A Fusion of Forces: A Preclinical Model to Study Mechanisms Impacted by Combination Biomaterial-Based Immunotherapy and Radiotherapy

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Objectives: Standard-of-care (SOC) treatment for head and neck squamous cell carcinomas (HNSCC) involves combinations of surgery and chemoradiotherapy (CRT). Severe acute and chronic side effects decreasing patients' quality of life could be de-escalated by combination with immunotherapy. We previously showed a single intratumoral injection of *STINGel* (a positively-charged multi-domain peptide loaded with STING agonist cyclic dinucleotide) or drug-mimicking *SynerGel* increased survival in HNSCC orthotopic mouse models. SOC RT may be utilized as a neoadjuvant to further delay tumor growth and enhance immunotherapy efficacy. We hypothesize that a novel biomaterial-based immunotherapy plus RT will enhance treatment efficacy via immune-modulation in HNSCC. Herein, we investigate the tumor immune microenvironment of heterotopic ROC1 tumor model and efficacy of a local immunotherapy targeting multiple tumor-promoting immune mechanisms for use with RT.

Experimental Methods: C57BL/6 mice were subcutaneously inoculated with $5x10^5$ ROC1 cells. Tissue and blood were harvested from ROC1 tumor-bearing mice and immune populations analyzed by flow cytometry. To study *SynerGel* treatment efficacy, established tumors were treated with single intratumoral injections of HBSS or different formulations of L-NIL-MDP hydrogel loaded with anti-PD-1, anti-CTLA4, or CDN. To study RT effect, fractionated RT (8Gy x 3) was performed on tumor-bearing mice.

Results: ROC1 tumors were sufficiently established in the murine flank. Efficacy study of ROC1 tumors treated with *SynerGel* alone or loaded with combinations of immune checkpoint inhibitors and CDN showed no differences in tumor growth or overall survival. RT of ROC1 tumor-bearing mice showed significant delayed tumor progression compared to untreated mice.

Conclusion: The success of irradiation treatment alone in delaying tumor growth is promising in its neoadjuvant potential to complement immunotherapy and improve efficacy. Alternative formulations of hydrogel-loaded treatment must be explored. Additionally, further investigation of the tumor microenvironment is needed to understand the mechanisms of immunosuppression that drive this treatment resistance.

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