

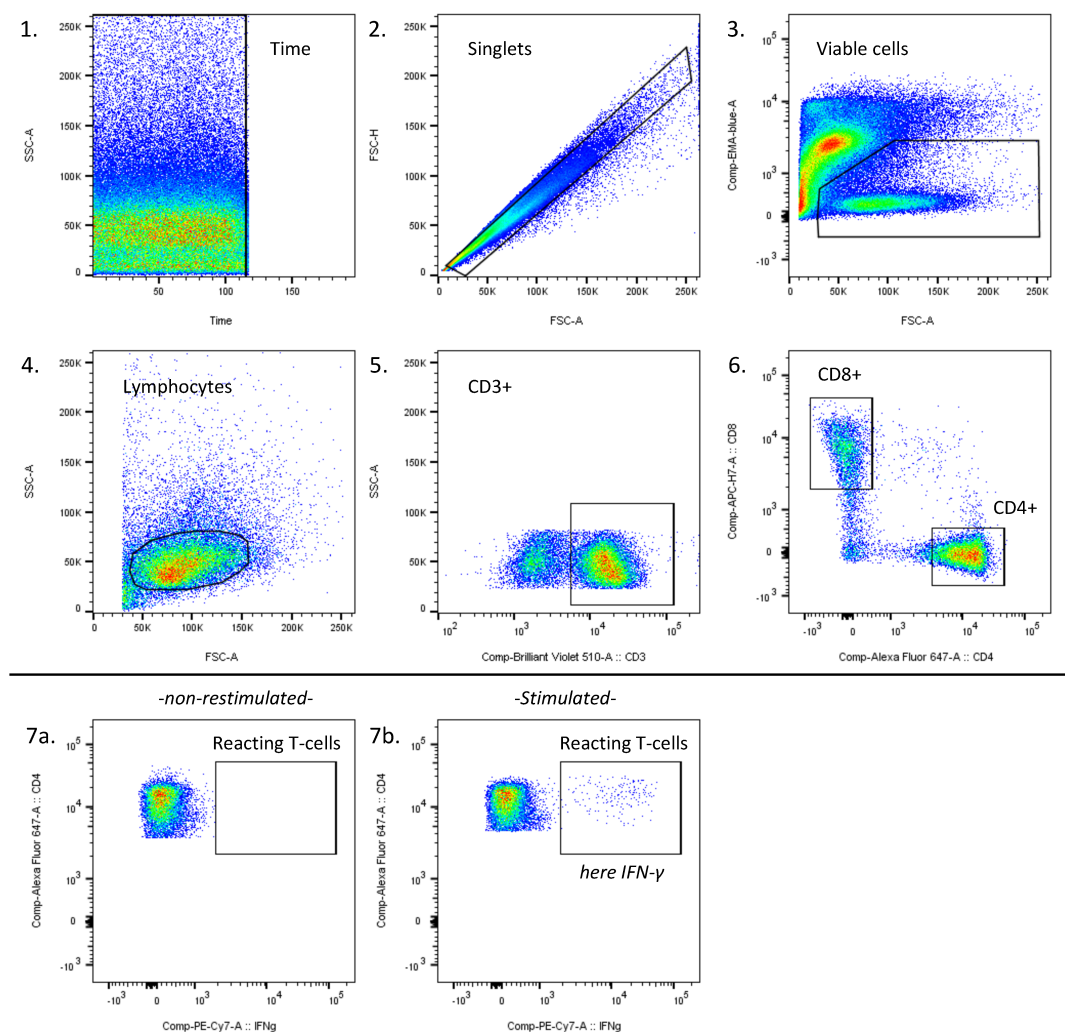
Early disappearance of tumor-reactive T cells from peripheral blood correlates with superior clinical outcomes in melanoma under anti-PD-1 therapy

