Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-22-1011

Reviewer A

・Cases of monotherapy and combination therapy were mixed. And ICI-chemotherapy cases accounted for the majority. Details of combination therapy were unknown. Is it a combination of ICI chemotherapy or a combination of ICIs? It is undesirable to mix data from ICI-monotherapy and ICI plus chemotherapy. If ICI plus chemotherapy is the majority, I think it would be better to analyze only the ICI plus chemotherapy group.

Reply 1: Thanks for your helpful suggestion. We add the details of combination therapy in supplementary table 1. (See chart file named Table, supplementary table 1). ASC is a relatively uncommon lung subtype. There is indeed a small number of samples for immunomonotherapy in this paper, which may be related to the fact that immunocombined therapy has become one of the standard treatment regiments for NSCLC. However, although the sample number of immune monotherapy is small, it is still very important to explore the efficacy of immune monotherapy, so we still carried out the analysis of single drug. The detailed baseline characteristics of patients administered ICI monotherapy and combination therapy are described in Supplementary Table 1.
Changes in the text: Chart file named supplementary table 1.

・The description of statistical analysis was unclear. I felt that you lacked knowledge and attention to statistics.
If your data are normally distributed, the unpaired Student’s t-test should be used to compare the two groups instead. However, the data in this report are assumed to take a non-Normal, or skewed, distribution. Therefore, you should use the Mann-Whitney's U test.
In “Follow-up and statistical analysis” section, you did not mention the test method used for comparison of PFS and OS. The log-rank test is generally used to compare PFS and OS.
"Age was identified as an independent prognostic factor of PFS. PS was identified an independent prognostic factor of OS (Figure 4B)."
Just because there is a significant difference in univariate analysis, don't call it an "independent" prognostic factor. In general, multivariate analysis should be performed.

Reply 2: Thank you for your comment. I'm very sorry that there is a problem with our statement. In our study, categorical data were compared using the Fisher’s test. And in “Follow-up and statistical analysis” section, Student’s t-test has been removed. In addition, Kaplan-Meier survival analysis was used for analyzing the survival of the patients, and Log-rank test was performed to compare the survival rates in relation to different prognostic factors.
Univariate and multivariate analysis was performed using the Cox regression model. Factors (age and PS) that were significant in univariate analysis of PFS were included in Cox multivariate regression analysis, which identified PS as independent prognostic factors for PFS in ASC patients who received ICI.
Changes in the text: Page 7, line 1335-136, 140-143.

Adenosquamous carcinoma is often difficult to diagnose with minute tissue specimens. Please indicate the biopsy method of the specimen used for diagnosis (Transbronchial lung biopsy, Computed tomography guided needle biopsy, resection, etc.)

Reply 3: Thank you for your comment. We added the biopsy method of the specimen used for diagnosis in table 1.
Changes in the text: Table 1 (in Page 20 line 439).

In TNM staging, the general rule is that patients with postoperative recurrence should follow preoperative staging. Specifically, patients with postoperative recurrence should be described as (post-operative) recurrence in staging.

Reply 4：We sincerely appreciate your comments. All the patients we included were advanced patients. All patients with postoperative recurrence developed distant metastasis, which was stage IV.
Changes in the text: No changes.

Page 12, line 263
I think “poor PS” is easier to understand than “higher PS scores”.

Reply 5: Thank you for your suggestion. We have modified our text as advised in Page 3, line62,65,73.
Changes in the text: Page 11, line 230.

Reviewer B

In this manuscript, the authors presented a retrospective analysis of 38 lung adenosquamous carcinoma patients treated with ICIs. The study is well designed; rigorous statistical analyses were implemented; results are clearly presented; and interpretations are adequate to support the conclusion. The manuscript is well organized and well written. The scope of the work is succinct yet covered all critical aspects including the following:
• Overall efficacy in the entire cohort
• Subgroup analysis with respect to line of therapy (1st line vs. 2+ line), ICI regimens (monotherapy vs. combination with chemotherapy), mutation status (EGFR/ALK altered vs. WT), and PD-L1 status (positive vs. negative)
• Multivariate analysis of relevant demographic and clinical variables
• irAE
To the best of my knowledge, this is the 1st such study on lung adenosquamous histology and therefore I’d recommend the manuscript to be published for sharing important results with the medical oncology community.

