Leukocyte Heterogeneity in Pancreatic Ductal Adenocarcinoma: Phenotypic and Spatial Features Associated with Clinical Outcome

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SUPPLEMENTARY DATA

SUPPLEMENTARY MATERIALS AND METHODS

ROI Selection

Whole-slide digital images of hematoxylin and PanCK staining from each tissue sample were annotated by a pathologist (S.H.) in Aperio ImageScope software (Leica Biosystems) to demarcate areas with different histopathology. Pathology annotations and CD45 immunoreactivity were used in combination to select leukocyte-infiltrated ROIs distributed throughout tissues. Areas of necrosis were excluded. ROIs containing invasive carcinoma ('T' regions) were evaluated in all specimens (1-4 ROIs per specimen). ROIs in the following histopathological regions were also analyzed in samples where they were identified: TAS located outside of annotated invasive carcinoma (1-2 ROIs per specimen), AN located outside of annotated invasive carcinoma (1-2 ROIs per specimen), LN (1-2 ROIs per specimen), pre-invasive dysplasia (1-3 ROIs per specimen), and TLS located within or outside annotated invasive carcinoma. For TLS analysis, TLS were manually identified by evaluation of hematoxylin, CD45, and CD20 immunoreactivity and assessed for MECA-79 positivity (n = 787 TLS total). All identified TLS were quantitatively analyzed for $CD45^+CD3^+$ (T cell) and $CD45^+CD20^+$ (B cell) density. A subset of

TLS (n = 173 total, 1-6 TLS ROIs per specimen) were randomly selected for full image cytometry analysis of myeloid, lymphoid, and functional mIHC panels. For HN pancreas, 3 ROIs were evaluated per specimen. ROI quantity, area, and placement were maintained across serial tissue sections in all samples to ensure the same regions were assessed with each antibody panel. Unless otherwise stated, data presented represent cumulative cell densities from multiple ROIs within an individual patient sample, grouped by histopathologic type. Cumulative density of each leukocyte population was calculated by: total cell number in all ROIs / total tissue area of all ROIs = cumulative cell density in mm^2.

For spatial mapping, proximity of individual ROIs to nearest annotated area of invasive carcinoma was manually measured in Aperio ImageScope software, and ROIs were assigned to spatial categories as shown in **Figure 2E**. Measurement criteria for these spatial categories were selected to most comprehensively encapsulate ROIs distributed across extensive regions of tissue. Sankey diagrams were generated with the alluvial R package (1).

Image Processing and Analysis

Image co-registration and processing were performed using methods adapted from our previously described workflow (2,3). ROIs from each single antibody stain were registered to the same regions on the hematoxylin-stained image using the detectSURFfeatures algorithm in the Computer Vision Toolbox in MATLAB version R2018b (The MathWorks, Inc., Natick, MA). This approach used an affine transformation, and only ROIs that were well-registered were included for downstream analysis. Image processing, cell quantification, and image cytometry were performed using Fiji, CellProfiler Version 3.5.1 (4), and FCS Express 6 Image Cytometry RUO (De Novo Software, Glendale, CA), respectively.

AEC chromogen signal was extracted in Fiji for cell quantification and visualization using a custom macro for color deconvolution. Briefly, the plugin Color_Deconvolution [H AEC] was used to separate hematoxylin, followed by postprocessing steps for signal cleaning and background elimination. The processed hematoxylin image was used to generate a nuclei binary mask. AEC signal was extracted with the NIH plugin RGB_to_CMYK to separate AEC signal into the Y channel (5). Signal extracted images were processed in CellProfiler to quantify single cell mean intensity measurements for each stain, scaled to a range of 0-1. The binary segmentation mask produced in Fiji was used to identify nuclei with the IdentifyPrimaryObjects module and mean intensity for each object for every marker measured using the MeasureObjectIntensity module. The output of these processes is mean signal intensity of every cell for each antibody stain, with data subsequently imported into FCS Express for manually gated single-cell image cytometry. Hierarchical cell classifications and image cytometry gating strategies are shown in **Figures 1C** and **S1A-C**. For mIHC visualization, single channel images were merged in pseudocolor using Fiji.

vTMA Analysis

1.0 mm vTMA cores were generated in Fiji and overlaid in Aperio ImageScope onto existing T ROIs that had been evaluated by mIHC (n = 5 treatment-naïve PDAC specimens). 19-28 vTMA cores were evaluated per specimen, depending on mIHC ROI area, and mIHC staining within each core was quantitatively evaluated by image cytometry in FCS Express. For each specimen, combinations of cores (1-18 vTMA cores per combination) were randomly sampled 100 times to generate a distribution of $CD3^+$ T cell, $CD20^+$ B cell, and $CD68^+$ monocyte/macrophage immunostaining per 'N' cores. These distributions were compared to a vTMA core reference mean (average percent positive cells from all

vTMA cores per patient) and a mIHC weighted mean (average percent positive cells from all mIHC T ROIs per patient, weighted by the number of vTMA cores contained within each ROI).

