Cell Reports Medicine, Volume *2*

Supplemental information

Response and recurrence correlates in individuals treated with neoadjuvant anti-PD-1 therapy for resectable oral cavity squamous cell carcinoma

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Supplemental Information

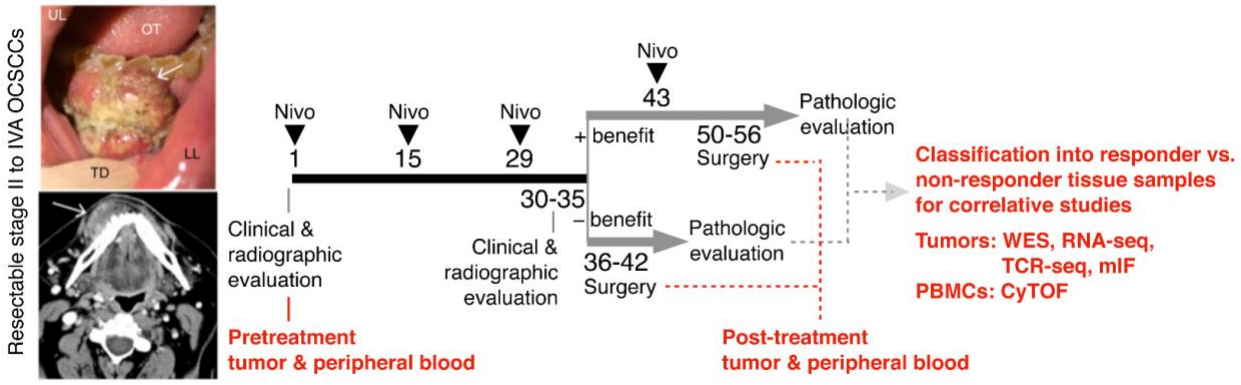
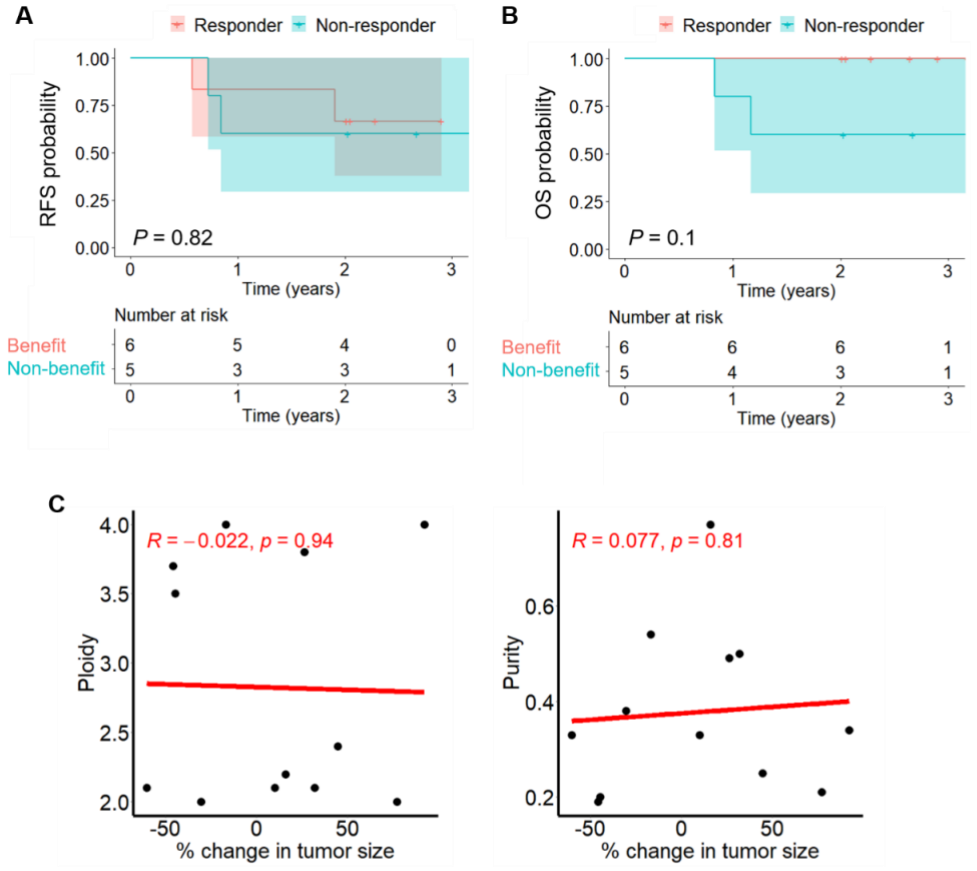


