Allogeneic stem cell transplantation (allo-SCT) is an effective, and sometimes even the only, curative therapy for acute myeloid leukemia (AML).” to “Based on genetic risk allogeneic stem cell transplantation (allo-SCT) is the only curative treatment for some forms of acute myeloid leukemia (AML)”. And we have corrected “Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective therapy for patients with acute myeloid leukemia (AML),” to “Based on genetic risk allogeneic stem cell transplantation (allo-SCT) is the only curative treatment for some forms of acute myeloid leukemia (AML),”. **(Revised manuscript; Abstract, Lines 27-28, Page 2; Introduction, Lines 52-53, Page 3)**.

Results

Was any molecular data available for risk stratification?

Reply: We appreciate your insightful comment. It is of great significance to find some molecular data available for risk stratification. Elihu H Estey and Fatemeh Pourrajab *et al.*[1,2] reported that at the molecular level, AML would be the consequence of

collaboration between at least three broad classes of gene alterations. Class I gene alterations were those aberrations that activating signal transduction pathways and enhancing proliferation with survival advantages of hematopoietic stem cells. This class of gene aberrations may activate the receptor tyrosine kinase FLT3 and Kit or the RAS-associated signaling pathway. Class II gene alterations may affect a master transcription factor or a protein involved in hematopoietic differentiation. Class III included those alterations that promoted epigenetic modifications of chromatin in a large area and affect further transcription factors or components of the transcriptional co-activation complexes whereby would confer malignant transformation to the HPCs and lead to overt AML (eg, DNMT3A and IDH1/2, involved in epigenetic regulation of chromatin and cellular processes). What's more, the negative effect of a FLT3-ITD in patients with an NPM1 mutation could be much more pronounced in patients with than without a DNMT3a mutation. Likewise, in patients with an NPM1 mutation presence of a RAS mutation improved survival more in the presence than the absence of a DNMT3 mutation. Due to the limited samples from the patients, there was no molecular data available for risk stratification in our study.

References

1. Pourrajab, F., Zare-Khormizi, M.R., Hashemi, A.S. & Hekmatimoghaddam, S. Genetic Characterization and Risk Stratification of Acute Myeloid Leukemia. *Cancer Manag Res* **12**, 2231-2253 (2020).
2. Estey, E.H. Acute myeloid leukemia: 2019 update on risk-stratification and management. *Am J Hematol* **93**, 1267-1291 (2018).