I do have a couple of suggestions for minor revision:
1.In Fig.1 and Fig.3, for each Kaplan-Meier curves, the upper-right corner table shows the median survival time, hazard ratio and p-value. Please clarify in the figure legend, hazard ratio is based what

comparison, e.g. 1st line vs. 2+ line (not 2+ line vs. 1st line), or PD-L1 positive vs. PD-L1 negative (not PD-L1 negative vs. PD-L1 positive).

Reply 1: We sincerely appreciate your comments and your recognition of our work. We have modified the figure legend as advised in Page19, line 419, 421, 423-424.
Changes in the text: Page19, line 419, 421, 423-424

2. A recent publication (https://pubmed.ncbi.nlm.nih.gov/34334296/) reported 79 advanced NSCLC with uncommon histology treated with ICI, the efficacy including ORR, DCR, PFS and OS. Although only 5 of the 79 patients are adenosquamous, the paper represents a similar effort to investigate ICI response in rare subtypes of NSCLC, and should be acknowledged in the introduction.

Reply 2: We sincerely appreciate your comments and your recognition of our work. We have supplemented this reference in Page 4, line 75-77.
Changes in the text: Page 4, line 75-77

Reviewer C

The authors retrospectively explored the efficacy of immunotherapy, including both ICI monotherapy and chemoimmunotherapy, for patients with advanced adenosquamous lung cancer. I agree that the theme of this study is clinically interesting and the findings can have some implications for future research. However, in my opinion, substantial revisions are warranted before considering this manuscript for publication. Please see my comments below.

Major comments:
1.Materials and methods: As for the pathological diagnosis of adenocarcinoma, please show more detailed explanation on it with brief descriptions on the WHO criteria. Were all cases clinically diagnosed as AdSq pathologically re-confirmed? Or some cases were excluded per WHO criteria despite of combined components of Ad and Sq? Also, please provide information on the samples (from biopsy or VATS etc.). The validity of the diagnosis of AdSq is a key points and clarification on this point is essential.

Reply 1: Thank you for your comment. We added the biopsy method of the specimen used for diagnosis in Table 1 (in Page 20 line 439). ASC is defined as a tumor type containing components of both squamous cell carcinoma (SCC) and adenocarcinoma (ADC), with each component comprising at least 10% of the tumor. All patients were pathologically confirmed with ASC according to the 2021 World Health Organization classification of lung tumors (in Page 5, line 90-93). All diagnoses were validated via immunohistochemical (IHC) analysis.
Changes in the text: Table 1 (in Page 20 line 439); Page 5, line 90-93.

2. Discussion: The majority of the study subjects received ICI as chemoimmunotherapy, not as ICI monotherapy. However, the authors failed to cite pivotal data from trials on chemoimmunotherapy,

mainly focusing on data of ICI monotherapy. Please carefully provide discussion and comparisons with preceding studies, differentiating ICI monotherapy and chemoimmunotherapy.

Reply 2: Thanks for your careful review. We provide discussion and comparisons with differentiating ICI monotherapy and chemoimmunotherapy as advised (see Page 12, line 256-263).
Changes in the text: Page 12, line 256-263

3. Conclusions, “ICI may serve as a promising~”: The conclusions the authors provided seems to be overinterpretation of their data and misleading. Only one patient (without data on PD-L1 expression) responded to ICI monotherapy and the main subjects were those received chemoimmunotherapy. It would not be surprising that some patients with AdSq respond to platinum doublet therapy.

Reply 3: Thanks for your careful review. We have modified conclusions as advised (see Page 3, line 47-48; Page 15, line 319).
Changes in the text: Page 3, line 47-48; Page 15, line 319.

4. Design: Considering the small sample size, especially for subgroups, of this study, the authors may be encouraged to present data as a case series. Swimmers’ plots for all the participants with sufficient information (ICI monotherapy or chemoimmunotherapy, PD-L1 expression, mutation status, line etc.) on each case might be more informative than KM curves for subgroups.