Molecular Status Determination

Molecular status of *KRAS, TP53, CDKN2A,* and *SMAD4* was determined for a subset of samples using integrated DNA sequencing and IHC approaches. For Cohort 1 samples, DNA was extracted from FFPE sections, and *KRAS, TP53,* and *CDKN2A* alterations were identified through DNA sequencing with either the 595-gene Tempus xT targeted cancer genome sequencing panel (Tempus, Chicago, IL), or the 124-gene GeneTrails Comprehensive Solid Tumor Panel clinical assay (Knight Diagnostics Laboratories, OHSU). *SMAD4* was detected by IHC as previously described (6), and slides were reviewed by three pathologists (J.A.N, A.D.C., S.A.V.) who jointly assigned Smad4 classification. For Cohort 2 samples, *KRAS* status was determined via combination of pyrosequencing and next generation sequencing; status of *CDKN2A, TP53,* and *SMAD4* were determined using a combination of next generation sequencing and IHC, as previously reported (6).

Unsupervised Analysis

Hierarchical clustering and heatmap generation was performed using the pheatmap R package (7). t-SNEs were generated in R with Rtsne (8). PCA was performed with the factoextra R package (9).

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SUPPLEMENTARY TABLES

Supplementary Table S1. Baseline characteristics of treatment-naïve patients with surgically resected PDAC

	Total	OHSU (Cohort 1)	DF/BWCC (Cohort 2)	*P*[a]
Number of subjects	104	46	58	
Women, n (%)	57 (55%)	24 (52%)	33 (57%)	0.69
Age in years, median (Q1, Q3)	64.0 (56.0, 71.5)	63.5 (58.0, 71.0)	64.5 (54.0, 72.0)	0.97
Tumor location, n (%)				
Head/Uncinate	81 (78%)	40 (87%)	41 (71%)	0.06
Body/Tail	23 (22%)	6 (13%)	17 (29%)	
Tumor size in cm, median (Q1, Q3)[b]	3.0 (2.5, 4.0)	3.4 (2.5, 4.5)	3.0 (2.5, 3.5)	0.23
AJCC 8th ed. pT stage, n (%)				
T1	11 (11%)	7 (15%)	4 (8%)	0.06
T2	62 (63%)	23 (50%)	39 (75%)	
T3	23 (24%)	15 (33%)	8 (15%)	
T4	2 (2%)	1 (2%)	1 (2%)	
Tx (cannot be assessed)	6	-	6	
AJCC 8th ed. pN stage, n (%)				
N0	28 (27%)	12 (26%)	16 (27%)	0.64
N1	38 (36.5%)	15 (33%)	23 (40%)	
N2	38 (36.5%)	19 (41%)	19 (33%)	
Tumor differentiation, n (%)				
Well/Moderately differentiated	53 (52%)	23 (51%)	30 (54%)	0.84
Poorly differentiated/Undifferentiated	48 (48%)	22 (49%)	26 (46%)	
Unknown	3	1	2	
Resection margin status, n (%)				
R0	43 (41%)	20 (44%)	23 (40%)	0.54
R1	60 (58%)	25 (54%)	35 (60%)	
R2	1 (1%)	1 (2%)	-	
Lymphovascular invasion, n (%)				
Negative	38 (38%)	11 (24%)	27 (50%)	0.01
Positive	61 (62%)	34 (76%)	27 (50%)	
Unknown	5	1	4	
Perineural invasion, n (%)				
Negative	12 (12%)	3 (7%)	9 (16%)	0.22
Positive	90 (88%)	42 (93%)	48 (84%)	
Unknown	2	1	1	
Adjuvant treatment, n (%)				
None	12 (11%)	5 (11%)	7 (12%)	0.50
Chemotherapy only	32 (31%)	17 (37%)	15 (26%)	
Radiation or chemoradiation only	10 (10%)	4 (9%)	6 (10%)	
Chemoradiation and chemotherapy	42 (40%)	15 (32%)	27 (47%)	
Other/Unknown	8 (8%)	5 (11%)	3 (5%)	
SMAD4, n (%)				0.11
Lost	56 (54%)	29 (63%)	27 (47%)	
Intact	48 (46%)	17 (37%)	31 (53%)	
KRAS, n (%)				0.18
Wild-type	6 (7.5%)	0	6 (10%)	
Mutant	74 (92.5%)	22 (100%)	52 (90%)	
Unknown	24	24	0	
TP53, n (%)				0.19
Wild-type	28 (36%)	5 (23%)	23 (41%)	
Altered	50 (64%)	17 (77%)	33 (59%)	
Unknown	26	24	2	
CDKN2A, n (%)				0.44
Intact	51 (65%)	16 (73%)	35 (62.5%)	
Lost	27 (35%)	6 (27%)	21 (37.5%)	
Unknown	26	24	2	

