Peer Review File

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Reviewer A

The article is not well presented and lacks enough data to support conclusions

Reply 1: Dear reviewer, thank you for your review comments. In this manuscript, we combined a multi-level GWAS summary data file to explore the efficacy of drug-targeting genes in non-small cell lung cancer (NSCLC) using Mendelian randomization. In addition, open databases were used for gene difference analysis and single-cell omics analysis to verify the potential role and prognostic value of genes in NSCLC, and databases such as DGIbd and Drugbank were also used to find out whether there is relevant evidence for drugs that have been validated in clinical studies and the value of drug remining. We hope that the above explanations in this manuscript could explain our research purpose clearly: to find potential drug targets for NSCLC at the genetic level.

Reviewer B

This study is intriguing as the authors employed a genome-wide Mendelian randomization approach to pinpoint new drug targets for non-small cell lung cancer, demonstrating clinical significance. I recommend a concise overview of established genetic drug targets for NSCLC and an examination of clinical challenges in uncovering additional genetic drug targets, as well as why genome-wide Mendelian randomization approach is optimal. Additionally, I urge the consideration of racial and ethnic disparities in source sample selection for GWAS data in the methodology.

Reply 2: Dear Reviewer, thank you for your interest in our manuscript and for your valuable comments and suggestions. We appreciate the time and effort you have taken to review our work, and we are grateful for your constructive feedback. Below, we address your specific comments and outline the changes made to the manuscript.

Comment 1: "I recommend a concise overview of established genetic drug targets for NSCLC and an examination of clinical challenges in uncovering additional genetic drug targets, as well as why genome-wide Mendelian randomization approach is optimal."

Response 1: We appreciate your recommendation to enhance the manuscript by including a concise overview of established genetic drug targets for non-small cell lung cancer (NSCLC), examining clinical challenges in uncovering additional genetic drug targets, and discussing the advantages of the genome-wide Mendelian randomization approach. We have revised the manuscript accordingly, and the following sections have been added or modified:

Overview of Established Genetic Drug Targets for NSCLC: In the revised manuscript, we have included a section that provides a concise overview of established

genetic drug targets for NSCLC. This section highlights key genetic alterations and the corresponding targeted therapies that have been developed. Notable targets such as EGFR, KRAS, ALK, ROS1, BRAF, MET, RET and so on have been discussed, along with their clinical implications and current therapeutic strategies.

Change in the text: Page 7, Line 85-94 (Introduction), as stated in the manuscript: " NSCLC is characterized by possessing various genetic drivers that tyrosine kinase inhibitors can specifically target. Established actionable drivers include the Epidermal Growth Factor Receptor (EGFR), Kirsten Rat Sarcoma Viral Oncogene (KRAS), Anaplastic Lymphoma Kinase (ALK), ROS Proto-Oncogene 1, Receptor Tyrosine Kinase (ROS1), B-Raf proto-oncogene, serine/threonine kinase (BRAF), MET Proto-Oncogene (MET), and RET proto-oncogene (RET) (1-7). Each of these gene alterations plays a crucial role in driving tumor growth and progression at the genetic level, and tyrosine kinase inhibitors tailored toward these genes have significantly improved patient outcomes by cutting off the driving power behind tumorigenesis. "

An examination of clinical challenges in uncovering additional genetic drug targets: To address this comment, we have included a dedicated section in the Discussion part of the manuscript. This section discusses various hurdles including tumor heterogeneity, the complexity of genetic interactions, and the limitations of current technologies. We have also explored the implications of intratumor heterogeneity and the tumor microenvironment in complicating the identification of new actionable genetic targets.

Change in the text: Page 19, Line 341-344 (Discussion). As discussed in the manuscript: " Despite advancements in targeted therapy, discovering new genetic targets remains challenging. Many barriers to the clinical success of targeted drugs include tumor heterogeneity, genetic complexity, and technological limitations."

The Rationale for Using Genome-Wide Mendelian Randomization in NSCLC Research: In response to the reviewer's suggestion, we have elaborated on the advantages of the Mendelian randomization (MR) approach in Discussion. This section explains how MR can help infer causality by using genetic variants as instrumental variables. We have detailed the strengths of this approach in overcoming confounding and reverse causation, which are significant limitations in traditional epidemiological studies.

Change in the text: Page 20, Line 369-373 (Discussion). As discussed in the manuscript: "Secondly, this study employed a novel MR approach, useing QTL-related SNPs as instrumental variables and anchoring of the druggable gene sites to translate drug effects into gene-level effects. By utilizing genetic variants as instrumental variables, MR can address confounding and reverse causation and provide more reliable insights into genetic associations."

Comment 2: "Additionally, I urge the consideration of racial and ethnic disparities in source sample selection for GWAS data in the methodology."

Response 2: We appreciate the reviewer's suggestion to consider racial and ethnic disparities in source sample selection for Genome-Wide Association Studies (GWAS) data. In response, we have stated in the limitation part of the discussion that there are some non-European people among the eQTL participants, which can be seen in Page 21, Line 389 (Discussion), as state: " Thirdly, the eQTL cohorts included some non-European individuals.".

We thank the reviewer for suggesting improvements to the overall structure of the

manuscript, which is indeed essential for standardizing the presentation of our study. Thank you once again for your valuable feedback.

1. Planchard D, Jänne PA, Cheng Y, et al. Osimertinib with or without Chemotherapy in EGFR-Mutated Advanced NSCLC. N Engl J Med 2023;389:1935-48.
2. Felip E, Shaw AT, Bearz A, et al. Intracranial and extracranial efficacy of lorlatinib in patients with ALK-positive non-small-cell lung cancer previously treated with second-generation ALK TKIs. Ann Oncol 2021;32:620-30.
3. Moro-Sibilot D, Cozic N, Pérol M, et al. Crizotinib in c-MET- or ROS1-positive NSCLC: results of the AcSé phase II trial. Ann Oncol 2019;30:1985-91.
4. Riely GJ, Smit EF, Ahn MJ, et al. Phase II, Open-Label Study of Encorafenib Plus Binimetinib in Patients With BRAF(V600)-Mutant Metastatic Non-Small-Cell Lung Cancer. J Clin Oncol 2023;41:3700-11.
5. Riedel R, Fassunke J, Scheel AH, et al. MET Fusions in NSCLC: Clinicopathologic Features and Response to MET Inhibition. J Thorac Oncol 2024;19:160-5.
6. Zhou C, Solomon B, Loong HH, et al. First-Line Selpercatinib or Chemotherapy and Pembrolizumab in RET Fusion-Positive NSCLC. N Engl J Med 2023;389:1839-50.
7. Hanna NH, Robinson AG, Temin S, et al. Therapy for Stage IV Non-Small-Cell Lung Cancer With Driver Alterations: ASCO and OH (CCO) Joint Guideline Update. J Clin Oncol 2021;39:1040-91.