Supplemental Online Content

Aggarwal C, Ben-Shachar R, Gao Y, et al. Assessment of tumor mutational burden and outcomes in patients with diverse advanced cancers treated with immunotherapy. *JAMA Netw Open*. 2023;6(5):e2311181. doi:10.1001/jamanetworkopen.2023.11181

This supplemental material has been provided by the authors to give readers additional information about their work.

eTable 1. Histologies by Cancer Type in the Evaluable Cohort (N=674)

	NSCLC	Bladder	HNSCC	Melanoma	CRC	Gastric	EC	Cervical
Adenocarcinoma	235 (71.2%)	3 (2.0%)	2 (2.1%)	0	39 (100.0%)	6 (85.7%)	4 (80.0%)	1 (100.0%)
Squamous cell carcinoma	65 (19.7%)	2 (1.3%)	90 (93.7%)	0	0	0	0	0
Transitional cell carcinoma	0	103 (69.5%)	0	0	0	0	0	0
Malignant melanoma, metastatic	0	0	0	34 (70.8%)	0	0	0	0
Non-small cell carcinoma	30 (9.1%)	1 (0.7%)	1 (1.04%)	0	0	0	0	0
Papillary transitional cell carcinoma	0	22 (14.9%)	0	0	0	0	0	0
Malignant melanoma	0	0	0	12 (25.0%)	0	0	0	0
Carcinoma	0	10 (6.8%)	0	0	0	0	0	0
Transitional cell carcinoma with squamous differentiation	0	2 (1.3%)	0	0	0	0	0	0
Nodular melanoma	0	0	0	1 (2.1%)	0	0	0	0
Endometrioid carcinoma	0	0	0	0	0	0	1 (20.0%)	0
Transitional cell carcinoma, micropapillary	0	1 (0.7%)	0	0	0	0	0	0
Signet ring cell carcinoma	0	0	0	0	0	1 (14.3%)	0	0
Transitional cell carcinoma, spindle cell	0	1 (0.7%)	0	0	0	0	0	0
Papillary carcinoma	0	1 (0.7%)	0	0	0	0	0	0
Carcinoma, undifferentiated	0	0	1 (1.04%)	0	0	0	0	0

Large cell carcinoma	0	0	1 (1.04%)	0	0	0	0	0
Papillary transitional cell carcinoma, non-invasive	0	1 (0.7%)	0	0	0	0	0	0
Superficial spreading melanoma	0	0	0	1 (2.1%)	0	0	0	0
Transitional cell carcinoma in situ	0	1 (0.7%)	0	0	0	0	0	0
Basaloid carcinoma	0	0	1 (1.04%)	0	0	0	0	0

eTable 2. FDA-Approved Medications Approved by Cancer Type and Line of Therapy Used for Cohort Definition

	NSCLC	Bladder	HNSCC	Melanoma	CRC	Gastric	EC	Cervical
pembrolizumab	1L	1L, 2L	1L, 2L	1L	1L, 2L	2L	2L	2L
atezolizumab	1L	1L						
cemiplimab	1L							
nivolumab		2L	2L	1L	1L, 2L	2L		
avelumab		2L						
ipilimumab				1L				
ipilimumab + nivolumab	1L			1L	1L, 2L			

eTable 3. End Point Event Definitions

End Point	Event Definition
OS	Death event ≥ two weeks after IO start
PFS	Earliest of: Progression or death event ≥ two weeks after IO start
TTP	Progression event ≥ two weeks after IO start

eTable 4. End Point Censoring Definitions

End Point	Right Censoring Definition
OS	Last known date
PFS	Earliest of: IO medication end date, date of ongoing IO treatment*, start of the next line of therapy, treatment discontinuation, or last known date. All patients with no last known date or event within one year of IO start for 1L and within 6 months for 2L will be censored.
TTP	Earliest of: IO medication end date, date of ongoing IO treatment*, start of the next line of therapy, treatment discontinuation, or last known date. All patients with no last known date or event within one year of IO start for 1L and within 6 months for 2L will be censored.

*This record is available only if no medication end date, treatment discontinuation, or start of next line therapy is available

eTable 5. Histologies by Cancer Type in the Prospective Cohort (N=403)

	NSCLC	Bladder	HNSCC	Melanoma	CRC	Gastric	EC
Adenocarcinoma	178 (73.6%)	2 (2.6%)	2 (4.7%)	0	23 (100.0%)	0	2 (66.7%)
Squamous cell carcinoma	41 (16.9%)	0	39 (90.7%)	0	0	0	0
Transitional cell carcinoma	0	56 (72.7%)	0	0	0	0	0
Malignant melanoma, metastatic	0	0	0	12 (85.7%)	0	0	0
Non-small cell carcinoma	23 (9.5%)	1 (1.3%)	0	0	0	0	0
Papillary transitional cell carcinoma	0	13 (16.9%)	0	0	0	0	0
Malignant melanoma	0	0	0	2 (14.3%)	0	0	0
Carcinoma	0	5 (6.5%)	0	0	0	0	0
Endometrioid carcinoma	0	0	0	0	0	0	1 (33.3%)
Signet ring cell carcinoma	0	0	0	0	0	1 (100.0%)	0
Carcinoma, undifferentiated	0	0	1 (2.3%)	0	0	0	0
Large cell carcinoma	0	0	1 (2.3%)	0	0	0	0

eTable 6. Medications by Cancer Type in the Evaluable Cohort (N=674)

