Background Tertiary lymphoid structures (TLS) are lymphoid aggregates that often form locally in tissues with chronic infection, autoimmune disease, and cancer. As such, TLS correlate with favorable prognosis in patients with solid tumors, including non-small-cell lung cancer (NSCLC). Further, TLS have recently been associated with superior response to immune checkpoint blockade (ICB).1, 2, 3 B cells are predominantly located within TLS and correlate with improved survival and ICB response.4 Despite the therapeutic promise of B cells and TLS, they have not been investigated as immunotherapeutic targets. Moreover, a mechanistic understanding of TLS formation and function in cancer is lacking.

Methods Our studies aim to interrogate unique factors that promote or inhibit TLS formation. First, we studied TLS in human lung adenocarcinoma using multispectral imaging and spatial transcriptomics (Nanostring Digital Spatial Profiler) to uncover known and unknown pathways that could improve TLS formation and subsequently B and T cell function. We paired these studies with murine models of lung adenocarcinoma. Specifically, we utilized a physiologically relevant, carcinogen (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNK) induced murine model of lung cancer that spontaneously forms TLS. A syngeneic tumor line derived from this model that can be orthotopically injected into the murine lung also forms high B cell infiltrate and TLS. We utilized these models to test if TLS induction and maturation were increased with an oncolytic virus that targets known TLS-initiating factors.

Results According to spatial transcriptomics, tumor-associated TLS have decreased TLS-initiating and maturation factors such as CXCL13, IL-21, CD40, and LTbeta/LIGHT in comparison to normal lymphoid tissues. Further, as TLS proximity to the tumor increases, there is an increased immune regulation surrounding TLS formation. Thus, using an oncolytic virus that targets CXCL13, IL-21, CD40 and LTbeta ligation or LIGHT, we observed increased TLS induction and tumor reduction.

Conclusions These studies will increase our understanding of TLS formation for improved immunotherapies in NSCLC patients and will potentially provide therapeutic interventions that could be administered prior to cancer development.

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