Reply 4: Thank you for your comment. We add the details of therapy in supplementary table 1. (see chart file named Table, supplementary table 1). And a Swimmer plot was generated to describe in detail the treatment outcomes of patients evaluated for PD-L1 expression status (Figure 2).
Changes in the text: Supplementary table 1 and Figure 2

5. Discussion: The authors failed to fully explain the limitations of this study.

Reply 5：Thank you for your kind reminder. We have added a fully explanation of the study's limitations
Changes in the text: Page 15, line 311- 315.

Minor comments:
1.Materials and methods: Please show the ID for IRB approval.

Reply 1: Thank you for your suggestion. We have added the ID for IRB approval as advised in Page 5, line 101.
Changes in the text: Page 5, line 101.

2. Materials and methods: Were all cases evaluated with CT scan every 2 cycles? It seems to be a little bit frequent as clinical practice. Or is this study post-hoc analysis from a prospective study?

Reply 2: Thank you for your kind reminder. Treatment efficacy was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) [1].

Changes in the text: No changes.

Reference: [1]Eisenhauer, EA, Therasse, P, Bogaerts, J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009; 2: 228-47.

3. Materials and methods: Objective responses were confirmed or not confirmed?

Reply 3：Thank you for your kind reminder. Objective responses were confirmed (see Page 6, line 111-119).
Changes in the text: No changes.

4. Table 1: What dose surgery (24 cases were recurrent after definitive surgery?) and radiotherapy (for what? Definitive or palliative?) exactly mean?
Reply 4: Thank you for your comment. The 24 patients who underwent surgery were those who had undergone radical surgery before and had a recurrence. Radiation therapy refers to having received radiation therapy prior to immunotherapy.
Changes in the text: No changes.

5. Table 1, Previous chemotherapy: The number of patients seems to be discordant with KM curves in figure 1.

Reply 5: Thank you for your comment. Chemotherapy in Table 1 refers to chemotherapy received prior to immunotherapy. A total of 7 people received chemotherapy before immunotherapy.
Changes in the text: No changes.

6. Table 1: Please provided more detailed information on chemotherapy regimens.

Reply 6: Thank you for your comment. Chemotherapy in Table 1 refers to chemotherapy received prior to immunotherapy. We add the details of chemotherapy in combination therapy in supplementary table 1. (see chart file named Table, supplementary table 1).
Changes in the text: Chart file named supplementary table 1.

7. Table 2: The AEs seems to be underreported as those for cohorts who received chemoimmunotherapy. Does this table only include irAE? If so, how did the authors differentiate AEs from cytotoxic agents from irAE (e.g., anemia, anorexia)?

Reply 6: Thank you for your comment. This table only include irAE. Toxicity was monitored based on Common Terminology Criteria for Adverse Events Version 5.0. Immune-related adverse events (irAEs). The diagnosis and severity of irAEs were based on clinical examinations and biological and imaging data. Scores of 1 to 5 were used for analysis of irAE grade by two or more independent medical professionals.
Changes in the text: No changes.

Reviewer D

The manuscript describes the clinical efficacy outcomes of ICIs for ASC in consideration with PD-L1 expression and irAEs occurrence in the patients. As the author mentioned in Limitation, the most important point is a limited number of patients to obtain definitive conclusions. Following points are raised.

1.The number of patients in subgroups should be given along with the p-values (Abstract, Lines 41 and 42) to let the readers notice that the statistical power is not enough.

Reply 1: Thanks for your careful review. We have modified our text as advised (see Page 2, line 39-41).
Changes in the text: Page 2, line 39-41.

2. No conclusion regarding the PD-L1 expression is given in Conclusion (Line 45), which should be given even if it was statistically negative.

Reply 2: Thanks for your careful review. We have modified our text as advised (see Page 3, line 47-48).
Changes in the text: We have added the “conclusion regarding the PD-L1 expression” in Page 3, line 47-48.

3. The need to obtain IC from patients waived (Line 95), probably this study was a retrospective observational study. Please give information about the basis of this decision – for example, please mention the clinical guideline or something that the reviewer confirm no need of individual IC. In such case, how the patients could know that their clinical data were used for this study?

Reply 3: Thank you for your comment. The need for informed consent was waived by the Institutional Review Board of the Zhejiang Cancer Hospital, because of the retrospective nature of the study.
Changes in the text: Page 5, line 101-102.

