TRANSLATIONAL LUNG CANCER RESEARCH

Peer Review File

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Reviewer A

General Comments: The manuscript presents data from the SEER registry on the characteristics and outcomes of combined small cell lung cancer (CSCLC) with comparison to other SCLC and NSCLC. This is a worthwhile project, but the presentation of data is not consistently clear and not as informative as it should be. The manuscript would greatly benefit from proofreading.

Comment: Thanks for your comments. Combined small cell lung cancer is a very special pathological type of pulmonary malignancies. Since it has both SCLC and NSCLC components, the biological behavior and progression are still unclear. Whether it acts like SCLC or NSCLC is a crucial question to be addressed. However, there are very few CSCLC patients have been diagnosed or recorded even in such worldwide project (SEER). Besides, some cases have to be filtered out during the data screening because of criteria and reasons including insufficient data, lacking of important information et.al. Therefore, the number of cases in final cohort is small. We have clarified the data selection process to make it more informative and clearer. Thank you.

Specific Comments:

1. Figures: Figure 1 on the selection process is missing and other figures are mislabeled. The figure labeled Figure 1 in the manuscript appears to actually be Figure 3, the multivariate survival analysis. Figure 2 is correct, but Figure 4 is labeled as Figure 3.

Comment: Thank you so much for your comment. We have clarified the data selection process and made a new Figure 1 for better demonstration of the selection process. All the figures have been labeled and re-cited in the manuscript.

2. Results, Clinical features: a) Text should focus on pointing out differences and similarities between CSCLC and other SCLC. b) In Table 1, the last column (Total) does not add anything to the paper. It would be more information for the last column to be a statistical analysis of CSCLC vs. SCLC.

Comment: Thanks for your suggestion. In table 1, the last column (Total) stands for the total SCLC patients which include both CSCLC and other types of SCLC. We have changed the title of second column from SCLC to other SCLC to clarify the difference from CSCLC and total SCLC. The main purpose of current study is to demonstrate the characteristics of CSCLC and try to figure out its biological and clinical features. Therefore, we intend to compare CSCLC to other types of SCLC. The total cohort of SCLC includes CSCLC, which makes it inappropriate to be compared with CSCLC directly. We have added the statistical analysis of CSCLC vs. SCLC in terms of clinical characteristics in Table 1.

3. Results, Survival outcomes by stage: a) The inclusion of cancer-specific survival (CSS) does not add useful information and only confuses the presentation. Overall survival (OS) is the only important survival statistic here. CSS should be deleted from the text and Figure 2. b) Prior to presenting survival by stage groupings, should present OS of the whole group of CSCLC vs. SCLC and CSCLC vs. NSCLC. c) The presentation of data and statistics in the text is not consistent. The same data should be presented in the same format for each of the stage groupings. Statistical analysis should be presented for both CSCLC vs. SCLC and CSCLS vs. NSCLC for each of the stage groupings. d) In Figure 2, it is not clear which comparison the stated p-value is for (CSCLS vs SCLC or CSCLC vs NSCLC). e) In the MVA figure (Figure 3, but included as Figure 1), the number of CSCLC cases is 785, but throughout the rest of the paper (and adding up rows on Figure 3) the number of cases is 784.

Comment: Thanks for the suggestion. We have checked the raw data and re-run the selection process again. The final number of primary cohort is 784. The article and table have been revised. The OS is the primary endpoint which is very important in the prognosis analysis. It is not only related to the severity of disease but also to the treatments for the disease. Therefore, we considered CSS would be an ideal complement for analyzing treatments for CSCLC. In order to avoid to confusion created by CSS, we put it to Figure S1. The p-values of comparisons between groups are presented in the new Figure 2. Thanks for your advice.

