The epithelium-stroma interface serves as a barrier to immune cell infiltration across tumor immune phenotypes in epithelial cancers

Xinwei Sher[1], Fredrick D Gootkind[1], Antoine Italiano[2], Florent Peyraud[2], Jean-Philippe Guégan[3], Guy T Clifton[1], Laura A Dillon[1]

[1]Parthenon Therapeutics, Boston, MA, USA; [2]Early Phase Trials and Sarcoma Unit, Institut Bergonié, Bordeaux, France; University of Bordeaux, Bordeaux, France; [3]Explicyte Immuno-Oncology, Bordeaux, France

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 indications (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 PathExplore™) for tissue segmentation and cell type classification. Computationally-extracted features for each image included lymphocyte density in cancer epithelium, in cancer stroma, and within varying distances (0-60 μm and 60-120 μm) from the epithelium-stroma interface (ESI) (Figure 1). Lymphocyte densities in different ESI distance bands, and their gradient of change across the ESI from the outer stroma to inner were calculated and compared within and between tumor types and by immune phenotype.

Results

Lymphocyte densities dropped by an average of 5-fold from the stroma side to the epithelium side of the ESI in all tumor types tested (Table 1, Figure 2). While the observed gradient across the ESI was greatest in excluded tumors (3.7-28.1 fold change compared to baseline), it was also observed in infiltrated (3.9-6.8 fold change) and desert tumors (2.7-22.3 fold change) (Table 1). The difference between excluded and infiltrated tumors was greatest in colorectal cancer, where the gradient fold-change compared to baseline was over four times greater in excluded than infiltrated tumors (28.1 vs 6.8). The most pronounced decrease in lymphocyte density occurs within 60 μm of the ESI (Figure 2), suggesting that barriers to lymphocyte infiltration occurred at the ESI and in the immediately adjacent stroma.

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.

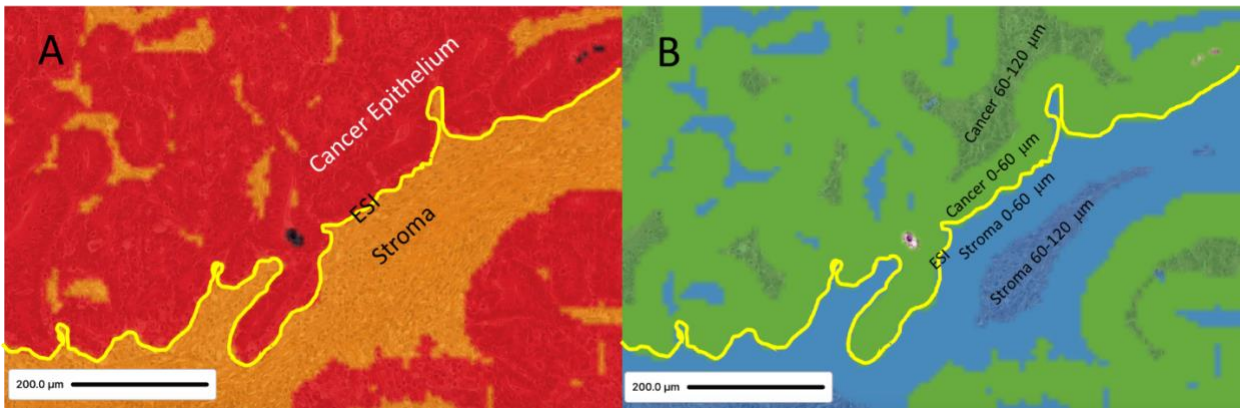

Figure 1. Colorectal cancer tumor segmentation example. Tumor sample A) segmented as epithelium (red), stroma (orange), and necrosis (black). An epithelium-stroma interface (ESI) is illustrated as a yellow line; B) segmented as ESI distance bands defined within 0-60 µm (solid) and 60-120 µm (textured) of the ESI in the cancer epithelium (green) and cancer stroma (blue).