	NSCLC	Bladder	HNSCC	Melanoma	CRC	Gastric	EC	Cervical
pembrolizumab	295 (89.4%)	118 (79.7%)	87 (90.6%)	14 (29.2%)	33 (84.6%)	7 (100.0%)	5 (100.0%)	1 (100.0%)
Ipilimumab + nivolumab	27 (8.2%)	0	0	23 (47.9%)	3 (7.7%)	0	0	0
nivolumab	0	6 (4.1%)	9 (9.4%)	10 (20.8%)	3 (7.7%)	0	0	0
atezolizumab	6 (1.8%)	11 (7.4%)	0	0	0	0	0	0
avelumab	0	13 (8.8%)	0	0	0	0	0	0
cemiplimab	2 (0.6%)	0	0	0	0	0	0	0
ipilimumab	0	0	0	1 (2.1%)	0	0	0	0

eTable 7. Medications by Cancer Type in the Prospective Cohort
(N = 403)

	NSCLC	Bladder	HNSCC	Melanoma	CRC	Gastric	EC
pembrolizumab	212 (87.60%)	61 (79.2%)	40 (93.0%)	3 (21.4%)	19 (82.6%)	1 (100.0%)	3 (100.0%)
Ipilimumab + nivolumab	25 (10.33%)	0	0	6 (42.9%)	2 (8.7%)	0	0
nivolumab	0	3 (3.9%)	3 (7.0%)	5 (35.7%)	2 (8.7%)	0	0
atezolizumab	3 (1.24%)	3 (3.9%)	0	0	0	0	0
avelumab	0	10 (13.0%)	0	0	0	0	0
cemiplimab	2 (0.83%)	0	0	0	0	0	0

eTable 8. Patient Summary in the Evaluable Cohort

(N = 674)

Clinical characteristic		**All Evaluable (N=674)**	**TMB-H Evaluable (N=206)**	**TMB-L Evaluable (N=468)**	**P Value**
Age at start of IO (yrs)	Median (range)	69.4 (28.6, 89.8)	69.6 (29.0, 89.7)	69.2 (28.6, 89.8)	0.95*
Sex, N (%)	Female	271 (40.2%)	84 (40.8%)	187 (40.0%)	0.91
Race/Ethnicity, N (%)	Asian	14 (2.1%)	3 (1.5%)	11 (2.4%)	0.02
	Black or African American	47 (7.0%)	20 (9.7%)	27 (5.8%)	
	Hispanic or Latino	15 (2.2%)	7 (3.4%)	8 (1.7%)	
	White	435 (64.5%)	116 (56.3%)	319 (68.2%)	
	Unknown	149 (22.1%)	53 (25.7%)	96 (20.5%)	
	Other	14 (2.1%)	7 (3.4%)	7 (1.4%)	
Smoking status, N (%)	Smoking data available	593 (88.0%)	180 (87.4%)	413 (88.2%)	
	Current or former smoker	472 (79.6%)	144 (80.0%)	328 (79.4%)	0.96
Histology, N (%)	Adenocarcinoma	290 (43.0%)	106 (43.0%)	184 (51.5%)	<0.001
	Transitional cell carcinoma	103 (15.3%)	27 (15.3%)	76 (13.1%)	
	Squamous cell carcinoma	157 (23.3%)	27 (23.3%)	130 (13.1%)	
	Other	124 (18.4%)	46 (18.4%)	78 (22.3%)	
Brain metastases, N (%)		124 (18.4%)	49 (23.8%)	75 (16.0%)	0.02
IO medication, N (%)	1L	537 (79.7%)	165 (80.1%)	372 (79.5%)	0.94

	Pembrolizumab	560 (83.1%)	169 (82.0%)	391 (83.5%)	0.71
Practice Setting, N (%)	Number of sites	300	130	240	
	AMCs	101 (33.7%)	41 (31.5%)	81 (33.8%)	0.75
	Community Clinic	199 (66.3%)	89 (68.5%)	159 (66.8%)	
Biomarker testing, N (%)	PD-L1 assessed	427 (63.4%)	133 (64.6%)	294 (62.8%)	
	PD-L1 Positive	293 (68.6%)	89 (66.9%)	204 (69.4%)	0.69
	PD-L1-Negative	134 (31.4%)	44 (33.1%)	90 (30.6%)	
	MSI-H	31 (4.6%)	18 (13.3%)	3 (0.7%)	<0.001

* Wilcoxon rank-sum test used. All other P Values were obtained using Pearson's chi-square test.

eTable 9. Patient Summary in the Evaluable Cohort by Cancer Type
(N = 674)

NSCLC		All Evaluable	Evaluable TMB-H	Evaluable TMB-L
Cohort Size	N	330	101	229
Age at start of IO, yrs	Median (range)	71.7, (28.6, 89.8)	68.8, (33.8, 88.3)	72.5, (28.6, 89.8)
Sex, N (%)	Female	169 (51.2%)	52 (51.5%)	117 (51.1%)
Race/Ethnicity, N (%)	Asian	5 (1.5%)	2 (2.0%)	3 (1.3%)
	Black or African American	34 (10.3%)	14 (13.9%)	20 (8.7%)
	Hispanic or Latino	6 (1.8%)	2 (2.0%)	4 (1.7%)
	White	225 (68.2%)	61 (60.4%)	164 (71.6%)
	Unknown	57 (17.3%)	21 (20.8%)	36 (15.7%)
	Other	3 (0.9%)	1 (1.0%)	2 (0.9%)
Smoking status, N (%)	Smoking data available	304 (92.1%)	94 (93.1%)	210 (91.7%)
	Current or former smoker	288 (94.7%)	93 (98.9%)	195 (92.9%)
Brain metastases, N (%)	Brain mets	97 (29.4%)	37 (36.6%)	60 (26.2%)
Histology, N (%)	Adenocarcinoma	235 (71.2%)	74 (73.2%)	161 (70.3%)
	Squamous cell carcinoma	65 (19.7%)	15 (14.9%)	50 (21.8%)
	Other	30 (9.1%)	12 (11.9%)	18 (7.9%)
IO medication, N (%)	1L	330 (100.0%)	101 (100.0%)	229 (100.0%)
	Pembrolizumab	295 (89.4%)	90 (89.1%)	205 (89.5%)
Practice Setting, N (%)	Number of sites	173	75	135
	AMCs	51 (29.5%)	20 (26.7%)	42 (31.1%)

