

Supplementary Figure Legends

T cell receptor repertoire characteristics both before and following immunotherapy correlate with clinical response in mesothelioma

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Fig. S1: Gating strategy.

Gating strategy for CD3⁺, PD1⁺ and PD1⁻ CD4⁺ T cells, PD1⁺ and PD1⁻ CD8⁺ T cells and Tregs.

Fig. S2: Obtained reads per sample.

Number of obtained TCR β reads per sample. Bars depict mean values and the standard deviations.

Fig. S3: Characteristics of the CD3⁺ TCR β clones after DC-treatment.

(A) Impact that different clonal sizes have on the CD3⁺ repertoires. Data for all individuals are plotted with mean and standard deviation using black bars for the repertoire pre-DC-therapy and white bars for the repertoire post-DC-therapy. (B) Heat map of the medians of the V β usage in the CD3⁺ samples pre and post-DC-therapy in all individuals. (C) Representative V-J circos plot for a single individual pre and post-DC-therapy. (D, E) Median CDR3 length in amino acids (D) and median CDR3 charge (E) for the CD3⁺ repertoires pre and post-DC-therapy. Each dot represents one individual.

Fig. S4: Correlation of similarity index and expansion in CD3 repertoire with clinics.

(A, B) Number of reads per CD3⁺ clones post-DC-therapy (x-axis) versus the Progression Free Survival in months (y-axis in A) and the Overall Survival in months (y-axis in B). Linear regression curve fitting, p values and r² are shown for each graph.

Fig. S5: Characteristics of the differentially expressed TCR β clones after DC-treatment.

(A, B) Median CDR3 charge (A) and median CDR3 length in amino acids (B) for the expanding, decreasing, newly appearing, disappearing and stable PD1⁺ CD4⁺, PD1⁺ CD8⁺

Table S1: Shared New TCR β Clones

	AA sequence	Clone size (%)	Patient	T cell subset	TRBV	TRBJ
Shared AA sequence	CASSYRNNEQFFG	0,9	PT6	CD4	6.2	2.1
		0,4	PT8	CD4	6.2	2.1
		0,35	PT3	CD4	6.2	2.1

and Tregs. clones. Each dot represents one individual. (C) Heat map of the medians of the V β usage in the expanding, decreasing, newly appearing, disappearing and stable PD1⁺ CD4⁺, PD1⁺ CD8⁺ and Tregs in all individuals. Significance of t-test is shown; * p < 0.05, ** p < 0.01.

Fig. S6: Clonal numbers of the CD3⁺ repertoire

(A-E) Number of expanding (A) decreasing (B), stable (C), newly appearing (D), and disappearing clones (E) after treatment of PD1⁺ CD4⁺ T cells, PD1⁺ CD8⁺ T cells, and Tregs. Significance of t-test is shown; ** p < 0.01 and **** p < 0.0001.

Fig. S7: Correlation of number of expanding clones with clinics.

(A, B, C) Overall Survival in months (y-axis) versus the number of expanding CD3⁺ clones (x-axis in A), PD1⁺ CD4⁺ clones (x-axis in B) and the number of expanding Tregs clones (x-axis in C). Linear regression curve fitting, p values and r² are shown for each graph.

		0,35	PT5	CD4	6.2	2.1
		0,3	PT2	CD4	6.2	2.1
		0,3	PT4	CD4	6.2	2.1
		0,35	PT3	Tregs	6.2	2.1
		0,2	PT8	Tregs	6.2	2.1
		0,15	PT4	Tregs	6.2	2.1
Shared AA sequence	CASSYRNDEQFFG	0,2	PT4	CD4	6.2	2.1
		0,1	PT8	CD4	6.2	2.1
Shared AA sequence	CASSFGGAGDTQYFG	0,35	PT4	CD4	12.3	2.3
		0,1	PT6	CD4	12.3	2.3
Shared AA sequence	CASSFRGRNTEAFFG	0,1	PT5	CD4	27	1.1
		0,1	PT8	CD4	27	1.1
Shared AA sequence	CASSLGTVNQPQHFG	0,2	PT8	CD4	7.8	1.5
		0,1	PT7	CD4	5.6	1.5
Shared AA sequence	CASSRGASTDTQYFG	0,15	PT1	CD4	7.9	2.3
		0,15	PT2	CD4	27	2.3
Shared AA sequence	CASSYSDRGYGYTFG	0,15	PT4	CD4	6.6	1.2
		0,1	PT6	CD4	6.6	1.2
Shared AA sequence	CASSLRGQGAYEQYFG	0,1	PT2	CD4	27	2.7
		0,1	PT6	CD4	6.5	2.7
Shared AA sequence	CASSYSRTGGGYGYTFG	0,1	PT5	CD4	6.5	1.2
		0,1	PT6	CD4	6.5	1.2
Shared AA sequence	CASSLGAQETQYFG	0,1	PT1	Tregs	5.1	2.5
		0,1	PT4	Tregs	11.2	2.5

Shared AA Shared AA sequence	CASSLGQRTDTQYFG	0,2	PT6	Tregs	5.1	2.3
		0,1	PT1	Tregs	5.1	2.3
Shared AA Shared AA sequence	CASSLRGNEQYFG	0,35	PT7	CD8	5.6	2.7
		0,1	PT6	CD8	5.6	2.7
Shared AA Shared AA sequence	CAIAEGPYQPQHFG	20,5	PT4	CD8	27	1.5
		0,1	PT9	CD8	27	1.5

Table S2: Correlations between clinical outcome and demographic data

	PFS		OS	
	r ²	p-value	r ²	p-value
age	0.021	0.71	0.081	0.457
gender	0.170	0.27	0.080	0.462
disease stage	0.203	0.22	0.446	0.049