[a] *P* value for Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables.
[b] Among 98/104 patients with available tumor size data.

Abbreviations: OHSU, Oregon Health & Science University; DF/BWCC, Dana-Farber/Brigham and Women's Cancer Center; Q1, 25th percentile; Q3, 75th

Supplementary Table S2. Baseline characteristics of presurgically-treated patients with resected PDAC

	OHSU (Cohort 1)
Number of subjects	13
Women, n (%)	4 (31%)
Age in years, median (Q1, Q3)	61.0 (56.0, 69.0)
Tumor location, n (%)	
Head/Uncinate	10 (77%)
Body/Tail	3 (23%)
Tumor size in cm, median (Q1, Q3)[a]	3.8 (2.9, 4.4)
AJCC 8th ed. pT stage, n (%)	
T1	3 (23%)
T2	3 (23%)
T3	5 (38%)
T4	2 (15%)
AJCC 8th ed. pN stage, n (%)	
N0	7 (54%)
N1	4 (31%)
N2	2 (15%)
Tumor differentiation, n (%)	
Well/Moderately differentiated	4 (31%)
Poorly differentiated/Undifferentiated	6 (46%)
Unknown	3 (23%)
Resection margin status, n (%)	
R0	5 (38%)
R1	7 (54%)
R2	1 (8%)
Lymphovascular invasion, n (%)	
Negative	7 (54%)
Positive	6 (46%)
Perineural invasion, n (%)	
Negative	0 (0%)
Positive	13 (100%)
Presurgical treatment, n (%)	
Chemotherapy only	6 (46%)
Radiation or chemoradiation only	1 (8%)
(Chemo)radiation and chemotherapy	6 (46%)
Unknown	0
Presurgical chemotherapy agent, n (%)	
FOLFIRINOX	3 (23%)
Gemcitabine/nab-paclitaxel	7 (54%)
Other combination	3 (23%)
Unknown	0 (0%)
Presurgical chemoradiation agent, n (%)	
No concurrent chemoradiotherapy	9 (69%)
Gemcitabine/nab-paclitaxel	1 (8%)
5-FU or capecitabine	3 (23%)
Histologic response to presurgical/neoadjuvant therapy, n (%)	
Poor or no response	3 (23%)
Moderate response	3 (23%)
Marked response (minimal residual disease)	6 (46%)
Unknown	1 (8%)
Adjuvant treatment, n (%)	
None	6 (46%)
Chemotherapy only	5 (38%)
Radiation or chemoradiation only	0 (0%)
Chemoradiation and chemotherapy	0 (0%)
Other/Unknown	2 (15%)

[a]Among 11/13 patients with available tumor size data
Abbreviations: Q1, 25th percentile; Q3, 75th percentile; AJCC, American Joint Committee on Cancer

Supplementary Table S3. Characteristics of pancreatic primary tumors and distant metastases from Cohort 3

	PICI (Cohort 3)[a]
Number of subjects	18
Women, n (%)	8 (44%)
Age in years, median (Q1, Q3)	65 (60, 69)
Primary tumor location, n (%)	
Head/Uncinate	56%
Body/Tail	44%
AJCC 8th ed. pM stage, n (%)[b]	
M0	10
M1	8
Tissue site, specimen type	
Pancreas, biopsy	3
Pancreas, surgical resection	6
Liver, biopsy	7
Lung, biopsy	1
Peritoneum, biopsy	1
Cancer treatment prior to specimen collection, n (%)	
None	16
Chemotherapy only	0
Radiation or chemoradiation only	0
Chemoradiation and chemotherapy	2

[a]Patients within this cohort were treated at multiple institutions participating in the PRINCE clinical trial (NCT03214250). Tissues evaluated herein are pre-study baseline tissues.
[b]pM staging at time of initial PDAC diagnosis.