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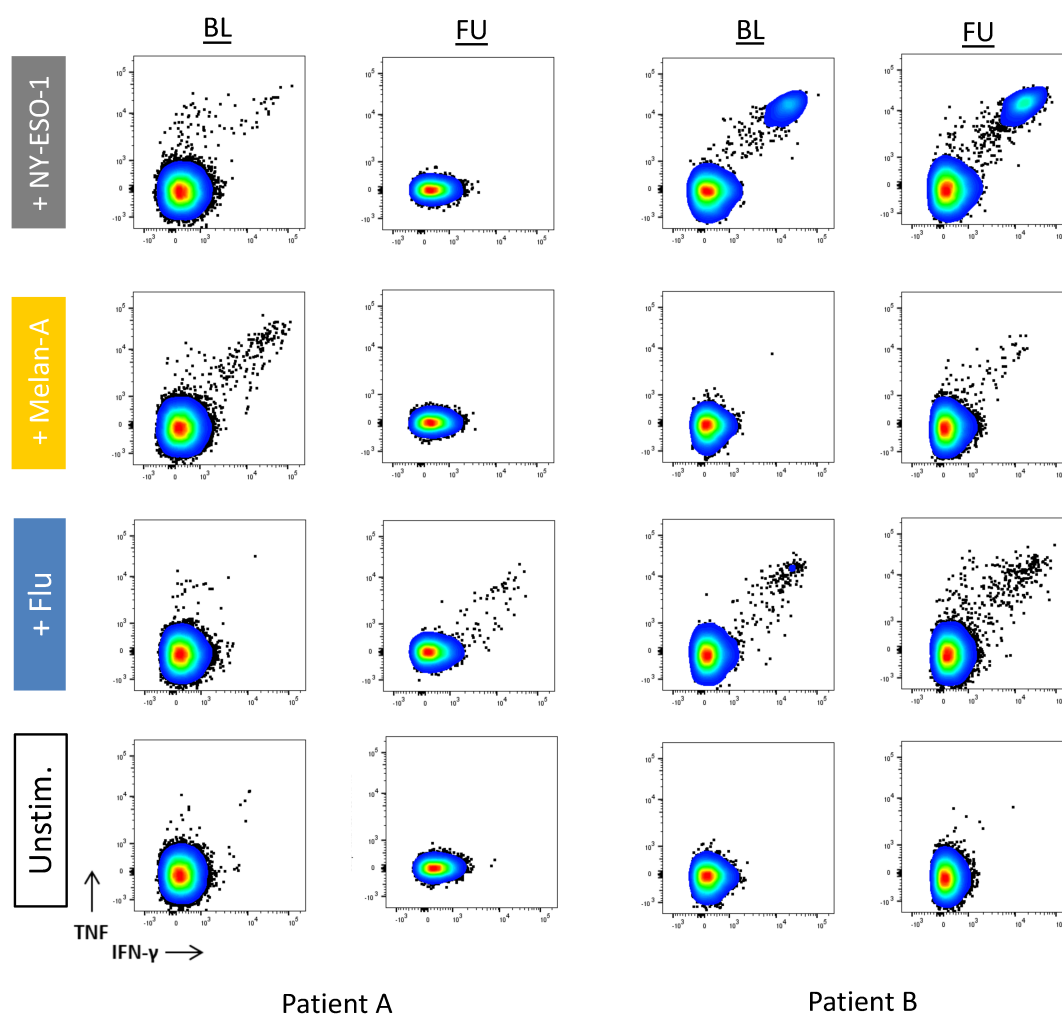
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Supplemental material