4. Isn’t it important to examine the timing of irAEs occurrence (when they occurred after ICI therapy)? Please add some discussions if necessary.

Reply 4: Thank you for your comment. irAEs occurred during or after immunotherapy.
Changes in the text: Page 6, line 127.

5. The authors examined only the univariate analysis for Cox regression (Line 135), probably because of less numbers of patients. Ideally, multivariate analysis should be done to consider possible correlations among covariates. This point – multivariate analysis could not be done because of not enough number of patients – should be stated in Limitation(s).

Reply 5: Thanks for your careful review. Univariate and multivariate analysis was performed using the Cox regression model. Factors (age and PS) that were significant in univariate analysis of PFS were included in Cox multivariate regression analysis, which identified PS as independent prognostic factors

for PFS in ASC patients who received ICI.
Changes in the text: Page 7, line 142-143; Page 11, line 227-230.

6. Also, the results of Cox regression – especially the description of significant factors (Lines 215, 217) should be carefully written in order not to misleads the readers.

Reply 6: We sincerely appreciate your kind suggestion. We describe the results of COX regression in more detail.
Changes in the text: We have modified our text as advised in Page 11, line 223-231.

Reviewer E

This is a very interesting and innovative study. Currently, there are few publications on this tumor. Adenosquamous lung carcinoma is regarded as more aggressive and carries a worse prognosis compared to adenocarcinoma and squamous cell carcinoma. The reported 5-year survival rate for adenosquamous lung carcinoma patients has varied between 6.2% and 25.4% [1-5]. Moreover, more than half adenosquamous lung carcinoma patients who underwent complete surgical resection experienced distant metastases including hilar lymph nodes, adrenal gland, bone, liver and distant brain metastases or local recurrence [1]. A previous study found that the amount o standard platinum-based doublet chemotherapy of advanced non-small cell lung cancer has limited efficacy, thus new therapies are needed.
In this study 11 patients (11/38 ASC) was identified PD-L1 positive.
For nonsquamous NSCLC, we currently have the randomized studies revealing safety and overall survival (OS) benefit of PD-(L)1 blockade plus chemotherapy: KEYNOTE-189, IMpower150, IMpower130, and CheckMate 9LA [6-9]. Now for patients in advanced NSCLC without a targetable genetic alteration, programmed death-(ligand) 1 (PD-[L]1) immune checkpoint blockade in combination with platinum-based chemotherapy has become a standard treatment. In the presented study, PD-1 inhibitors were used, such as: sintilimab, tislelizumab and pembrolizumab. In phase 3 trial ORIENT-11 study, that evaluated sintilimab in patient (n=397) with advanced (stage IIIB to IV)non-squamous NSCLC patients with no previous systemic treatment, novel programmed cell death protein-1 (PD-1) inhibitor in combination with chemotherapy, significant results have been achieved: median progression-free survival (PFS) was significantly prolonged in the sintilimab combination group compared with the placebo combination group (8.9 mo versus 5.0 mo; hazard ratio [HR]: 0.48; $p < 0.00001$)[10].In phase 3 trial ORIENT-12 sintilimab plus GP (n=543) reveals clinical benefit than GP alone as first-line therapy in patients with locally advanced or metastatic sqNSCLC [11]. In study ORIENT-11 and ORIENT-12 the toxicity was acceptable, and no new unexpected safety signals were observed [10,11]. In phase 3,open-label,randomized,multicenter clinical trial RATIONALE 307 (n=355), adding tislelizumab to chemotherapy was associated with significantly prolonged IRC-assessed PFS, higher IRC-assessed ORRs, and a manageable safety/tolerability profile in patients with advanced sq-NSCLC.Trial was in in China with treatment-naive, histologically confirmed locally advanced (stage IIIB) or metastatic (stage IV) squamous -NSCLC[12]. In study RATIONALE 304, a randomized phase 3 trial (n=332) addition of tislelizumab to chemotherapy resulted in significantly

prolonged PFS, higher response rates, and longer response duration compared with chemotherapy alone, identifying a new potential option for first-line treatment of advanced nsq-NSCLC (stage IIIB and IV) [13].

