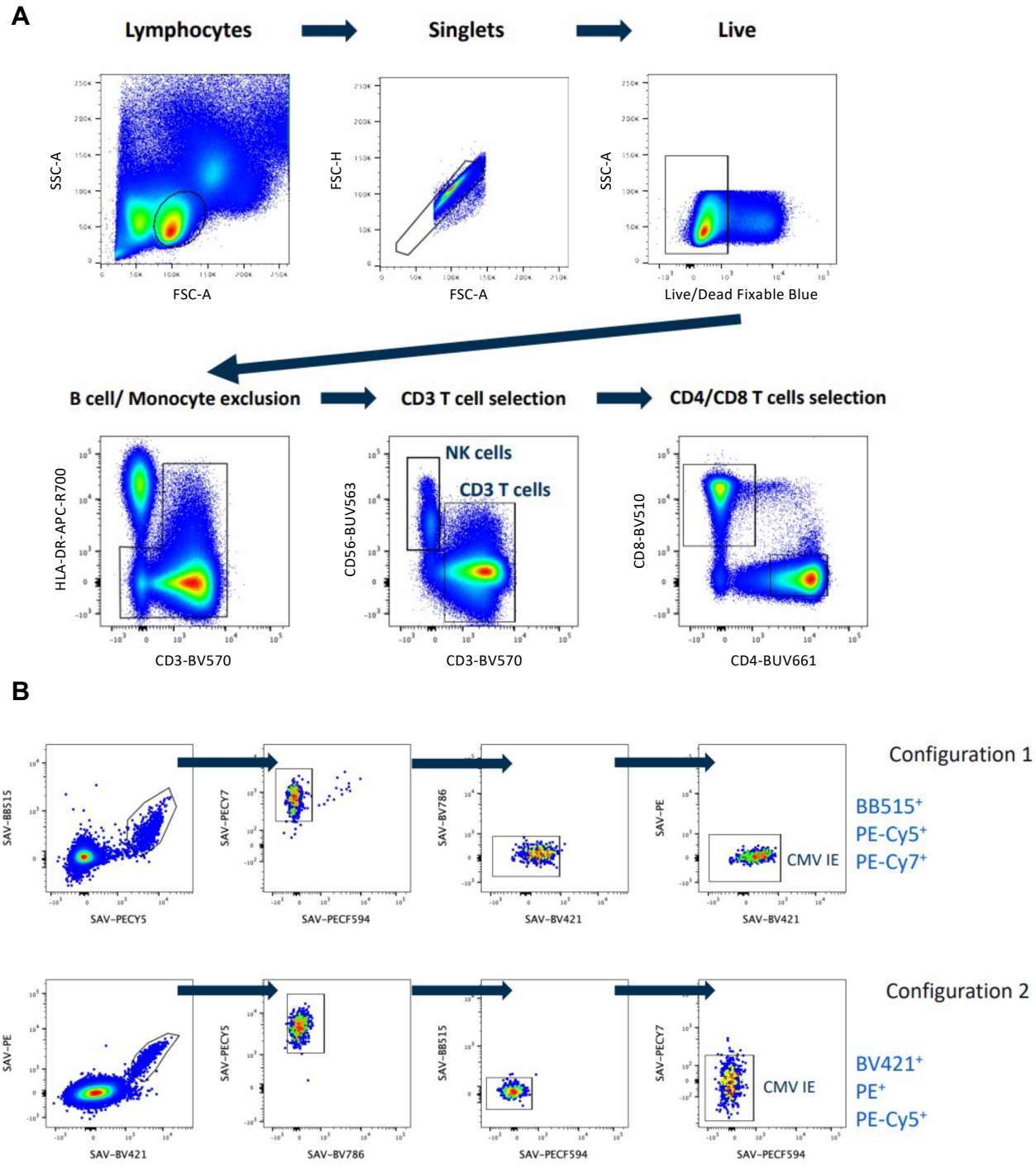
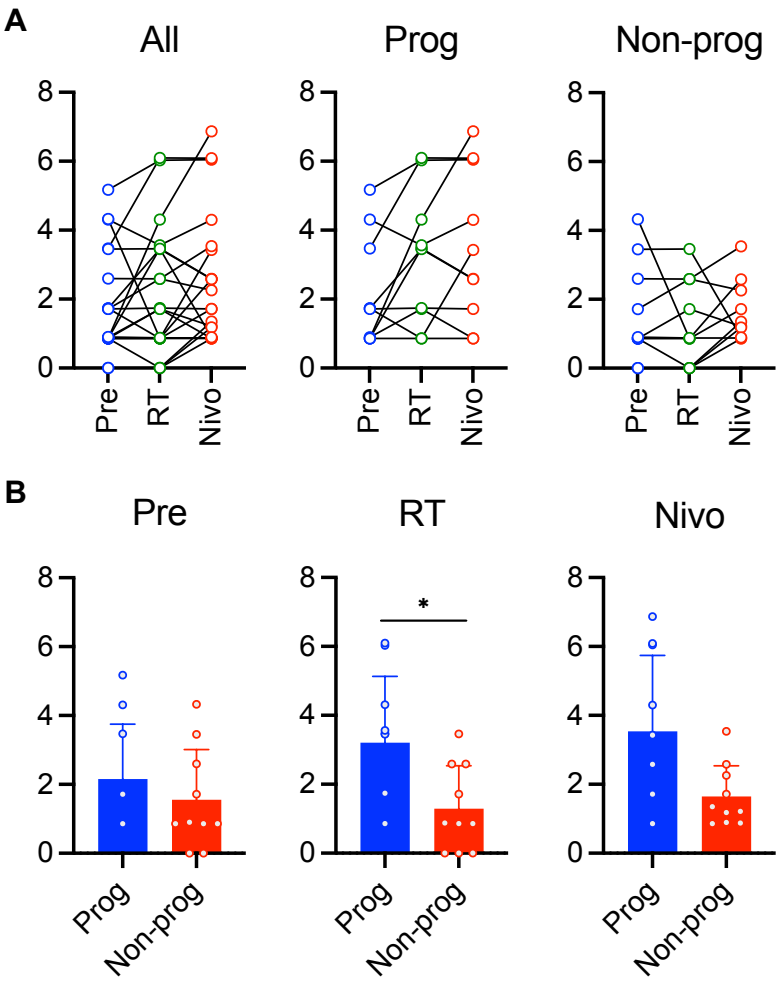
Supplementary Fig. S1