4. Results, Survival by treatment: a) Need to do a better job of describing treatments. Groupings should be surgery alone, surgery + chemotherapy, chemotherapy alone, radiotherapy alone (if applicable), chemoradiotherapy, and surgery, chemotherapy and radiotherapy. Saying just adjuvant chemotherapy is confusing as this could be after surgery or after radiotherapy. b) In the overall assessment of the data, you need to consider the reasons why treatment might be done, particularly for patients with stage IIA-IIIA or IIIB-IV when they had surgery. All of these people must have had clinical stage I disease that was found to be more extensive during surgery or on pathologic examination of surgical samples. They would not have gone to surgery if they had clinical stage III or IV disease. They are a very different population from someone who had clinical stage III or IV disease, so they will have better survival just because they had lower bulk of disease starting out - may not have anything to do with treatment.

Comment: We believe your comments are reasonable and very inspiring. We have made a table 2 to demonstrate the number of CSCLC patients in different treatments groups stratified by tumor stages. We believe this table would be clear to indicate the preference and propensity of treatments the patients had received. We also regrouped the treatment arms in figure 4 and showed more detailed KM comparisons among them. In order to minimize the influence on survival analysis, some treatment groups, which only had very few patients, were not included in the comparisons. We shared the same understanding with you that some advanced stage patients might be accidentally

diagnosed as higher stages during or after the surgeries. Unfortunately, the SEER database did not offer such detailed information. In the analysis, we noticed that trimodality treatment could bring remarkable better prognosis to stage IIIB-IV patients. These patients might benefit from debulking surgeries.

5. Discussion: The retrospective nature of this analysis is a major limitation that needs to be included in the discussion. As noted above, the selection bias involved in selecting treatments could affect outcomes even more than the treatment that was received, so making statements about what treatment is better is clearly confounded by selection. This is a major problem for stage III and IV patients who underwent surgery, so saying "In local and distant advanced CSCLC patients, we found that trimodality treatment … could improve OS" is not just misleading, but dangerously wrong, and could lead to substantial inappropriate treatment and harm for future patients. Even the final conclusion that "surgical treatment is crucial in early stage CSCLC" is overstated (though I do believe based on all data available that surgery is appropriate for stage I patients with SCLC and CSCLC).

Comment: Thanks for your suggestion. We believe that retrospective analysis is the major limitation as well. Since CSCLC has a relatively low incidence, it is difficult to perform a prospective study or even a propensity score matching study which would potential eliminate useful data. Therefore, treatment selection bias definitely exists. As we can see that early stage patients tend to receive surgical treatments while advanced stage patients are mostly administered chemotherapy and chemoradiation. As a matter of fact, we would try to show and state what the data looks like rather than give any assertive suggestion or recommendation. Therefore, we rewrite the discussion and conclusion to make them more objective. Thank you again for your advice.

Reviewer B

In this study, Authors provide a valid study on a rare entity (combined small cell lung cancer, CSCLC). The manuscript is well written, clear and informative. I have only minor comments.

Results

As this is a very detailed cohort of a rare entity, I ask Authors if it is possible to provide additional data:

Would it be possible for Authors to obtain the smoking histories of patients?

Is it possible to retrace the precise combined NSCLC histology to SCLC, allowing the diagnosis of CSCLC?

Have any patient in the cohort being tested for molecular aberrations typical of NSCLC (EGFR, ALK…), with particular regard to non/light smoker patients?

Comment: Thank you for your comments. We understand that smoking history is a risk factor in SCLC. Therefore, it is also crucial to investigate its importance in CSCLC. Unfortunately, the SEER

database does not provide smoking history.

We believed that the diagnosis of CSCLC is very difficult from the biopsy specimens, since the amount of tissue is limited. Therefore, some cases of CSCLC would have been mis-diagnosed as SCLC or NSCLC. We do have a plan to retrace the precise histology. Thanks for the suggestion.

The SEER database does not provide molecular aberrations. But according to the previous studies or case reports, the molecular content of NSCLC in CSCLC is relatively independent. It means whether it has SCLC content or not does not quite influence the molecular aberrations of NSCLC content. Therefore, molecular targeting treatment could be considered if it is appropriate.

Survival Outcomes of CSCLC in Different Treatments

With regard to stage IIA to IIIA patients (Fig. 3B): what authors mean with "adjuvant chemotherapy": patients who have undergone surgery and then adjuvant chemotherapy? Other? Please specify both in the text and in the Figure.

