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Reviewer A

The authors described that this retrospective study showed that the median TMB for six long-term survival (LTS) patients (≥4years) with stage IIB or IIIA small cell lung cancer (SCLC) was high and five short-term survival (STS) patients (<2 years) with stage IA or IB SCLC was low. FAT3 mutation could serve as useful prognostic biomarkers for LTS in resected SCLC.
Major comments:
1. The focus of the test is interesting, but the overall number is too small for statistical analysis. Although operable small cell lung cancer patients are very rare, the study would be suggestive if it were a multicenter study and the analysis were more precise.
Reply: Thank you for this very insightful comment. Your suggestion gives us a solution to make up for the small sample size, we tried, but the workload of case screening and follow-up is heavy, and it is difficult to implement.

2. For TMB and FAT3 mutation, there is no mention of what the difference was between OS of 4 years or more and 4 years or less in stage IIB-III. Similarly, there is no information on the comparison of TMB and FAT3 mutation in Stage I with OS of 2 years or more vs. less than 2 years. If you have not done the tests, you should consider those results first. If you had done the tests, it is unnatural that they are not mentioned.
Reply: We gratefully appreciate for your constructive suggestion. The results would have been more rigorous if we had followed your suggestion. Our aim of the study is to explore the genetic characteristics of long-term survival in surgically resected IIB-III stage SCLC, the population with high-risk relapse, theoretically. We did not detect the genetic characteristics of all 52 patients, but only Stage IIB, IIIA patients obtaining long-term survival and LTS and stage I patients suffering short-term survival as control.

Reviewer B

I have reviewed the manuscript titled "Tumor Mutation Burden and FAT3 Mutation Influences Long-term Survival in Surgically Resected Small-cell Lung Cancer" submitted by Hailing Lian. This study investigated the relationship between FAT3 gene mutations and survival in small-cell lung cancer, presenting significant findings that contribute to the current understanding of genetic influences on cancer prognosis.

Overall, the manuscript is well structured and addresses an important topic in oncology. However, I have identified a key area that requires further clarification and detail to strengthen the manuscript's scientific rigor.

Key points for revision: The manuscript currently lacks information on the use of immune checkpoint inhibitors (ICIs) among the study participants. Previous studies have demonstrated that ICIs are particularly effective in patients with high FAT3 gene mutations. Therefore, including details on whether ICIs were administered to the patients, and if so, analyzing the impact of ICI treatment on survival outcomes, is essential. This addition will provide a more comprehensive understanding of the factors contributing to the observed survival benefits.
Reply: We thank the reviewer for the great suggestions. In this article, the subjects are early-stage patients underwent surgery and adjuvant chemotherapy, who does not need immunotherapy according to treatment guidelines. We are collecting genetic characteristics of long-term survival after immunotherapy in ES-SCLC (extensive-stage SCLC, current standard first-line therapeutic regimen as immunotherapy combined with chemotherapy), we will further pay attention to whether FAT3 and TMB are key prognostic factors in our ongoing study.

Recommendations: Patient Treatment Information: The authors should specify whether any patients with high FAT3 gene mutations received ICIs.
Reply: Patient Treatment Information: surgery and adjuvant chemotherapy.

Statistical Analysis: If relevant, the manuscript should include a comparative analysis of survival outcomes between patients treated with ICIs and those who were not.
Reply: None of the patients received immunotherapy.
Changes in the text: We added some content to the discussion "Our study focuses on the genetic characteristics of LTS postoperative patients and does not involve immunotherapy efficacy. The correlation of *FAT3* gene mutations and the potential therapeutic benefits of immune checkpoint inhibitors (ICIs) in ES-SCLC is well worth further discussion." (see Page 11, line 361-364)

By incorporating these revisions, the manuscript will be better positioned to provide valuable insights into the role of FAT3 gene mutations and the potential therapeutic benefits of ICIs in small-cell lung cancer.
Reply: Thank you for the direction. The study of long-term survival after immunotherapy is ongoing.

Reviewer C

This paper is interesting in focus the biomarker of SCLC. However, there are three major points for improvement in order to be published in this journal.

First, it is unclear just in Figure1 how they had chosen eleven patients. They have to describe the stage, the overall survival and the availability of tissue of all 52 patients. Also, is the number of Long-term survivors wrong?

Reply: We are sorry we didn't explain the procedure clearly. 52 patients underwent surgery without neoadjuvant chemotherapy before surgery and were diagnosed small cell lung cancer by pathology. And the errors in the diagram have been modified.

Changes in the text: "All 52 patients were retrospectively collected, diagnosed with conventional SCLC, and underwent surgery no neoadjuvant chemotherapy or chemoradiotherapy at Zhejiang Cancer Hospital (Hangzhou, China) between April 2008 and December 2017." (see Page 4, line 118-121); The numerical error in Figure 1 has been corrected. (see Page 15, line 496)

Second, they need to showed the correct information about the TMB value in each groups (STS and LTS). TMB values that they described in Table2 don't match to Fig2. For example, they show that the TMB value is 5.2 mutations/Mb in the No11 patient, but the minimum TMB value in Fig2 is about 20mutatins/Mb. And if they want to indicate to distribution of TMB in each group (STS and LTS), they have to use the box plot.