Supplementary Fig. S1. Gating strategy in flow cytometry. (A) A classical gating strategy. (B) Identification of antigen-specific CD8(+) T cells: Triple coding, 2 configuration staining. The detection criteria were descried in METHODS.

Supplementary Fig. S2



Supplementary Fig. S2. Tumor mutational burden (TMB) score during treatment. TMB analysis using circulating tumor DNA derived from peripheral blood plasma. Summarized TMB score was analyzed during treatment (A) and between progressors (Prog) and non-progressors (Non-prog) (B). *p<0.05. Error bars represent ± SD. All, all patients; Nivo, after nivolumab administrations; Pre, before treatment; RT, after radiotherapy.

Supplementary Table S1. Antibodies used in the highly multiplexed flow cytometric analysis for immune cell subset discrimination and phenotypic profiling.

Antibody target	Fluorochrome	Antibody clone	Vendor
TIGIT	BUV395	741182	BD Biosciences
CD25	BUV615	2A3	BD Biosciences
KLRG1	PerCP-eFluor™ 710	13F12F2	eBioscience™
PD-1	BV650	EH12	BD Biosciences
TIM-3	BV711	7D3	BD Biosciences
CD160	Alexa Fluor® 647	BY55	BD Biosciences
CD56	BUV563	NCAM16.2	BD Biosciences
CD4	BUV661	SK3	BD Biosciences
CD45RO	BUV737	UCHL1	BD Biosciences
CD27	BUV805	L128	BD Biosciences
CXCR5	BV480	RF8B2	BD Biosciences
CD8	BV510	RPA-T8	BioLegend
CD3	BV570	UCHT1	BioLegend
CD127	BV605	HIL-7R-M21	BD Biosciences
HLA-DR	APC-R700	G46-6	BD Biosciences
CD39	APC/Fire 750	A1	BioLegend

Supplementary Table S2. A list of 56 epitopes restricted to HLA-A02:01 or HLA-A24:02, which included 46 TAAs and 10 virus antigens.

