

## TUMOR-DIRECTED IMMUNORADIOTHERAPY ENHANCES SURVEILLANCE FROM THE SENTINEL LYMPH NODE

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**Background** PD-1 inhibition (PD1i) has demonstrated no benefit for locally advanced HNSCC, and emerging neoadjuvant PD1i window of opportunity trial data yield promising but limited responses. Our previous work demonstrates that ablating tumor draining lymphatics compromises the response to immune-oncology therapy.<sup>1</sup> In concert, a recently completed phase I trial investigating neoadjuvant immunoradiotherapy (IRT) for treatment-naïve HNSCC demonstrated a 60% major pathologic response and 100% clinical-to-pathologic downstaging (NCT03247712). Accordingly, we hypothesize that lymphatic-preserving immune oncology therapy can potentiate PD1i and promote antitumor immunity by enhancing surveillance along the tumor-immune-lymphatic axis.

**Methods** To explore this, we employed our recently characterized tobacco-signature, orthotopic murine oral squamous cell carcinoma models, one of which matches the immune infiltrate and PD1i response of human disease and the other of which is immune-cold with limited response to PD1i. In both models we define an IRT scheme, using low-dose tumor-directed radiotherapy (td-RT), that achieves complete response and confers durable immunity.

**Results** Mechanistically, we observe that successful td-IRT potentiates the response to PD1i by coordinating antitumor immunity across the tumor and regional lymphatics – specifically, the sentinel lymph node (SLN) – suggesting an active

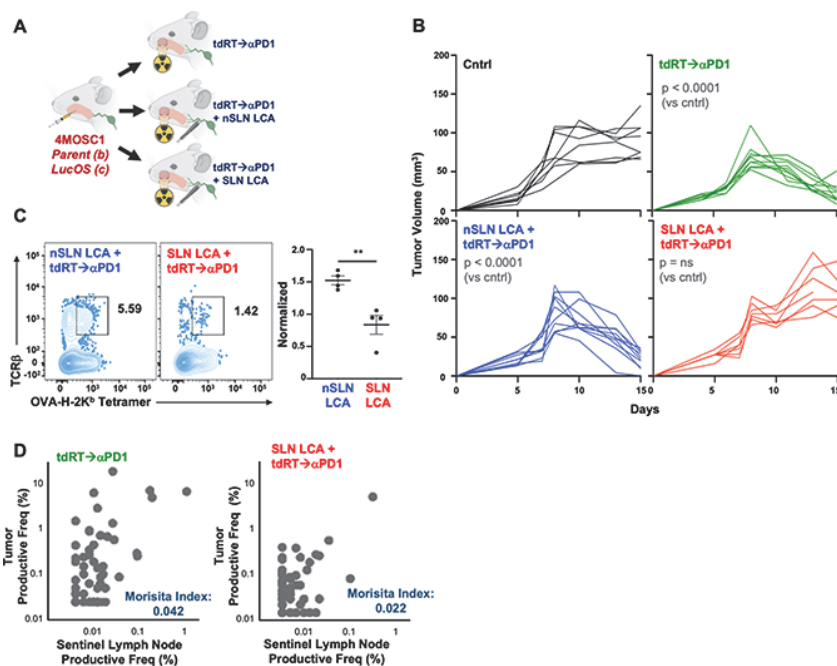
process of locoregional antitumor immunosurveillance. To study surveillance across the tumor-SLN axis, we map the locoregional lymphatics joining the primary tumor to its SLN and develop models to selectively ablate these lymphatic channels. Interestingly, ablation of the lymphatic channels that link the tumor and SLN is sufficient to block the tumor IRT response, leading to a restriction of cytotoxic, tumor-antigen specific CD8 T cells from the TIME and an overall reduced antitumor T cell repertoire (figure 1). To identify the requisite immune effectors that transit across tumor-SLN axis to mediate the td-IRT response, we employ tamoxifen-inducible reporter animal models in which we spatiotemporally label immune effectors during td-IRT with or without lymphatic channel ablation. Using CITE-sequencing, we comprehensively profile the dynamics of locoregional antitumor immunosurveillance at single cell resolution, finding that activated, migratory myeloid effector cells transiting between the SLN and tumor are critical for T cell priming and clonotypic expansion; and, ultimately, the successful tumor response to IRT.

**Conclusions** We demonstrate that targeting tumors with stereotactic radiation and PD1i while sparing draining lymphatics enhances anticancer immunity by promoting locoregional immunosurveillance between the tumor and sentinel lymph node, resulting in significantly improved responses. Overall, this work elucidates the mechanistic underpinnings of successful tumor responses to immune oncology therapy, which can immediately inform the design of next-generation therapies for HNSCC.

### REFERENCE

- Saddawi-Konefka R, et al. Lymphatic-preserving treatment sequencing with immune checkpoint inhibition unleashes cDC1-dependent antitumor immunity in HNSCC. *Nat Commun* 2022;**13**:4298.

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**Abstract 884 Figure 1** (A) Cartoon schema showing 4MOSC1 (B) parent or (C) luciferase-ovalbumin (LucOS) buccal-tumor bearing WT animals treated with tumor-directed radiation (tdRT) followed by pD-1 with or without lymphatic channel ablation (LCA) to the sentinel lymph node (SLN) or non-SLN. (B) Tumor kinetics after tdRT → PD1 ± LCA to the SLN or nSLN. (C) Quantification of tumor-infiltrating Ova-specific CD8 T cells after tdRT → PD1 ± LCA to the SLN or nSLN. (D) TCR-sequencing showing the TCR productive frequency (%) and similarity score [Morista Index] between the tumor and SLN after tdRT → PD1 (left) ± LCA (right).