Comment: Thanks for your suggestion. In order to demonstrate the treatment data more specifically, we have added Table 2 to indicate the number of each treatment or treatment group in the cohort. Moreover, we re-evaluated the data and revised the Figure 4. In the current study, we defined adjuvant chemotherapy as surgery in combination with chemotherapy, which included adjuvant and neo-adjuvant chemotherapy. Unfortunately, SEER does not provide the regimen or sequence of chemotherapy. Therefore, it is difficult to investigate the different outcomes between adjuvant and neo-adjuvant chemotherapy.

With regard to stage IIIB and IV (Fig. 3C): can Authors specify which kind of radiation therapy is included in "chemoradiation"? Thoracic RT with chemo for IIIB and/or thoracic RT after chemo for stage IV (consolidation, see Slotman Lancet 2014 for SCLC) and/or prophylactic cranial irradiation (PCI) for stage IIIB/IV after chemo? All possible details that can be driven from the database can be useful.

Moreover, can Authors specify on "trimodality"? Surgery + chemo/RT in stage IIIB-IV? This seems quite strange.

Comment: Thanks for your suggestion. According to SEER records, radiation probably means thoracic RT. There is no information indicating the radiation therapy is prophylactic cranial irradiation. We understand and certainly agree with your idea that prophylactic cranial irradiation is crucial in advanced stage SCLC. Owing to the lacking of such data, it is difficult to elucidate this problem in the current study. Therefore, we would not try to recommend PCI in stage IIIB-IV CSCLC patients. Referring to trimodality, it means surgery+chemo+RT. But we share the same consideration with you that the surgery for stage IIIB-IV is not radical. In another word, some patients were accidentally diagnosed as stage IIIB-IV during the surgery or after surgery. Therefore, the surgeries they have received are probably salvage or debulking ones. Considering this, we would not encourage radical invasive treatment for advanced stage patients. But the effect on the prognosis

outcomes from debulking surgeries could not be ignored from the current data. Further studies are warranted to solve this problem.

Do Authors have information concerning the administration of PCI both in “early” and “advanced” stages of CSCLC?

Comment: Thanks for your question. It is the same question which is in our minds. Unfortunately, we do not have such data. The database provides radiation data including sequence and method. The radiation position, radiation dosage or duration was not provided. We would try to cooperate with the Department of Radiation to do the further investigations on the sequence and dosage. Thanks for your advice.

Ideally, survival curves should contain number at risk. If this is possible, it would be of interest if Authors could include them. If not, Authors are invited to include the total number of patients for each group (=f or each curve), putting it close to the text of the figure.

Comment: Thanks for your suggestion. We think this is a really good idea. As you can see that some figures contain many curves, we believe it would be overwhelming if we add too many contents in the figure. The total numbers of patients for each group are indicated in the tables including the numbers of CSCLC, other SCLC, NSCLC and different treatment groups. Thank you very much.

Reviewer C

Dr. He and colleagues conducted a large-scale retrospective study on clinical features, treatments and outcomes, including OS and cancer specific survival, in a cohort of combined sclc patients from SEER database. Based on the lack of clear evidence in this special and rare histological population, this work provides a piece of new information. However, some considerations and revisions are needed.

- Line 113: what is the value of Figure 1? Clinical and pathological characteristics are reported in table 1. What does mean Figure 1? Was it a multivariable analysis?? other?? Anyway, some information is missing in the figure

Comment: Thanks for your comment. Maybe we have submitted the wrong figure. Figure 1 is the selection process. Figure 2 shows the overall survival of CSCLC, other SCLC and NSCLC in different stages. Figure 3 shows the multivariable analysis. Figure 4 shows the overall survival of patients in different treatment arms stratified by stages. We have revised the manuscript and the figures. They would be clearer and more informative.

- Lines 135-136: poor differentiated and undifferentiated refer to CSCLC and not to patients

Comment: Thanks for your suggestion. We have revised the manuscript and highlighted in red.