HLA-A*02:01				
No	**HLA allele**	**Peptide sequence**	**Antigen**	**Ref**
TAA				
1	HLA-A*02:01	PLFDFSWLSL	Bcl-2 208-217	#1
2	HLA-A*02:01	WLSLKTLLSL	Bcl-2 214-223	#1
3	HLA-A*02:01	CLPSPSTPV	BMI1 271-279	#2
4	HLA-A*02:01	TLQDIVYKL	BMI1 74-82	#2
5	HLA-A*02:01	YLSGANLNL	Carcinogenic Embryonic Antigen (CEA) 571-579	#3
6	HLA-A*02:01	YLNTVQPTCV	EGF-R 1138-1147	#4
7	HLA-A*02:01	TLADFDPRV	EphA2	#5
8	HLA-A*02:01	LIAHNQVRQV	HER-2/neu (85–94)	#6
9	HLA-A*02:01	KIFGSLAFL	HER-2/neu 369-377	#7
10	HLA-A*02:01	KLMSSNSTDL	HSP105 234-243	#8
11	HLA-A*02:01	GLYDGMEHL	MAGEA-10 254-262	#9
12	HLA-A*02:01	FLWGPRALV	MAGEA3 271-279	#10
13	HLA-A*02:01	GVYDGREHTV	MAGE-A4 230–239	#11
14	HLA-A*02:01	LLFGLALIEV	MAGE-C2 191-200	#12
15	HLA-A*02:01	VLPLTVAEV	Mesothelin 530–538	#13
16	HLA-A*02:01	SLLFLLFSL	MSLN mesothelin	#13
17	HLA-A*02:01	YLFFYRKSV	hTERT 572-580	#14
18	HLA-A*02:01	LLLLTVLTV	MUC-1 12-20	#15
19	HLA-A*02:01	SLLMWITQV	NY-ESO-1 157-165	#16
20	HLA-A*02:01	SLPPPGTRV	p53 149-157	#17
21	HLA-A*02:01	VLDGLDVLL	PRAME 100-108	#18
22	HLA-A*02:01	LMLGEFLKL	Survivin 96-104	#19
23	HLA-A*02:01	ILAKFLHWL	Telomerase 540-548	#20
24	HLA-A*02:01	RMFPNAPYL	WT-1 126-134 (Wilms tumor)	#21
Virus				
1	HLA-A*02:01	NLVPMVATV	pp65/CMV	#22
2	HLA-A*02:01	VLEETSVML	IE-1/CMV	#22
3	HLA-A*02:01	CLGGLLTMV	LMP-2A/EBV	#23
4	HLA-A*02:01	GILGFVFTL	MP/Influenza	#24