Reply: Thanks for your suggestion, we have checked the data and revised Figure 2 into the box plot.

Changes in the text: Figure 2 has been modified. (see Page 16, line 502)

Third, Figures 3 and 5 are too insufficient to describe the Kaplan-Meier curve and do not seem necessary.

Reply: Thank you for your suggestion, and we agree with your points. The sample size in our study is small and may not be representative. However, the results of KM curve analysis can provide some research conclusions and provide important reference value for the further accumulation of cases.

Reviewer D

In the manuscript entitled "Tumor Mutation Burden and FAT3 Mutation Influences Long-term Survival in Surgically Resected Small-cell Lung Cancer", the authors study a cohort of resected Small Cell Lung cancer patients with either short or long term survival. Via NGS testing, they assessed TMB and the genomic landscape of these tumors, and found non-significant trends for TMB and FAT3 mutations differing between survival groups.

Overall, this is an interesting study with a not-often studied subset of resected small cell carcinoma patients. However, there are some major shortcomings of this study that should be addressed:

1-From the total cohort of 52 resected Small Cell Carcinomas, only 11 were included in this study: 6 LTS and 5 STS. These "n's" are too small to make any significant conclusions, as reflected in the statistical tests not reaching statistical significance. The authors state that limiting funds precluded testing a larger cohort, but that unfortunately is not justification for publishing preliminary results on an insufficiently large cohort.

Reply: Thank you for this very insightful comment. It is the limitation of the article. Our aim is to explore the genetic characteristics of long-term survival in surgically resected IIB-III stage SCLC, the population with high-risk relapse, theoretically. As a result, the number of patients meeting these criteria is very small, with only six LTS cases in the 10 years (from April 2008 to December 2017). Your comments remind me to restate the original intention and significance of our research

Changes in the text: We added “The intention of our study is to explore the genetic characteristics of LTS in surgically resected IIB-III stage SCLC, the population with high-risk relapse, theoretically. As a result, the number of patients meeting these criteria is rare.” We deleted “The sample sizes of similar studies range from 40 to 50 cases (1,2). In our study, 52 SCLC patients who underwent surgery were preliminarily eligible. Because of the limitation of our research funds, we ultimately selected 11 patients between the LTS and STS cohorts to explore any genomic differences.” (see Page 11, line 343-345)

2-How did the authors decide on 4 years as the cutoff for LTS, and 2 years for the cutoff for STS? Is there a reference for use of that timeframe for long/short term survival for small cell carcinoma? It seems a bit arbitrary.

Reply: Thank you for your advice. We referred to the article (3) (J Thorac Oncol. 2019 Jul;14(7):1286-1295. PMID: 31078775) on the definition of LTS (OS ≥ 4year). We will try to explain this definition. At present, researchers define the time points of long-term survival according to the median OS in different tumor types and different tumor stages. The median OS of metastatic small cell lung cancer was about 10 months. Previous studies defined the long-term survival of metastatic small cell lung cancer as OS > 24 months and the short-term survival as 2-8 weeks (Next-Generation Sequencing May Discriminate Extreme Long-term versus Short-term Survival in Patients with Metastatic Small Cell Lung Cancer (SCLC). Lohinai Z, et al. Transl Oncol. 2019. PMID: 31476386). It is reported that the median survival of stage IIB lung cancer after surgical treatment is about 24 months, and that of stage IIIA lung cancer is about 21.7 months. Therefore, we defined long-term survival as twice the median OS, and chose 4 years as the time point of long-term survival. (Wakeam E, Acuna SA, Leighl NB, et al. Surgery versus chemotherapy and radiotherapy for early and locally advanced small cell lung

cancer: a propensity-matched analysis of survival. Lung Cancer 2017;109:78—88).Also, it is reported that the median survival of stage I after surgery is 38.6-62 months. (Engelhardt KE, Coughlin JM, DeCamp MM, et al. Survival after adjuvant radiation therapy in localized small cell lung cancer treated with complete resection. J Thorac Cardiovasc Surg 2019;158:1665—77 [e2].). So, we defined the short survival as half of the median OS, and chose 2 years as the time point of short survival.

3-What about the ~34 patients with "intermediate" survival of between 2 and 4 years? The authors could consider testing this cohort as well, to see if there are: FAT3 mutations in this group (which would argue against the potential significance as a LTS predictor), TMB...is there an intermediate TMB level between the STS and LTS cohort? If there is a gradient from low to high seen, that would better support the notion that TMB is possibly related to survival.

Reply: Thank you very much for the important comments and constructive suggestions. We explain a little bit more as follows. Among the 52 patients we collected, according to the convention of disease recurrence, most of stage IA-IB patients did not recurrence, achieving long survival. However， 5 stage IA-IB patients early recrudesced, and we collected them in the STS. Patients with stage III had a high recurrence risk, but 8 patients had no recurrence or later recurrence, achieving long survival; 6 patients with sufficient specimen were collected in LTS. Not all of 34 patients' survival between 2 and 4 years.

