

Supplemental Table 1. Transcription levels of CD8 α , granzyme B (GZMB), FoxP3, PD-L1, CTL- and Treg-attracting chemokines, as well as ratios of CD8 α /FoxP3, GZMB/FoxP3, CCL5/CCL22 and CXCL10/CCL22 (N=6 patients) were measured using RT-PCR (TaqMan). All gene expression levels were normalized for HPRT1 housekeeping gene in each sample.

Gene Expression Normalized for HPRT1																		
Gene:	CD8a		GZMB		FOXP3		PD-L1		CCL5		CXCL9		CXCL10		CCL22		CXCL12	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
PT3	0.157	0.341667	0.015333	0.038667	0.0337	0.037	0.036266	0.045793	0.24	0.493667	1.135667	1.999667	0.105667	0.349667	0.002	0.003667	0.087667	0.054667
PT4	0.006	0.091333	*	0	0.003	0.001	0.001667	0.002333	0.004667	0.027667	0.006	0.014333	0.010667	0.013667	0.001333	0.005667	0.131	0.406
PT5	0.03867	0.274	0.012	0.169667	0.0593	0.008667	0.006667	0.006667	0.078667	0.239333	0.033667	0.011333	0.051	0.707667	0.012667	0.0003	0.644667	0.494333
PT6	0.06467	2.081667	0.024333	0.179333	4.2623	0.066	0.005	0.002333	0.353333	2.262667	3.013667	1.817333	0.441	1.663667	1.078	0.298	0.144667	0.034667
PT7	0.144	0.573667	0.028	0.119	0.0217	0.044	1.68E-05	0	0.466	2.968333	0.643	3.920333	0.595333	1.285333	0.006667	0.006667	0.783	0.586333
PT8	0.159	0.104	0.084333	0.060333	0.0393	0.012667	0.003259	0.002404	0.881667	0.682333	1.681667	0.762	0.409	0.201	0.023667	0.004333	1.078	0.298

Ratios								
Genes:	CD8/FOXP3		GZMB/FOXP3		CCL5/CCL22		CXCL10/CCL22	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
PT3	4.663366	9.234235	0.455445	1.045045	120	134.6351	52.83335	95.36277
PT4	6	91.3333	0.4	3	3.500026	4.882325	8.000027	2.411751
PT5	0.651691	31.61526	0.202247	19.57685	6.210529	797.7778	4.026316	2358.889
PT6	0.015172	31.5404	0.005709	2.717172	0.327767	7.592841	0.409091	5.582774
PT7	6.646144	13.03788	1.292306	2.704545	69.9	445.2478	89.29999	192.799
PT8	4.042373	8.210524	2.144067	4.763156	37.25352	157.4627	17.28169	46.38497

* Since the readout was under the limit of detection, 0.0004 was used for estimating a fold-change.

Supplemental Table 2. Adverse events, definitely, probably or possibly related to CKM, as determined by CTCAEv5.0.

Adverse events definitely, probably, or possibly related to CKM	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)	Grade 5 N (%)
Hematologic					
ITP	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Gastrointestinal					
Mouth bleeding	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Nausea	1 (12.5)	3 (37.5)	0 (0.0)	0 (0.0)	0 (0.0)
Vomiting	1 (12.5)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
General					
Chills	6 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	2 (25.0)	2 (25.0)	1 (12.5)	0 (0.0)	0 (0.0)
Malaise	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Pain	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pyrexia	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Laboratory					
ALT increase	3 (37.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AST increase	3 (37.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ALP increase	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Lymphocyte count decrease	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Neutrophil count decrease	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Platelet count decrease	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)
WBC count decrease	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Metabolism and Nutrition					
Decreased appetite	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Neurological					
Dizziness	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Headache	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Taste disorder	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Cutaneous					
Night sweats	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Petechiae	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Rash	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Vascular					
Hot flashes	3 (37.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hypotension	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abbreviations: ALP- alkaline phosphatase; ALT- alanine aminotransferase; AST- aspartate transaminase; ITP- immune thrombocytopenic purpura; WBC- white blood cell					

Supplemental Table 3. Adverse events, definitely, probably, or possibly related to pembrolizumab as determined by CTCAEv5.0

Adverse events definitely, probably, or possible related to pembrolizumab	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)	Grade 5 N (%)
Hematologic					
ITP	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Ophthalmic					
Eye disorder	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gastrointestinal					
Mouth bleeding	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
General					
Fatigue	0 (0.0)	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)
Laboratory					
ALP increase	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Lymphocyte count decrease	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Platelet count decrease	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)
Metabolism and Nutrition					
Myalgia	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Respiratory					
Dyspnea	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Hypoxia	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Pneumonitis	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)
Cutaneous					
Petechiae	1 (12.5)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Pruritis	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Rash	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Abbreviation: ALP- alkaline phosphatase, ITP- immune thrombocytopenic purpura					

