
Multi-parametric atlas of the pre-metastatic liver for prediction of metastatic outcome in early-stage pancreatic cancer

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Supplementary Figure S1

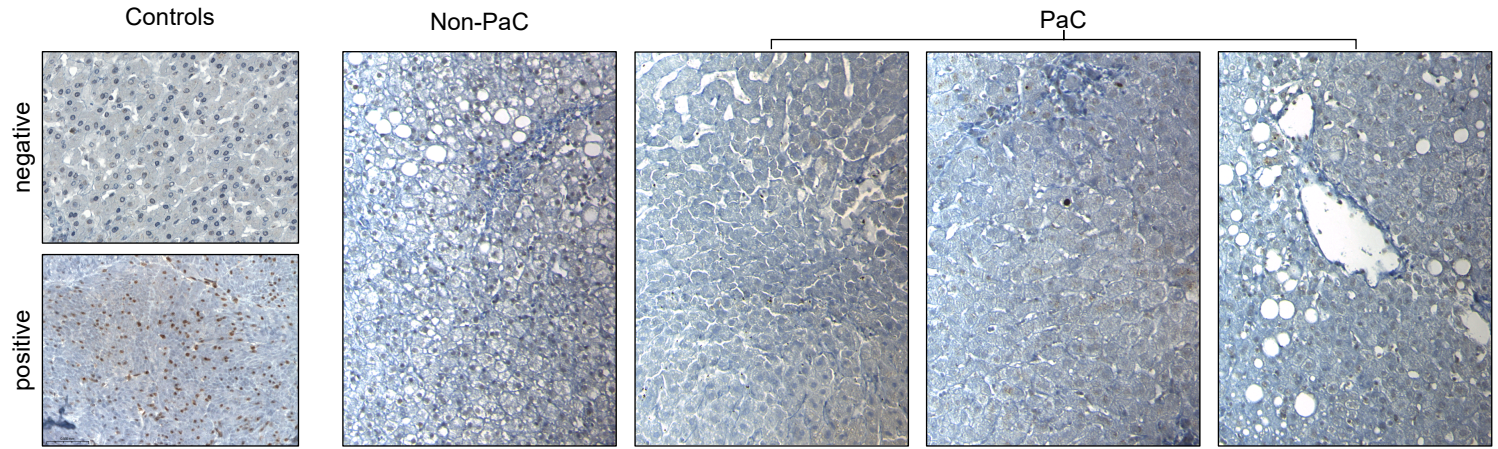


Fig. S1 | Immunohistochemical staining for p53. Liver biopsies were evaluated by H&E and p53 immunohistochemical staining, without identification of micrometastases. Representative sections from one Non-PaC and three PaC patients are shown. Rare hepatocytes (as shown in the middle PaC panel) or biliary epithelial cells were noted to stain positively for p53, but no malignant cells were identified. Unrelated normal liver tissue and a colon cancer case were used as negative and positive controls (left panels).

1 Supplementary Tables

2 Supplementary Table S1. Clinicopathological characteristics of all patients

Variable	Non-PaC		PaC		%
		%			
Patients in group	19		49		
Male	8	42%	32		65%
Age - Median (Range)	69.6	(48.7-86.8)	68.0		(49.0-87.0)
BMI - Median (Range)	29.1	(20.4-37.8)	26.9		(19.5-39.0)
Biliary obstruction*	2	11%	31		63%
Preop. Biliary drainage	1	5%	20		41%
Histologic subtype					
IPMN	10	56%	Conventional PDAC	40	82%
PanIN 1-2 +/- retention cyst	4	22%	Adenosquamous PDAC	7	14%
Low grade PaNET	2	11%	Undifferentiated anaplastic Ca	1	2%
Serous cystadenoma	1	5.5%	Pancreatobiliary Ca	1	2%
Ampullary adenoma	2	11%			
IPMN Grade			PaC Differentiation		
Low	4	22%	Well	2	4%
Intermediate	4	22%	Moderate	30	61%
High	3	17%	Poor	15	31%
			Undifferentiated	1	2%
			Unspecified	1	2%
T-stage (per AJCC 8th ed.)					
			1 (≤ 2 cm)	5	10%
			2 (>2 and ≤ 4 cm)	33	67%
			3 (>4 cm)	10	21%
			4 (invasion of great vessels)	1	2%
N-stage (per AJCC 8th ed.)					
			0 (LN -ve)	14	29%
			1 (1-3 +ve LNs)	19	39%
			2 (>3 +ve LNs)	16	33%
Lymphovascular invasion	-			37	76%
Perineural invasion	-			48	98%

* Biliary obstruction was defined by the presence of either (a) clinical/biochemical (based on abnormal total bilirubin) evidence of jaundice, or (b) preoperative biliary drainage procedure for the relief of biliary obstruction.