In this study 11patients (11/38 ASC) was EGFR mutation.

The collected group of patients is heterogeneous in terms of genetic changes in the tumor. In addition, a prior study showed an adenosquamous lung carcinoma patient harboring EGFR-sensitizing mutation had a remarkable response to gefitinib [14]. Therefore, EGFR tyrosine kinase inhibitors would be a reasonable therapeutic option to adenosquamous lung carcinoma patients due to the relatively high frequency of EGFR mutations in this cohort. Recent advancements in EGFR mutation targeted therapy led to a major paradigm shift in the treatment of non-small cell lung cancer. EGFR-sensitizing mutations are strongly associated with robust responses to EGFR tyrosine kinase inhibitors (EGFR-TKI) and improved progression-free survival (PFS). However, EGFR mutations are most common in Asian patients, nonsmokers, females and those with adenocarcinoma histology [15]. In squamous cell carcinoma, the EGFR mutation rate is reported to be approximately 5% [16]. Although several small studies have indicated that the frequency of EGFR mutation in adenosquamous lung carcinoma ranges from 15% to 44% in the East Asian population, the exact prevalence of EGFR mutation in adenosquamous lung carcinoma is still not clear.

However, its biological behaviors based on clinicopathological factors are not well understood. According to the proportion of glandular and squamous components, ASC could be divided into AC and SCC predominant subtypes. A few study reported that predominant subtype to be associated with prognosis of ASC. However, due to its rarity, no definitive clinical conclusion. Thirty nine patients of the study cohort [17]were with AC-predominant ASC and 29 with SCC-predominant ASC. This study showed that the two different pathological subtypes of ASC were with different radiologic findings and prognosis characteristics. AC-predominant ASC were more commonly presented with air bronchogram, and were with a better prognosis than SCC-predominant ASC [17].

Studies with a much larger sample size and longer duration of follow-up are still necessary to confirm these results. I hope that the presented study is the beginning of creating precise recommendations for the management of ASC.Perhaps the treatment of ASC will depend on the dominant component in microscopic and genetic examination.

References:

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Cancer ; (2020) 20:520 https://doi.org/10.1186/s12885-020-06972-5.

103- how many patients with which drug?

Reply 1: Thank you for your comment. We add the details of drugs in supplementary table 1. (See chart file named Table, supplementary table 1).
Changes in the text: See chart file named Table, supplementary table 1.

148- mutations EGFR in which exons?

Reply 2: Thank you for your comment. We add the details of EGFR mutation in table 1.
Changes in the text: Table 1 (in Page 20 line 439); Page 8, line 159-160.

154- 17 patients received first- line immunotherapy: was immunotherapy used in 1-line treatment as monotherapy or in combination with chemotherapy?

Reply 3: Thanks for your helpful suggestion. We add the details of monotherapy and combination therapy in supplementary table 1. (See chart file named Table, supplementary table 1).
Changes in the text: Chart file named supplementary table 1.

167- monotherapy or in combination with chemotherapy?

Reply 4: Thanks for your helpful suggestion. We add the details of monotherapy and combination therapy in supplementary table 1. (See chart file named Table, supplementary table 1). Combination immunotherapy is a combination of ICI and chemotherapy.
Changes in the text: Chart file named supplementary table 1.

168- monotherapy or in combination with chemotherapy?
Figure 4: chemotherapy- is it chemotherapy in the early lines of treatment, or is it current treatment as a combination therapy?

Reply 5: Thank you for your recognition of our work. Chemotherapy it is chemotherapy in the early lines of treatment in Figure 4.
Changes in the text: No changes.

Reviewer F

These authors demonstrate the efficacy and toxicity of immune checkpoint inhibitors in patients with adenosquamous carcinoma, a relatively rare pathological type of primary lung cancer. This is a retrospective study of 38 of his ASC patients from multiple thoracic cancer centers. The authors found a significant relationship between PDL-1 expression in ASCs and their response to ICI.

1.The authors referred to RECIST rather than iRECIST for ICI response assessment. Please provide a comment in the discussion section as to why this is the case.

