<u>Peer-Review Report</u>

Peer Review of "Selection of the Optimal L-asparaginase II Against Acute Lymphoblastic Leukemia: An In Silico Approach"

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This is a peer-review report submitted for the paper "Selection of the Optimal L-asparaginase II Against Acute Lymphoblastic Leukemia: An In Silico Approach".

Round 1 Review

General Comments

This paper [1] aimed to investigate whether asnB from *E Coli* and *Erwinia* is the best asparaginase for the therapeutic treatment of acute lymphoblastic leukemia by using asnB sequence of *E Coli* to search for homologous proteins in other bacteria and archaea phyla. The authors mention that asnB with the lowest Michaelis Menten constant (K_m) and the lowest immunogenicity is to be considered the most suitable enzyme. A phylogenetic tree was created, after which homology modeling was conducted, followed by docking to identify the binding energies to determine the relationship between binding energy and K_m. The technical aspects of the paper are adequately conveyed, and the in silico method is appropriate to answer the question. However, I have a few comments that need to be clarified.

Specific Comments

Major Comments

1. Perhaps, explain what blastp does to provide more insight.

2. It would be beneficial to include a figure that displays the sequence alignment of the query sequence along with the similarity percentage.

3. The discussion is well-written, although could benefit from including other relevant studies/prior works to support your results.

4. Conclusion is relatively weak. Please consider revising it and sufficiently summarizing the Methods and Results.

Minor Comments

1. Section 2.2: Please define DOPE and SOAP before abbreviating.

2. Discussion: It is mentioned that only 6 of 10 species could have a K_m value assigned to a certain sequence, please mention these 6 species.

3. Discussion: The sentence "Thus it can be predicted that an enzyme with better kinetics that currently commercially available asparaginase can be cloned from Streptomyces species" is a bit ambiguous. Please rewrite this sentence.

Round 2 Review

General Comments

The authors have addressed all reviewer comments and improved the manuscript. I have no further comments.

Conflicts of Interest

No conflict declared.

Reference

1. Baral A, Gorkhali R, Basnet B, Koirala S, Bhattarai HS. Selection of the optimal L-asparaginase II against acute lymphoblastic leukemia: an in silico approach. JMIRx Med 2021 Sep 7;2(3):e29844 [FREE Full text] [doi: 10.2196/29844]

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