**Peer Review File**

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**Reviewer A**
**Comments:**

The authors have submitted an editorial on the efficacies of different types of neoadjuvant treatment for esophageal squamous cell cancer.

Comment 1. Although the study by Liu and colleagues is a nice analysis, it is a very small cohort. I think that this editiorial should highlight that limitation more. The authors have a very brief mention of the cohort size but should expand on this.

Reply 1: Thank you for the reviewer's comments, we have revised manuscript.

Changes in the text: "A larger cohort is essential for robustness, additional aspects warrant consideration. The application of single-cell RNA sequencing (scRNA-seq) introduces specific challenges, including potential biases and complexities in data interpretation. Moreover, the heterogeneity within esophageal squamous cell carcinoma (ESCC) itself may pose challenges in extrapolating findings to broader contexts. To address these concerns, future studies should explore the generalizability of scRNA-seq across different cancer types and acknowledge the intricacies of ESCC heterogeneity."

Comment 2. Some of the language is too elaborate and should be edited. For example, lines 22-23 say "employs cutting-edge single-cell RNA sequencing to meticulously delve into the TME of locally advanced ESCC." It would be easier to read if the authors just removed the word "meticulously." There are multiple other examples throughout the manuscript.

Reply 2: Thank you for the reviewer's comment. We have revised the manuscript thoroughly to make it more readable.

Comment 3. The authors should give a brief overview about what single-cell RNA sequencing is for readers that are not familiar. Two or three sentences about the technology and how it differs from total RNA sequencing.

Reply 3: Thank you for the reviewer's comment. We have revised the manuscript accordingly by adding the introduction of the scRNA-seq technology and how it differs from total RNA sequencing.
Changes in the text: "Single-cell RNA sequencing (scRNA-seq) is a powerful technique

that allows researchers to analyze gene expression at the individual cell level. Unlike traditional bulk RNA sequencing, which provides an average expression profile for a population of cells, scRNA-seq captures the transcriptome of each individual cell. This enables the identification of cellular heterogeneity within a sample, unveiling variations in gene expression patterns among different cells. By offering a more detailed and precise understanding of cellular diversity, scRNA-seq contributes to uncovering detailed insights into complex biological processes and disease mechanisms."

**Reviewer B**
**Comments:**

I would like to thank the editors for the opportunity to review this very interesting editorial commentary. The authors quite nicely describe the identification of CD8+ Tex-SPRY1 cells as a potential predictive biomarker for the use of neoadjuvant immune checkpoint blockade in patients with ESCC as well as the interactions with proinflammatory macrophages, and TLS-related B cells described by Liu et al Cancer Cell 2023. PD-L1 expression is quite an imperfect biomarker, so the potential for improved methods to predict outcomes is eagerly awaited. However, I would recommend a deeper discussion regarding the limitations of the study as well as potential next steps beyond simply identifying that the trial was a relatively small sample size and that larger cohorts are needed. What other limitations of the study might be considered and what challenges of the broad applicability of scRNAseq exist?

Reply: Thank you for the reviewer's comments, we have revised manuscript.
Changes in the text: "A larger cohort is essential for robustness, additional aspects warrant consideration. The application of single-cell RNA sequencing (scRNA-seq) introduces specific challenges, including potential biases and complexities in data interpretation. Moreover, the heterogeneity within esophageal squamous cell carcinoma (ESCC) itself may pose challenges in extrapolating findings to broader contexts. To address these concerns, future studies should explore the generalizability of scRNA-seq across different cancer types and acknowledge the intricacies of ESCC heterogeneity."