Supplementary Table 1. Multiplex IHC Panel

Order of Addition	Marker	Clone	Vendor	Catalog #	Antibody Dilution	OPAL Dye	OPAL Dilution
1	CD8	SP57	Roche	5937248001	1x	570	1:400
2	CD68	KP1	DAKO	76535	1:100	620	1:100
3	FOXP3	236A/E7	abcam	ab20034	1:500	690	1:100
4	CD20	L26	Inter Medico	BC-CM004A	1:1000	540	1:100
5	CD3	2GV6	Roche	5278422001	1x	650	1:400
6	PanCK	AE1/AE3	Inter Medico	BC-CM011B	1:500	520	1:100
7	Spectral DAPI	n/a	Akoya Biosciences	FP1490	n/a	n/a	n/a

Supplementary Table 2. Baseline characteristics of entire INSPIRE Cohort (n=106).

Characteristic	n=106
Age	
Median (Min,Max)	59.4 (21.1,81.8)
Sex	
Female	66 (62)
Male	40 (38)
Ethnicity	
American Indian or Alaska Native	1 (1)
Asian	14 (13)
Black or African American	3 (3)
Native Hawaiian or other pacific islander	2 (2)
Unknown or not reported	2 (2)
White	84 (79)
Cohort	
A	19 (18)
B	22 (21)

1

C	21 (20)
D	12 (11)
E	32 (30)
Stage at diagnosis	
1	8 (8)
2	23 (22)
3	25 (24)
4	38 (36)
X	12 (11)
History of autoimmune disease	
No	101 (96)
Yes	4 (4)
Missing	**1**
PD-L1 percentage	
0	52 (50)
1-10	26 (25)
11-25	8 (8)
26-49	4 (4)
50+	13 (13)
Missing	**3**
PD-L1	
zero	52 (50)
positive	51 (50)
Missing	**3**
PD-L1 percentage	
Mean (sd)	15 (30.1)
Median (Min,Max)	0 (0,100)
Missing	**3**
TMB	
Mean (sd)	5.7 (19.7)
Median (Min,Max)	2 (0.5,187)
Missing	**12**
log TMB	
Mean (sd)	0.9 (1)
Median (Min,Max)	0.7 (-0.8,5.2)
Missing	**12**
Follow-up (months)	
Median (Min,Max)	12.6 (0.6, 58.1)

2

Supplementary Table 3. Baseline characteristics of evaluable patients for Δcholine$_{C3}$ analysis (n=81).

Characteristic	n=81
Age	
Median (Min,Max)	60.9 (21.1,81.8)
Sex	
Female	44 (54)
Male	37 (46)
Ethnicity	
American Indian or Alaska Native	0 (0)
Asian	11 (14)
Black or African American	3 (4)
Native Hawaiian or other pacific islander	0 (0)
Unknown or not reported	1 (1)
White	66 (81)
Cohort	
A	17 (21)
B	12 (15)
C	12 (15)
D	11 (14)
E	29 (36)
Stage at diagnosis	
1	7 (9)
2	18 (22)
3	13 (16)
4	31 (38)
X	12 (15)
History of autoimmune disease	
No	78 (98)
Yes	2 (3)
Missing	**1**
PD-L1 percentage	
0	38 (47)
1-10	22 (27)
11-25	5 (6)
26-49	3 (4)
50+	13 (16)
PD-L1	

3

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zero	38 (47)
positive	43 (53)
PD-L1 percentage	
Mean (sd)	17.8 (33.1)
Median (Min,Max)	1 (0,100)
TMB	
Mean (sd)	6.7 (22.3)
Median (Min,Max)	2 (0.5,187)
Missing	8
log TMB	
Mean (sd)	1 (1.1)
Median (Min,Max)	0.7 (-0.8,5.2)
Missing	8
Follow-up (months)	
Median (Min,Max)	17.8 (2.3,58.1)

Supplementary Table 4. Baseline characteristics of evaluable patients for both $\Delta choline_{C3}$ and $\Delta ctDNA_{C3}$ analysis (n=72).

Characteristic	n=72
Age	
Median (Min,Max)	60.2 (21.1,81.8)
Sex	
Female	39 (54)
Male	33 (46)
Ethnicity	
American Indian or Alaska Native	0 (0)
Asian	8 (11)
Black or African American	2 (3)
Native Hawaiian or other pacific islander	0 (0)
Unknown or not reported	1 (1)
White	61 (85)
Cohort	
A	14 (19)
B	10 (14)
C	10 (14)
D	10 (14)
E	28 (39)
Stage at diagnosis	
1	6 (8)

4

2	17 (24)
3	12 (17)
4	26 (36)
X	11 (15)
History of autoimmune disease	
No	70 (97)
Yes	2 (3)
PD-L1 percentage	
0	35 (49)
1-10	20 (28)
11-25	5 (7)
26-49	3 (4)
50+	9 (12)
PD-L1	
zero	35 (49)
positive	37 (51)
PD-L1 percentage	
Mean (sd)	15.3 (31.1)
Median (Min,Max)	1 (0,100)
TMB	
Mean (sd)	6.7 (22.4)
Median (Min,Max)	2 (0.5,187)
log TMB	
Mean (sd)	1 (1.1)
Median (Min,Max)	0.7 (-0.8,5.2)
Follow-up (months)	
Median (Min,Max)	18 (2.3,58.1)

Supplementary Table 5. Baseline characteristics of evaluable patients with a decrease in ctDNA at cycle 3 (n=33) and stratified by Δcholine$_{C3}$. Fisher's exact test and Wilcoxon's rank sum test were used to compare categorical and continuous variables, respectively, between the two groups.