	Community Clinic	122 (70.5%)	55 (73.3%)	93 (68.9%)
Biomarker testing, N (%)	PD-L1 assessed	224 (67.9%)	74 (73.3%)	150 (65.5%)
	PD-L1 Positive	196 (87.5%)	63 (85.1%)	133 (88.7%)
	PD-L1-Negative	28 (12.5%)	11 (14.9%)	17 (11.3%)
	MSI-H	0 (0.0%)	0 (0.0%)	0 (0.0%)
Bladder		**All Evaluable**	**Evaluable TMB-H**	**Evaluable TMB-L**
Cohort Size	N	148	38	110
Age at start of IO, yrs	Median (range)	69.2 (39.3, 89.7)	73.9 (48.0, 89.7)	68.5 (39.3, 87.6)
Sex, N (%)	Female	34 (23.0%)	9 (23.7%)	25 (22.7%)
Race/Ethnicity, N (%)	Asian	3 (2.0%)	2 (5.3%)	1 (0.9%)
	Black or African American	6 (4.1%)	2 (5.3%)	4 (3.6%)
	Hispanic or Latino	5 (3.4%)	4 (10.5%)	1 (0.9%)
	White	96 (64.9%)	19 (50.0%)	77 (70.0%)
	Unknown	35 (23.6%)	11 (28.9%)	24 (21.8%)
	Other	3 (2.0%)	0	3 (2.7%)
Smoking status, N (%)	Smoking data available	128 (86.5%	33 (86.8%)	95 (86.4%)
	Current or former smoker	91 (71.1%)	22 (66.7%)	69 (72.6%)
Brain metastases, N (%)	Brain mets	8 (5.4%)	3 (7.9%)	5 (4.5%)
Histology, N (%)	Transitional Cell Carcinoma	103 (69.6%)	27 (71.0%)	76 (69.1%)
	Other	40 (27.0%)	9 (23.7%)	31 (28.2%)
	Adenocarcinoma	3 (2.0%)	0	3 (2.7%)

	Squamous cell carcinoma	2 (1.4%)	2 (5.3%)	0
IO medication, N (%)	1L	89 (60.1%)	23 (60.5%)	66 (60.0%)
	Pembrolizumab	118 (79.7%)	31 (81.6%)	87 (79.1%)
Practice Setting, N (%)	Number of sites	91	33	71
	AMCs	35 (38.5%)	12 (36.4%)	27 (38.0%)
	Community Clinic	56 (61.5%)	21 (63.6%)	44 (62.0%)
Biomarker testing, N (%)	PD-L1 assessed	91 (61.5%)	23 (60.5%)	68 (61.8%)
	PD-L1 Positive	46 (50.5%)	14 (60.9%)	32 (47.1%)
	PD-L1-Negative	45 (49.5%)	9 (39.1%)	36 (52.9%)
	MSI-H	1 (0.7%)	1 (2.6%)	0 (0.0%)
HNSCC		**All Evaluable**	**Evaluable TMB-H**	**Evaluable TMB-L**
Cohort Size	N	96	12	84
Age at start of IO, yrs	Median (range)	65.9 (35.8, 87.2)	69.2 (51.5, 75.9)	65.8 (35.8, 87.2)
Sex, N (%)	Female	24 (25.0%)	4 (33.3%)	20 (23.8%)
Race/Ethnicity, N (%)	Asian	2 (20.1%)	0	2 (2.4%)
	Black or African American	3 (3.1%)	2 (16.7%)	1 (1.2%)
	White	62 (64.6%)	6 (50.0%)	56 (66.7%)
	Unknown	27 (28.1%)	3 (25.0%)	24 (28.6%)
	Other	2 (2.1%)	1 (8.3%)	1 (1.2%)
Smoking status, N (%)	Smoking data available	81 (84.4%)	8 (66.7%)	73 (86.9%)
	Current or former smoker	56 (69.1%)	7 (87.5%)	49 (67.1%)
Brain metastases, N (%)	Brain mets	4 (4.2%)	1 (8.3%)	3 (3.6%)