Pancreatic biopsies (n = 3) are from patients with Stage IV disease; pancreatic surgical resections are from different patients (n = 6) with Stage II disease at time of tissue collection. Liver, lung, and peritoneum biopsies (n = 9 total) are confirmed metastatic PDAC and are not matched to primary pancreatic tumors in this cohort.

Abbreviations: PICI, Parker Institute for Cancer Immunotherapy; Q1, 25th percentile; Q3, 75th percentile; AJCC, American Joint Committee on Cancer

Supplementary Table S4. mIHC Antibody Panels

Myeloid Panel

	Pre-AR	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7
Primary Antibody	**Hematoxylin**	**CD68**	**DC-SIGN**	**DC-LAMP**	**CD45**	**CD66b**	**HLA-DPB1**	**CD163**
RRID	N/A	AB_306119	AB_1121347	AB_2827532	AB_467274	AB_396066	AB_2827533	AB_10982556
Clone	N/A	PG-M1	DC-28	1010E1.01	HI30	G10F5	EPR11226	10D6
Vendor	Dako	Abcam	Santa Cruz	Novus Biologicals	ThermoFisher	BD Biosciences	Abcam	ThermoFisher
Catalog #	S3301	ab783	sc-65740	DDX0191P-100	14-0459-82	555723	ab157210	MA5-11458
Concentration	N/A	1:50	1:100	1:100	1:100	1:200	1:20,000	1:100
Incubation	1 min @ RT	30 min @ RT	30 min @ RT	30 min @ RT	60 min @ RT	O/N @ 4ºC	30 min @ RT	30 min @ RT

	Cycle 8	Cycle 9	Cycle 10	Cycle 11	Cycle 12
Primary Antibody	**PD-L1**	**CD3/CD20/NKp46**	**Tryptase**	**αSMA**	**Pan Cytokeratin**
RRID	AB_2687655	see footnote	AB_303023	AB_2223021	AB_777047
Clone	E1L3N	see footnote	AA1	Polyclonal	AE1/AE3
Vendor	Cell Signaling	see footnote	Abcam	Abcam	Abcam
Catalog #	13684S	see footnote	ab2378	ab5694	ab27988
Concentration	1:100	see footnote	1:20,000	1:200	1:2000
Incubation	O/N @ 4ºC	30 min @ RT	30 min @ RT	30 min @ RT	30 min @ RT

Lymphoid Panel

	Pre-AR	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7
Primary Antibody	**Hematoxylin**	**PD-1**	**CD3**	**RORγt**	**NKp46**	**CD45**	**CD8α**	**T-bet**
RRID	N/A	AB_881954	AB_149922	AB_11205416	AB_2149153	AB_467274	AB_11000353	AB_2616022
Clone	N/A	NAT105	SP7	6F3.1	195314	HI30	C8/144B	D6N8B
Vendor	Dako	Abcam	ThermoFisher	Millipore Sigma	R&D Systems	ThermoFisher	ThermoFisher	Cell Signaling
Catalog #	S3301	ab52587	RM-9107-S	MABF81	MAB1850	14-0459-82	MA5-13473	13232S
Concentration	N/A	1:50	1:150	1:200	1:20	1:100	1:100	1:500
Incubation	1 min @ RT	30 min @ RT	30 min @ RT	30 min @ RT	30 min @ RT	60 min @ RT	30 min @ RT	O/N @ 4ºC

