Peer Review File

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Reviewer A

This manuscript reports on clinical data and genomic profiling of a very small cohort of 31 patients with relapsed neuroendocrine small cell lung cancer (SCLC). The main problem of this manuscript is the lack of novelty and the lack of clinical impact on the findings concerning repetitive somatic (or germ-line) variants.

Major

Since the landmark publication of George J, 2015 in Nature it is well known that SCLC typically reveals destructive genomic alterations/mutation in all 4 alleles of p53 and RB; and further that many other somatic repetitive mutations may occur.

The clinical data show poor outcome of these patients, which is known since approximately 30 years and true for all eographic regions world-wide.

Importantly, such additional somatic mutations do not cause genomic dependence and do not cause vulnerability to targeted therapies, i.e. for EGFR-TKIs in the case of EGFR mutations. Such the conclusions of the authors are misleading.

An astonishingly high frequency of mutations was observed in HRR genes (32% BRCA2, 13% ATM, and 10% FANCA). It is not clear whether these mutations refer to clearly pathogenic mutations (category 5) and whether their is biallelic inactivation causing an HRD-positive tumor phenotype.

Reply 1: We thank the reviewer for their objective and positive comments. Our studies first exhibited comprehensive genomic profiling of relapsed SCLC, identifying several candidate genes, and briefly analyzed the association of survival and genomic alterations. Like **Reviewer A** said, several somatic mutations do not cause genomic dependence and do not cause vulnerability to targeted therapies in SCLC, i.e. for EGFR-TKIs in the case of EGFR mutations. Our study also exhibited a poorer prognosis of patients with EGFR mutations than NSCLC: After EGFR tyrosine kinase inhibitor (TKI) treatment, a male patient (19-del) exhibited a PFS of only 1.87 months, and the PFS of 2 females (L858R) were 5.1 and 7.7 months, respectively.

Additionally, in this cohort of relapsed SCLC, apart from the most common TP53 and RB1 mutations, high-frequency mutations in the DNA damage repair pathway were also observed including BRCA2 (32%), ATM (13%), and FANCA (10%), which typically indicate those patients are otherwise sensitive to the blockade of DNA damage repair response (DDR). However, two patients with confirmed BRCA2 mutations failed to benefit significantly from

olaparib monotherapy, achieving only 2.5 and 3.1 months of disease responses, respectively. Previous studies have shown (1,2) that DDR inhibitor can synergise with immune checkpoint inhibitors e.g. PD-1 or PD-L1 via the cGas-STING-IFN stimulation, which makes them a promising option for recurrent SCLC with DDR vulnerabilities.

Changes in the text: In order to avoid the misleading conclusions, we added sentences in ***Discussion*** part (see Page 10, lines 28-30 and page 11, lines 7-9).

Reference:

1.Zhang N, Gao Y, Huang Z, et al. PARP inhibitor plus radiotherapy reshapes an inflamed tumor microenvironment that sensitizes small cell lung cancer to the anti-PD-1 immunotherapy. Cancer Lett. 2022;545:215852.

2.Sen T, Rodriguez BL, Chen L, et al. Targeting DNA Damage Response Promotes Antitumor Immunity through STING-Mediated T-cell Activation in Small Cell Lung Cancer. Cancer Discov. 2019;9:646-661.

Reviewer B

General comments:

In this study, the authors evaluated blood samples and revealed genomic profiling of relapsed SCLC patients. Some of the observations are interesting, but there are several concerns that the authors need to address.

Specific comments:

i) Results (page 7, lines 8-12). The authors described that TMB is lower than previously reported data (references 15-17). The reviewers think those references' data were obtained from tissue samples, not blood samples. Thus, the authors should select adequate references and correct the description related to TMB throughout the whole manuscript.

Reply 1: Thanks for the comments. We have updated references and corrected the description related to TMB throughout the whole manuscript.