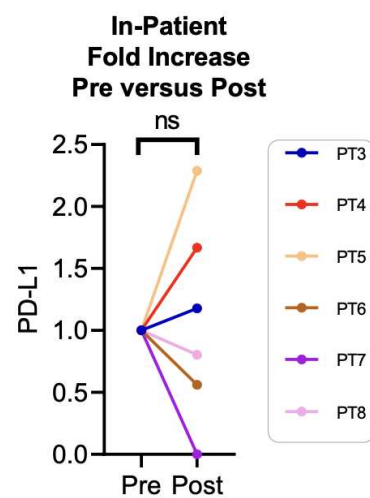
Supplemental Table 4. Demographic and clinical characteristics and treatment response of patients on the study. A total of 8 patients were enrolled on this study with two patients (PT2 and PT3) receiving prior treatments in the metastatic setting. Four patients were alive at the data cut-off of January 24, 2023. Best response assessments by RECISTv1.1 and irRECIST were concordant for all patients.

PT	Age at Dx	Race	PD-L1 (%)	Sites of metastasis at treatment initiation	Prior lines of systemic treatment for metastatic disease	Pre- & Post-CKM tissue analyzed	BR (RECIST v1.1)	Time to BR (days)	BR (irRECIST)	Response Duration (days)	Status
1	74	C	10	chest wall, lungs, pleura	0	No	PD*	119	irPD		A
2	42	C	0	LN, muscle, pleura, skin	4	No	PD	45	irPD		D
3	33	AA	10	bone, LN, s/c tissue	1	Yes	SD	50 [#]	irSD	69	D
4	66	C	0	breast, liver	0	Yes	PD	56	irPD		D
5	38	C	0	LN, liver, lung, peritoneum	0	Yes	PD	42	irPD		D
6	69	C	5	LN	0	Yes	SD	60	irSD	105	A
7	63	C	10	LN, s/c tissue	0	Yes	SD	58	irSD	68	A
8	36	C	1	bone, liver, lung, LN, s/c tissue	0	Yes	PD	51	irPD		A

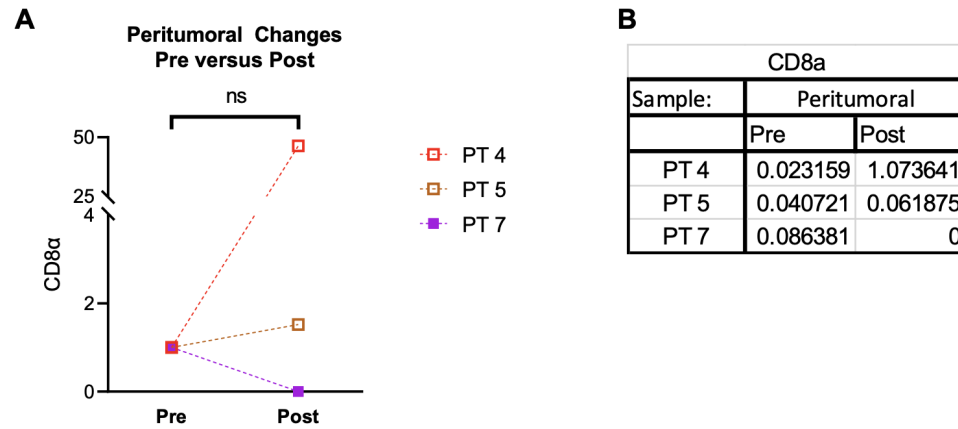
* PT1 continued to have PR after PD per RECIST v1.1 and continues pembrolizumab until the data cut-off date.
[#] Unscheduled scan performed after 50 days for thrombocytopenia and shortness of breath showed evidence of response

Abbreviations: A- alive; AA- African American; BR- best response; C- Caucasian; CKM- chemokine modulation; D- dead; Dx- diagnosis; LN- lymph node; PD- progressive disease; PR- partial response; s/c- subcutaneous

Supplemental Figure 1. PD-L1 expression levels in pre- and post-CKM treatment tumor biopsies were measured using RT-PCR (N=6 patients). Data show in-patient fold increases (post- versus pre-treatment; relative to HPRT1) for PD-L1 gene transcripts.



Supplemental Figure 2. Transcription levels of CD8 α (N=3 patients) in paired peritumoral biopsies obtained pre- and post-CKM treatment were evaluated by RT-PCR and expressed as (A) in-patient fold increases (post- versus pre-treatment; relative to HPRT1) and (B) raw values (relative to HPRT1).



Supplemental Figure 3. Changes in the immune cell subsets circulating in the peripheral blood were measured by (A) multi-parameter flow cytometry and (B) RT-PCR (N = 7). Patient 3, who later developed ITP, demonstrated the highest relative percentage of CD3⁺CD8⁺ T cells in the blood by multi-parameter flow cytometry at baseline, day 2, day 7 and week 3. RT-PCR measured expression of CD8 α in PBMCs at baseline, day 2, day 7, and week 3 and showed analogous changes.