	Cycle 8	Cycle 9	Cycle 10	Cycle 11	Cycle 12	Cycle 13	Cycle 14
Primary Antibody	**GATA3**	**PD-L1**	**Foxp3**	**CD20**	**Smad4**	**αSMA**	**Pan Cytokeratin**
RRID	AB_10895444	AB_2687655	AB_467556	AB_1139386	AB_627905	AB_2223021	AB_777047
Clone	L50-823	E1L3N	236A/E7	SP32	B-8	Polyclonal	AE1/AE3
Vendor	BioCare	Cell Signaling	ThermoFisher	Abcam	Santa Cruz	Abcam	Abcam
Catalog #	CM405A	13684S	14-4777-82	ab64088	sc-7966	ab5694	ab27988
Concentration	1:50	1:100	1:40	1:1000	1:50	1:200	1:2000
Incubation	O/N @ 4ºC	O/N @ 4ºC	30 min @ RT	60 min @ RT	O/N @ 4ºC	30 min @ RT	30 min @ RT

Functional Panel

	Pre-AR	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7
	Round 1	Round 1	Round 1	Round 1	Round 1	Round 1	Round 1	Round 1
Primary Antibody	**Hematoxylin**	**PD-1**	**CD138**	**CD68**	**CD38**	**CD45**	**IDO**	**CD8α**
RRID	N/A	AB_881954	AB_10987019	AB_306119	AB_10986743	AB_467274	AB_1977068	AB_11000353
Clone	N/A	NAT105	MI15	PG-M1	38C03 (SPC32)	HI30	1F8.2	C8/144B
Vendor	Dako	Abcam	ThermoFisher	Abcam	ThermoFisher	ThermoFisher	Millipore Sigma	ThermoFisher
Catalog #	S3301	ab52587	MA5-12400	ab783	MA5-14413	14-0459-82	MAB10009	MA5-13473
Concentration	N/A	1:50	1:20	1:50	1:100	1:100	1:100	1:100
Incubation	1 min @ RT	30 min @ RT	30 min @ RT	30 min @ RT	30 min @ RT	60 min @ RT	30 min @ RT	30 min @ RT
		Round 2	Round 2	Round 2	Round 2	Round 2	Round 2	Round 2
Primary Antibody		**PD-L1**	**CD4**	**CD3**	**T-bet**	**Granzyme B**	**CD278 (ICOS)**	**CD27**
RRID		AB_2687655	AB_2335982	AB_149922	AB_2616022	AB_304251	AB_2827535	AB_2827537
Clone		E1L3N	SP35	SP7	D6N8B	Polyclonal	SP98	Polyclonal
Vendor		Cell Signaling	Ventana	ThermoFisher	Cell Signaling	Abcam	LifeSpan Bio	Novus Biologicals
Catalog #		13684S	790-4423	RM-9107-S	13232S	ab4059	LS-C210350-500	NBP2-38434
Concentration		1:100	1:4	1:150	1:500	1:200	1:100	1:500
Incubation		O/N @ 4ºC	30 min @ RT	30 min @ RT	O/N @ 4ºC	30 min @ RT	30 min @ RT	30 min @ RT

	Cycle 8	Cycle 9	Cycle 10	Cycle 11	Cycle 12
	Round 1	Round 1	Round 1	Round 1	Round 1
Primary Antibody	**CD5**	**IgD**	**CD20**	**Pan Cytokeratin**	**PNAd (MECA-79)**
RRID	AB_10985112	AB_10974228	AB_1139386	AB_777047	AB_395099
Clone	4C7	EPR6146	SP32	AE1/AE3	MECA-79
Vendor	ThermoFisher	Abcam	Abcam	Abcam	BD Biosciences
Catalog #	MA5-13308	ab124795	ab64088	ab27988	553863
Concentration	1:40	1:200	1:1000	1:2000	1:500
Incubation	O/N @ 4ºC	O/N @ 4ºC	60 min @ RT	30 min @ RT	O/N @ 4ºC
	Round 2	Round 2	Round 2	Round 2	Round 2
Primary Antibody	**EOMES (Tbr2)**	---	---	**Ki67**	---
RRID	AB_10806889			AB_1158031	
Clone	Polyclonal			SP6	
Vendor	Millipore Sigma			Millipore Sigma	
Catalog #	AB2283			275R-14	
Concentration	1:1000			1:500	
Incubation	30 min @ RT			30 min @ RT	

a. In Myeloid panel Cycle 9, antibodies were applied simultaneously in a cocktail. CD3 (Clone SP7, ThermoFisher RM-9107-S, 1:150), CD20 (Clone: SP32, Abcam ab64088 , 1:1000), NKp46 (Clone 195314, R&D Systems MAB1850, 1:20)

Abbreviations: AR, antigen retrieval; RRID, Research Resource Identifier; αSMA, alpha smooth muscle actin