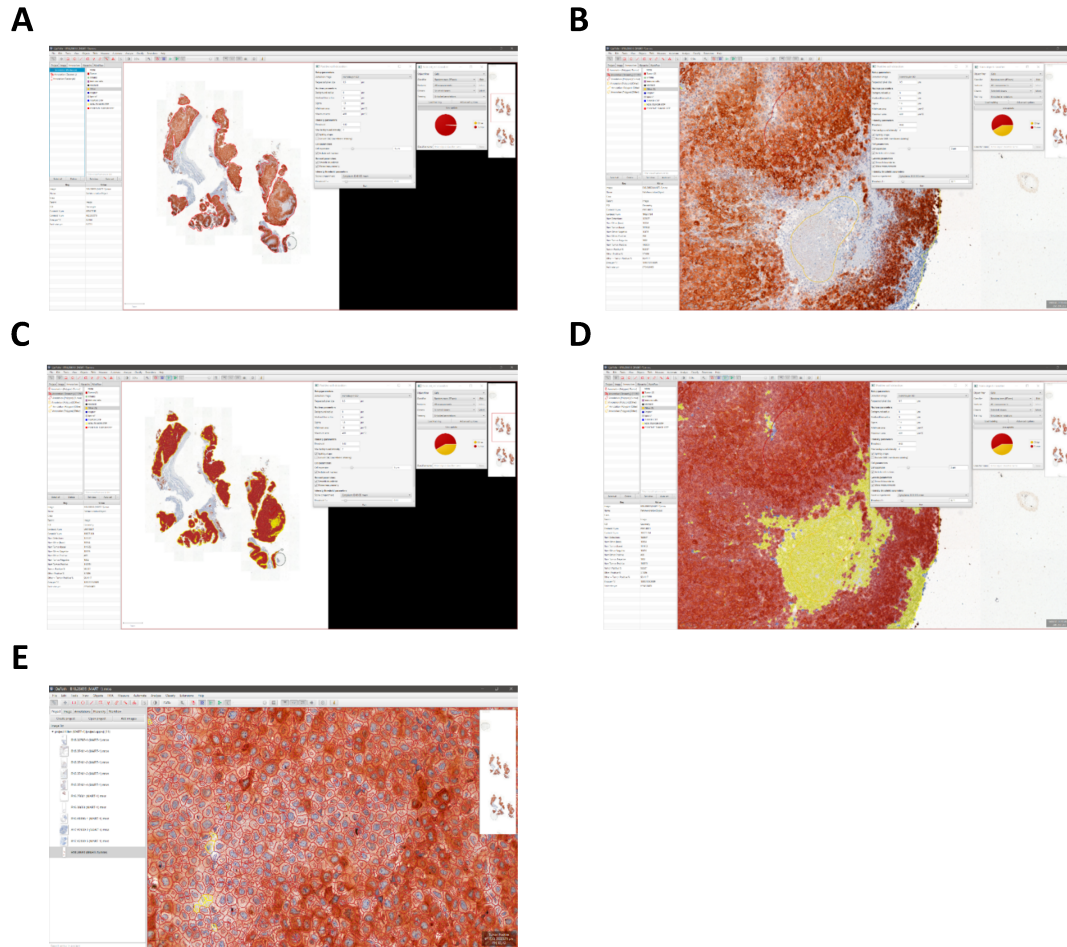


Supplemental Figure 1: Gating strategy for flow cytometry data analysis. First a time gate was used to select the measured cells and to monitor for eventually occurring pressure variability of the system. Next, single cells were identified by plotting FSC-H against FSC-A. Dead cells (high proportions expected after 13 day culture of PBMCs while only stimulating antigen specific T cell clones) and debris was excluded using the DNA intercalator ethidium bromide monoazide (EMA). A morphological gate identified the viable lymphocyte population. T cells were identified as expressing CD3 on their surface and were further subdivided into CD4+ and CD8+ cells. Both were then separately examined for the expression of functional markers (here as example the expression of IFN- γ) using a non-restimulated control.

