Editorial Commentary

Unraveling the intricacies of neoadjuvant immune checkpoint blockade in esophageal squamous cell carcinoma: a comprehensive single-cell perspective

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Comment on: Liu Z, Zhang Y, Ma N, *et al.* Progenitor-like exhausted SPRY1(+)CD8(+) T cells potentiate responsiveness to neoadjuvant PD-1 blockade in esophageal squamous cell carcinoma. Cancer Cell 2023;41:1852-1870.e9.

Keywords: Esophageal squamous cell carcinoma (ESCC); neoadjuvant immune checkpoint blockade (neoadjuvant ICB); tumor microenvironment (TME)

Submitted Nov 17, 2023. Accepted for publication Jan 18, 2024. Published online Feb 20, 2024.
doi: 10.21037/jtd-23-1771
View this article at: https://dx.doi.org/10.21037/jtd-23-1771

Esophageal squamous cell carcinoma (ESCC) stands as a lethal disease, ranking as the seventh leading cause of cancer-related mortality worldwide (1,2). Despite the current therapeutic standards employing neoadjuvant chemoradiotherapy followed by surgery for locally advanced ESCC, the clinical benefits remain suboptimal (3,4). The evolving landscape of cancer treatment has witnessed the emergence of immunotherapy, particularly immune checkpoint blockade (ICB), as a promising avenue for addressing the challenges posed by advanced esophageal cancer (5). Neoadjuvant ICB (NICB) therapy before esophagectomy has surfaced as a potential solution, and in this editorial, we aim to dissect the details of this approach, offering insights from a comprehensive single-cell perspective.

The recent study by Liu *et al.* utilized cutting-edge single-cell RNA sequencing (scRNA-seq) to thoroughly examine the tumor microenvironment (TME) of locally advanced ESCC undergoing NICB therapy (6). 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. This sophisticated approach reveals a subset of exhausted $CD8^+$ T cells expressing SPRY1 with a progenitor phenotype. The identification of this distinctive profile associated with heightened responsiveness to NICB unveils SPRY1 as a potential biomarker for guiding patient selection in ESCC immunotherapy. The findings usher in a deeper understanding of the mechanisms supporting NICB sensitivity, laying the foundation for more personalized and effective treatment strategies in ESCC.

The comprehensive exploration of the TME dynamics is a hallmark of this study. Tumor specimens collected from patients participating in a phase II clinical trial investigating the efficacy of NICB before esophagectomy serve as a valuable resource for the single-cell analysis. The NICB regimen, comprising anti-programmed cell death 1 (anti-PD-1) plus chemotherapy, is chosen for its established safety and efficacy in advanced, inoperable ESCC. The utilization of scRNA-seq on paired pre-treatment and post-

treatment tumors provides a detailed cell atlas, allowing for a detailed evaluation of the changes induced by NICB.

The evaluation of NICB efficacy, revealing complete regression (CR) in three patients and non-complete regression/response (NCR) in four patients, is a pivotal observation. Genomic analysis confirming typical ESCC alterations in these patients aligns with the broader molecular landscape of this aggressive cancer. The scRNA-seq analysis identifies immune and non-immune cell clusters shared across patients, treatments, and responses. However, the CR group stands out, demonstrating a significant change in TME appearance, suggesting a potential NICB-induced cellular remodeling effect. Principal components analysis further reveals a pattern indicative of a successful antitumor response in the CR group.

Digging deeper into the specific $CD8^+$ exhausted T cell (Tex) populations, the study observes higher PD-1 expression in the CR group, emphasizing the pivotal role of $CD8^+$ T cells in NICB for ESCC. The identification of two transcriptionally heterogeneous $CD8^+$ Tex clusters, $CD8^+$ Tex-SPRY1 and $CD8^+$ Tex-XAF1, adds granularity to the analysis. $CD8^+$ Tex-SPRY1, with elevated SPRY1 expression and a progenitor-like phenotype, emerges as a distinct subset associated with heightened antitumor responses. Trajectory analysis delineates the progression from early-stage exhausted cells with a progenitor phenotype ($CD8^+$ Tex-SPRY1) to a more terminally exhausted state ($CD8^+$ Tex-XAF1), offering insights into the complex landscape of ESCC immunotherapy response.

Building upon these findings, the study explores the predictive value of $CD8^+$ Tex-SPRY1 cells as a biomarker for NICB outcomes. In the discovery cohort, a higher proportion of $CD8^+$ Tex-SPRY1 cells in CR tumors before treatment correlates significantly with NICB-induced tumor response, proposing its potential as a predictive biomarker. Validation cohorts further support these observations, demonstrating the effectiveness of $CD8^+$ Tex-SPRY1 cells across different treatment subgroups. Importantly, $CD8^+$ Tex-SPRY1 cells surpass programmed death-ligand 1 (PD-L1) as a predictive biomarker, underlining their robustness in guiding NICB benefits in ESCC.