Histology, N (%)	Squamous cell carcinoma	90 (93.7%)	10 (83.3%)	80 (95.2%)
	Other	4 (4.2%)	1 (8.3%)	3 (3.6%)
	Adenocarcinoma	2 (2.1%)	1 (8.3%)	1 (1.2%)
IO medication, N (%)	1L	51 (53.1%)	3 (25.0%)	48 (57.1%)
	Pembrolizumab	87 (90.6%)	10 (83.3%)	77 (91.7%)
Practice Setting, N (%)	Number of sites	81	12	73
	AMCs	34 (42.0%)	6 (50.0%)	31 (42.5%)
	Community Clinic	47 (58.0%)	6 (50.0%)	42 (57.5%)
Biomarker testing, N (%)	PD-L1 assessed	58 (60.4%)	10 (83.3%)	48 (57.1%)
	PD-L1 Positive	35 (60.3%)	5 (50.0%)	30 (62.5%)
	PD-L1-Negative	23 (39.7%)	5 (50.0%)	18 (37.5%)
	MSI-H	1 (1.0%)	0 (0.0%)	1 (1.2%)
Melanoma		**All Evaluable**	**Evaluable TMB-H**	**Evaluable TMB-L**
Cohort Size	N	48	23	25
Age at start of IO (yrs)	Median (range)	63.2, (34.0, 89.3)	67.2, (34.8, 89.3)	61.5, (34.0, 85.3)
Sex, N (%)	Female	20 (41.7%)	5 (21.7%)	15 (60.0%)
Race/Ethnicity, N (%)	Asian	1 (2.1%)	0	1 (4.0%)
	Hispanic or Latino	1 (2.1%)	0	1 (4.0%
	White	25 (52.1%)	14 (60.9%)	11 (44.0%)
	Unknown	16 (33.3%)	7 (30.4%)	9 (36.0%)
	Other	5 (10.4%)	2 (8.7%)	3 (12.0%)
	Smoking data available	40 (83.3%)	19 (82.6%)	21 (84.0%)

Smoking status, N (%)	Current or former smoker	16 (40.0%)	8 (42.1%)	8 (38.1%)
Brain metastases, N (%)	Brain mets	11 (22.9%)	6 (26.1%)	5 (20.0%)
Histology, N (%)	Other	48 (100.0%)	23 (100.0%)	25 (100.0%)
IO medication, N (%)	1L	48 (100.0%)	23 (100.0%)	25 (100.0%)
	Pembrolizumab	14 (29.2%)	10 (43.5%)	4 (16.0%)
Practice Setting, N (%)	Number of sites	33	17	18
	AMCs	8 (24.2%)	5 (29.4%)	4 (22.2%)
	Community Clinic	25 (75.8%)	12 (70.6%)	14 (77.8%)
Biomarker testing, N (%)	PD-L1 assessed	20 (41.7%)	8 (34.8%)	12 (48.0%)
	PD-L1 Positive	10 (50.0%)	4 (50.0%)	6 (50.0%)
	PD-L1-Negative	10 (50.0%)	4 (50.0%)	6 (50.0%)
	MSI-H	1 (2.1%)	1 (4.3%)	0 (0.0%)
CRC		**All Evaluable**	**Evaluable TMB-H**	**Evaluable TMB-L**
Cohort Size	N	39	27	12
Age at start of IO (yrs)	Median (range)	63.1 (29.0, 88.1)	65.4 (29.0, 87.8)	60.6 (36.7, 88.1)
Sex, N (%)	Female	16 (41.0%)	10 (37.0%)	6 (50.0%)
Race/Ethnicity, N (%)	Asian	3 (7.7%)	1 (3.7%)	2 (16.7%)
	Black or African American	2 (5.1%)	1 (3.7%)	1 (8.3%)
	Hispanic or Latino	1 (2.6%)	1 (3.7%)	0
	White	22 (56.4%)	14 (51.9%)	8 (66.7%)
	Unknown	10 (25.6%)	9 (33.3%)	1 (8.3%)
	Other	1 (2.6%)	1 (3.7%)	0

Smoking status, N (%)	Smoking data available	30 (76.9%)	22 (81.5%)	8 (66.7%)
	Current or former smoker	16 (53.3%)	12 (54.5%)	4 (50.0%)
Brain metastases, N (%)	Brain mets	2 (5.1%)	1 (3.7%)	1 (8.3%)
Histology, N (%)	Adenocarcinoma	39 (100.0%)	27 (100.0%)	12 (100.0%)
IO medication, N (%)	1L	19 (48.7%)	15 (55.6%)	4 (33.3%)
	Pembrolizumab	33 (84.6%)	23 (85.2%)	10 (83.3%)
Practice Setting, N (%)	Number of sites	35	25	12
	AMCs	11 (31.4%)	9 (36.0%)	4 (33.3%)
	Community Clinic	24 (68.6%)	16 (64.0%)	8 (66.7%)
Biomarker testing, N (%)	PD-L1 assessed	23 (59.0%)	15 (55.6%)	8 (66.7%)
	PD-L1 Positive	5 (21.7%)	3 (20.0%)	2 (25.0%)
	PD-L1-Negative	18 (78.3%)	12 (80.0%)	6 (75.0%)
	MSI-H	24 (61.5%)	23 (85.2%)	1 (8.3%)
Gastric		**All Evaluable**	**Evaluable TMB-H**	**Evaluable TMB-L**
Cohort Size	N	7	1	6
Age at start of IO, yrs	Median (range)	66.4 (50.8, 70.9)	70.9 (70.9, 70.9)	66.2 (50.8, 70.2)
Sex, N (%)	Female	2 (28.6%)	0 (0.0%)	2 (33.3%)
Race/Ethnicity, N (%)	Hispanic or Latino	2 (28.6%)	0	2 (33.3%)
	White	3 (42.9%)	1 (100.0%)	2 (33.3%)
	Unknown	2 (28.6%)	0	2 (33.3%)
Smoking status, N (%)	Smoking data available	5 (71.4%)	1 (100.0%)	4 (66.7%)
	Current or former smoker	3 (60.0%)	1 (100.0%)	2 (50.0%)