Supplementary Table S2. Genes deregulated in bulk liver specimens from PaC patients compared to Non-PaC controls



UP-REGULATED			DOWN-REGULATED		
Gene	logFold Δ	Adj. <i>p</i> -value	Gene	logFold Δ	Adj. <i>p</i> -value
SLC51B	3.93787948	0.00448147	CYP17A1	-1.0103352	0.07589972
TRIM31	3.49098287	0.00397538	LGR6	-1.0234902	0.06223514
AKR1B10	2.97314024	0.01446193	TMEM100	-1.0375946	0.00397538
DBNDD1	2.88751556	0.00174362	DND1	-1.0509621	0.0962492
LOXL4	2.64932872	0.00364002	NDRG4	-1.0518643	0.04026881
CCL20	2.52647527	0.02737509	SCN7A	-1.0709203	0.03969302
HS3ST2	2.45403359	0.00740081	PRIMA1	-1.0788986	0.09587573
KCNJ5	2.21516757	0.00019795	PRSS35	-1.1045353	0.04608003
CCAT1	2.17740423	0.00099447	EDAR	-1.1435971	0.03464416
TREM2	2.16918565	0.02317979	GPR153	-1.2163746	0.03488404
UBD	2.16910164	0.00397538	COL28A1	-1.2183629	0.00986282
HKDC1	2.00760492	0.06612741	LDLRAD4-AS1	-1.2400806	0.07411398
MMP7	1.89845078	0.01197887	GGACT	-1.2525449	0.00059414
ROS1	1.87763751	0.02080977	MAMDC2	-1.2538878	0.04718306
DTNA	1.84022918	0.01394864	DDIT4	-1.2970125	0.03964432
KB-1615E4.2	1.70741829	0.01846313	GRIK3	-1.3524865	0.09685178
FAM169B	1.65426671	0.04742395	CSPG4	-1.3737184	0.04986076
GDF15	1.64302043	0.02788377	NEGR1	-1.3771335	0.0962492
GPNMB	1.58404955	0.0049062	ATP1A2	-1.4016051	0.03464416
CFTR	1.56449261	0.01283695	CBLN3	-1.4528987	0.06462747
TTC9	1.54047658	0.01101693	GPM6B	-1.4540314	0.01509086
CAPG	1.51911956	0.03916409	MROH2A	-1.4806942	0.05843885
CCL18	1.50330877	0.0962492	NRXN1	-1.5111126	0.07650124
IQGAP3	1.50251607	0.03484719	L1CAM	-1.5306355	0.09035285
VTCN1	1.44951155	0.09703832	SORCS1	-1.5340123	0.06925869
CXCL10	1.44638522	0.07703202	RNF157	-1.5841558	0.02164629
PCDP1	1.35769229	0.02537807	SLC5A7	-1.7322185	0.09577506
ANLN	1.35474966	0.08072348	SLC26A3	-1.7886442	0.08350883
RGS4	1.35095853	0.02737509	XKR4	-1.8827735	0.0251224
RAB42	1.33778471	0.02080977	SPOCK3	-1.9496298	0.05907239
PLA2G7	1.33148196	0.00059414	SCUBE1	-2.2067337	0.00174362
FOXMI	1.33053878	0.08072348	AC004593.3	-2.7687667	0.00239657
CLSPN	1.31353181	0.08350536			
SERPINE1	1.30110444	0.05907239			
KNDC1	1.23685908	0.08529973			
TMEM45B	1.21427725	0.03068519			
IL32	1.19596025	0.09042697			
EYS	1.19356936	0.06999612			
MKI67	1.17803338	0.06612741			
TLR7	1.09348062	0.09614234			
IGSF3	1.06421415	0.08350536			
IFIT2	1.0504882	0.04026881			
AJUBA	1.02782625	0.0403852			
ADM2	1.02233956	0.04986076			

Comparisons performed with the Wald test implemented using the DESeq2 package, with adjustment for multiple comparisons.