Figure S1. Design of clinical trial and correlative study, Related to STAR Methods.

Patients with surgically resectable, HPV-negative, locoregionally advanced (stage II to IVA) oral-cavity squamous cell carcinoma were enrolled. Patient #6 pretreatment clinical and radiographic (CT) images are shown as an example. Pretreatment clinical and radiographic tumor measurements along with tumor and peripheral blood sampling were performed before patients received 3 mg/kg of nivolumab every two weeks for three doses. After these three doses, patients' initial tumor responses were categorized into benefit (partial response or stable disease) vs. non-benefit (progression) based on repeat clinical and radiographic assessments between days 30 to 35. In the case of disease progression by RECIST 1.1 criteria, patients proceeded directly to surgery between days 36 to 42. In the case of patient benefit, patients received a 4th dose of nivolumab on day 43 before proceeding to surgery between day 50 to 56. Pathology-based changes in tumor size were determined by comparing final tumor size by pathologic evaluation to the initial pretreatment tumor size by radiographic imaging. These tumor size changes were used to classify tumors or patients into, respectively, responsive or responders versus non-responsive or non-responders. Specifically, since only three to four doses of bi-weekly nivolumab were given, responders were defined as patients who derived clinical benefit (complete response, partial response, and stable disease per RECIST 1.1), and non-responders were defined as patients who derived no clinical benefit (progression per RECIST 1.1). Surgical tumor samples represent post-treatment tumors; peripheral blood samples were collected again at the time of surgery. Red texts refer to this correlative study. Molecular analyses based on responder versus non-responder status were performed on the pretreatment and post-treatment tumors and PBMCs isolated from peripheral blood. Additional tumor samples were collected at the times of recurrences post-neoadjuvant nivolumab treatment and post-surgery. Longitudinal tumor samples (pretreatment versus recurrence or post-treatment versus recurrence) were analyzed for tumor evolution by WES or by mIF.



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ID	HLA-A		HLA-B		HLA-C		Zygosity	HLA mutation
Pt01-Pre	HLA-A*26:01	HLA-A*32:01	HLA-B*14:01	HLA-B*45:01	HLA-C*06:02	HLA-C*08:02	Hetero	0
Pt02-Pre	HLA-A*03:01	HLA-A*23:01	HLA-B*56:01	HLA-B*44:03	HLA-C*04:01	HLA-C*01:02	Hetero	0
Pt03-Pre	HLA-A*03:01	HLA-A*01:01	HLA-B*08:01	HLA-B*47:01	HLA-C*07:01	HLA-C*06:02	Hetero	0
Pt04-Pre	HLA-A*11:01	HLA-A*02:01	HLA-B*07:02	HLA-B*51:01	HLA-C*07:02	HLA-C*16:01	Hetero	0
Pt05-Pre	HLA-A*11:01	HLA-A*02:01	HLA-B*18:01	HLA-B*27:05	HLA-C*05:01	HLA-C*01:02	Hetero	0
Pt06-Pre	HLA-A*01:01	HLA-A*31:01	HLA-B*35:01	HLA-B*51:01	HLA-C*04:01	HLA-C*04:01	Homo	0
Pt07-Pre	HLA-A*03:01	HLA-A*26:01	HLA-B*07:02	HLA-B*14:02	HLA-C*07:02	HLA-C*08:02	Hetero	1
Pt08-Pre	HLA-A*01:01	HLA-A*24:02	HLA-B*08:01	HLA-B*35:03	HLA-C*07:01	HLA-C*12:03	Hetero	0
Pt09-Pre	HLA-A*02:01	HLA-A*02:01	HLA-B*07:02	HLA-B*07:02	HLA-C*07:02	HLA-C*07:02	Homo	0
Pt10-Pre	HLA-A*02:01	HLA-A*02:01	HLA-B*35:01	HLA-B*51:01	HLA-C*15:02	HLA-C*04:01	Homo	0
Pt12-Pre	HLA-A*02:01	HLA-A*02:01	HLA-B*07:02	HLA-B*07:02	HLA-C*07:02	HLA-C*05:01	Homo	0
Pt14-Pre	HLA-A*11:01	HLA-A*02:01	HLA-B*40:01	HLA-B*44:03	HLA-C*04:01	HLA-C*03:04	Hetero	0

