**Peer Review File**

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**Reviewer A**

*Comment 1*

*Line 80: Azvudine (FNC) is a synthetic nucleoside analog and a broad-spectrum oral RNA small-molecule antiviral drug, initially developed in China treatment for mild and common COVID-19 may shorten the for HIV-1 treatment*

*I apologize for the confusion. Based on the information provided, it seems that the original sentence is indeed unclear and lacks proper context regarding both COVID-19 and HIV-1 treatment.*

**Response:** Thank you for bringing this to our attention. We apologize for the oversight. The sentence appears to have been inadvertently fragmented. To clarify, Azvudine (FNC) is a synthetic nucleoside analog and represents a broad-spectrum oral RNA small-molecule antiviral drug. It was originally developed in China for the treatment of HIV-1 infection. We have rectified the error to ensure clarity in the revised manuscript.

**Changes in the text:** Azvudine (FNC) is a synthetic nucleoside analog and represents a broad-spectrum oral RNA small-molecule antiviral drug. It was originally developed in China for the treatment of human immunodeficiency virus type 1 (HIV-1) infected adult patients with a high viral load. (Page 4, lines 83-86)

*Comment 2*

*FNC, once inside the host cell, undergoes phosphorylation by kinase to convert into an active compound, the nucleoside triphosphate*

*This phrase will be rearranged and placed at the beginning of the introduction*

**Response:** Thank you for your suggestion. We have accordingly moved this sentence, along with several sentences after it, to the designated location in the revised manuscript, aiming to enhance the coherence and logical flow of the paper.

**Changes in the text:** Azvudine (FNC) is a synthetic nucleoside analog and represents a broad-spectrum oral RNA small-molecule antiviral drug. It was originally developed in China for the treatment of human immunodeficiency virus type 1 (HIV-1) infected adult patients with a high viral load. FNC, once inside the host cell, undergoes phosphorylation by kinase to convert into an active compound, the nucleoside triphosphate. This active form disrupts viral RNA synthesis during replication, thereby inhibiting the virus's ability to reproduce. FNC also acts on the viral RNA-dependent RNA polymerase, terminating viral RNA synthesis during reverse transcription, which further impedes viral replication. After administration, FNC is primarily distributed to the thymus, where it undergoes triple phosphorylation and boosts the immune response. (Page 4, lines 83-93)

*Comment 3*

*After administration, FNC is primarily distributed to the thymus, where it undergoes triple*

**Response:** Thank you for your comment. We have accordingly moved this sentence, along with several sentences after it, to the designated location in the revised manuscript, aiming to enhance the coherence and logical flow of the paper.

**Changes in the text:** FNC, once inside the host cell, undergoes phosphorylation by kinase to convert into an active compound, the nucleoside triphosphate. This active form disrupts viral RNA synthesis during replication, thereby inhibiting the virus's ability to reproduce. FNC also acts on the viral RNA-dependent RNA polymerase, terminating viral RNA synthesis during reverse transcription, which further impedes viral replication. After administration, FNC is primarily distributed to the thymus, where it undergoes triple phosphorylation and boosts the immune response. (Page 4, lines 86-93)

*The sample size (n=13) is too small to draw conclusive outcomes, warranting a revision in the language used and raising concerns about the validity of the results. Consequently, a thorough discussion becomes challenging due to the limited data.*

**Response:** We genuinely appreciate the reviewer's feedback regarding the sample size of our study. We acknowledge the limitations that come with a small sample size and understand the concerns raised regarding drawing conclusive outcomes. In the revised manuscript, we have explicitly stated the small sample size as one of the limitations in our discussion. To address this, we have added the phrase "future studies with larger sample sizes are necessary" as a way to underscore the need for further research to validate our preliminary findings. Furthermore, we have modified the language used to describe our results. Instead of presenting definitive conclusions, we have chosen a more cautious tone. We hope these amendments address the concerns raised and enhance the clarity and accuracy of our paper. Once again, we thank the reviewer for their insightful feedback.