- What is the staging system to which the authors refer? Please, specify

Comment: The included patients were diagnosed from 2004-2016, which overlapped AJCC TNM 6th and 7th Staging system. Therefore, we extracted the data of tumor size and extension, and recoded the patients from 6th to 7th staging system. Therefore, the staging system we referred in the current study is TNM 7th Staging system. Thanks for your comment.

- Lines 158-164: Figure of multivariate analysis needs to be included along with p values. “Other carachteristics including race, sex..etc” should be placed before the sentence “In terms of treatment, patients who...). This sentence refers to multivariate analysis. All p values should be included.

Comment: The new figure 3 which shows the multivariate analysis has included the p values. We also revised the manuscript. Thanks for your suggestion.

- Line 166: It would be advisable to specify how many patients with stage IA-B CSCLC underwent surgery and to which kind of surgery, evaluating outcomes also through this stratification (for example, lobectomy vs segmentectomy)

Comment: This is a really good comment. We have made a table 2 to demonstrate the number of patients in different treatment arms in each stage. There are 31 patients received sublobectomy and 91 patients received lobectomy in stage IA-IB. No difference was observed between these surgical methods. The KM curve was shown in Figure S2.

- Line 168: add information regarding the number of patients who received chemotherapy only

Comment: Please see Table 2.

- Lines 170-171: the authors should add the number of patients who received adjuvant chemotherapy and the p value

Comment: Please see Table 2. The p value is 0.034 which is shown in the revised manuscript.

- Lines 174-176: how many patients received chemotherapy alone, chemoradiotherapy and adjuvant chemotherapy in stage IIA-IIIA CSCLC??

Comment: Please see Table 2.

- Lines 176-177: what is the p value?

Comment: The p value is added to the revised manuscript. Thanks for your suggestion.

- Line 177: how many patients received trimodality therapy and among them could the authors provide information on the kind of surgery (lobectomies, pneumonectomies, segmentectomies)?

Comment: Please see Table 2.

- Lines 180-181: how many patients received trimodality strategy in stage IIIB-IV CSCLC??? I think its numeber is quite limited, so the comparison with the other two groups (chemotherapy and chemoradiation) is unlikely

Comment: Please see Table 2. Thanks for you comment. We share the same opinion with you. Surgery is not routinely recommended in stage IIIB-IV patients. In another word, some patients

were accidentally diagnosed as stage IIIB-IV during the surgery or after surgery. Therefore, the surgeries they have received are probably salvage or debulking ones. Considering this, we would not encourage radical invasive treatment for advanced stage patients. But the effect on the prognosis outcomes from debulking surgeries could not be ignored from the current data. Further studies are warranted to solve this problem.

- Lines 182-183: how many patients recedived chemotherapy and chemoradiation? What is the p value?

Comment: Thanks for your comments. In order to address these questions, we have made Table 2 to demonstrate the number of patients in each treatment arms. Besides, we also revised Figure 3 and manuscript. The p values would be shown in Figure 4.

- Lines 248-253: this sentence is too strong. SCLC transformation from NSCLC is a distinct entity based on the phenotype change as mechanism of secondary resistance under the selective pressure on EGFR-TKI therapy. Combined SCLC is more likely another biological entity. I suggest removing this sentence.

Comment: Thanks for your suggestion. We agree with your comment that primary CSCLC is more likely another biological entity. Whether it is resistant to EGFR-Tki is a compete different story from SCLC transformation. We deleted this sentence.

- Different grammatical errors and typos throughout the text are to be corrected

Comment: Thanks for your suggestion. We would revise the manuscript.

- I agree with the conclusions of the authors, although trimodality could be a potential treatment strategy in selected patients whose features are to be defined yet.

Comment: Thanks for your suggestion. As we can see that early stage patients tend to receive surgical treatments while advanced stage patients are mostly administered chemotherapy and chemoradiation. Some studies reported that surgical treatments such as debulking surgery or even lobectomy were effective in oligometastatic patients and M1a (malignant pleural effusion) patients. Therefore, further studies are warranted to identify the potential treatment strategy for the specific patients. Thank you very much.