HLA-A*24:02				
No	**HLA allele**	**Peptide sequence**	**Antigen**	**Ref**
TAA				
1	HLA-A*24:02	DYLQYVLQI	BCL-2A1	#25
2	HLA-A*24:02	EYRALQLHL	Carbonic anhydrase CA9 219-227	#26
3	HLA-A*24:02	TYACFVSNL	Carcinogenic Embryonic Antigen (CEA) 652-660	#27
4	HLA-A*24:02	IYTWIEDHF	FOXM1 262-270	#28
5	HLA-A*24:02	EYILSLEEL	Glycipan 3	#29
6	HLA-A*24:02	TYLPTNASL	HER-2/neu 63-71	#30
7	HLA-A*24:02	KYYLRVRPLL	KIF20A	#28, 31
8	HLA-A*24:02	VYLRVRPLL	KIF20A 67-75	#28, 31
9	HLA-A*24:02	EYLQLVFGI	MAGEA2 156-164	#32
10	HLA-A*24:02	IMPKAGLLI	MAGE-A3	#33
11	HLA-A*24:02	TFPDLESEF	MAGEA3 97-105	#33
12	HLA-A*24:02	EYCPGGNLF	MELK 87-95 (93N)	#34
13	HLA-A*24:02	DYLNEWGSRF	p-Cadherin	#35
14	HLA-A*24:02	AYACNTSTL	Survivin 80-88	#36
15	HLA-A*24:02	VYGFVRACL	hTRT 461-469	#37
16	HLA-A*24:02	SYRNEIAYL	TTK protein kinase 551-559	#38
17	HLA-A*24:02	RYCNLEGPPI	ULRC10/LY6K-177	#28
18	HLA-A*24:02	SYGVLLWEI	VEGFR1-1084	#39
19	HLA-A*24:02	RFVPDGNRI	VEGFR2-169	#39
20	HLA-A*24:02	CYTWNQMNL	WT1	#40
21	HLA-A*24:02	EYYELFVNI	DEPDC1-294	#28
22	HLA-A*24:02	KTVNELQNL	IMP3-508	#38
Virus				
1	HLA-A*24:02	QYDPVAALF	pp65/CMV	#28
2	HLA-A*24:02	VYALPLKML	pp65/CMV	#41
3	HLA-A*24:02	AYAQKIFKI	IE-1/CMV	#42
4	HLA-A*24:02	TYGPVFMSL	LMP2/EBV	#43
5	HLA-A*24:02	DYCNVLNKEF	BRLF1/EBV	#44
6	HLA-A*24:02	TYQWIIRNW	PB2/Influenza	#45

HLA, human leukocyte antigen; Ref, reference; TAA, tumor-associated antigen.

Supplementary Table S3. Summary of treatments administered prior to the treatment protocol of CIRCUIT trial.

	1st treatment	2nd treatment	3rd treatment	4th treatment
P1	SOX	RAM + PTX		
P2	SOX	RAM + PTX		
P3	DS	SP	RAM + PTX	
P4	DS	XP + HER	RAM + nab-PTX	
P5	SP	RAM + nab-PTX		
P6	SP	SOX	RAM + PTX	
P7	SP	XP + HER	RAM + PTX	DS
P8	SOX	RAM + nab-PTX		
P9	SP	RAM + PTX		
P10	XP + HER	XELOX + HER	RAM + PTX	RAM + nab-PTX
P11	XP + HER	RAM + nab-PTX		
P12	DCS	RAM + nab-PTX		
P13	DCS	biweekly CPT-11	weekly PTX	
P14	SOX	PTX + RAM		
P15	XP + HER	RAM + nab-PTX		
P16	SOX	nab-PTX	RAM + nab-PTX	
P17	SOX	RAM + PTX		
P18	XP + HER	SP + HER	RAM + PTX	
P19	SP	RAM + nab-PTX		
P20	SP	RAM + PTX	LAK	

Cases with italicized case number were survivors alive as of the data of confirmation of survival.
CDDP, cisplatin; CPT-11, irinotecan; DTX, docetaxel; HER, trastuzumab; LAK, lymphokine activated killer; nab-PTX, nanoparticle albumin-bound paclitaxel; PTX, paclitaxel; RAM, ramucirumab; DCS, docetaxel + cisplatin + tegafur/gimeracil/oteracil; DS, docetaxel + tegafur/gimeracil/oteracil; SOX, tegafur/gimeracil/oteracil + oxaliplatin; SP, tegafur/gimeracil/oteracil + cisplatin; XELOX, capecitabine + oxaliplatin; XP, capecitabine + cisplatin.

Supplementary Table S4. The basic stat of the TCR repertoire analysis.