**Changes in the text:** Our study, while offering preliminary insights into the potential benefits of FNC against the Omicron BA.5.1.3 subvariant, is not without its limitations. Foremost, the small sample size of 13 patients restricted the generalizability of our findings to a broader population, and results should be interpreted with caution. The absence of a true control group poses another limitation. The diversity in treatments challenged the robustness of our comparative analysis, as there wasn't a consistent standard of care against which FNC's effects could be directly measured. Furthermore, the retrospective nature of our study inherently carried potential biases, such as selection bias and data incompleteness. While our findings provided an initial indication of FNC's potential utility, larger, prospective, randomized controlled trials are essential to validate and build upon these observations. (Pages 18-19, lines 443-453)

***Reviewer B***

*Interesting paper regarding an interesting potential use of an HIV medication FNC administered intranasally as treatment for COVID-19.*

*Small sample size with 13 subjects analyzed.*

*No true control as a comparator arm, given treatments were diverse.*

**Response:** We greatly appreciate the reviewer's observation regarding the sample size and the lack of a true control arm in our study. We concur with these observations and have duly mentioned these concerns in the discussion section of our paper.

**Changes in the text:** Our study, while offering preliminary insights into the potential benefits of FNC against the Omicron BA.5.1.3 subvariant, is not without its limitations. Foremost, the small sample size of 13 patients restricted the generalizability of our findings to a broader population, and results should be interpreted with caution. The absence of a true control group poses another limitation. The diversity in treatments challenged the robustness of our comparative analysis, as there wasn't a consistent standard of care against which FNC's effects could be directly measured. Furthermore, the retrospective nature of our study inherently carried potential biases, such as selection bias and data incompleteness. While our findings provided an initial indication of FNC's potential utility, larger, prospective, randomized controlled trials are essential to validate and build upon these observations. (Pages 18-19, lines 443-453)

*The conclusion of FNC "reducing ICU stay duration" needs to be explained, as it is not clear, given it states there is no statistical difference when analyzing NAAT positivity: "When we consider the interval from the first positive to the first negative test for the 13 ICU patients who were part of the FNC and no-FNC groups, there were no significant differences even without considering groupings,".*

**Response:** We appreciate the reviewer's astute observation and feedback on our conclusion regarding FNC's potential in "reducing ICU stay duration". Indeed, while Figure 4A presents a numerical difference in ICU stay between the FNC and non-FNC groups, this discrepancy does not achieve statistical significance (P=0.22). We have thus revised our conclusions and descriptions in the manuscript, eliminating overstated claims. Specifically, phrases such as "duration of ICU stay was effectively shortened" have been modified to more accurately reflect the observed numerical differences without implying statistical significance.

**Changes in the text:** Among the 13 patients in this cohort, there appeared to be a reduction in the duration of ICU stay, though no significant differences were evident between the FNC and non-FNC groups. (Page 16, lines 377-380)

*Is the study controlled for co-morbidities, other treatments utilized for COVID-19? If so, please clarify this in the Methods portion. It is not clear how to distinguish which patients improved from FNC alone, vs. potentially a variety of combination therapies. If not, this should be explained as a limitation of the study. Perhaps can make a table of the 13 subjects and clinical features, to clarify which subject had which symptoms, co-morbidities, etc. Perhaps a cumulative table would be more helpful to define individual characteristics, rather than separated tables, as it is unclear which variable applies to which patient.*

**Response:** Thank you for your valuable comments and suggestions. We recognize the

importance of detailing co-morbidities and other treatments received by the patients, as these factors could indeed influence the outcomes observed. Given the limited sample size, it may be better to provide detailed information about each patient rather than performing comparisons between groups. Therefore, we have now included a comprehensive table (the new Table 1) in the revised manuscript that delineates the clinical and imaging features of the 13 patients, and the initial Tables 1 & 2 were removed. This will offer a clearer view of individual patient characteristics and their respective treatments. In addition, treatment regimens presented in Table 3 were checked and revised. Considering the limited sample size, p values were removed.

*Please fix this sentence grammar as not clear: "mild and 82 common COVID-19 may shorten the for HIV-1 treatment"*

**Response:** Thank you for bringing this to our attention. We apologize for the oversight. The sentence appears to have been inadvertently fragmented. To clarify, Azvudine (FNC) is a synthetic nucleoside analog and represents a broad-spectrum oral RNA small-molecule antiviral drug. It was originally developed in China for the treatment of HIV-1 infection. We have rectified the error to ensure clarity in the revised manuscript.

**Changes in the text:** Azvudine (FNC) is a synthetic nucleoside analog and represents a broad-spectrum oral RNA small-molecule antiviral drug. It was originally developed in China for the treatment of human immunodeficiency virus type 1 (HIV-1) infected adult patients with a high viral load. (Page 4, lines 83-86)

*In this line 84, "It means nucleic acid negative conversion" perhaps can instead say "its mechanism is via"*

**Response:** Thank you for the recommendation. We have taken your suggestion into account and revised the sentence.

**Changes in the text:** Its mechanism is via nucleic acid negative conversion (NANC), particularly by shortening the time required for NANC in mild to moderate cases compared to standard antiviral therapy. (Page 4, lines 94-96)

*Change no-FNC to non-FNC throughout.*

**Response:** Thank you for pointing this out. We have made the recommended change, replacing "no-FNC" with "non-FNC" throughout the manuscript. We appreciate your attention to detail and guidance in ensuring clarity and consistency in our paper.