Figure S2. Association of response status with patient survival, tumor purity, tumor ploidy, and tumor HLA I genotypes, Related to Figure 1

(A to B) Kaplan–Meier (KM) curves of RFS (A) and OS (B) comparing responder (n = 6) versus non-responder (n = 5) patients. P-values, Wilcoxon rank-sum test. Pt12 was not included in this analysis due to short/lost follow-up.

(C) Pearson correlations of ploidy and purity of pretreatment tumors with pathology-based changes in tumor sizes.

(D) HLA-I (HLA-A, HLA-B and HLA-C) genotypes of pretreatment tumors in each patient.

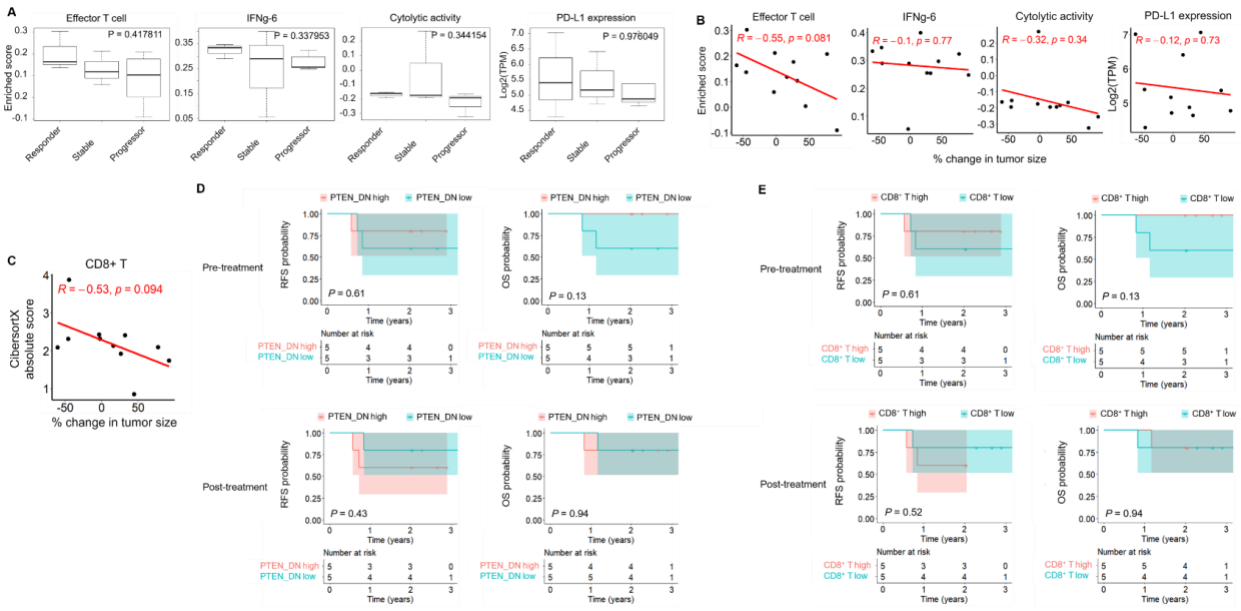

Figure S3. Association of tumor-based, immune-related signatures with response and survival status, Related to Figure 3

(A) Levels of enrichment (effector T-cell signature, IFNG-6 genes signature, cytolytic activity signature) and *PD-L1* expression in pretreatment tumors of responders (n = 3) versus stable diseases (n = 3) versus progressors (n = 5); P-values, Kruskal–Wallis test.