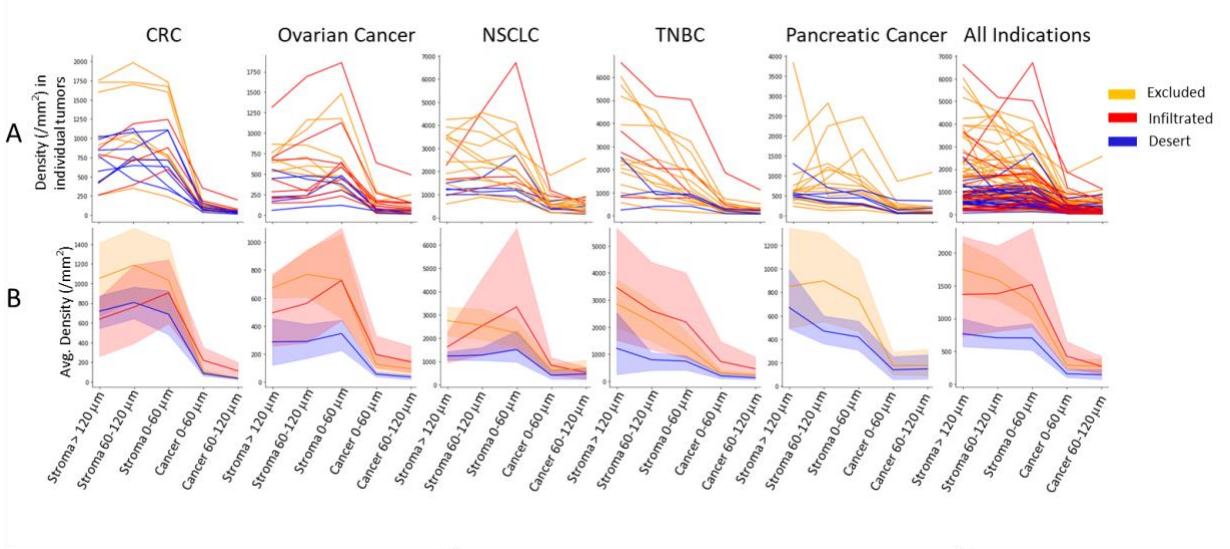

Figure 2. Lymphocyte densities in bands of cancer epithelium and cancer stroma at different distances (0-60 µm or 60-120 µm) from the epithelium-stroma interface (ESI) by tumor type and for all indications together. A) Lymphocyte densities by ESI distance band for individual tumors, colored by immune phenotype categorization. B) Averages and confidence intervals of lymphocyte densities bands by immune phenotype.

CRC: Colorectal Cancer; NSCLC: Non-Small Cell Lung Cancer; TNBC: Triple Negative Breast Cancer

		Cancer 60-120 μm	Cancer 0-60 μm	Stroma 0-60 μm	Stroma 60-120 μm	Stroma > 120 μm	Difference between Excluded and Infiltrated
CRC	Excluded	1	2.3	24.5	28.1	25.0	4.2
	Infiltrated	1	2.0	8.0	6.8	5.7	
	Desert	1	2.4	19.0	22.3	19.8	
Ovarian	Excluded	1	1.3	7.7	8.1	7.1	2.1
	Infiltrated	1	1.4	5.0	3.9	3.4	
	Desert	1	1.6	9.5	8.0	7.9	
NSCLC	Excluded	1	0.9	3.2	3.7	4.0	0.8
	Infiltrated	1	1.6	6.2	4.7	3.0	
	Desert	1	0.9	3.2	2.7	2.7	
TNBC	Excluded	1	1.4	5.8	9.7	12.6	1.7
	Infiltrated	1	1.6	4.7	5.6	7.5	
	Desert	1	1.6	5.9	6.3	9.6	
Pancreatic	Excluded	1	1.0	4.2	5.1	4.8	NA
	Desert	1	0.9	2.9	3.2	4.6	
All Indications	Excluded	1	1.1	4.6	5.9	6.5	1.2
	Infiltrated	1	1.6	5.5	5.0	5.0	
	Desert	1	1.1	5.0	5.0	5.4	

Table 1. Average fold change difference of lymphocyte density in distance bands - 0-60 μm or 60-120 μm - from the epithelium-stroma interface (ESI) on the cancer or stroma sides of the ESI, as compared to baseline. Baseline is defined as the Cancer 60-120 μm ESI distance band. 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.