Supplementary Table S3. Gene set enrichment analysis (GSEA) in bulk liver specimens from PaC patients compared to Non-PaC controls



A. Enriched in PaC

	Gene Set <i>(follow link to MSigDB)</i>	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	HALLMARK MITOTIC SPINDLE	197	0.45	1.70	0.020	0.504	0.225	4943
2	HALLMARK INTERFERON GAMMA RESPONSE	197	0.59	1.69	0.020	0.270	0.237	4958
3	HALLMARK ALLOGRAFT REJECTION	191	0.53	1.66	0.043	0.240	0.288	6240
4	HALLMARK INTERFERON ALPHA RESPONSE	93	0.62	1.61	0.036	0.208	0.367	6063

B. Enriched in Non-PaC

none

Supplementary Table S4. Gene set enrichment analysis (GSEA) in bulk liver specimens from PaC patients comparing patients with distant recurrence to those with no evidence of disease (NED).



Enriched in patients with distant recurrence

	Gene Set (<i>follow link to MSigDB</i>)	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	HALLMARK_PI3K_AKT_MTOR_SIGNALING	100	0.39	1.55	0.036	0.242	0.446	9149
2	HALLMARK_E2F_TARGETS	195	0.49	1.54	0.093	0.233	0.471	7305
3	HALLMARK_SPERMATOGENESIS	109	0.37	1.52	0.028	0.240	0.501	4608
4	HALLMARK_INTERFERON_ALPHA_RESPONSE	93	0.58	1.50	0.048	0.247	0.534	9369
5	HALLMARK_KRAS_SIGNALING_DN	157	0.40	1.45	0.033	0.199	0.591	8602

Enriched in NED patients
none

31 **Supplementary Table S5. Differentially expressed genes in PaC patients with**  vs 
 32 **Extrahepatic (EHM) metastasis, compared to NED.**

UP-REGULATED					
Gene	logFold Δ	Adj. <i>p</i> -value	Gene	logFold Δ	Adj. <i>p</i> -value
LOXL4	3.38272757	0.095318788	FGR	1.13773169	0.07501625
IRF4	3.05077569	0.003874647	HDX	1.13449732	0.049025534
TMC4	2.67901617	0.06049485	ADAMTS1	1.11776522	0.070805804
GDNF	2.58310020	0.0408066	PLK2	1.10538464	0.015380088
IP6K3	2.46929398	0.051969902	IFI16	1.09799465	0.053840123
RASD1	2.45417477	0.011444336	PPP1R18	1.08673207	0.090660286
ETV7	2.32807078	0.01947401	SOD2	1.07338711	0.042541574
SLCO4A1	2.31051432	0.051846372	MYO1G	1.01742132	0.086640108
RGS9	2.27978933	0.000553294	NCOA7	1.00610837	0.047486481
DNAH5	2.26959019	0.015683479			
G0S2	2.12203885	0.014052084			
IL1RL1	2.07080291	0.015326322			
ADAMTS4	2.06763641	0.053840123			
ACKR3	2.01135817	0.006331992			
TMPRSS3	1.98596804	0.092234919			
NOD2	1.88500411	0.063128333			
KLHL29	1.85139859	0.004921512			
HAPLN3	1.81777801	0.047486481			
WIPF3	1.78243082	0.014052084			
IL1RN	1.76977354	0.08489918			
SLC11A1	1.75556083	0.079649249			
GFPT2	1.74654127	0.070805804			
MEFV	1.72660825	0.051846372			
MX1	1.72415493	0.024686976			
SLC22A15	1.72337457	0.049428133			
SYTL3	1.70267733	0.090172812			
ELF3	1.62126140	0.051846372			
FAT1	1.58798844	0.031110441			
RALGDS	1.55986995	0.080958875			
ADAMTS9	1.54308731	0.051846372			
ITGAX	1.50630327	0.06089242			
JAK3	1.50331742	0.019574209			
NR1D1	1.47209679	0.036581449			
TGFB3	1.40349814	0.006735664			
GOLM1	1.39660559	0.079569459			
MLKL	1.39267168	0.014052084			
PFKP	1.38184003	0.047486481			
RUNX1	1.36637315	0.017375988			
IFI6	1.30472579	0.066651409			
HK2	1.30132979	0.021253867			
ARNTL2	1.29264807	0.004921512			
CDC7	1.28358455	0.015683479			
MCAM	1.22123274	0.076183944			
OSMR	1.21735998	0.014052084			
CD274	1.21256674	0.026981961			
YBX3	1.17807169	0.037576482			
MTHFD2	1.15081395	0.051969902			
EMR2	1.15049637	0.049258092			
			DOWN-REGULATED		
			MYCL	-1.08622412	0.051846372
			EPB41L1	-1.76927696	0.0408066