No.	Time-points	Number of Tcells (/μl)	Read count*	Clones detected**
P1	Pre	1,254	176,416	19,036
	RT	753	79,805	9,340
	Nivo	1,341	145,840	11,736
P2	Pre	1,537	95,004	5,695
	RT	590	110,225	7,616
	Nivo	486	151,817	10,846
P3	Pre	1,662	82,744	14,113
	RT	1,261	155,266	14,712
	Nivo	1,135	135,140	14,865
P4	Pre	1,736	128,091	29,428
	RT	1,414	163,078	25,169
	Nivo	740	148,483	20,388
P5	Pre	1,527	162,449	18,826
	RT	951	172,581	14,099
	Nivo	933	199,660	17,414
P6	Pre	1,655	136,482	13,819
	RT	1,311	198,776	13,315
	Nivo	1,551	90,151	11,556
P7	Pre	1,537	190,299	22,150
	RT	2,559	177,283	15,672
	Nivo	1,645	213,904	24,269
P8	Pre	802	199,918	16,872
	RT	866	153,061	11,582
	Nivo	715	160,164	12,388
P9	Pre	1,820	145,748	11,723
	RT	1,530	187,995	13,579
	Nivo	1,701	124,525	8,903
P10	Pre	1,142	170,026	12,313
	RT	688	73,234	5,078
	Nivo	1,018	148,645	9,878
P11	Pre	1,798	154,719	19,370
	RT	869	88,178	10,969
	Nivo	1,106	109,059	13,926
P12	Pre	938	190,391	17,392
	RT	682	154,430	12,064
	Nivo	555	220,345	15,937
P13	Pre	2,901	141,057	27,821
	RT	1,728	115,057	10,061
	Nivo	1,631	88,628	8,327
P14	Pre	1,889	121,126	12,969
	RT	963	139,119	11,230
	Nivo	1,003	129,620	15,382
P15	Pre	1,686	67,061	17,153
	RT	1,728	133,858	18,429
	Nivo	1,125	146,390	19,982
P16	Pre	2,080	193,173	20,107
	RT	1,441	167,697	18,615
	Nivo	1,562	174,364	19,708
P17	Pre	1,279	82,554	9,868
	RT	1,138	164,487	15,120
	Nivo	1,192	125,863	14,995
P18	Pre	1,114	120,606	20,709
	RT	774	148,523	18,021
	Nivo	892	80,862	18,129
P19	Pre	1,027	164,485	21,588
	RT	726	231,327	23,541
	Nivo	1,095	181,699	22,006
P20	Pre	1,261	170,012	18,767
	RT	842	99,198	8,051
	Nivo	844	135,481	10,619

Cases with italicized case number were survivors alive as of the data of confirmation of survival. *Read count (assigned reads): Number of reads in which TCR genes were aligned with reference sequences. **Clones detected (assigned reads): Number of reads with unique combination. TCR, T cell receptor.

Supplementary Table S5. Concentration of ctDNA.

No.	Time-points	ctDNA concentration (ng/μl)
P1	Pre	24.2
	RT	58.8
	Nivo	51.2
P2	Pre	1.38
	RT	0.688
	Nivo	1.31
P3	Pre	1.84
	RT	1.82
	Nivo	5.24
P4	Pre	2.62
	RT	1.74
	Nivo	3.26
P5	Pre	2.2
	RT	2.5
	Nivo	4.18
P6	Pre	2.84
	RT	2.84
	Nivo	6.8
P7	Pre	10.2
	RT	13.3
	Nivo	25.2
P8	Pre	0.968
	RT	0.954
	Nivo	1.16
P9	Pre	0.982
	RT	1.52
	Nivo	1.04
P10	Pre	8.36
	RT	8.14
	Nivo	10.6
P11	Pre	1.47
	RT	1.33
	Nivo	1.68
P12	Pre	2.2
	RT	1.41
	Nivo	1.22
P13	Pre	1.62
	RT	2.2
	Nivo	6.08
P14	Pre	2.36
	RT	1.19
	Nivo	1.3
P15	Pre	1.99
	RT	1
	Nivo	0.21
P16	Pre	1.17
	RT	2.42
	Nivo	1.45
P17	Pre	2.82
	RT	2.06
	Nivo	2.06
P18	Pre	0.8
	RT	1.07
	Nivo	1.07
P19	Pre	0.916
	RT	0.704
	Nivo	0.566
P20	Pre	1.69
	RT	1.73
	Nivo	2.18

Cases with italicized case number were survivors alive as of the data of confirmation of survival. ctDNA, circulating tumor DNA.

