Quantifying Spatiotemporal Immune Dynamics Following Chemoradiation

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ABSTRACT

Purpose:
Head and neck squamous cell carcinoma (HNSCC) is a clinically aggressive malignancy with heterogeneous responses to chemoradiation, driven in part by dynamic immune remodeling. While immune infiltration has been studied at different time points, the spatiotemporal organization and communication of immune cells across time following chemoradiation remain poorly defined. This study longitudinally characterizes immune infiltration and immune network topology following chemoradiation, linking early innate responses to adaptive immune coordination and endpoint immune states.

Methods:
Using the aggressive MOC2 murine oral squamous cell carcinoma model, 25 *CCR2*$^{+/-}$ or mice (6–11 weeks) were inoculated with 30,000 tumor cells in the right buccal mucosa. At tumor volumes of ~50 mm^3, mice received 8 Gy radiation with 5 mg/kg cisplatin. Tumors were harvested at days 1 (n=7), 3 (n=6), and 7 (n=5) post-treatment, and at humane endpoint (≥10 mm in any dimension; n=7). H&E tumors slides were digitized and underwent pathomic analysis. Deep learning framework detected, segmented, and classified nuclei to generate spatial graphs, where nodes represent cells and edges encode spatial proximity. High-throughput pathomic features quantified immune infiltration, and immune network connectivity.

Results:
Immune infiltration increased from day 1 to day 7, with immune-to– non-immune cell density rising from 4.14×10^{-8} to 3.79×10^{-7} cells/μm^2, followed by reduced immune presence at endpoint (1.79×10^{-8} cells/μm^2). Tumors with higher immune infiltration were smaller (inverse exponential correlation; R^2=0.963), consistent with enhanced antitumor immunity, whereas larger tumors exhibited diminished immune presence suggesting immune escape. Graph-based analysis demonstrated increasing mean K -core values through day 7, indicating enhanced immune network connectivity consistent with a transition from innate to adaptive immunity. Subsequent decline at endpoint suggested immune dysregulation and chronic inflammation.

Conclusion:
Immune response to chemoradiation is both quantitatively and topologically dynamic. Ongoing studies involve increasing sample size, incorporating immunohistochemistry and RNA sequencing to improve robustness and further define immune cell–specific interactions.

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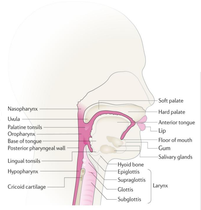
INTRODUCTION

- Head and neck squamous cell carcinoma (HNSCC) represents roughly 4% of all cancer cases worldwide and is the 7th most common cancer.[1]
- Despite available therapies, the overall 5-year survival rate remains below 50%, highlighting its aggressive nature and the need for improved treatment strategies.
- Standard care typically includes radiation therapy, cisplatin-based chemotherapy, and surgical intervention when feasible.
- Treatment response is highly heterogeneous demonstrating the need to understand its dynamic nature
- Immunotherapy, continues to show limited success in HNSCC, underscoring the urgency for better predictive tools and therapeutic approaches.[2]
- Pathomic analysis on digital pathology helps characterize the spatial relationships between cells in the tumor microenvironment proving quantitative measurements that extend beyond cell abundance.[3]
- This study is focused on investigating the innate immune response to treatment and to elucidate the connection between innate and adaptive immunity.



METHODS AND MATERIALS

- 25 CCR2$^{+/-}$ mice were inoculated with 30,000 cells of MOC2 in PBS
- When tumors reached ~50mm^3 mice received 8Gy radiation with 5mg/kg cisplatin
- Following treatment mice were monitored thrice weekly and were sacrificed 1 (n=7),3 (n=8) or 7 (n=5) days post chemoradiation or followed until endpoint (≥10mm in any direction n=7)
- Tumor tissue was collected; H&E stained and underwent pathomic analysis
- Deep learning model detected, segmented and classified nuclei to generate spatial graphs

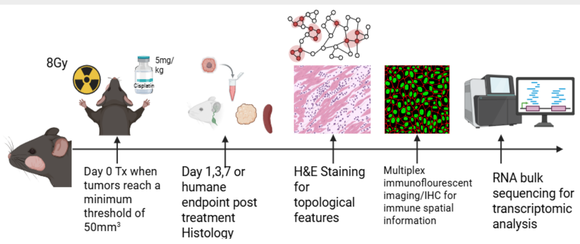


Figure 1: Experimental workflow, mice were inducted with an aggressive Moc2 cells which were allowed to grow to approximately 50mm^3 . At that point they were treated with 8Gy radiation and 5mg/kg cisplatin. Following treatment, they were sacrificed at Day 1,3,7 or humane endpoint. Tumor tissue was extracted and H&E Staining to observe topological features. Future work will include IHC to understand how specific immune cells interact as well as RNA sequencing to uncover the molecular drivers to these topological changes