Supplementary Table S5. Summary of tissue area analyzed, median cell counts, and median cell densities across histopathological regions

	Tumor (T)	Tumor Adjacent Stroma (TAS)	Tumor Adjacent Normal (AN)	Tertiary Lymphoid Structure (TLS)[a]	Healthy Normal Pancreas (HN)
Total # ROIs Analyzed	407	162	50	173	18
Avg. Area Analyzed per Specimen (mm^2)	20.2	8.8	3.2	0.7	11.1
Cell Counts per Patient, Median (IQR)					
$PanCK^+$	22883 (11938-33497)	3255 (1066-8450)	4532 (2193-7366)	25 (4-80)	20285 (16323-24624)
$CD8^+$ T cell	1192 (469-2682)	979 (388-1973)	207 (95-445)	576 (300-1317)	599 (330-724)
Th0 T cell	1356 (477-3019)	684 (371-1604)	100 (44-184)	624 (278-1345)	91 (79-206)
Th1 T cell	50 (14-115)	27 (8-71)	4 (2-9)	17 (3-39)	7 (4-9)
Th2 T cell	110 (36-291)	103 (27-301)	13 (2-25)	201 (45-551)	1 (0-3)
Th17 T cell	16 (6-62)	7 (2-24)	2 (0-4)	3 (1-12)	7 (3-11)
Treg	288 (86-576)	119 (47-296)	9 (3-31)	48 (21-113)	2 (1-5)
$CD20^+$ B cell	344 (120-795)	252 (81-500)	34 (11-76)	1857 (721-3850)	13 (3-42)
Plasma cell	11 (1-70)	3 (1-12)	0 (0-2)	1 (0-3)	not evaluated
Plasmablast	54 (12-234)	26 (11-72)	5 (0-9)	2 (0-8)	not evaluated
Mast cell	337 (143-829)	216 (97-454)	171 (64-324)	13 (5-27)	154 (90-177)
Neutrophil/Eosinophil	754 (265-2647)	257 (109-953)	379 (145-913)	13 (5-33)	68 (27-97)
$DC\text{-}LAMP^+$ DC	27 (12-56)	15 (5-29)	15 (5-43)	16 (4-43)	1 (0-2)
$DC\text{-}LAMP^-$ DC	1062 (305-2585)	686 (287-1559)	591 (181-1408)	81 (10-224)	9 (4-23)
$CD163^+$ Mono/Macrophage	996 (437-2367)	476 (167-1184)	350 (101-857)	19 (8-52)	1053 (889-1184)
$CD163^-$ Mono/Macrophage	676 (315-1570)	190 (95-515)	212 (100-485)	10 (4-26)	111 (37-204)
Cell Density[b] per Patient, Median (IQR)					
$PanCK^+$	995 (660-1526)	391 (210-841)	1645 (1150-2061)	41 (16-111)	1873 (1717-1940)
$CD8^+$ T cell	76 (31-142)	131 (66-240)	83 (43-128)	1250 (896-2435)	40 (28-47)
Th0 T cell	77 (34-134)	99 (48-165)	29 (17-58)	1111 (634-1927)	11 (7-16)
Th1 T cell	3 (1-6)	4 (1-9)	2 (1-2)	28 (9-79)	1 (0-1)
Th2 T cell	7 (2-18)	14 (4-35)	4 (1-9)	474 (165-814)	0 (0-0)
Th17 T cell	1 (0-4)	1 (0-2)	1 (0-2)	6 (1-19)	1 (0-1)
Treg	14 (5-26)	17 (7-32)	4 (1-7)	104 (52-195)	0 (0-0)
$CD20^+$ B cell	21 (8-42)	28 (14-52)	10 (6-19)	4230 (3069-5202)	1 (0-5)
Plasma cell	2 (0-10)	0 (0-2)	0 (0-1)	0 (0-5)	not evaluated
Plasmablast	2 (0-7)	3 (1-9)	1 (0-3)	4 (0-11)	not evaluated
Mast cell	21 (10-42)	28 (18-49)	18 (6-25)	21 (11-53)	11 (9-13)
Neutrophil/Eosinophil	49 (17-149)	49 (13-121)	13 (8-24)	25 (11-56)	5 (2-11)
$DC\text{-}LAMP^+$ DC	1 (1-4)	2 (1-4)	1 (1-6)	33 (16-65)	0 (0-0)
$DC\text{-}LAMP^-$ DC	69 (21-161)	99 (44-180)	64 (21-149)	129 (29-513)	1 (0-2)
$CD163^+$ Mono/Macrophage	61 (26-118)	63 (27-119)	25 (9-73)	49 (17-112)	78 (73-92)
$CD163^-$ Mono/Macrophage	39 (20-68)	26 (14-54)	15 (6-40)	24 (11-53)	10 (6-17)

