**Development and Induction of Tertiary Lymphoid Structures in Lung Cancer for Improved Immunotherapeutics**

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Tertiary lymphoid structures (TLS) are ectopic immune structures that often form locally in cancer. TLS correlate with favorable prognosis in patients with solid tumors, including lung cancer. Further, TLS are associated with superior response to immune checkpoint blockade (ICB). B cells are predominantly located within TLS and correlate with improved survival and ICB response. Despite the therapeutic promise of B cells and TLS, they have not been investigated as immunotherapeutic targets. Moreover, the mechanisms of TLS development remain poorly understood due to a paucity of murine models with spontaneous TLS formation. Thus, our project utilizes a carcinogen (NNK) induced murine model of lung adenocarcinoma (LUAD) that recapitulates human LUAD and spontaneously develops TLS with approximately 20-30% of TLS containing germinal centers (GCs). In this model, TLS maturity i.e. GC formation is associated with an increase in tumor-infiltrating immune cells while the size of tumor correlates with the number of anti-tumor immune cells within TLS. According to our temporal assessment of TLS formation, we learned that B cells are the first to arrive for initial TLS formation, subsequently followed by T cells. Furthermore, inhibition of TLS formation via B cell depletion demonstrated a loss of effective antitumor immunity and increased tumor size, indicating the critical role of B cells in TLS formation and activity. In parallel with our mouse studies, our lab evaluates the complexity of TLS within human LUAD using multispectral imaging and spatial transcriptomics to uncover pathways that could improve TLS formation and subsequently immune cell function. According to our spatial transcriptomic data, TLS in patients can have incomplete expression of inducing factors such as LIGHT/LTβ, CXCL13, CD40 ligand, and IL-21. Thus, we have generated an oncolytic virus (OV) which can deliver these factors while also generating immunogenic antigens and stromal space for TLS to thrive. We are utilizing two syngeneic lung cancer murine models to test our OV; (1) FVBW-17, derived from NNK induced LUAD model, and (2) Lewis lung carcinoma (LLC). These studies will increase our mechanistic understanding of TLS development for improved immunotherapies and will potentially provide new therapeutic interventions to treat lung cancer patients.