Brain metastases, N (%)	Brain mets	1 (14.3%)	0 (0.0%)	1 (16.7%)
Histology, N (%)	Other	1 (14.3%)	0	1 (16.7%)
	Adenocarcinoma	6 (85.7%)	1 (100.0%)	5 (83.3%)
IO medication, N (%)	1L	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Pembrolizumab	7 (100.0%)	1 (100.0%)	6 (100.0%)
Practice Setting, N (%)	Number of sites	7	1	6
	AMCs	5 (71.4%)	0 (0.0%)	5 (83.3%)
	Community Clinic	2 (28.6%)	1 (100.0%)	1 (16.7%)
Biomarker testing, N (%)	PD-L1 assessed	7 (100.0%)	1 (100.0%)	6 (100.0%)
	PD-L1 Positive	1 (14.3%)	0 (0.0%)	1 (16.7%)
	PD-L1-Negative	6 (85.7%)	1 (100.0%)	5 (83.3%)
	MSI-H	0 (0.0%)	0 (0.0%)	0 (0.0%)

eTable 10. Patient Summary in the Prospective Cohort by Cancer Type
(N = 403)

NSCLC		**All Prospective**	**Prospective TMB-H**	**Prospective TMB-L**
Cohort Size	N	242	79	163
Age at start of IO, yrs	Median (range)	72.4 (43.7, 89.8)	68.8 (51.4, 88.3)	74.4 (43.7, 89.8)
Sex, N (%)	Female	129 (53.3%)	42 (53.2%)	87 (53.4%)
Race/Ethnicity, N (%)	Asian	4 (1.7%)	2 (2.5%)	2 (1.2%)
	Black or African American	26 (10.7%)	11 (13.9%)	15 (9.2%)
	Hispanic or Latino	6 (2.5%)	2 (2.5%)	4 (2.5%)
	White	168 (69.4%)	52 (65.8%)	116 (71.2%)
	Unknown	35 (14.5%)	11 (13.9%)	24 (14.7%)
	Other	3 (1.2%)	1 (1.3%)	2 (1.2%)
Smoking status, N (%)	Smoking data available	232 (95.9%)	75 (94.9%)	157 (96.3%)
	Current or former smoker	223 (96.1%)	74 (98.7%)	149 (94.9%)
Brain metastases, N (%)	Brain mets	75 (31.0%)	31 (39.2%)	44 (27.0%)
Histology, N (%)	Adenocarcinoma	178 (73.6%)	62 (78.5%)	116 (71.2%)
	Squamous cell carcinoma	41 (16.9%)	9 (11.4%)	32 (19.6%)
	Other	23 (9.5%)	8 (10.1%)	15 (9.2%)
	1L	242 (100.0%)	79 (100.0%)	163 (100.0%)

IO medication, N (%)	Pembrolizumab	212 (87.6%)	69 (87.3%)	143 (87.7%)
	Number of sites	127	58	96
Practice Setting, N (%)	AMCs	29 (22.8%)	10 (17.2%)	25 (26.0%)
	Community Clinic	98 (77.2%)	48 (82.8%)	71 (74.0%)
Biomarker testing, N (%)	PD-L1 assessed	189 (78.1%)	65 (82.3%)	124 (76.1%)
	PD-L1 Positive	164 (86.8%)	54 (83.1%)	110 (88.7%)
	PD-L1-Negative	25 (13.2%)	11 (16.9%)	14 (11.3%)
	MSI-H	0 (0.0%)	0 (0.0%)	0 (0.0%)
Bladder		**All Prospective**	**Prospective TMB-H**	**Prospective TMB-L**
Cohort Size	N	77	22	55
Age at start of IO, yrs	Median (range)	68.6 (43.8, 89.7)	74.3 (48.0, 89.7)	67.1 (43.8, 87.6)
Sex, N (%)	Female	15 (19.5%)	4 (18.2%)	11 (20.0%)
Race/Ethnicity, N (%)	Asian	2 (2.6%)	0	2 (3.6%)
	Black or African American	2 (2.6%)	0	2 (3.6%)
	Hispanic or Latino	4 (5.2%)	3 (13.6%)	1 (1.8%)
	White	48 (62.3%)	11 (50.0%)	37 (67.3%)
	Unknown	18 (23.4%)	6 (27.3%)	12 (21.8%)
	Other	3 (3.9%)	2 (9.1%)	1 (1.8%)
	Smoking data available	67 (87.0%)	19 (86.4%)	48 (87.3%)

Smoking status, N (%)	Current or former smoker	49 (73.1%)	11 (57.9%)	38 (79.2%)
Brain metastases, N (%)	Brain mets	5 (6.5%)	1 (4.5%)	4 (7.3%)
Histology, N (%)	Transitional Cell Carcinoma	56 (72.7%)	17 (77.3%)	39 (70.9%)
	Other	19 (24.7%)	5 (22.7%)	14 (25.5%)
	Adenocarcinoma	2 (2.6%)	0	2 (3.6%)
IO medication, N (%)	1L	41 (53.2%)	13 (59.1%)	28 (50.9%)
	Pembrolizumab	61 (79.2%)	17 (77.3%)	44 (80.0%)
Practice Setting, N (%)	Number of sites	52	21	40
	AMCs	17 (32.7%)	8 (38.1%)	13 (32.5%)
	Community Clinic	35 (67.3%)	13 (61.9%)	27 (67.5%)
Biomarker testing, N (%)	PD-L1 assessed	50 (64.9%)	15 (68.2%)	35 (63.6%)
	PD-L1 Positive	29 (58.0%)	8 (53.3%)	21 (60.0%)
	PD-L1-Negative	21 (42.0%)	7 (46.7%)	14 (40.0%)
	MSI-H	0 (0.0%)	0 (0.0%)	0 (0.0%)
HNSCC		**All Prospective**	**Prospective TMB-H**	**Prospective TMB-L**
Cohort Size	N	43	8	35
Age at start of IO (yrs)	Median (range)	68.7 (46.1, 87.2)	69.2 (51.5, 75.9)	68.6 (46.1, 87.2)
Sex, N (%)	Female	13 (30.2%)	3 (37.5%)	10 (28.6%)