You are right. If our conclusion is to be verified, it needs a larger cohort and multi-centers data, which is more difficult to implement. We are collecting genetic characteristics of long survival after immunotherapy in ES-SCLC, we will further pay attention to whether FAT3 and TMB are key prognostic factors.

Reviewer E

Authors aimed to provide possible prognosis factors to patients with small-cell lung cancer who were surgically treated.

Although well-intended, several aspects need to be addressed:

Abstract: background does not contain the study design. It is mentioned that 52 subjects were included, but only 11 were analyzed, so, final sample size was 11, not 52.

Reply: We completed the revision in accordance with the above requirements.

Changes in the text: The section has been changed to "The present study screened 11 patients from 52 patients with SCLC who underwent surgery at Zhejiang Cancer Hospital from April 2008 to December 2017". (see Page 2, line 44-45)

Background: there's been novel molecular features in SCLC and they were not approached in the main text. Text needs to be updated. Also, the text doesn't follow the current guidelines for these patients' treatments.

Reply: Thank you for this very insightful comment. We have added the novel molecular features and made some revisions about treatments.

The current guidelines are mentioned in the background. ES-SCLC current guidelines recommend first-line treatment is immunotherapy combined with chemotherapy. LS-SCLC guidelines recommend treatment is chemoradiotherapy and preventive intracranial irradiation (PCI). For early stage SCLC(T1-2N0M0), guidelines recommend treatment is surgery and adjuvant chemotherapy.

Changes in the text: We added "New SCLC subtypes based on key transcription regulators ASCL1-high (SCLC-A), NEUROD1-high (SCLC-N), POU2F3-high (SCLC-P), and YAP1-high (SCLC-Y) as well as on certain inflammatory characteristics（SCLC-I）were defined and analyzed in relation to tumor evolution or immunotherapy effect.(4)Prognostic relevance of transcription subtypes in surgically resected SCLC found high POU2F3 expression is associated with improved survival whereas elevated ASCL1 expression is an independent negative prognosticator (5). But the mechanism of long survival is still not convincingly explained". (see Page 3, line 93-100)

Methods: the methods section has different information from the discussion. First, authors mention that they excluded subjects from final analyses due to DNA damage and neoadjuvant therapy, later, on the discussion they said they only performed 11 NGS due to lack of proper funding. Information got confused. Please revise that.

Reply: thanks for your careful checks.

Changes in the text: We have changed this section to "All 52 patients in our analysis were diagnosed with conventional SCLC, and underwent surgery no neoadjuvant chemotherapy or chemoradiotherapy at Zhejiang Cancer Hospital (Hangzhou, China) between April 2008 and December 2017, and were followed up for at least 2 years." (see Page 4, line 118-121)

Patients characteristics: age was not described as mean or median. Regarding smoking history, the stratification of the variable is different from what was reported at the table.

Reply: Thanks for your correction. We have made the modification in the corresponding position.

Changes in the text: We have changed this section to "The median age of patients in the LTS group was 57 years (range, 49-63 years), while that of patients in the STS group was 57.8 years (range, 38-76 years). Six patients were smokers, and the five remaining patients had no history of smoking" (see Page 6, line 168-188)

Line 208: English error. I believe authors tried to say, “in four” and not “in the four”.

Reply: Thanks for your correction. We feel sorry for our error.

Changes in the text: we have modified our text as advised “The *FAT3* gene was only detected in four LTS patients”. (see Page 7, line 219)

Discussion:

- No mention to selection bias of the study sample, no mention of patients with SCLC and no history of smoking (which is highly unusual)

Reply: We sincerely appreciate the valuable comments

Changes in the text: we have modified our text as advised. “Selection bias of the study sample is inevitable, such as gender and smoking history”. (see Page 11, line 350-351)

- Try to compare data to other studies that had much larger sample sizes than this paper. Again, final sample is 11, not 52. I suggest rephrasing it in the main text.

Reply: In the third paragraph of the discussion section, we analyzed and compared the correlation between TMB and prognosis with other three studies (33,34,35). We corrected for 11 patients in both the abstract and the text.

Changes in the text: we have modified our text as advised. “our results were very consistent with previous research findings, high TMB having a better prognosis in surgically resected SCLC”. (see Page 11, line 348-350)

- There’s not enough data to provide a statement that “FAT3 mutation and survival of resected SCLC patients” as mentioned on lines 312-313. Authors must be careful with that.

Reply: After careful consideration and discussion, we deleted this sentence.

- Authors must be careful when saying the study design is strong. I suggest reviewing the paragraph starting on line 322.

Reply: Thank you for your careful and sincere suggestions.

Changes in the text: we have modified our text as advised. “Our study derived retrenchment from its design and specific patient selection”. (see Page 10, line 329)