(B) Pearson correlations of pathology-based changes in tumor sizes with levels of enrichment (effector T-cell signature, IFNG-6 genes signature, and cytolytic activity signature) and *PD-L1* expression in pretreatment tumors.

(C) Pearson correlations of pathology-based changes in tumor sizes with pretreatment levels of $CD8^+$ T cell.

(D) KM curves of RFS and OS comparing tumors with high enrichment scores of PTEN_DN signature (≥ median enrichment score, n = 5) versus tumors with low enrichment scores (< median enrichment score, n = 5) before (top) or after (bottom) neoadjuvant nivolumab treatment; P-values, two-sided log-rank test. Pt12 was not included in this analysis.

(E) KM curves of RFS and OS comparing tumors with high infiltration levels of $CD8^+$ T cell (≥ median infiltration level, n = 5) versus tumors with low infiltration levels (< median infiltration level, n = 5) before (top) or after (bottom) neoadjuvant nivolumab treatment; P-values, two-sided log-rank test. Pt12 was not included in this analysis.

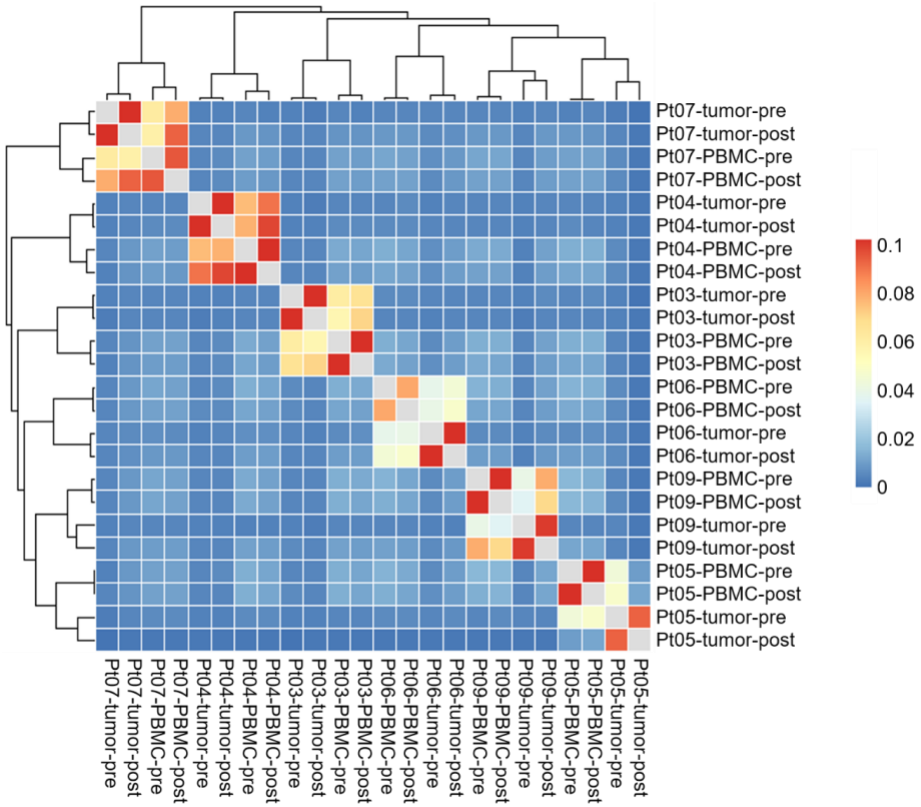

Figure S4. Heatmap of Jaccard indices of TCRβ repertoires between pairs of tissue samples as indicated, Related to Figure 4