33 Comparisons performed with the Wald test implemented using the DESeq2 package, with adjustment for multiple
 34 comparisons.

Supplementary Table S6. Gene set enrichment analysis (GSEA) in bulk liver specimens of PaC patients comparing patients with distant extrahepatic metastasis (EHM) to those with no evidence of disease (NED)



A. Enriched in EHM

	Gene Set (follow link to MSigDB)	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	HALLMARK TNFA SIGNALING VIA NFKB	197	0.66	3.25	0.000	0.000	0.000	6265
2	HALLMARK INTERFERON GAMMA RESPONSE	197	0.65	3.22	0.000	0.000	0.000	5673
3	HALLMARK INTERFERON ALPHA RESPONSE	93	0.66	2.90	0.000	0.000	0.000	5430
4	HALLMARK IL6 JAK STAT3 SIGNALING	81	0.67	2.85	0.000	0.000	0.000	3712
5	HALLMARK INFLAMMATORY RESPONSE	194	0.54	2.63	0.000	0.000	0.000	7092
6	HALLMARK ALLOGRAFT REJECTION	191	0.49	2.38	0.000	0.000	0.000	8198
7	HALLMARK P53 PATHWAY	188	0.46	2.26	0.000	0.000	0.000	7889
8	HALLMARK APOPTOSIS	158	0.46	2.20	0.000	0.000	0.000	5905
9	HALLMARK IL2 STAT5 SIGNALING	193	0.43	2.13	0.000	0.000	0.000	7585
10	HALLMARK HYPOXIA	186	0.44	2.11	0.000	0.000	0.000	5617
11	HALLMARK KRAS SIGNALING UP	189	0.43	2.09	0.000	0.000	0.000	6650
12	HALLMARK COMPLEMENT	197	0.41	2.03	0.000	0.000	0.000	7079
13	HALLMARK G2M CHECKPOINT	189	0.41	2.01	0.000	0.000	0.000	10219
14	HALLMARK MITOTIC SPINDLE	197	0.39	1.92	0.000	0.000	0.001	9694
15	HALLMARK EPITHELIAL MESENCHYMAL TRANSITION	194	0.39	1.92	0.000	0.000	0.001	7825
16	HALLMARK TGF BETA SIGNALING	54	0.48	1.92	0.002	0.000	0.001	7053
17	HALLMARK APICAL JUNCTION	186	0.39	1.89	0.000	0.000	0.001	6808
18	HALLMARK E2F TARGETS	195	0.39	1.89	0.000	0.000	0.001	8850
19	HALLMARK ESTROGEN RESPONSE EARLY	192	0.37	1.80	0.000	0.001	0.009	6762
20	HALLMARK UV RESPONSE UP	151	0.37	1.76	0.000	0.001	0.014	5821
21	HALLMARK_PI3K_AKT_MTOR_SIGNALING	100	0.37	1.67	0.002	0.003	0.041	8260
22	HALLMARK_HEME_METABOLISM	188	0.34	1.65	0.000	0.003	0.050	5492
23	HALLMARK_MTORC1_SIGNALING	195	0.33	1.62	0.000	0.004	0.067	5792
24	HALLMARK_UV_RESPONSE_DN	138	0.34	1.59	0.002	0.007	0.099	6985
25	HALLMARK_MYC_TARGETS_V2	58	0.39	1.58	0.018	0.007	0.117	8175
26	HALLMARK_UNFOLDED_PROTEIN_RESPONSE	107	0.35	1.55	0.008	0.009	0.146	8967
27	HALLMARK_ANGIOGENESIS	36	0.41	1.49	0.035	0.016	0.244	8477
28	HALLMARK_SPERMATOGENESIS	109	0.29	1.31	0.050	0.086	0.807	4115