Race/ Ethnicity, N (%)	Asian	2 (4.7%)	0	2 (5.7%)
	White	30 (69.8%)	5 (62.5%)	25 (71.4%)
	Unknown	9 (20.9%)	2 (25.0%)	7 (20.0%)
	Other	2 (4.7%)	1 (12.5%)	1 (2.9%)
Smoking status, N (%)	Smoking data available	37 (86.0%)	6 (75.0%)	31 (88.6%)
	Current or former smoker	25 (67.6%)	5 (83.3%)	20 (64.5%)
Brain metastases, N (%)	Brain mets	2 (4.7%)	1 (12.5%)	1 (2.9%)
Histology, N (%)	Squamous cell carcinoma	39 (90.7%)	7 (87.5%)	32 (91.4%)
	Adenocarcinoma	2 (4.7%)	1 (12.5%)	1 (2.9%)
	Other	2 (4.7%)	0	2 (5.7%)
IO medication, N (%)	1L	26 (60.5%)	2 (25.0%)	24 (68.6%)
	Pembrolizumab	40 (93.0%)	7 (87.5%)	33 (94.3%)
Practice Setting, N (%)	Number of sites	38	8	32
	AMCs	16 (42.1%)	6 (75.0%)	12 (37.5%)
	Community Clinic	22 (57.9%)	2 (25.0%)	20 (62.5%)
Biomarker testing, N (%)	PD-L1 assessed	35 (81.4%)	6 (75.0%)	29 (82.9%)
	PD-L1 Positive	22 (62.9%)	4 (66.7%)	18 (62.1%)
	PD-L1-Negative	13 (37.1%)	2 (33.3%)	11 (37.9%)
	MSI-H	1 (2.3%)	0 (0.0%)	1 (2.9%)
Melanoma		**All Prospective**	**Prospective TMB-H**	**Prospective TMB-L**

Cohort Size	N	14	7	7
Age at start of IO, yrs	Median (range)	60.2 (34.0, 89.3)	60.6 (34.8, 89.3)	59.8 (34.0, 76.3)
Sex, N (%)	Female	7 (50.0%)	3 (42.9%)	4 (57.1%)
Race/Ethnicity, N (%)	Asian	1 (7.1%)	0	1 (14.3%)
	White	8 (57.1%)	4 (57.1%)	4 (57.1%)
	Unknown	3 (21.4%)	2 (28.6%)	1 (14.3%)
	Other	2 (14.3%)	1 (14.3%)	1 (14.3%)
Smoking status, N (%)	Smoking data available	12 (85.7%)	7 (100.0%)	5 (71.4%)
	Current or former smoker	4 (33.3%)	3 (42.9%)	1 (20.0%)
Brain metastases, N (%)	Brain mets	4 (28.6%)	3 (42.9%)	1 (14.3%)
Histology, N (%)	Other	14 (100.0%)	7 (100.0%)	7 (100.0%)
IO medication, N (%)	1L	14 (100.0%)	7 (100.0%)	7 (100.0%)
	Pembrolizumab	3 (21.4%)	3 (42.9%)	0 (0.0%)
Practice Setting, N (%)	Number of sites	8	5	4
	AMCs	1 (12.5%)	1 (20.0%)	1 (25.0%)
	Community Clinic	7 (87.5%)	4 (80.0%)	3 (75.0%)
Biomarker testing, N (%)	PD-L1 assessed	5 (35.7%)	3 (42.9%)	2 (28.6%)
	PD-L1 Positive	3 (60.0%)	2 (66.7%)	1 (50.0%)
	PD-L1-Negative	2 (40.0%)	1 (33.3%)	1 (50.0%)
	MSI-H	1 (7.1%)	1 (14.3%)	0 (0.0%)

CRC		All Prospective	Prospective TMB-H	Prospective TMB-L
Cohort Size	N	23	17	6
Age at start of IO (yrs)	Median (range)	73.1 (38.3, 88.1)	74.0 (38.3, 87.8)	57.4 (48.2, 88.1)
Sex, N (%)	Female	12 (52.2%)	7 (41.2%)	5 (83.3%)
Race/Ethnicity, N	Asian	2 (8.7%)	1 (5.9%)	1 (16.7%)
	Black or African American	2 (8.7%)	1 (5.9%)	1 (16.7%)
	White	13 (56.5%)	10 (58.8%)	3 (50.0%)
	Unknown	6 (26.1%)	5 (29.4%)	1 (16.7%)
Smoking status, N (%)	Smoking data available	21 (91.3%)	16 (94.1%)	5 (83.0%)
	Current or former smoker	12 (52.7%)	10 (62.5%)	2 (40.0%)
Brain metastases, N (%)	Brain mets	0 (0.0%)	0 (0.0%)	0 (0.0%)
Histology, N	Adenocarcinoma	23 (100.0%)	17 (100.0%)	6 (100.0%)
IO medication, N (%)	1L	13 (56.5%)	11 (64.7%)	2 (33.3%)
	Pembrolizumab	19 (82.6%)	13 (76.5%)	6 (100.0%)
	Number of sites	20	16	6
Practice Setting, N (%)	AMCs	6 (30.0%)	5 (31.2%)	3 (50.0%)
	Community Clinic	14 (70.0%)	11 (68.8%)	3 (50.0%)
Biomarker testing, N (%)	PD-L1 assessed	13 (56.5%)	10 (58.8%)	3 (50.0%)
	PD-L1 Positive	3 (23.1%)	2 (20.0%)	1 (33.3%)
	PD-L1-Negative	10 (76.9%)	8 (80.0%)	2 (66.7%)