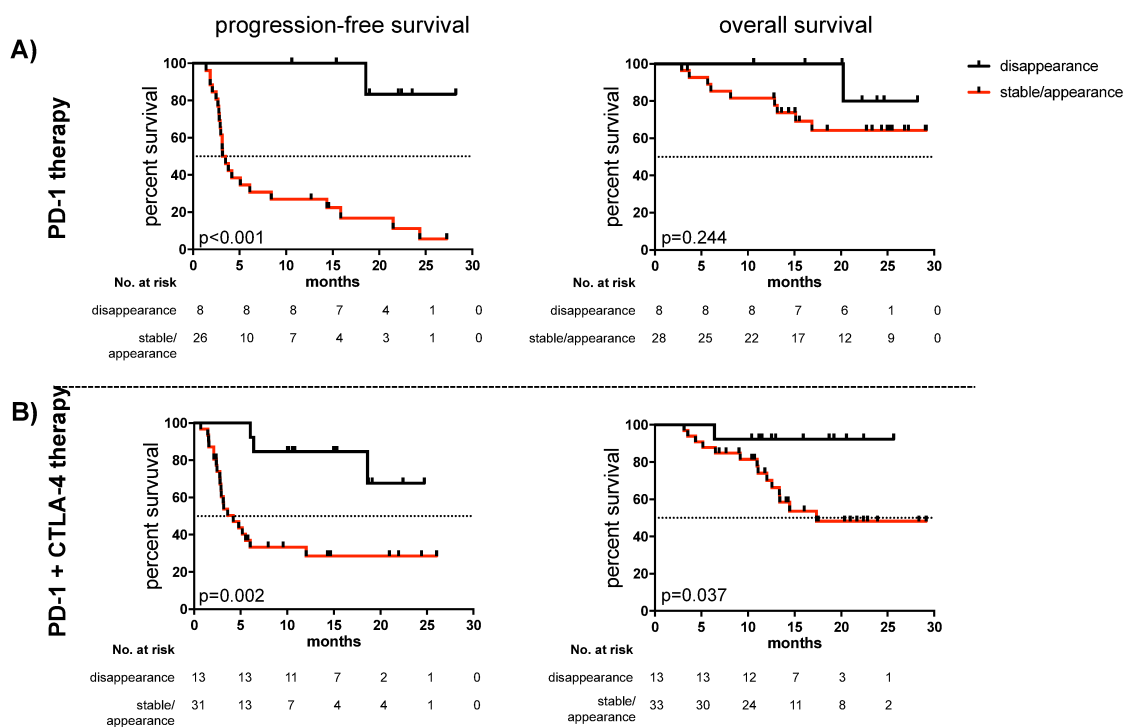


Supplemental Figure 2: Definition of T cell responses: IFN- γ (x-axis) and TNF (y-axis) production by CD4+ T cells activated with either NY-ESO-1-derived, Melan-A-derived, Influenza-derived peptides or left unstimulated (Unstim.). Examples of 2 patients (A & B) are shown before start of ICB (BL) and under therapy (FU).

In patient A, both NY-ESO-1- and Melan A-reactive T cells disappeared from the blood, whereas in patient B, Melan A-reactive T cells appeared during therapy, and NY-ESO-1-reactive T-cells remained present. For both patients, Flu-reactive T-cells were not affected.



Supplemental Figure 3: Morphometric analysis of Melan A expression using QuPath software. In a first step the region of interest is selected for morphometric analysis (red contour) (A), followed by the classification of the detected cells in “tumor cells” (red contour), and “non-tumor cells” (yellow contour) (B). The result is a visual representation of Melan A-positive tumor cells (red mask), Melan A-negative tumor cells (blue mask), and non-tumor cells (yellow mask) following image processing using the cell detection and classification parameters previously defined by the user (C, D, E).



Supplemental Figure 4: Correlation of dynamics of NY-ESO-1- / Melan-A-reactive T cells from the circulation with clinical outcome of patients under PD-1 blockade alone A) or in combination with CTLA-4 inhibition B) in a combined analysis of cohort A and B. Probability of overall survival (right panel) and progression-free survival (left panel) among patients with disappearing (black arm) or appearing/stable (red arm) TAA-specific T cells. Progression-free survival could not be evaluated for 4 patients.

