Background
B cell infiltrate is a common feature of High Grade Serous Ovarian Cancer (HGSOC) and ~15% of patients contain B and T cell-rich tertiary lymphoid structures (TLS) which organize adaptive immunity and correlate with immunotherapy response in other cancer types. Increased B cells, plasma cells, and TLS correlate with improved prognosis in HGSOC. Sadly, current immunotherapies have exhibited poor response in ovarian cancer (~10%), highlighting the need for synergistic or complementary strategies. This research aims to identify underlying stromal and tumor-intrinsic factors promoting TLS formation and maturation in the ovarian tumor microenvironment. We hypothesize that pro-tumorigenic cancer associated mesenchymal stem cells (CA-MSC) hinder TLS, while BRCA mutant tumors promote TLS via DNA damage response. Increasing number and maturity of TLS could boost the poor response of HGSOC patients to immunotherapy.

Methods
HGSOC clinical samples were obtained with patient consent through the Pitt Biospecimen Core and clinician collaboration. Original Vectra MoTIF multiplex immunofluorescence panels were designed to capture immune phenotypes and consensus markers of TLS maturity. Digital image analysis was used to analyze TLS presence and maturity and CA-MSC to MSC ratios. These features are correlated with clinical annotations. Digital Spatial Profiling (DSP) using whole transcriptome libraries was conducted on n=16 HGSOC samples to provide a spatially resolved transcriptional signature of TLS, B cells, other immune cells and stroma. Analysis was performed in collaboration with the HCC Cancer Bioinformatics Services.

Results
We report the development of a new TLS scoring strategy which relies on multiplex immunofluorescence and spatial transcriptomics. This approach was applied to a large cohort of HGSOC patient samples. Using multiplex immunofluorescence panels, we report the frequency, composition and maturity of TLS across HGSOC anatomical sites (fallopian tube, ovary, omentum), noting an unexpected enrichment of mature structures in tumors residing in the fallopian tubes. Additionally, this analysis reveals a role for BRCA mutations in TLS dynamics. We also quantified CA-MSC burden within tumors using a novel multiplex panel, allowing correlation of CA-MSC to MSC ratio with TLS presence. Complementing this imaging data, we used spatial transcriptomics (DSP) to produce highly precise transcriptional signatures of TLS, TLS-resident versus non-resident B lymphocytes, and TLS-adjacent versus TLS-distal stromal signatures.

Conclusions
This work contributes a comprehensive and widely applicable TLS scoring strategy. Its application reveals stromal and tumor-intrinsic factors involved with TLS maturation, revealing plausible strategies for promoting the development of these anti-tumor structures, with the ultimate goal of increasing immunotherapy efficacy, and extending patient survival.

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Ethics Approval
This study obtained ethics approval under IRB: STUDY19060197. Specifically, "Prognostic Marker: Acquisition of Blood Samples and Tissue for Research Purposes (UPCI 07-058)”. Patient samples utilized were collected with patient consent.