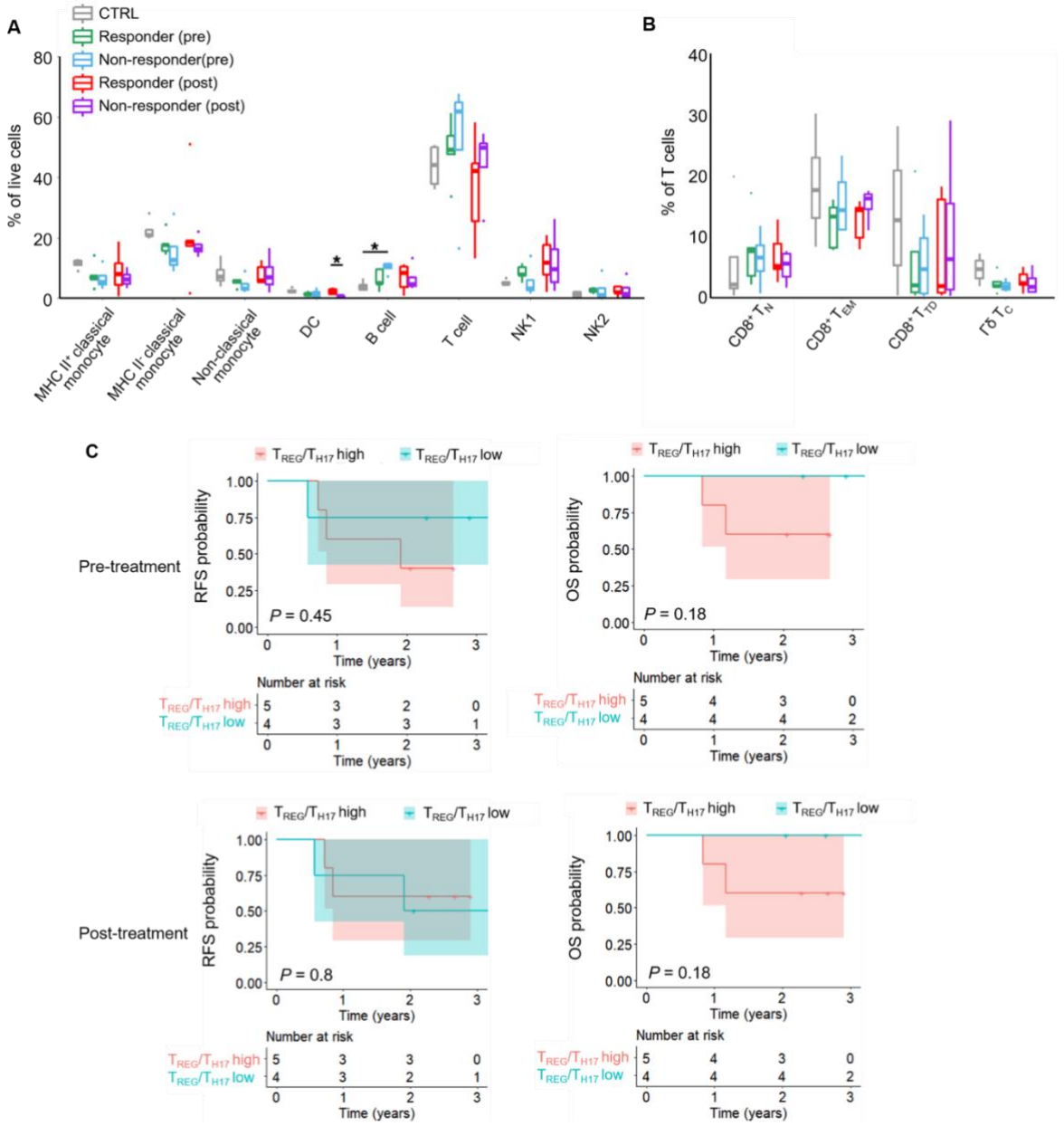

Figure S5. Association of pre- and post-treatment peripheral blood immune cell types with response or survival status, Related to Figure 4

(A and B) Frequencies of major immune cell types in all live cells (A) and $CD8^+$ T-cell subsets and γδ T cells in the T-cell population (B) in responders versus non-responders, before and after neoadjuvant nivolumab treatment. P-value, Student's *t* test, *p<0.05.