Supplemental Table 1: Prior therapies in the combined cohorts

factor	patients			1-year survival rate (%)	overall survival		
	n	%	% dead		HZ	95% CI	P (log-rank)
prior therapy							
yes	45	40.5	40.0	73.3	1.07	0.57 – 1.98	0.838
no	66	59.5	34.8	76.5	1		
checkpoint therapy							
yes	18	16.2	50.0	66.7	1.23	0.85 – 1.78	0.276
no	93	83.8	34.4	76.9	1		
radiation							
yes	14	12.6	35.7	71.4	0.94	0.37 – 2.41	0.902
no	97	87.4	37.1	75.5	1		
chemotherapy							
yes	8	7.2	50.0	62.5	1.15	0.69 – 1.93	0.590
no	103	92.8	35.9	76.1	1		
MAP-kinase inhibitors							
yes	13	11.7	53.8	61.5	1.39	0.92 – 2.09	0.107
no	98	88.3	34.3	76.7	1		

Supplemental Table 2: Subsequent therapies of patients with discontinuation of treatment

factor	patients			1-year survival rate (%)	overall survival		
	n	%	% dead		HZ	95% CI	P (log-rank)
subsequent therapy							
yes	57	51.4	43.9	78.4	1.39	0.74 – 2.60	0.306
no	54	48.6	29.6	71.3	1		
checkpoint therapy							
yes	16	28.1	43.7	80.8	1.13	0.47 – 2.73	0.788
no	41	71.9	43.9	77.4	1		
radiation							
yes	27	47.4	55.6	81.3	1.94	0.84 – 4.43	0.111
no	30	52.6	33.3	75.5	1		
chemotherapy							
yes	13	22.8	69.2	61.5	2.47	1.08 – 5.66	0.027
no	44	77.2	36.4	83.8	1		
MAP-kinase inhibitors							
yes	17	29.8	35.3	81.9	0.59	0.23 – 1.50	0.263
no	40	70.2	47.5	76.9	1		

Supplemental Table 3: Univariate analyses of NY-ESO-1-, Melan-A- and Flu-reactive T cell populations before start of ICB.

	feature	patients			1-year survival rate		2-year survival rate		overall survival			progression free survival					
		n	%	% dead	%	95% CI	%	95% CI	HR	95% CI	p (log-rank)	median PFS [months]	median PFS 95% CI	HR PFS	HR PFS 95% CI	p (log-rank)	
	NY-ESO-1-reactive T cells																
cohort A	present	61	84.7	37.7	71.1	65.1 – 77.1	54.7	47.0 – 62.4	0.83	0.31 – 2.18	0.702	5	0.7 – 9.3	0.63	0.29 – 1.37	0.238	
	absent	11	15.3	45.4	54.5	39.5 – 69.5	54.5	39.5 – 69.5	1			3	1.5 – 4.5	1			
cohort B	present	37	94.9	32.4	86.3	80.6 – 92.0	69.4	61.2 – 77.6	1.12	0.14 – 8.82	0.912	6	2.2 – 9.7	2.06	0.28 – 15.31	0.470	
	absent	2	5.1	50.0	100	-	50.0	14.6 – 85.4	1			2	n.a.	1			
	Melan-A-reactive T cells																
cohort A	present	42	60.0	35.7	70.8	63.7 – 77.9	57.4	48.2 – 66.6	1.16	0.54 – 2.47	0.708	5	0.0 – 11.2	0.75	0.42 – 1.34	0.326	
	absent	28	40.0	42.9	65.9	56.6 – 75.2	50.7	40.1 – 61.3	1			3	1.1 – 4.9	1			
cohort B	present	31	79.5	32.3	86.8	80.7 – 92.9	70.8	61.9 – 79.7	0.99	0.27 – 3.63	0.985	8	4.3 – 11.7	0.91	0.34 – 2.44	0.851	
	absent	8	20.5	37.5	87.5	75.8 – 99.2	58.3	39.7 – 76.9	1			3	0.2 – 5.8	1			
	Flu-reactive T cells																
cohort A	present	70	97.2	38.6	67.5	61.8 – 73.2	55.9	49.0 – 62.8	0.78	0.10 – 5.74	0.804	4	2.1 – 5.8	1.52	0.21 – 11.09	0.673	
	absent	2	2.8	50.0	100	n.a.	0	0	1			3	n.a.	1			
cohort B	present	38	97.4	31.6	86.7	81.1 – 92.3	70.3	62.3 – 78.3	0.36	0.05 – 2.83	0.311	6	2.2 – 9.8	0.82	0.11 – 6.13	0.846	
	absent	1	2.6	100	100	n.a.	0	0	1			2	n.a.	1			

