Background: Tumor immune phenotypes - immune-infiltrated, immune-desert, and immune-excluded, characterized by the presence and distribution of lymphocytes in the tumor bed - are associated with patient response to immune checkpoint inhibitor therapy. Understanding the distribution of lymphocyte density at the cancer epithelium-stroma boundary can further our understanding of immune phenotypes and provide insights into how barriers to lymphocyte entry into the cancer epithelium may impact therapeutic response to immunotherapy.

Methods: Human tumor samples (n=102) from 5 tumor indicia (colorectal, ovarian, non-small cell lung, triple negative breast, and pancreatic cancer) were classified as infiltrated, desert, or excluded by pathologist assessment. H&E-stained whole-slide images were further analyzed using AI-powered tumor microenvironment (TME) models developed by PathAI (Boston, MA; commercially available as PathExploreTM) for tissue segmentation and cell type classification. Computationally-extracted features for each image included lymphocyte density in cancer epithelium, in cancer stroma, and within the gradient fold-change compared to baseline. Baseline is defined as the Cancer 60-120 μm distance bands. Values are reported by tumor type and immune phenotype, and by immune phenotype for all indications together. The gradient difference between excluded and infiltrated tumors is determined by the ratio of fold changes of lymphocyte density at Stroma 60–120 μm compared to baseline.

Conclusions: This analysis of H&E-based spatial features revealed that barriers to lymphocyte infiltration exist at the transition between cancer epithelium and stroma in tumors of all immune phenotypes. While the gradient in lymphocyte density from stroma to cancer epithelium was much lower in tumors classified as infiltrated than excluded, the presence of this gradient even in non-excluded tumors suggests that therapeutics which seek to address barriers to lymphocyte infiltration may benefit patients with all tumor immune phenotypes.