Supplementary Table S6. Frequency and cell count of TAA- and virus-specific CD8(+) T cells.

HLA allele			HLA-A24:02					HLA-A02:01				
Antigen			TAA [% (cell count)]			Virus [% (cell count)]		TAA [% (cell count)]		Virus [% (cell count)]		
			MELK 87-95	p-Cadherin	DEPDC1-294	pp65/CMV	IE-1/CMV	hTERT 572-580	PRAME 100-108	pp65/CMV	IE-1/CMV	MP/Influenza
Peptide sequence			EYCPGGNLF	DYLNEWGSRF	EYYELFVNI	QYDPVAALF	AYAQKIFKI	YLFFYRKSV	VLDGLDVLL	NLVPMVATV	VLEETSVML	GILGFVFTL
Prog	P2	Pre								3.74 (244)		
	(73)	RT							0.0035 (5)	6.44 (9125)	0.0057 (8)	
	(PD)	Nivo								10.8 (13094)		
	P3	Pre				0.031 (43)						
	(83)	RT				0.039 (27)						
	(PD)	Nivo				0.056 (54)						
	P4	Pre				0.0021 (4)	0.0054 (10)					
	(95)	RT					0.011 (10)					
	(PD)	Nivo										
	P6	Pre					0.0036 (19)					
	(158)	RT	0.0032 (13)				0.0056 (23)					
	(PD)	Nivo	0.0068 (28)				0.0046 (19)					
	P7	Pre				0.28 (450)						
	(167)	RT				0.42 (447)						
	(PD)	Nivo				0.61 (955)						
	P8	Pre		0.0033 (4)								
	(174)	RT										
	(PD)	Nivo		0.0051 (10)								
	P9	Pre				0.0077 (23)	0.74 (2277)					
	(202)	RT				0.085 (16)	0.28 (52)					
	(PD)	Nivo				0.064 (200)	0.68 (2119)					
	P10	Pre								1.47 (6476)		0.0043 (19)
	(290)	RT								1.29 (2232)		0.0045 (8)
	(PD)	Nivo								1.24 (1955)		
Non-prog	P11	Pre										
	(303)	RT										
	(SD)	Nivo				0.0035 (12)						
	P12	Pre					0.001 (4)					
	(330)	RT										
	(SD)	Nivo										
	P13	Pre					0.0018 (12)	0.002 (13)		0.46 (3025)		0.0027 (18)
	(342)	RT				0.0012 (6)	0.0017 (8)	0.00085 (4)		0.25 (1189)		0.0023 (11)
	(SD)	Nivo					0.0034 (13)	0.0038 (15)		0.30 (1133)		0.0016 (6)
	P14	Pre	0.0049 (14)									
	(435)	RT	0.019 (10)									
	(PR)	Nivo	0.009 (17)									
	P15	Pre				0.057 (73)	2.7 (3489)					
	(651)	RT				0.089 (83)	2.5 (2356)					
	(CR)	Nivo				0.051 (31)	1.69 (1050)					
	P17	Pre								0.073 (156)		
	(1111)	RT								0.15 (198)		
	(SD)	Nivo								0.037 (68)		
	P18	Pre					0.004 (7)					
	(1118)	RT			0.0078 (7)							
	(CR)	Nivo										
	P19	Pre					0.05 (106)					
	(1160)	RT	0.014 (13)	0.024 (22)			0.12 (111)					
	(CR)	Nivo	0.015 (7)				0.23 (105)					
	P20	Pre	0.0058 (9)				0.011 (17)					
	(1489)	RT										
	(PR)	Nivo					0.0059 (7)					

Cases with italicized case number were survivors alive as of the data of confirmation of survival. Overall survival for each patient is shown below the patient's identification number and best overall response for each patient is also shown below overall survival. HLA, human leukocyte antigen; Prog, progressors; Non-prog, non-progressors; TAA, tumor-associated antigen.

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