Changes in the text: In order to avoid the misleading conclusions, we have modified our text (see Page 7, line 9 and Page 14, reference 20).

ii) Results (page 8, lines 20-22). Based on what genomic profile did the author use an anti-angiogenetic TKI?

Reply 2: Thanks for the comments. Anti-angiogenetic TKIs have been already approved for third-line treatment of SCLC patients regardless of the presence or absence of driver mutations. The genomic profile of the three patients (No. 9, 11, and 14) receiving anti-angiogenic TKI treatment was exhibited in Figure 1.

Changes in the text: We have modified our text as advised (see Page 8, line 21).

iii) Conclusions. Please summarize more adequately and shortly.

Reply 3: Thanks for the comments.

Changes in the text: We have modified our Conclusions as advised (see Page 11, lines 19-22).

iii) Table. The authors include the number of "31" in every characteristic of patients, but those are unnecessary.

Reply 4: Thanks for the comments.

Changes in the text: We have deleted the number of "31" in Table 1 as advised (see Pages 15-16, Table 1).

v) Keyword. What is "breast cancer2"?

Reply 5: Thanks for the comment. We are very sorry for the mistake leading to misunderstanding.

Changes in the text: We have modified the errors in Keywords (see Page 2, line 27).

Reviewer C

The authors present the data of genomic profiling of 31 relapsed SCLC. The data are of interest because they deal with an important unmet need. The manuscript is well written. However, there are some points to reconsider before publishing:

- Abstract: please remove the word "eventually" in the first sentence

Reply 1: Thanks for the comment.

Changes in the text: We have modified our text as advised (see Page 2, line 2).

- Please provide more detailed information on the method you used for NGS. What DNA was used (whole blood? ctDNA?)

Reply 2: Thanks for the comment.

Changes in the text: We have added more detailed information on the method for NGS (see Page 5, lines 12-14).

- Where there any samples with no results in the testing due to technical reasons? Normally nearly 100% of SCLCs harbour TP53 and/or RB1 mutations. The frequency in your cohort is lower. Why?

Reply 3: Thanks for the comment. In our cohort, among the 31 blood samples used for DNA extraction, no differences were observed in the quality of sequencing results. All 31 relapsed SCLC specimens (100%) harbored at least 1 genomic alteration. Indeed, TP53 and RB1

mutations were reported the most frequent genetic alterations but its mutation frequency is not 100% (1). Our cohort also exhibited TP53 and RB1 mutations were the most frequent genomic alterations, whose lower frequency than those references' data might be partly attributed to relapsed SCLC.

Changes in the text: We have modified our text and highlighted them (see Page 7, lines 6-7 and lines 15-17; Page 9, line 33 and Page 10, lines 1-2).

Reference:

1.Sivakumar S, Moore JA, Montesion M, et al. Integrative Analysis of a Large Real-World Cohort of Small Cell Lung Cancer Identifies Distinct Genetic Subtypes and Insights into Histologic Transformation. Cancer Discov. 2023;13:1572-1591.

- please discuss the high rate of EGFR-Mutations in your cohort. Are these cases transformations of EGFR-Mutation Adeno-carcinomas?

Reply 4: Thanks for the comment. In our cohort, there are 3 patients (No. 18, 1, and 21) with common EGFR mutation. And none of these cases is transformation of EGFR-Mutation Adeno-carcinomas. The high rate of EGFR-Mutations in our cohort is partly explained by a small sample study, which inevitably resulted in a selection bias.

Changes in the text: We have modified our text as advised (see Page 11, lines 3-5).

- Please discuss also the new genomic classification of SCLC subtypes (ASCL1, NEUROD1, POU2F3 and YAP1) in the context of your findings.

Reply 5: Thanks for the suggestion. The new classification of SCLC subtypes is based on expression of ASCL1, NEUROD1, POU2F3 and YAP1. Unfortunately, no expectational gene expression were detected in our cohort.

Changes in the text: We have added discussion on the new classification of SCLC subtypes (see Page 9, lines 4-7).