*Cannot infer lab values--would delete line 268.*

**Response:** Thank you for your suggestion. We concur with your recommendation and have removed line 268. In the revised manuscript, we now only mention the ultra-sensitive CRP and procalcitonin levels without speculating on the IL-6 and serum troponin levels. We appreciate your feedback in ensuring the accuracy and clarity of our findings.

**Changes in the text:** In the non-FNC group, data for IL-6 and serum troponin levels were unavailable for one patient. Nonetheless, this patient exhibited significantly elevated ultra-sensitive CRP and procalcitonin levels during the same period. (Page 11, lines 254-256)

*When it is stated, "Conversely, patients in the FNC group demonstrated lower inflammatory markers and D-dimers," please clarify the timing labs were collected.*

**Response:** Thank you for pointing this out. The laboratory test results presented in Table 2 were obtained after 5-day treatment. This has been clarified in the revised manuscript.

**Changes in the text:** Conversely, patients in the FNC group demonstrated lower inflammatory markers and D-dimers after 5-day treatment. Detailed laboratory results are presented in Table 2. (Page 11, lines 260-261)

*Please clarify the duration of FNC.*

**Response:** Thank you for your inquiry. The duration for FNC administration was clarified in the Methods.

**Changes in the text:** The duration for FNC administration lasted 5-9 days until the patient achieved nucleic acid negative conversion. (Page 7, lines 153-155)

*Change in lines 304-305 positive COVID-19 test to positive SARS-CoV-2 test.*

**Response:** Thank you for pointing that out. We have made the necessary changes in lines 304-305, replacing "positive COVID-19 test" with "positive SARS-CoV-2 test" as suggested.

**Changes in the text:** Subgroups A1 and A2 were formed based on the interval between the first positive SARS-CoV-2 test and the initiation of FNC, with A1 receiving FNC within 5 days and A2 after 5 days. (Page 12, lines 292-294)

*In line 358, please clarify what you are comparing to: "emerged that younger male patients had a more expedient recovery period."*

**Response:** Thank you for bringing this to our attention. In line 358, we intended to refer to the data presented in Figure 3E and 3F. Specifically, Figure 3E illustrates that younger patients (those aged ≤ 60 years) experienced a shorter NANC time in comparison to their counterparts aged 60-80 and those aged ≥80. Similarly, as demonstrated in Figure 3F, male patients had a shorter NANC time when compared to females. We have made the necessary adjustments in the manuscript to provide clarity on this comparison.

**Changes in the text:** As shown in Figures 3E and 3F, younger patients (those aged ≤ 60 years) experienced a shorter NANC time in comparison to their counterparts aged 60-80 years and those aged ≥80 years. Similarly, male patients had a shorter NANC time when compared to females. (Page 14, lines 345-348)

*Please clarify what this means and how this conclusion was reached: "none of the four baseline variables were deemed significant"*

**Response:** Thank you for pointing this out. In our study, when we referred to "none of the four baseline variables were deemed significant," we meant that upon analysis, there was no statistically significant difference in outcomes between groups when segmented based on these four baseline variables: severity of COVID-19, age, sex, and comorbidities. It is pertinent to note, however, that the lack of observed statistical significance can be attributed, in part, to the small sample size of our study.

**Changes in the text:** Therefore, for these six ICU patients receiving FNC, none of the four baseline variables (severity of COVID-19, age, sex, and comorbidities) were deemed significant. (Pages 15, lines 350-352)

*Clarify what " numerical advantage for" means as unclear.*

**Response:** Thank you for seeking clarity. By "numerical advantage for," we are referring to observed differences in the measured values or outcomes between groups. While these differences did not reach statistical significance, likely due to the small sample size, they were still present in a numerical sense and might suggest potential clinical benefits.

**Changes in the text:** While these differences did not reach statistical significance, likely because of our small sample size, there was a noticeable numerical trend favoring patients aged under 60. This might be influenced by the tendency for younger male patients to contract the disease, suggesting potential clinical benefits. (Page 15, lines 352-355)

*Once the aforementioned portions are revised, then the Discussion will need to be clarified.*

**Response:** Thank you for the feedback. We have made the necessary revisions as suggested in the respective sections of the manuscript. Following these modifications, we have also made appropriate adjustments to the Discussion section to ensure clarity and coherence with the updated content. We believe these changes now provide a more accurate representation of our findings and address the concerns raised.