	MSI-H	16 (69.6%)	15 (88.2%)	1 (16.7%)

eTable 11. Univariate Cox-Proportional Hazards Models

Cohort	coxPH formula	HR	Upper_CI	P Value
Prospective NSCLC	OS ~ TMB	0.67	1.00	0.05
Prospective Pembrolizumab treated	OS ~ TMB + strata(LoT)	0.67	0.94	0.03
Prospective non-Pembrolizumab treated	OS ~ TMB + strata(LoT)	0.37	0.85	0.03
Prospective NSCLC	OS ~ PD-L1	0.55*	0.96	0.04

*HR is for PD-L1 > 50% vs. PD-L1 < 1%. The HR for PD-L1 1-49% vs. PD-L1 < 1% was 0.55 (P=0.064). The HR for PD-L1 unknown vs. PD-L1 < 1% was 0.44 (P=0.016).

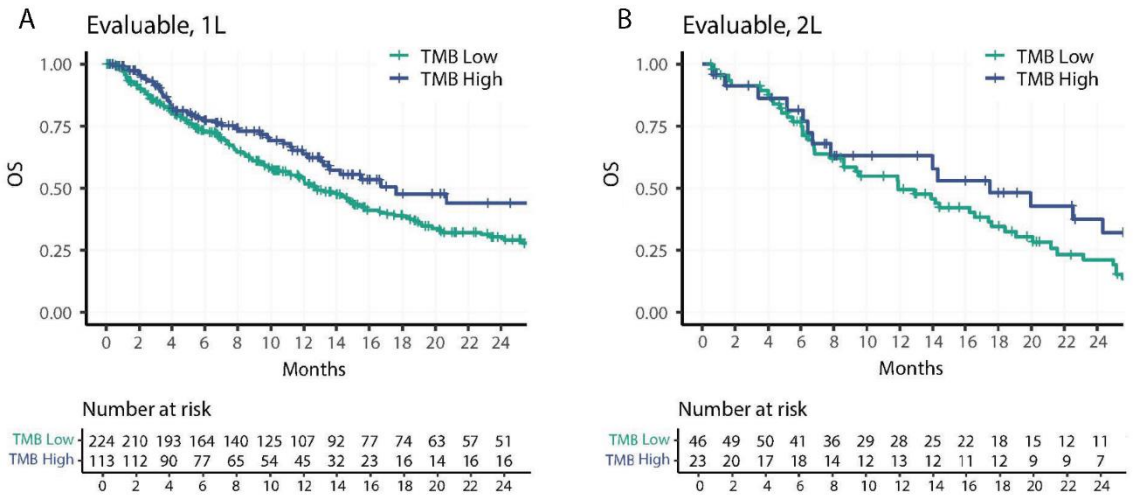
eTable 12. Multivariable Cox-Proportional Hazards Models

Cohort	Survival Formula	Variable	HR	One-sided UCB	P Value
Prospective	OS ~ TMB + PD-L1 classification (posi. vs. neg vs. unknown) + MSI classification (MSI-H vs. MSS) + strata(LoT)	TMB high vs low	0.67	0.92	0.02
		PDL1 Pos. vs Neg	1.03		
		PDL1 Unknown vs. Neg	0.92		
		MSI-H Vs MSS	0.46		
Prospective NSCLC	OS ~ TMB + PD-L1 classification (positive vs. neg vs. unknown)	TMB high Vs low	0.65	0.97	0.04
		PD-L1 >50% vs PD-L1 < 1%	0.52		
		PD-L1 1-49% vs PD-L1 < 1%	0.54		
		PD-L1 Unknown vs PD-L1 < 1%	0.42		