The study's extension into external validation cohorts, including ESCC treated with anti-PD-1 alone, reaffirms the specificity of $CD8^+$ Tex-SPRY1 as an immunotherapy biomarker. High $CD8^+$ Tex-SPRY1 signature expression significantly correlates with better clinical response and improved overall survival, outperforming PD-L1. The specificity of $CD8^+$ Tex-SPRY1 as a predictive biomarker for immunotherapy in ESCC is further emphasized by the lack of significant association with non-immunotherapy cohorts, such as those treated with neoadjuvant chemoradiotherapy and upfront surgery.

Moving beyond the tumor-centric perspective, the study delves into the biological importance of $CD8^+$ Tex-SPRY1 cells. Adoptive cell transfer experiments in a tumor-bearing mouse model demonstrate enhanced anti-tumor responses, particularly in the presence of anti-PD-1 therapy, validating scRNA-seq findings. The exploration of non-invasive biomarkers, such as SPRY1 expression in peripheral blood from ESCC patients, further bolsters the potential clinical relevance of $CD8^+$ Tex-SPRY1 cells.

Moreover, the investigation into the interplay between $CD8^+$ Tex-SPRY1 cells and macrophages adds a layer of complexity to the understanding of immunotherapy outcomes in ESCC. The identification of distinct macrophage subsets, particularly those exhibiting a proinflammatory/M1 polarization enriched in CR tumors, emphasizes the potential synergy between $CD8^+$ Tex-SPRY1 cells and the immune-activated macrophages. The ligand-receptor interactions, with $CD8^+$ Tex-SPRY1 cells emerging as pivotal players, highlight the complex communication within the TME. IFNG and TNF produced by $CD8^+$ Tex-SPRY1 cells are identified as crucial regulators inducing a proinflammatory macrophage phenotype, unveiling potential mechanisms for improved antitumor immunity in ESCC.

The study's exploration of B cells and tertiary lymphoid structures (TLS) adds another layer to the multifaceted immune response in ESCC. The activated state of B cells in pre-treatment tumors of the CR group, particularly within TLS, signifies their potential contribution to ESCC immunotherapy. The significant increase in TLS activity post-NICB treatment, especially in the CR group, aligns with the enhanced disease-free survival observed in ESCC undergoing upfront surgery. The study's functional enrichment analysis of B cell subsets further emphasizes their dual significance in immunotherapy and ESCC prognosis.

In conclusion, this comprehensive study provides a detailed and multidimensional perspective on NICB in ESCC. The integration of scRNA-seq into the evaluation of treatment response and the identification of $CD8^+$ Tex-SPRY1 cells as a potential predictive biomarker open new avenues for personalized and effective immunotherapeutic strategies. The complex interactions among $CD8^+$ Tex-SPRY1 cells, proinflammatory macrophages, and TLS-

related B cells shed light on the collaborative efforts within the TME to enhance antitumor immunity.

While the study of Liu *et al.* offers valuable insights, it is essential to acknowledge certain limitations, including the small sample size and disease-specific considerations (6). A larger cohort is essential for robustness, additional aspects warrant consideration. The application of scRNA-seq introduces specific challenges, including potential biases and complexities in data interpretation. Moreover, the heterogeneity within 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. The journey from bench to bedside is a complex one, and this study serves as a significant step forward in unraveling the complexities of ESCC immunotherapy. As we navigate the era of precision medicine, the identification of predictive biomarkers and the elucidation of intricate immune cell interactions pave the way for more effective and tailored treatment approaches in the relentless battle against ESCC.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Journal of Thoracic Disease*. The article has undergone external peer review.

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1771/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1771/coif). The author has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. Abnet CC, Arnold M, Wei WQ. Epidemiology of Esophageal Squamous Cell Carcinoma. Gastroenterology 2018;154:360-73.
3. Eyck BM, van Lanschot JJB, Hulshof MCCM, et al. Ten-Year Outcome of Neoadjuvant Chemoradiotherapy Plus Surgery for Esophageal Cancer: The Randomized Controlled CROSS Trial. J Clin Oncol 2021;39:1995-2004.
4. Liu S, Wen J, Yang H, et al. Recurrence patterns after neoadjuvant chemoradiotherapy compared with surgery alone in oesophageal squamous cell carcinoma: results from the multicenter phase III trial NEOCRTEC5010. Eur J Cancer 2020;138:113-21.
5. Liu J, Yang Y, Liu Z, et al. Multicenter, single-arm, phase II trial of camrelizumab and chemotherapy as neoadjuvant treatment for locally advanced esophageal squamous cell carcinoma. J Immunother Cancer 2022;10:e004291.
6. Liu Z, Zhang Y, Ma N, et al. Progenitor-like exhausted SPRY1(+)CD8(+) T cells potentiate responsiveness to neoadjuvant PD-1 blockade in esophageal squamous cell carcinoma. Cancer Cell 2023;41:1852-1870.e9.

Cite this article as: Zou G, Anwar J, Pizzi MP, Abdelhakeem A. Unraveling the intricacies of neoadjuvant immune checkpoint blockade in esophageal squamous cell carcinoma: a comprehensive single-cell perspective. J Thorac Dis 2024;16(2):826-828. doi: 10.21037/jtd-23-1771