High prevalence of immune exclusion in cancer as determined by pathologist assessment and image analysis

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Background
Immune infiltrated tumors have high levels of lymphocytes contacting tumor cells and are more responsive to checkpoint inhibitors. Tumors with few lymphocytes in contact with tumor cells can be divided into desert or excluded phenotypes based on lymphocyte absence/paucity or restriction to the peritumoral stroma, respectively. Standard methods to systematically identify and characterize immune exclusion for patient stratification are lacking.

Methods
Slides from colorectal (CRC), non-small cell lung (NSCLC), ovarian (OC), pancreatic (PDAC), and triple-negative breast cancers (TNBC), and leiomyosarcoma (LMS) and undifferentiated pleomorphic sarcoma (UPS) were stained with multiplex IHC (mIHC) for CD8 and panCK. Pathologist assessment (PA) was done to classify the tumors as: desert, with a paucity of CD8 T cells; excluded, with CD8 T cells not penetrating the tumor parenchyma; and infiltrated, with CD8 T cells within the tumor parenchyma. For the carcinomas, adjacent sections were stained with a multiplex immunofluorescence (mIF) panel containing CD8 and a tumor cell marker. Image analysis (IA) was performed on the mIF images to quantify CD8 cell density in the tumor parenchyma and stroma to categorize immune phenotypes.

Results
Immune phenotypes were classified for 143 samples based on PA of mIHC images and 103 samples by IA of mIF images (see Table 1). Immune exclusion as determined by both PA and IA was highest in CRC, PDAC, and TNBC. IA differed from PA in 25 (24.3%) cases. Pathologist review of the discordant cases revealed discrepancies were generally due to tumor heterogeneity, thresholding, assessment of cells at the tumor-stroma boundaries, necrosis, and artifacts.

Conclusion
Immune exclusion is highly prevalent in the examined carcinoma types. IA-based approaches, guided by pathologist input, offer promise to quantitatively determine tumor immune phenotypes in a quick and systematic way to guide patients to the most effective therapy.

Table 1:

		Pathologist Assessment Classification			Image Analysis Classification		
Tumor Type	**n**	**Desert (%)**	**Excluded (%)**	**Infiltrated (%)**	**Desert (%)**	**Excluded (%)**	**Infiltrated (%)**
CRC	20	3 (15.0)	14 (70.0)	3 (15.0)	6 (30.0)	11 (55.0)	3 (15.0)
NSCLC	21	6 (28.6)	12 (57.1)	3 (14.3)	6 (28.6)	9 (42.9)	6 (28.6)
OC	20	3 (15.0)	9 (45.0)	8 (40.0)	9 (45.0)	3 (15.0)	8 (40.0)
PDAC	21	6 (28.6)	14 (66.7)	1 (4.8)	7 (33.3)	12 (57.1)	2 (9.5)
TNBC	21	3 (14.3)	15 (71.4)	3 (14.3)	4 (19.0)	10 (47.6)	7 (33.3)
LMS	20	3 (15.0)	1 (5.0)	16 (80.0)	NA	NA	NA
UPS	20	4 (20.0)	1 (5.0)	15 (75.0)	NA	NA	NA
Total	143/103	28 (19.6)	66 (46.2)	49 (34.3)	32 (31.1)	45 (43.7)	26 (25.2)