Dear Editor and Reviewers,

We are very pleased to have your comments concerning our manuscript entitles "*Pneumocystis jirovecii* pneumonia diagnosed by next-generation sequencing of bronchoscopic alveolar lavage fluid：A case report" (Manuscript NO: 79340). Thank the editor and reviewers for taking time out of your busy schedule to review our paper and provide constructive comments on it. Those comments are all vaiuable and helpful. We have studied all comments carefully and made corrections based on them.

The responses to the reviewers's comments are presented following:

Reviewer #1：The article is within the scope of the journal and deals with an interesting topic. It is well written. reading is fluent However, it cannot be accepted in the current state. Some aspects need to be improved: a) A section on the state of the art should be included. b) The materials and methods used should be better explained. c) A discussion section should be included where the results of the work presented are compared with other similar works, showing the advantages and limitations. d) The conclusions must indicate the scientific contribution of the article and establish a set of lines of future work.

Response: Thank you for your encouraging comment! We add the materials and methods of this technology and details of its application in FINAL DIAGNOSIS. In addition, we explain the current status of NGS of BALF for the diagnosis of rare infections and compare the advantages and disadvantages of this technology with other related technologies in DISCUSSION. In the end, in CONCLUSION, we add the scientific significance of this article.

Please see below for point-by-point responses:

a) A section on the state of the art should be included.

Response:Traditional diagnostic methods, such as smear microscopy and induced sputum culture, have a low positive rate for the diagnosis of *Pneumocystis jirovecii* infection. Lung tissue biopsy is a means of trauma examination, which is difficult to carry out clinically. Bronchoalveolar lavage fluid (BAL) can provide targeted

sampling of the lower respiratory tract. The positive rate of diagnosis can reach 90% ~ 99%. As a new detection method independent of microbial culture, NGS is a second-generation gene sequencing technology with the advantages of high throughput, wide coverage and high accuracy. NGS can directly detect the nucleic acid sequence of pathogenic bacteria in the samples and determine their type and proportion . It can be used for the detection of not only a variety of pathogens, such as bacteria, fungi, viruses, and parasites, but also a variety of specimens, such as sputum, blood, cerebrospinal fluid, alveolar lavage fluid, and tissue. Compared with the traditional culture method, NGS has higher detection rate and higher negative predictive value. At present, NGS has been successfully used to diagnose and treat difficult and critical infectious diseases, identify unknown pathogens, monitor drug-resistant genes, carry out epidemiological follow-up investigations, etc.

b) The materials and methods used should be better explained.

Response:A bronchoscopy lavage fluid NGS test was thus performed on March 25, 2022. The patient underwent bronchoscopy, and the fiberoptic bronchoscope reached the trachea and bronchi of both lungs. Irrigation with sterile saline was performed repeatedly in the left lower lobe bronchus and right lower lobe bronchus. The collected samples were then sent to the laboratory for NGS testing and analysis, which revealed *Pneumocystis jirovecii* infection.

c) A discussion section should be included where the results of the work presented are compared with other similar works, showing the advantages and limitations.

Response: PJP diagnosis is difficult. The clinical features of PJP are not specific, and the diagnosis mainly depends on the detection of pathogens. X-ray and CT chest examinations lack specificity in PJP; Microscopic inspection is commonly utilized to confirm PJP, and clinical specimens typically employed include induced sputum, bronchoalveolar lavage fluid, and lung tissue biopsy, but induced sputum culture has a low positive rate, and lung tissue biopsy is traumatic and difficult to carry out clinically. The bronchoscopic alveolar lavage fluid (BALF) technique allows targeted

sampling of the lower respiratory tract with a diagnostic positivity rate of 90% to 99% compared to sputum analysis. Polymerase chain reaction (PCR) of alveolar lavage fluid samples is a reliable method for diagnosing PJP, with some studies indicating that PCR has a sensitivity of ≥97% and a negative predictive value of ≥99% .

NGS is a second-generation gene sequencing technology with the advantages of high throughput, wide coverage and high accuracy. NGS has been successfully used to diagnose and treat difficult and critical infectious diseases, identify unknown pathogens, monitor drug resistance genes, and in epidemiological follow-up investigations, to name a few. NGS detection of alveolar lavage fluid can offer a rapid diagnosis and determine the correct anti-infection treatment. Notably, BALF's second-generation sequencing is more sensitive and specific than older approaches in diagnosing HIV-negative PJP . NGS still faces many problems with a widespread application. Diagnosis by NGS testing needs to be combined with host factors, and chest CT findings. NGS pathogen test can only identify the pathogen, but cannot detect antimicrobial susceptibility. In addition, the current research on NGS testing of atypical respiratory pathogens is mostly in the form of case reports and clinical studies with a small sample size. Further clinical studies with a larger sample size are required to compare its sensitivity and specificity with traditional detection methods.

d) The conclusions must indicate the scientific contribution of the article and establish a set of lines of future work.

With the widespread use of anti-tumor immunosuppressants, the risks of lung infection with atypical bacteria or fungi in cancer patients have increased, and existing traditional diagnostic procedures make such infections difficult to diagnose. Therefore, in the event that tumor patients experience lung infection after anti-tumor treatment and the effect of conventional diagnosis and treatment are unsatisfactory, evaluating BALF with NGS technology can be used to detect pathogens and determine the correct treatment plan for such patients. Collectively, we believe that this approach is promising in the early diagnosis of such infections and deserves more clinical attention.

Reviewer #2：I concern and want to review related to the imaging of the patient. Pneumocitis jerovici was actually have the sign mimicking another diagnosis. Regarding this issue, I want to give some suggestions below : 1. Author should mention the sign found in CT scan or chest x-ray to define Pneumocitis jerovici although it has been supported by other modality of diagnosis (laboratory based) 2. I suggest the author to include the clear imaging related to the diagnosis. It would help the reader to differenciate and confirm the diagnosis of Pneumocitis jerovici.

Response: We thank you for the critical comments and helpful suggestions.The typical imaging feature of *Pneumocystis jiroveci*i pneumonia (PJP) in HIV-infected patients is diffuse ground-glass opacity in both lungs centered on the pulmonary hilum, with or without pneumatocele, progressing towards patchy and diffuse consolidation in both lungs; some patients show interstitial changes.Imaging features of non-HIV-related PJP are atypical, We add supplementary figures of this patient to further illustrate the CT signs of PJP.

Revision reviewer：The paper can be accepted in current form

Response: Thanks for your comments.

Thank you for your consideration!