B. Enriched in NED

	Gene Set (follow link to MSigDB)	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	HALLMARK BILE ACID METABOLISM	112	-0.50	-2.39	0.000	0.000	0.000	7867
2	HALLMARK OXIDATIVE PHOSPHORYLATION	183	-0.43	-2.19	0.000	0.000	0.000	9947
3	HALLMARK FATTY ACID METABOLISM	152	-0.37	-1.83	0.000	0.000	0.005	8044
4	HALLMARK PEROXISOME	101	-0.35	-1.61	0.006	0.004	0.050	8207
5	HALLMARK XENOBIOTIC METABOLISM	194	-0.29	-1.47	0.002	0.017	0.222	8071

Supplementary Table S7. Gene set enrichment analysis (GSEA) in bulk liver specimens of PaC patients comparing patients with late liver metastasis (LiM>6) to those with no evidence of disease (NED)



A. Enriched in LiM>6

	Gene Set (follow link to MSigDB)	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	HALLMARK_E2F_TARGETS	195	0.45	2.22	0.000	0.000	0.000	8005
2	HALLMARK_G2M_CHECKPOINT	189	0.46	2.19	0.000	0.000	0.000	7703
3	HALLMARK_P53_PATHWAY	188	0.37	1.78	0.000	0.004	0.006	6173
4	HALLMARK_PI3K_AKT_MTOR_SIGNALING	100	0.40	1.77	0.000	0.003	0.006	9682
5	HALLMARK_MITOTIC_SPINDLE	197	0.33	1.62	0.000	0.019	0.046	8619
6	HALLMARK_MYC_TARGETS_V1	194	0.33	1.60	0.000	0.019	0.058	8745
7	HALLMARK_GLYCOLYSIS	195	0.31	1.50	0.003	0.047	0.158	7230
8	HALLMARK_HEME_METABOLISM	188	0.31	1.50	0.003	0.041	0.158	7295
9	HALLMARK_ESTROGEN_RESPONSE_EARLY	192	0.30	1.46	0.003	0.053	0.225	4582
10	HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	47	0.38	1.44	0.030	0.055	0.259	4132
11	HALLMARK_SPERMATOGENESIS	109	0.32	1.43	0.018	0.051	0.265	3581
12	HALLMARK_ANDROGEN_RESPONSE	94	0.33	1.42	0.015	0.054	0.299	5413
13	HALLMARK_HYPOXIA	186	0.29	1.40	0.011	0.058	0.344	7008
14	HALLMARK_KRAS_SIGNALING_DN	157	0.28	1.32	0.025	0.114	0.576	5822

B. Enriched in NED

	Gene Set (follow link to MSigDB)	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	HALLMARK_BILE_ACID_METABOLISM	112	-0.36	-1.53	0.005	0.084	0.177	9458

Supplementary Table S8. Clinicopathological characteristics of patients subjected to single-cell RNA sequencing of hepatic CD45⁺ cells.

Variable	Non-PaC		PaC		%
Patients in group	3		5		
Male	1	33%	5		100%
Age - Median (Range)	65.1	(52.3-70)	66.9		(58.6-84.1)
Biliary obstruction*	0	0%	3		60%
Preop. Biliary drainage	0	0%	2		40%
Histologic subtype					
IPMN	1	33%	Conventional PDAC	3	60%
Low grade PaNET	1	33%	Adenosquamous PDAC	1	20%
Ampullary adenoma	1	33%	Pancreatobiliary Ca	1	20%
IPMN Grade			PaC Differentiation		
Low	1	100%	Well	0	0%
Intermediate	0	0%	Moderate	4	80%
High	0	0%	Poor	1	20%
T-stage (per AJCC 8th ed.)					
			1 (≤2cm)	1	20%
			2 (>2 and ≤4cm)	3	60%
			3 (>4cm)	1	20%
			4 (invasion of great vessels)	0	0%
N-stage (per AJCC 8th ed.)					
					32
			0 (LN -ve)	3	60%
			1 (1-3 +ve LNs)	2	40%
			2 (>3 +ve LNs)	0	0%
Lymphovascular invasion	-			4	80%
Perineural invasion	-			4	80%