Supplemental Table 4: Modelling peripheral TAA-reactive T cell signatures under ICB.

	factor	patients			1-year survival rate		2-year survival rate		overall survival			progression free survival					
		n	%	% dead	%	95% CI	%	95% CI	HR	95% CI	P (log-rank)	Median PFS [months]	Median PFS 95% CI	HR PFS	HR PFS 95% CI	P (log rank)	
	TAA-reactive T cells																
cohort A	disappearance	15	30.0	13.3	93.3	86.9 – 99.7	77.8	62.6 – 93.0	0.25	0.09 - 0.67	0.045	not reached	n.a.	0.13	0.06– 0.27	<0.001	
	appearance/stable	35	70.0	42.9	66.5	58.2 – 74.8	50.6	41.1 – 60.1	1			3	2.1 – 3.9	1			
cohort B	disappearance	6	18.8	0.0	100	n.a.	100	n.a.	0.03	0.00 – 63.69	0.160	not reached	n.a.	0.14	0.05 – 0.37	0.020	
	appearance/stable	26	81.3	30.8	88.1	81.6 – 94.6	64.1	53.7 – 74.5	1			5	1.2 – 8.8	1			
	Flu-reactive T cells																
cohort A	disappearance	7	14.0	57.1	57.1	38.4 – 75.8	28.6	6.3 – 50.9	2.18	0.51 – 9.24	0.162	4	0.0 – 7.8	1.43	0.48 – 4.27	0.457	
	appearance/stable	43	86.0	30.2	77.4	70.7 – 84.1	64.1	55.8 – 72.4	1			6	0.0 – 18.0	1			
cohort B	disappearance	8	25.0	25.0	85.7	72.5 – 98.9	64.3	43.3 – 85.3	1.14	0.22 – 5.98	0.872	3	n.a.	0.92	0.31 – 2.74	0.887	
	appearance/stable	24	75.0	25.0	91.7	86.1 – 97.3	71.3	61.2 – 81.4	1			8	0.0 – 19.0	1			

Supplemental Table 5: Univariate analyses of demographic data before start of ICB.

