Peer Review File

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Reviewer A

Comment 1: Patial tandem duplications (or deletions) of the KMT2A (MLL) gene region is not equivalent to an KMT2A gene rearrangement. While these results are interesting, the conclusion that concurrent ETV6:RUNX1 fusion and KMT2A rearrangement is not accurate. Perhaps the authors could reframe the purpose of the manuscript, but as is, it is not suitable for publication.

Reply 1: Thanks for your suggestion. Actually, the KMT2A gene is located on chromosome 11q23 and it can be rearranged to generate partial tandem duplications, so partial tandem duplications of the KMT2A gene region belong to an KMT2A gene rearrangement (BMC Cancer. 2022 Jan 3;22(1):11.doi: 10.1186/s12885-021-09051-5). Importantly, concurrent ETV6:RUNX1 fusion and KMT2A rearrangement is rare among children ALL, and to explore the outcome of the type of ALL in critical, which showed different treatment protocol.

However, we agree that deletions of the KMT2A (MLL) gene region is not equivalent to an KMT2A gene rearrangement, so we replaced "KMT2A rearrangement" with "KMT2A aberration" in our revised manuscript (see the whole revised manuscript).

Reviewer B

Comment 2: Authors report on 4 ALL cases with MLL intragenic rearrangements (not translocations) occurring exclusively in cases harboring ETV6-RUNX1. They also provide a literature review. This finding is not novel, but it is important to stress that not all MLL rearrangements are indicative of poor prognosis. Besides, it may help call attention to the participation of an altered MLL in the leukemogenic process driven by ETV6-RUNX1.

Reply 2: Thanks for your useful suggestion. We also agree that MLL rearrangements might be the secondary genetic abnormality of ETV6-RUNX1. In addition, Gagnon et al use the whole genome sequencing to elucidate and characterize this case of coexistence, and they reveals that the apparent fusion between MLL and AF9 by FISH was not predicted to result in a bona fide MLL-AF9 fusion and would not be expected to alter the intrinsic MLL regulatory mechanisms and confer leukemogenic potential, while it would be expected to result in a lack of protein production from this allele. We believe Gagnon et al study had better persuasiveness, and we put their study in our revised study (see Page 6, line 171-181).

Comment 3: The English is not good and needs major revision.

Reply 3: We are sorry for our poor English. To improve our language, we invite a native English speaker to help us to polish the language.

Comment 4: As a suggestion to the authors, discussion could be improved by trying to find in the literature how ETV6-RUNX1 and MLL-r could relate to one another. Perhaps the

occurrence of this MLL intragenic alterations may simply relate to the presence of RSS sequences in the MLL. It is known that ETV6-RUNX1 driven ALL is associated with increased RAG-driven mutations (Nat Genet. 2014 Feb;46(2):116-25. doi: 10.1038/ng.2874.).

Reply 4: Thanks for your useful suggestion. We also agree that MLL rearrangements might be the secondary genetic abnormality of ETV6-RUNX1. In addition, Gagnon et al use the whole genome sequencing to elucidate and characterize this case of coexistence, and they reveals that the apparent fusion between MLL and AF9 by FISH was not predicted to result in a bona fide MLL-AF9 fusion and would not be expected to alter the intrinsic MLL regulatory mechanisms and confer leukemogenic potential, while it would be expected to result in a lack of protein production from this allele (2022 Sep;63(9):2243-2246.doi: 10.1080/10428194.2022.2064991.). We believe Gagnon et al study had better persuasiveness, and we put their study in our revised study (see Page 6, line 171-181).

Reviewer C

Kun-yin Qiu et al. has documented a case report regarding coexistence of ETV6-RUNX1 and MLL rearrangement among pediatric acute lymphoblastic leukemia.

This is considered an important report because ALL patients with MLL rearrangement usually have poor prognosis and sometimes undergo allogeneic stem cell transplantation. However, coexistence of ETV6-RUNX1 improves prognosis and patients could avoid unnecessary invasive treatments such as allogeneic stem cell transplantation.

Comment 5: Authors should show the results of flow cytometric analysis. CD10 is prognostic factor, and however, the patients with ETV6-RUNX1 usually are positive for CD10, the patients with MLL rearrangement are negative for CD10. (Blood Rev. 2012; 26: 123-35)

Reply 5: Thanks for your nice comment. In fact, by flow cytometry, the four patients all expressed CD10. We have modified our text as advised (see Page 2, line 54; Page 3, line 71,86; Page 4, line 100).

Comment 6: The authors comment that seven patients were positive for both ETV6-RUNX1 and MLL rearrangement in abstract. Why the authors described only four cases?

Reply 6: Thanks for pointing out this mistake. We are sorry for this mistake. Actually, only 4 patients in our study were positive for both ETV6-RUNX1 and MLL rearrangement. Now we correct our mistake in our revised abstract (see Page 1, line 23).

Comment 7: Authors should present the searched words in PubMed

Reply 7: Thanks for your suggestion. Now we added the sentence “With the following keywords of "ETV6-RUNX1" " MLL" “children” and "acute lymphoblastic leukemia" as keywords, the literature was searched from PubMed database” in our revised manuscript (see Page 1, line 25-26).

Comment 8: Abbreviations are usually defined at the first use (Ex: MRD, PTD, AL, CR, PTD)

Reply 8: Thanks a lot. Your suggestion is really important to improve our manuscript. Now we defined at the first use for all the abbreviation in our revised manuscript.