IPMN: Intraductal papillary mucinous neoplasm; PaNET: Pancreatic neuroendocrine tumor

57 **Supplementary Table S9. Genes deregulated in T cell cluster by scRNAseq.**

UP-REGULATED		DOWN-REGULATED	
Gene	Adj. <i>p</i> -value	Gene	Adj. <i>p</i> -value
RPS4Y1	0	HOPX	6.88E-24
MIR24-2	3.62E-46	FGFBP2	4.01E-17
CEBPD	4.66E-60	RPS4X	1.09E-166
CD8A	9.29E-103	CCL3	0.02023019
KLF6	4.52E-57	RPL17	6.00E-72
CD8B	1.67E-50	ZNF90	1.36E-44
CD27	4.40E-47	RPSAP58	1.71E-57
SPOCK2	9.38E-40	LGALS1	7.35E-24
DUSP4	3.53E-41	RPL36A	2.81E-121
RGCC	1.88E-15	EIF5A	4.56E-56
MYADM	2.65E-27	RPS10	3.77E-156
HIST1H4C	3.68E-29	KLRF1	5.42E-35
APOBEC3G	2.54E-28	IFITM1	5.34E-81
ITM2C	6.10E-25	CCL3L1	9.08E-09
AMICA1	1.72E-25	NBEAL1	1.50E-117
H1FX	3.45E-28	MT2A	1
CTSW	5.56E-46	RPS26	0
EIF1AY	3.49E-42	XCL1	5.87E-45
CD97	9.52E-22	IFNG	3.15E-43
RNF213	2.09E-31	MT1X	7.86E-06
ERAP2	2.09E-35	XCL2	2.21E-53
SNORD3B-2	1.35E-38	XIST	0
KIAA1551	3.95E-24	GNLY	5.84E-75
KLRG1	1.01E-20		
CXCR4	3.51E-33		

58 Analysis was performed using the Wilcoxon's rank sum test. P-values are provided after adjustment for multiple
 59 comparisons with the Bonferroni method.

60

Supplementary Table S10. Features included in the four models predicting each metastatic outcome.

Model	Prediction	Included features
m1	Liver metastasis (LiM>6mo)	• CD45+ cell count IHC • CD8+ T cell lobular infiltration IHC – manual score [Scattered vs (Few/Widespread)] • SORT1 gene • IRF4 gene • Liver creatine (metabolomics)
m2	Early LiM (LiM<6mo)	• NET area (IF) • NR1D1 gene • SORT1 gene
m3	Distant extrahepatic (EHM)	• RGS9 gene • NR1D1 gene • OSMR gene • PPP1R18 gene
m4	No evidence of disease (NED) for at least 21 months	• IBA1+ cell portal infiltration IHC – manual score [(Mod/Severe) vs (Absent/Scattered/Mild)] • RGS9 gene

IHC, Immunohistochemistry; IF, Immunofluorescence

65 **Supplementary Table S11. Prediction of individual patient output using the combined**
 66 **model (N=41).**