(C) KM curves of RFS and OS comparing tumors with high T_{REG}/Th17 ratios (≥ median ratio, n = 5) versus tumors with low T_{REG}/Th17 ratios (< median ratio, n = 4); P-values, two-sided log-rank test. Pt12 was not included in this analysis.

		All patients (n=12)	Response (n=4)	Stable (n=4)	Progression (n=4)	p value
Age, yrs	mean +/-SD	64+/-8.0	61.1 +/- 1.2	65.2 +/- 9.0	65.2 +/- 11.8	0.75
	median (range)	62 (48-78)	61.1 (59-62)	62.1 (58-78)	68.6 (48-75)	
Gender (%)	Male	5 (42)	2 (50)	1 (25)	2 (50)	0.1
	Female	7 (58)	2 (50)	3 (75)	2 (50)	
Smoking status (%)	Current	6 (67)	2 (50)	1 (25)	3 (75)	0.75
	Former	3 (22)	1 (25)	1 (25)	1 (25)	
	Never	3 (11)	1 (25)	2 (50)	0 (0)	
Alcohol use (drinks/week)	mean +/-SD	13.2 +/-27.4	7 +/- 5.7	7+/-9.9	25.9 +/- 48.1	0.58
	median (range)	5.5 (0-98)	7 (0-14)	3.5 (0-21)	4 (0-98)	
ECOG status	0	5 (42)	2 (50)	0 (0)	3 (75)	0.1
	1	7 (58)	2 (50)	4 (100)	1 (25)	
T stage (%)	T2	3 (25)	2 (50)	0 (0)	1 (25)	0.75
	T3	3 (25)	0 (0)	3 (75)		
	T4a	6 (50)	2 (50)	1 (25)	3 (75)	
N stage (%)	N0	4 (33)	2 (50)	0 (0)	2 (40)	0.15
	N1	4 (33)	1 (25)	1 (25)	2 (40)	
	N2b	2 (16)	0 (0)	2 (50)	0 (0)	
	N2c	2 (16)	1 (25)	1 (25)	0 (0)	
Clinical stage (%)	II	3 (25)	2 (50)	0 (0)	1 (25)	0.53
	III	1 (8)	0 (0)	1 (25)	0 (0)	
	IVA	8 (67)	2 (50)	3 (75)	3 (75)	
Tumor size (greatest dimension), cm	mean +/- SD	3.6 +/- 1.1	3.7 +/- 1.1	3.9 +/- 1.1	3.2 +/- 1.4	0.38
	median (range)	3.1 (2.1-5.4)	3.6 (2.8-5.0)	3.7 (3.0-5.4)	2.8 (2.1-5.1)	
Histologic grade	Well	1 (8)	1 (25)	0 (0)	0 (0)	0.27
	Mod to Well	1 (8)	1 (25)	0 (0)	0 (0)	
	Moderate	5 (43)	1 (25)	3 (75)	1 (25)	
	Mod to Poor	3 (25)	0 (0)	1 (25)	2 (25)	
	Poor	1 (8)	1 (25)	0 (0)	0 (0)	
	Spindle	1 (8)	0 (0)	0 (0)	1 (25)	

Table S1. Patient and tissue characteristics, Related to STAR Methods.