Reply 1: Thanks for your helpful suggestion. In practice, although the iRECIST is used for ICI response assessment, the RECIST1.1 criterion is still used as the main criterion for evaluating the efficacy of solid tumors. There are also many studies using RECIST1.1 for ICI response assessment(1-3).
Changes in the text: No changes.

1 Derosa, L, Hellmann, MD, Spaziano, M, et al. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Ann Oncol 2018; 6: 1437-1444.

2 Rodriguez, CP, Wu, QV, Voutsinas, J, et al. A Phase II Trial of Pembrolizumab and Vorinostat in Recurrent Metastatic Head and Neck Squamous Cell Carcinomas and Salivary Gland Cancer. Clin Cancer Res 2020; 4: 837-845.

3 Grimm, MO, Schmitz-Drager, BJ, Zimmermann, U, et al. Tailored Immunotherapy Approach With Nivolumab in Advanced Transitional Cell Carcinoma. J Clin Oncol 2022; 19: 2128-2137.

2. Fewer than half of the patients had PD-L1 expression assessed. What is the reason?

Reply 2: Thanks for your careful review. Due to the small sample size, only some patients were tested for PD-L1.
Changes in the text: No changes.

3. Detailed results may not be repeated in discussion sessions (eg lines 261, 285, 290). please discuss the results compared to previous studies and make your points.

Reply 2: Thanks for your careful review. We have modified our text as advised (see Page 5, line 124).
Changes in the text: Page 13, line 275-279; Page 14, line 299-301; Page 14, line 304-305.

Reviewer G

The authors are conducting a retrospective study of the usefulness of immune checkpoint inhibitors for ASCs.
However, it is difficult to discuss the usefulness of immune checkpoint inhibitors in this study, where the number of patients in each group is extremely small.

Reply 1: Thank you for your comments. The sample size of ASC patients with ICI treatment was insufficient. However, in view of the lack of clinical studies to date, our findings provided a set of useful guidelines on the utility of ICIs as a treatment option for lung ASC.

Changes in the text: No changes.

Reviewer H

In this retrospective study, the authors evaluated the efficacy and toxicity of immunotherapy for adenosquamous lung cancer patients. The manuscript is well written, and the results are clearly presented. The reviewers have a few concerns that the authors need to address.

i)Follow-up and statistical analysis (lines, 132-134). It would be better to change the OS definition from "the date of confirmed ASC" to "day one of immunotherapy" when the authors want to see the clinical outcomes of immunotherapy.

Reply 1: Thanks for your careful review. We have modified the OS definition as advised (see Page 7, line 138-139).
Changes in the text: Page 7, line 138-139; all OS values.

ii) Results (lines, 188-189). Do the authors have more details about KRAS and MET mutation? Are these KRAS G12C and MET exon 14 skipping mutations？ If so, it would be better to include patients with KRAS and MET mutation in the subgroup with the mutant.

Reply 2: Thanks for your careful review. In KRAS mutation-positive patients, there were 3 patients with KRAS G12C mutation and 1 with KRAS G12D and G12S mutations. There were 1 patient with MET mutation and 1 patient with MET amplification. We have modified include patients with KRAS and MET mutation in the subgroup with the mutant. (see Page 9, line 196-198).
Changes in the text: Page 9, line 196-198;

iii) Results (lines, 191-194). This study showed no efficacy (ORR, DCR, PFS) differences between the mutant and non-mutant patients. In contrast, it is widely accepted that immunotherapy is less effective for mutant patients. Please describe these points in the discussion.

Reply 3: Thanks for your careful review. We have added relevant content in the article (see Page 12-13, line 263-267).
Changes in the text: Page 12-13, line 263-267

iv) The initiation of immunotherapy for EGFR or ALK-positive patients was after the disease progression with EGFR or ALK-TKI? Please describe the above in the manuscripts because these concerns influence the OS time.

Reply 4: Thanks for your careful review. We have changed the OS definition from "the date of confirmed ASC" to "day one of immunotherapy". Ten patients received targeted therapy prior to immunotherapy (Table 1).
Changes in the text: Changed the OS definition from "the date of confirmed ASC" to "day one of

immunotherapy".

v) The reviewers could not find the supplementary Table 1.

Reply 5: Thank you for your comments. We will reconfirm that supplementary table 1 has been uploaded
Changes in the text: No changes.