ID	1 st Recur. Site	Time to 1 st Recur (mo)	LiM	Time to LiM or Length of follow up (mo)	Actual Recur. Pattern	Predicted Recur. Pattern	Remarks
PT3	Liver	4.5	Yes	4.5	Early LiM	Early LiM	
PT6	Liver	4.1	Yes	4.1	Early LiM	Early LiM	
PT9	Liver	1.3	Yes	1.3	Early LiM	Early LiM	
PT11	Liver	1.8	Yes	1.8	Early LiM	Early LiM	
PT34	Liver	4.5	Yes	4.5	Early LiM	Early LiM	
PT43	Liver	1.6	Yes	1.6	Early LiM	NED	
PT53	Liver + Lung	3.2	Yes	3.2	Early LiM	Early LiM	Synchronous EHM and LiM
PT58	Liver	1.2	Yes	1.2	Early LiM	Early LiM	
PT67	Liver	5.9	Yes	5.9	Early LiM	Early LiM	
PT78	Liver	1.8	Yes	1.8	Early LiM	Early LiM	
PT1	Liver	8.3	Yes	8.3	Late LiM	Late LiM	
PT13	Liver + Local	7.3	Yes	7.3	Late LiM	Late LiM	
PT16	Liver	8.4	Yes	8.4	Late LiM	Early LiM	LiM at 8.4mo detected by PET, not visible on CT before or after PET but may have been present earlier than performance of PET
PT23	Liver + Local	32.3	Yes	32.3	Late LiM	Late LiM	Synchronous EHM and LiM
PT27	Lung, then Liver	4.2	Yes	9.6	Late LiM	NED	Initially found to have multiple sub-centimeter lung nodules, which progressed, with development of lymph node and liver metastases
PT32	Cervical LN, then Liver	8.7	Yes	10.1	Late LiM	Late LiM	Cervical LN identified by palpation at 8.7mo (biopsy-confirmed), then found to have multiple LiM on CT at 10mo
PT49	Liver	9.8	Yes	9.8	Late LiM	Late LiM	
PT57	Liver	10.4	Yes	10.4	Late LiM	Late LiM	
PT64	Liver	11.3	Yes	11.3	Late LiM	Late LiM	
PT71	Liver	17.2	Yes	17.2	Late LiM	Late LiM	
PT76	Liver + Spleen	7.2	Yes	7.2	Late LiM	Late LiM	
PT77	Liver	19.6	Yes	19.6	Late LiM	Late LiM	
PT85	Liver + Local	6.0	Yes	6.0	Late LiM	EHM	
PT2	Peritoneum	13.8	No	35.6	EHM	EHM	
PT10	Peritoneum	22.0	No	22.1	EHM	EHM	
PT37	Lung	1.9	No	15.7	EHM	EHM	
PT46	Lung	4.3	No	4.3	EHM	EHM	Patient died at 6.9mo
PT47	Lung	11.4	No	25.5	EHM	Early LiM	
PT48	Peritoneum	14.2	No	26.1	EHM	EHM	
PT75	Lung	17.9	No	36.0	EHM	Late LiM	
PT84	Lung	34.4	No	34.4	EHM	EHM	
PT91	Local + LN + Peritoneum	7.5	No	15.9	EHM	Late LiM	
PT7	None	-	No	36.0	NED	NED	
PT14	None	-	No	36.0	NED	NED	
PT15	None	-	No	34.7	NED	NED	
PT24	None	-	No	35.3	NED	Early LiM	
PT30	None	-	No	35.2	NED	NED	
PT41	None	-	No	36.0	NED	NED	
PT44	None	-	No	36.0	NED	Late LiM	
PT50	None	-	No	36.0	NED	NED	
PT65	None	-	No	20.6	NED	NED	

67 **Supplementary Table S12. Antibodies used for IMC.**

Epitope	Metal tag	Antibody vendor	Antibody catalog	Antibody clone	Antibody lot	Antibody dilution
Histone H3	In113	Cell Signaling Technology	4499BF	D1H2	17	1:400
FOXP3	Gd155	Invitrogen	14-4777-82	236A/E7	2378013	1:25
CD4	Gd156	Abcam	ab181724	EPR6855	GR3285644-12	1:50
NKG2A	Tb159	Abcam	ab273516	EPR23737-127	GR3410074-2	1:25
CD8a	Dy162	eBioscience	14-0085-82	C8/144B	2132595	1:100
ARG1	Dy164	Cell Signalling	89872SF	D4E3M	1	1:50
CD3	Er170	Fluidigm	3170019D	Polyclonal. C-Terminal	2202221-16	1:100
TRDC	Yb171	Santa Cruz	sc-100289	H-41	L1321	1:50
KRT8/18	Yb174	Fluidigm	3174022D	C51	372007	1:100
DNA1	Ir191	Fluidigm	201192B	DNA intercalator		1:300
DNA2	Ir193	Fluidigm	201192B	DNA intercalator		1:300

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