Figure Legends

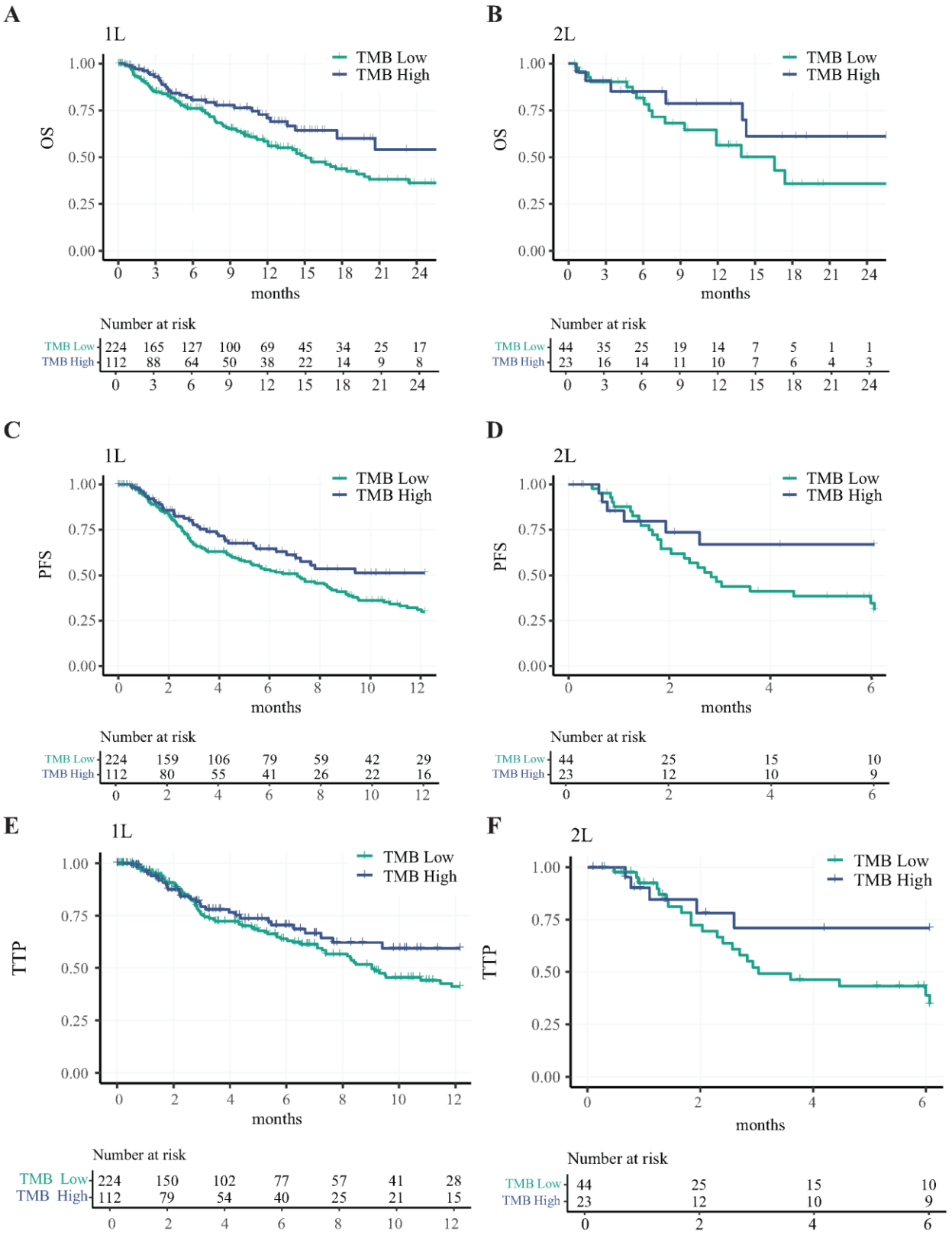
eFigure 1. Kaplan-Meier (KM) Analysis of OS by TMB Status in the Evaluable Cohort in First-Line and Second-Line Treated Patients

(a) KM plot of OS in the evaluable cohort treated with IO at 1L. (b) KM plot of OS in the evaluable cohort treated with IO at 2L.



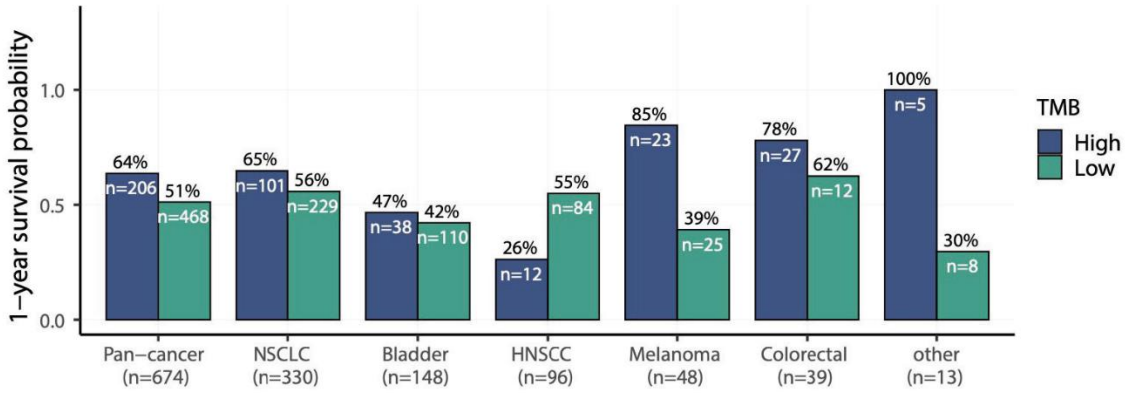
eFigure 2. Kaplan-Meier (KM) Analysis of Clinical Outcomes by TMB Status in the Prospective Cohort in First-Line and Second-Line Treated Patients

(a) OS in the prospective cohort treated with IO at 1L. (b) OS in the prospective cohort treated with IO at 2L. (c) PFS in the prospective cohort treated with IO at 1L. (d) PFS in the prospective cohort treated with IO at 2L. (e) TTP in the prospective cohort treated with IO at 1L. (f) TTP in the prospective cohort treated with IO at 2L.



eFigure 3. Assessment of Overall Survival by TMB-Status in Each Cancer Indication in the Evaluable Cohort

1-year survival probability in each cancer type by TMB status estimated from KM analysis in the evaluable cohort.



eFigure 4. Kaplan-Meier (KM) Analysis of Clinical Outcomes by TMB Status in the Prospective Cohort in Pembrolizumab and Non-pembrolizumab Treated Patients

(a) KM analysis of OS by TMB status in pembrolizumab treated patients. (b) KM analysis of OS by TMB status in non-pembrolizumab ICI treated patients.