	feature	patients			1-year survival rate		2-year survival rate		overall survival			progression free survival					
		n	%	% dead	%	95% CI	%	95% CI	HR	95% CI	p (log-rank test)	median PFS [months]	median PFS 95% CI	HR PFS	HR PFS 95% CI	p (log-rank test)	
	age [years]																
cohort A	≥68	38	52.8	36.8	70.3	62.7 – 77.8	56.7	47.2 – 66.2	0.86	0.41 – 1.80	0.684	5	0.0 – 10.3	0.61	0.35 – 1.07	0.084	
	<68	34	47.2	41.2	66.1	57.8 – 74.4	52.6	42.6 – 62.6	1			4	1.8 – 6.2	1			
cohort B	≥68	14	35.9	50.0	71.4	59.3 – 83.5	42.9	28.0 – 57.8	2.69	0.90 – 8.05	0.066	3	n.a.	1.04	0.44 – 2.44	0.956	
	<68	25	64.1	24.0	95.7	91.4 – 100	81.1	72.5 – 89.7	1			8	4.6 – 11.4	1			
	sex																
cohort A	female	28	38.9	38.6	71.4	62.9 – 79.9	54.8	43.7 – 65.9	0.97	0.45 – 2.07	0.936	4	0.0 – 8.3	0.98	0.55 – 1.73	0.942	
	male	44	61.1	37.8	65.9	58.4 – 73.4	54.3	45.5 – 63.1	1			4	2.3 – 5.7	1			
cohort B	female	13	33.3	30.8	83.9	73.5 – 94.3	62.9	47.9 – 77.9	0.92	0.28 – 3.00	0.891	2	0.9 – 3.1	1.46	0.62 – 3.45	0.379	
	male	26	66.7	34.6	88.5	82.2 – 94.8	70.4	60.8 – 80.0	1			8	3.3 – 12.7	1			
	M category																
cohort A	M1a or b	32	44.4	40.6	71.3	63.2 – 79.4	54.9	45.0 – 64.8	0.94	0.44 – 1.97	0.861	3	0.8 – 5.2	1.24	0.71 – 2.18	0.448	
	M1c	40	55.6	37.5	66.2	58.5 – 73.9	55.4	45.8 – 65.0	1			7	1.1 – 12.9	1			
cohort B	M1a or b	5	12.8	40.0	80.0	62.1 – 97.9	40.0	10.3 – 69.7	1.29	0.28 – 5.84	0.743	undefined	n.a.	0.44	0.10 – 1.86	0.247	
	M1c	34	87.2	32.3	88.0	82.4 – 93.6	70.3	61.9 – 78.7	1			5	1.4 – 8.6	1			
	LDH																
cohort A	ULN	32	44.4	37.5	75.0	67.3 – 82.7	54.9	44.3 – 65.5	0.95	0.45 – 2.01	0.891	3	1.3 – 4.7	1.18	0.67 – 2.09	0.550	
	LLN	40	55.6	40.0	63.3	55.4 – 71.2	54.8	45.8 – 63.8	1			6	2.3 – 9.7	1			
cohort B	ULN	15	39.5	33.3	86.7	77.9 – 95.5	70.9	58.5 – 83.3	0.86	0.27 – 2.75	0.797	4	0.2 – 7.8	1.21	0.53 – 2.76	0.648	
	LLN	23	59.5	30.4	86.7	79.5 – 93.8	64.6	53.6 – 75.6	1			8	0.0 – 19.8	1			

Abbreviations: ULN, upper limit of normal; LLN, lower limit of normal

Supplemental Table 6: Combined modelling of cohort A's and B's peripheral TAA-reactive T cell signatures under ICB.

factor	patients			1-year survival rate		2-year survival rate		overall survival			1-year PFS		progression free survival		
	n	%	% dead	%	95% CI	%	95% CI	HR	95% CI	p(log-rank)	%	95% CI	HR PFS	HR PFS 95% CI	p (log rank)
disappearance	21	25.6	9.5	95.2	90.6 – 99.8	83.3	71.4 – 95.2	0.21	0.05 – 0.91	0.021	90.5	84.1 – 96.9	0.13	0.05 – 0.36	<0.001
appearance/stable	61	74.4	37.7	75.9	70.3 – 81.5	56.5	49.4 – 63.6	1			27.9	21.8 – 34.0	1		

Supplemental Table 7: Combined modelling of alterations in peripheral TAA-reactive T cell signatures in cohort A and B under ICB.

factor	patients			1-year survival rate		2-year survival rate		1-year PFS	
	n	%	% dead	%	95% CI	%	95% CI	%	95% CI
both disappear	5	6.1	0	100	n.a.	100	n.a.	100	n.a.
one disappears	16	19.5	12.5	93.8	87.7 – 99.9	80.4	67.0 – 93.8	87.5	79.2 – 95.8
appearance/stable	61	74.4	37.7	75.9	70.3 – 81.5	56.5	49.4 – 63.6	27.9	21.8 – 34.0