Covariate	Full Sample (n=33)	Increase in choline (n=20)	Decrease in choline (n=13)	p-value
Age				0.41
Mean (sd)	61.2 (11.6)	59.4 (12.6)	63.9 (9.6)	
Median (Min,Max)	65.1 (34.1,78.2)	63.3 (34.1,74.8)	65.7 (45.0,78.2)	
Sex				1
Female	19 (58)	12 (60)	7 (54)	

5

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Male	14 (42)	8 (40)	6 (46)	
Ethnicity				0.62
American Indian or Alaska Native	0 (0)	0 (0)	0 (0)	
Asian	5 (15)	2 (10)	3 (23)	
Black or African American	1 (3)	1 (5)	0 (0)	
Native Hawaiian or other pacific islander	0 (0)	0 (0)	0 (0)	
Unknown or not reported	0 (0)	0 (0)	0 (0)	
White	27 (82)	17 (85)	10 (77)	
Cohort				0.16
A	6 (18)	1 (5)	5 (38)	
B	4 (12)	2 (10)	2 (15)	
C	5 (15)	4 (20)	1 (8)	
D	8 (24)	6 (30)	2 (15)	
E	10 (30)	7 (35)	3 (23)	
Stage at diagnosis				0.61
1	3 (9)	3 (15)	0 (0)	
2	7 (21)	3 (15)	4 (31)	
3	6 (18)	4 (20)	2 (15)	
4	13 (39)	8 (40)	5 (38)	
X	4 (12)	2 (10)	2 (15)	
History of autoimmune disease				1
No	33 (100)	20 (100)	13 (100)	
Yes	0 (0)	0 (0)	0 (0)	
PD-L1				0.17
zero	15 (45)	7 (35)	8 (62)	
positive	18 (55)	13 (65)	5 (38)	
PD-L1 percentage				0.3
0	15 (45)	7 (35)	8 (62)	
1-10	6 (18)	3 (15)	3 (23)	
11-25	4 (12)	4 (20)	0 (0)	
26-49	1 (3)	1 (5)	0 (0)	
50+	7 (21)	5 (25)	2 (15)	
PD-L1 percentage				0.15
Mean (sd)	23.3 (38.1)	28.2 (39.9)	15.7 (35.4)	
Median (Min,Max)	1 (0,100)	6.5 (0.0,100.0)	0 (0,100)	
TMB				0.52
Mean (sd)	11.1 (32.6)	15.4 (41.5)	4.7 (4.2)	
Median (Min,Max)	2.1 (0.6,187.0)	1.7 (0.6,187.0)	3.1 (1.0,15.3)	
log TMB				0.52
Mean (sd)	1.2 (1.3)	1.2 (1.6)	1.2 (0.9)	
Median (Min,Max)	0.8 (-0.5,5.2)	0.5 (-0.5,5.2)	1.1 (0.0,2.7)	

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Follow-up (months)				0.0071
Median (Min,Max)	35.4 (2.3,52.8)	40.3 (5.0,52.8)	18.2 (2.3,51.9)	

Supplementary Table 6. Patient demographics, clinical and treatment characteristics of the patients in the validation (LIBERATE) cohort in early phase clinical trials (n=51).

Characteristic	n=51
Age	
Median (Min,Max)	60 (29-79)
Gender	
Female	25 (49)
Male	26 (51)
Treatment	
PD-1 monotherapy	4 (8)
Combination with any checkpoint inhibitor (non PD-1)	25 (49)
Combination with ERK inhibitor	10 (19)
Combination with Interleukins	6 (12)
Combination with other immunomodulators	6 (12)
Cancer Type	
Sarcoma	7 (13)
Colorectal	6 (11)
Breast	4 (8)
Mesothelioma	4 (8)
Melanoma	3 (6)
Cholangiocarcinoma	3 (6)
Renal	3 (6)
Endometrium	3 (6)
Head and Neck (Squamous)	2 (4)
Cervix	2 (4)
Prostate	2 (4)
Others (1 case each): suprarrenal, esophagus, cutaneous adenocarcinoma, gastric, neuroendocrine tumor, anal, conjuctival melanoma, small cell lung carcinoma, nasopharynx, ovarian, pancreas, salivary gland	12 (24)
Treatment Response	

7

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Complete Response	3 (6)
Partial Response	4 (8)
Stable Disease	8 (16)
Progressive Disease	36 (70)
Clinical Benefit Rate	15 (29)

8