ID	Outcome	Radiological response (scan 1 vs scan 2)	Pathologic response Scan 1 vs path	Status	Status of the last observation	RFS (Yrs)	OS (Yrs)
Pt01	Responder	-8.3333	-45.6522	0	Alive with evidence of disease	0.5753	3.1808
Pt02	Progressor	4.4177	44.5783	0	Alive with evidence of disease	3.2630	3.2932
Pt03	Progressor	16.3522	32.0755	0	Alive without evidence of disease	2.6685	2.6685
Pt04	Progressor	20.4327	92.3077	1	Dead of disease	0.8466	1.1699
Pt05	Responder	-4.1522	-44.6367	0	Alive without evidence of disease	2.8986	2.8986
Pt06	Responder	-15.3937	-29.4947	0	Alive with evidence of disease	1.9096	2.6411
Pt07	Progressor	14.8688	26.3362	1	Dead of disease	0.7260	0.8329
Pt08	Progressor	108.9552	62.6866	1	Alive without evidence of disease	2.0274	2.0274
Pt09	Responder	-16.3347	-60.1594	0	Alive without evidence of disease	2.0521	2.0521
Pt10	Stable	13.7050	16.1440	0	Alive without evidence of disease	2.2822	2.2822
Pt12	Stable	1.5177	-2.1922	0	Alive without evidence of disease	0.2767	0.2767
Pt14	Stable	21.4171	-3.3816	0	Alive without evidence of disease	2.0164	2.0164

Table S2. Tumor response and survival patterns, Related to STAR Methods.

Patient	WES (Tumor)	RNA-seq (Tumor)	CyTOF (PBMC)	TCR-seq (Tumor)	TCR-seq (PBMC)
Pt01-Pre	✓	✓	✓		
Pt01-Post		✓	✓		
Pt01-Recur	✓	✓	✓		
Pt02-Pre	✓	✓	✓		
Pt02-Post		✓	✓		
Pt03-Pre	✓	✓	✓	✓	✓
Pt03-Post		✓	✓	✓	✓
Pt04-Pre	✓	✓	✓	✓	✓
Pt04-Post		✓	✓	✓	✓
Pt05-Pre	✓	✓	✓	✓	✓
Pt05-Post		✓	✓	✓	✓
Pt06-Pre	✓		✓	✓	✓
Pt06-Post			✓	✓	✓
Pt06-Recur1	✓				
Pt06-Recur2	✓				
Pt07-Pre	✓	✓	✓	✓	✓
Pt07-Post		✓	✓	✓	✓
Pt07-Recur	✓				
Pt08-Pre	✓	✓			
Pt08-Post		✓			
Pt09-Pre	✓	✓	✓	✓	✓
Pt09-Post		✓	✓	✓	✓
Pt10-Pre	✓	✓	✓		
Pt10-Post		✓	✓		
Pt12-Pre	✓	✓			
Pt12-Post		✓			
Pt14-Pre	✓	✓			
Pt14-Post		✓			

Table S3. Multi-omic data and associated tissues and time points, Related to STAR Methods.

IDs	Tumor coverage (mean)	Normal coverage (mean)	Tumor purity	Ploidy	Number of SNV	Number of INDEL	Mutation burden/MB
Pt01-Pre	439.57	444.88	0.19	3.7	129	12	2.35
Pt01-Recur	560.35	444.88	0.22	3.6	214	8	3.70
Pt02-Pre	407.03	385.94	0.25	2.4	410	44	7.57
Pt03-Pre	507.06	580.79	0.5	2.1	353	18	6.18
Pt04-Pre	565.37	497.63	0.34	4	268	13	4.68
Pt05-Pre	639.93	496.08	0.2	3.5	377	21	6.63
Pt06-Pre	532.61	674.54	0.38	2	291	29	5.33
Pt06-Recur1	618.75	674.54	0.2	1.6	280	69	5.82
Pt06-Recur2	380.18	674.54	0.96	1.7	215	66	4.68
Pt07-Pre	446.95	590.39	0.49	3.8	310	18	5.47
Pt07-Recur	638.25	590.39	0.2	1.8	455	53	8.47
Pt08-Pre	406.22	536.89	0.21	2	349	10	5.98
Pt09-Pre	370.15	436.67	0.33	2.1	539	20	9.32
Pt10-Pre	636.78	577.63	0.77	2.2	412	25	7.28
Pt12-Pre	579.19	519.47	0.33	2.1	21	10	0.52
Pt14-Pre	556.59	577.7	0.54	4	483	37	8.67

Table S4. Whole-exome sequence data characteristics, Related to Figure 1.