**Changes in the text:** Our study, while offering preliminary insights into the potential benefits of FNC against the Omicron BA.5.1.3 subvariant, is not without its limitations. Foremost, the small sample size of 13 patients restricted the generalizability of our findings to a broader population, and results should be interpreted with caution. The absence of a true control group poses another limitation. The diversity in treatments challenged the robustness of our comparative analysis, as there wasn't a consistent standard of care against which FNC's effects could be directly measured. Furthermore, the retrospective nature of our study inherently carried potential biases, such as selection bias and data incompleteness. While our findings provided an initial indication of FNC's potential utility, larger, prospective, randomized controlled trials are essential to validate and build upon these observations.

In summary, these findings imply that FNC may be a feasible treatment option for the novel coronavirus omicron BA.5.1.3 variant, with no significant side effects. Moreover, FNC did not impact the time to NANC in the special population infected with SARS-CoV-2 omicron subvariant in the ICU and exhibited a commendable safety profile. It is anticipated that a national multi-center clinical study will be conducted to further determine the real-world effectiveness of FNC, which could potentially confer greater benefits to younger patients. Although these results offer a glimmer of hope to COVID-19 patients, further research remains imperative. (Pages 18-19, lines 443-461)

**Reviewer C**

1. The citations of Ref. 10 and Ref. 20 in the main text were missing. Please indicate where you would like to cite them in the main text. The references should be cited in order of their appearance in the text.

**Response:** We are sorry for that. The initial references #10 and #20 have been removed

in the last revision. In addition, we have checked the references throughout the manuscript to ensure that they are appropriately cited.

2. The authors mentioned "studies...", while no reference was cited. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.
*Despite ongoing global vaccination efforts, recent studies reveal a significant decline in the humoral immune response to SARS-CoV-2 over time, implying a sustained risk of infection post-immunization.*
*Existing studies suggest that in the early stages of COVID-19, patients typically present with normal or diminished total leukocyte counts in peripheral blood.*

**Response:** We have added a systematic review for the first statement, and added 5 references for the second one.

3. The authors mentioned "a study...", while no reference was cited. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.
*A phase III clinical study has reported that the 4-day NANC rate of FNC is 100% .*

**Response:** We added a reference to back the statement. Besides, we have corrected this sentence as "A small randomized clinical trial has reported that the 4-day NANC rate of FNC is 100%".

4. The authors mentioned "future studies...", while reference 25 was cited. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.
*Additionally, future studies with larger sample sizes are necessary (25)*

**Response:** The reference was removed.

5. Table 2
Please indicate how the data are presented in Tables. For example, Data are presented as No. (%). Data are presented as mean ± standard deviation, median (interquartile range), or number (frequency).

**Response:** The data are presented as mean ± standard deviation (range). It was clarified as a footnote.

6. ALL abbreviations used in each table/figure or table/figure description should be defined in a footnote below the corresponding table/figure. Please check all figures/tables and provide correspondingly.
For example
FNC in Table 4
AZ in Figure 2
AZ, FNC in Figure 3
AZ, FNC in Figure 4

**Response:** We have revised Figures 2-4 and verified all abbreviations in the Tables and Figures.

7. Figure 2A-E

Please provide the unit for the x-axis

**Response:** The x-axis is revised as “Treatment time, day”.

8. Figure 2B

It seems that the groups (≤60 and 60~80) both covered 60 and the groups (60~80 and ≥80) both covered 80. Please make sure that the grouping is correct.

**Response:** The groups were <60, 60-80, and >80. It was corrected in Figure 2.

9. Figure 3A-F

Please provide the unit for the x-axis

**Response:** The x-axis is revised as “Treatment time, day”.

10. Figure 3B, 3E

It seems that the groups (≤60 and 60~80) both covered 60 and the groups (60~80 and ≥80) both covered 80. Please make sure that the grouping is correct.

**Response:** The groups were <60, 60-80, and >80. It was corrected in Figure 3.

11. Figure 4B-D

Please provide the unit for the x-axis

**Response:** The x-axis is revised as “Treatment time, day”.

12. Figure 4B, 4C

It seems that the groups (≤60 and 60~80) both covered 60 and the groups (60~80 and ≥80) both covered 80. Please make sure that the grouping is correct.

**Response:** The groups were <60, 60-80, and >80. It was corrected in Figure 4.