RESULTS

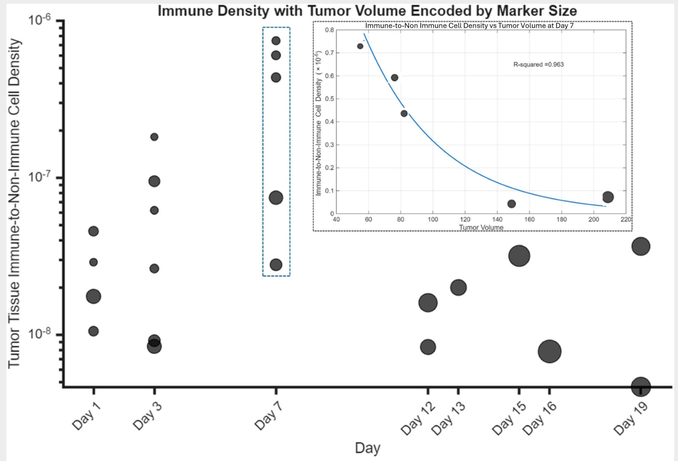


Figure 2:Immune-to-non-immune cell density plotted over time, with the relative size of each point corresponding to tumor volume at the time of sacrifice. The inset shows the correlation between immune-to-non-immune cell density as a function of tumor volume, approximated by an exponential curve (R2=0.963).

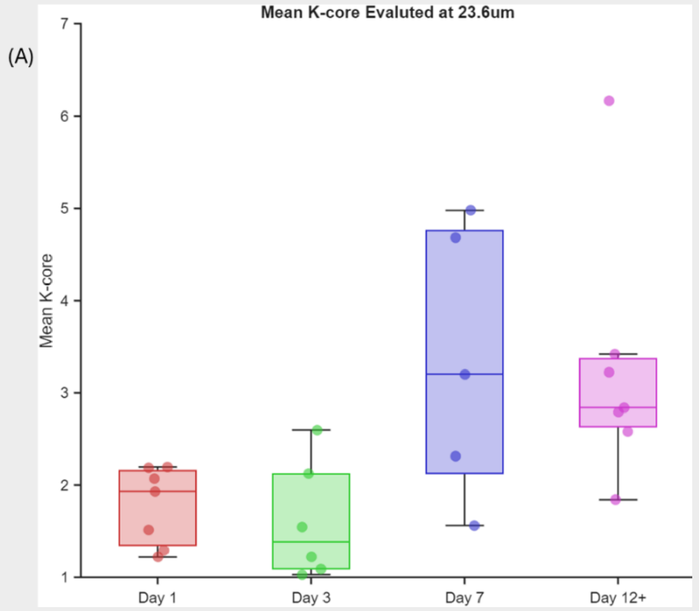


Figure 3: Differences in mean K-core value as a function of time. (A) Box and whisker plots comparing the mean K-core value of the graph networks over time. (B,C,D,E) Immune cell graph network (yellow) overlayed on the H&E stain, which segments tumor cells (red) and immune cells (blue).

DISCUSSION

- Digital Pathology provides spatial characterization of immune cells distribution withing the tumor microenvironment over time.
- Between day 1-7 there was an increase in the immune infiltrating density suggesting progressive immune infiltration into the tumor as the immune system is initially activated by chemoradiation and recruiting other immune cells.
- A greater immune-tumor cell density indicated greater overall tumor control at that given time point. There was an exponential trend between immune-tumor cell density and volume.
- Extending beyond immune cell counts, between day 1-7 there was an increase in K-core which describes the level of subnetworks of a given core structure. This suggests the transition from the innate response to an adaptive immune response, where immune cells are communicating with each other.

CONCLUSIONS

By performing tissue analysis at pre-defined endpoints and at humane endpoint, this study quantified the immune dynamics post chemoradiation. Digital pathology demonstrated progressive immune infiltration and increasing immune-tumor cell interactions over time. Pathomic network features such as K-core revealed dynamic spatial organization and communication among immune cells. Together, these finding highlight the value of spatial pathomic analysis for characterizing the temporal remodeling of the tumor microenvironment and suggest that higher order spatial features may serve as informative biomarkers to treatment response and tumor control.

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