[a]TLS data included here reflect the subset TLS ROIs fully analyzed in all mIHC panels. See Materials & Methods for additional detail.
[b]Cell density reported as cells per mm^2
Abbreviations: IQR, interquartile range

Supplementary Table S6. Overview of individual ROIs analyzed from PDACs in Cohorts 1 and 2, by histopathology type and location

	Histopathology Type					
	Tumor (T)	**Tumor Adjacent Stroma (TAS)**	**Adjacent Normal (AN)**	**Tertiary Lymphoid Structure (TLS)**	**Lymph Node (LN)**	**Dysplasia**
Location[a]: Intratumoral	312 (Tx Naïve) 40 (PST)	0 (Tx Naïve) 0 (PST)	0 (Tx Naïve) 0 (PST)	67 (Tx Naïve) 1 (PST)	2 (Tx Naïve)* 0 (PST)	6 (Tx Naïve)* 0 (PST)
Location: Border	----	38 (Tx Naïve) 5 (PST)	----	46 (Tx Naïve) 4 (PST)	1 (Tx Naïve) 0 (PST)	8 (Tx Naïve) 0 (PST)
Location: Spanning Border-Distal	----	26 (Tx Naïve) 4 (PST)	2 (Tx Naïve) 0 (PST)	1 (Tx Naïve) 2 (PST)	----	----
Location: Distal	----	72 (Tx Naïve) 16 (PST)	42 (Tx Naïve) 3 (PST)	42 (Tx Naïve) 11 (PST)	26 (Tx Naïve) 2 (PST)	14 (Tx Naïve) 0 (PST)
Total ROIs Analyzed (by Histopathology Type)	**312** (Tx Naïve) **40** (PST)	**136** (Tx Naïve) **25** (PST)	**44** (Tx Naïve) **3** (PST)	**156** (Tx Naïve) **18** (PST)	**29** (Tx Naïve) **2** (PST)	**28** (Tx Naïve) **0** (PST)

[a] Definitions of location categories are provided in Figure 2 and Materials & Methods. Table contents relate to data presented in Figure 2E-G, Figure 7E[#], and Supplementary Figure S7E[#].

[*] These ROIs were annotated by a pathologist to be surrounded by invasive carcinoma and fell within broader. "Tumor" pathology annotations, thereby leading to their location classification as Intratumoral

[#]Indicated figures do not incorporate data from LN or Dysplasia ROIs, as these histopathology types were scarce or absent in PST tissue specimens.

Abbreviations: ROI, region of interest; Tx, treatment; PST, presurgically treated

Supplementary Table S7. Correlations between intratumoral leukocyte density, tumor characteristics, and survival

	Leukocyte Density (Tumor)			*P*[a]
	Tertile 1 (32-438 leukocytes/mm^2)	**Tertile 2** (441-727 leukocytes/mm^2)	**Tertile 3** (733-2576 leukocytes/mm^2)	
AJCC 8th ed. pT stage, n (%)				0.49
T1 (n=11)	2	5	4	
T2 (n=62)	19	23	20	
T3 (n=23)	10	6	7	
T4 (n=2)	2	0	0	
AJCC 8th ed. pN stage, n (%)				0.97
N0 (n=28)	9	10	9	
N1 (n=38)	11	13	14	
N2 (n=38)	14	12	12	
Tumor differentiation, n (%)				0.80
Well/Moderately differentiated (n=53)	17	20	16	
Poorly differentiated/Undifferentiated (n=48)	17	15	16	
Lymphovascular invasion, n (%)				0.84
Negative (n=38)	13	13	12	
Positive (n=60)	17	21	22	
OS				0.49
No. patients/No. event	34/33	35/28	35/31	
Median survival, months	20.4	22.1	23.1	
DFS				0.28
No. patients/No. event	34/30	35/25	35/31	
Median survival, months	11.1	14.3	10.9	

[a]*P* value for Fisher's exact test for categorical variables and for log-rank test for OS and DFS.
Data reported within table is from treatment-naïve PDACs from Cohorts 1 and 2.

Supplementary Table S8. Correlations between tumor adjacent stroma (TAS) leukocyte density, tumor characteristics, and survival

	Leukocyte Density (TAS)			***P*[a]**
	Tertile 1 (162-562 leukocytes/mm^2)	**Tertile 2** (572-1077 leukocytes/mm^2)	**Tertile 3** (1095-3116 leukocytes/mm^2)	
AJCC 8th ed. pT stage, n (%)				0.22
T1 (n=9)	1	4	4	
T2 (n=49)	17	14	18	
T3 (n=17)	6	8	3	
T4 (n=2)	2	0	0	
AJCC 8th ed. pN stage, n (%)				1.00
N0 (n=23)	7	8	8	
N1 (n=31)	11	10	10	
N2 (n=27)	9	9	9	
Tumor differentiation, n (%)				0.16
Well/Moderately differentiated (n=46)	12	19	15	
Poorly differentiated/Undifferentiated (n=34)	15	8	11	
Lymphovascular invasion, n (%)				1.00
Negative (n=32)	11	11	10	
Positive (n=47)	15	16	16	
OS				0.80
No. patients/No. event	27/24	27/24	27/24	
Median survival, months	21.2	19.1	23.1	
DFS				0.98
No. patients/No. event	27/22	27/20	27/24	
Median survival, months	13.7	14.3	13.6	

[a]*P* value for Fisher's exact test for categorical variables and for log-rank test for OS and DFS.
Data reported within table is from treatment-naïve PDACs from Cohorts 1 and 2.

Supplementary Table S9. Correlations between tumor cluster[a], tumor characteristics, and survival

	Tumor Cluster			*P*[b]
	Mixed	**Myeloid**	**Lymphoid**	
AJCC 8th ed. pT stage, n (%)				0.60
T1 (n=11)	4	5	2	
T2 (n=62)	25	20	17	
T3 (n=23)	9	5	9	
T4 (n=2)	2	0	0	
AJCC 8th ed. pN stage, n (%)				0.82
N0 (n=28)	11	10	7	
N1 (n=38)	14	11	13	
N2 (n=38)	17	9	12	
Tumor differentiation, n (%)				0.15
Well/Moderately differentiated (n=53)	18	20	15	
Poorly differentiated/Undifferentiated (n=48)	23	10	15	
Lymphovascular invasion, n (%)				0.14
Negative (n=38)	12	15	11	
Positive (n=61)	29	13	19	
OS				0.20
No. patients/No. event	42/38	30/25	32/29	
Median survival, months	20.1	22.6	21.0	
DFS				0.94
No. patients/No. event	42/32	30/25	32/29	
Median survival, months	11.5	10.9	13.0	

[a] Unsupervised hierarchical clustering of patients based on tumor immune composition in Figure 4

[b] *P* value for Fisher's exact test for categorical variables and for log-rank test for OS and DFS.

Supplementary Table S10. Correlation between tumor adjacent stroma cluster[a], tumor characteristics, and survival

	TAS Cluster			*P*[b]
	Mixed	**Myeloid**	**Lymphoid**	
AJCC 8th ed. pT stage, n (%)				0.30
T1 (n=9)	1	1	7	
T2 (n=47)	12	17	18	
T3 (n=18)	7	5	6	
T4 (n=2)	0	1	1	
AJCC 8th ed. pN stage, n (%)				0.79
N0 (n=23)	5	7	11	
N1 (n=31)	11	10	10	
N2 (n=26)	7	8	11	
Tumor differentiation, n (%)				0.65
Well/Moderately differentiated (n=47)	15	14	18	
Poorly differentiated/Undifferentiated (n=32)	7	11	14	
Lymphovascular invasion, n (%)				0.92
Negative (n=32)	9	11	12	
Positive (n=47)	14	14	19	
OS				0.67
No. patients/No. event	23/21	25/21	33/30	
Median survival, months	24.4	22.1	19.1	
DFS				0.17
No. patients/No. event	23/22	25/17	33/28	
Median survival, months	13.8	15.9	10.2	

[a] Refers to unsupervised hierarchical clustering of patients based on stromal immune composition in Supplementary Figure S4

[b] *P* value for Fisher's exact test for categorical variables and for log-rank test for OS and DFS