Supplementary materials: Clinical Activity of an hTERT-Specific Cancer Vaccine (Vx-001) in "Immune Desert" NSCLC

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Table S1. Correlation of TAIC with CD3- and CD8- TILs.

	Tumor-associated immune cells (TAIC)		
Covariant	Low	High	*p*-value[a]
CD3-TILs			
Low	30[b] (68.2%[c])	15 (24.6%)	
High	14 (31.8%)	46 (75.4%)	<0.001
CD8-TILs			
Low	29 (65.9%)	13 (22.8%)	
High	15 (34.1%)	44 (77.2%)	<0.001

[a] Fisher's Exact test. [b] Number of patients. [c] % percentage within TAIC.

Table S2. Correlation of PD-L1, TAIC, CD3- TILs, CD8-TILs and GZMB-TILs with patients' clinicopathological data.

	Gender			Age			Prior treatment response			Histology			Smoking		
Covariant	Male	Female	*p*[a]	<65y	≥65y	*p*[a]	OR[d]	SD[e]	*p*[a]	NSQ/MH[f]	SQ[g]	*p*[a]	No	Yes	*p*[a]
PD-L1															
Negative	75[b] (53.7%[c])	26 (19.8%)		48 (36.6%)	53 (40.5%)		46 (35.1%)	55 (42.0%)		59 (45.0%)	42 (32.1%)		9 (6.9%)	92 (70.2%)	
Positive	20 (15.3%)	10 (7.6%)	0.486	15 (11.5%)	15 (11.5%)	0.838	19 (14.5%)	11 (8.4%)	0.100	16 (12.2%)	14 (10.7%)	0.677	4 (3.1%)	26 (19.8%)	0.493
TAIC															
Low	40 (30.5%)	20 (15.3%)		32 (24.4%)	28 (21.4%)		31 (23.7%)	29 (22.1%)		35 (26.7%)	25 (19.1%)		6 (4.6%)	54 (41.2%)	
High	55 (42.0%)	16 (12.2%)	0.176	31 (23.7%)	40 (30.5%)	0.296	34 (26.0%)	37 (28.2%)	0.727	40 (30.5%)	31 (23.7%)	0.861	7 (5.3%)	64 (48.9%)	1.000
CD3-TILs															
Low	35 (33.3%)	10 (9.5%)		22 (21.0%)	23 (21.9%)		23 (21.9%)	22 (21.0%)		27 (25.7%)	18 (17.1%)		5 (4.8%)	40 (38.1%)	
High	43 (41.0%)	17 (16.2%)	0.508	30 (28.6%)	30 (28.6%)	1.000	32 (30.5%)	28 (26.7%)	0.846	32 (30.5%)	28 (26.7%)	0.554	6 (5.7%)	54 (51.4%)	1.000
CD8-TILs															
Low	32 (31.7%)	10 (9.9%)		21 (20.8%)	21 (20.8%)		22 (21.8%)	20 (19.8%)		23 (22.8%)	19 (18.8%)		5 (5.0%)	37 (36.6%)	
High	41 (40.6%)	18 (17.8%)	0.506	28 (27.7%)	31 (30.7%)	0.842	27 (26.7%)	32 (31.7%)	0.549	31 (30.7%)	28 (27.7%)	0.842	5 (5.0%)	54 (53.5%)	0.738
GZMB-TILs															
Low	51 (60.0%)	19 (22.4%)		30 (35.3%)	40 (47.1%)		36 (42.4%)	34 (40.0%)		39 (45.9%)	31 (36.5%)		6 (7.1%)	64 (75.3%)	
High	11 (12.9%)	4 (4.7%)	1.000	10 (11.8%)	5 (5.9%)	0.153	7 (8.2%)	8 (9.4%)	0.782	8 (9.4%)	7 (8.2%)	1.000	2 (2.4%)	13 (15.3%)	0.626

[a] Fisher's Exact test, [b] Number of patients, [c] % percentage of total patients, [d] Optimal response, [e] Stable disease, [f] Non-squamous/mixed histology, [g] Squamous.

Table S3. Distribution of gender, age, response to previous treatment, tumor histology and smoking behaviour in Vx-001 and placebo-treated arms.

	Vaccination		
Covariant	Placebo	Vx-001	*p*-value[a]
Gender			
Male	51[b] (38.9%[c])	44 (33.6%)	
Female	17 (13.0%)	19 (14.5%)	0.560
Age			
<65y	32 (24.4%)	31 (23.7%)	
≥65y	36 (27.5%)	32 (24.4%)	0.862
Response to previous treatment			
OR[d]	38 (29.0%)	27 (20.6%)	
SD[e]	30 (22.9%)	36 (27.5%)	0.163
Histology			
NSQ/MH[f]	41 (31.3%)	34 (26.0%)	
SQ[g]	27 (20.6%)	29 (22.1%)	0.485
Smoking			
No	7 (5.3%)	6 (4.6%)	
Yes	61 (46.6%)	57 (43.5%)	1.000

[a] Fisher's Exact test, [b] Number of patients, [c] % percentage of total patients, [d] Optimal response, [e] Stable disease, [f] non-squamous/mixed histology, [g] squamous.

Table S4. OS in patients with PD-L1(+), TAIC high, CD3-TIL high, CD8-TIL high or GZMB-TIL high tumors.

| **Patients**	OS (months)		HR[a]	95% CI[b]	log-rank p value
	Placebo	Vx-001			
PD-L1(+) (n=30)	24.9	7.9	1.646	0.647-4.186	0.291
TAIC high (n=71)	16.3	14.3	1.463	0.850-2.517	0.166
CD3-TIL high (n=60)	16.3	10.0	1.599	0.895-2.856	0.109
CD8-TIL high (n=59)	16.3	12.7	1.501	0.832-2.709	0.174
GZMB-TIL high (n=15)	21.0	4.6	3.751	1.022-13.77	0.034

[a] Hazard Ratio, [b] 95% confidence interval of the estimated HR.

Table S5. TTF in patients with PD-L1(+), TAIC high, CD3-TIL high, CD8-TIL high or GZMB-TIL high tumors.

Patients	TTF (months)		HR[a]	95% CI[b]	log-rank p value
	Placebo	Vx-001			
PD-L1(+) (n=30)	3.0	3.5	1.344	0.615-2.934	0.437
TAIC high (n=71)	2.8	2.3	1.040	0.639-1.693	0.869
CD3-TIL high (n=60)	3.0	2.3	1.201	0.701-2.058	0.484
CD8-TIL high (n=59)	3.0	2.3	1.208	0.705-2.068	0.473
GZMB-TIL high (n=15)	2.8	2.0	1.043	0.362-3.005	0.936

[a] Hazard Ratio, [b] 95% confidence interval of the estimated HR.

CD8/GZMB double staining
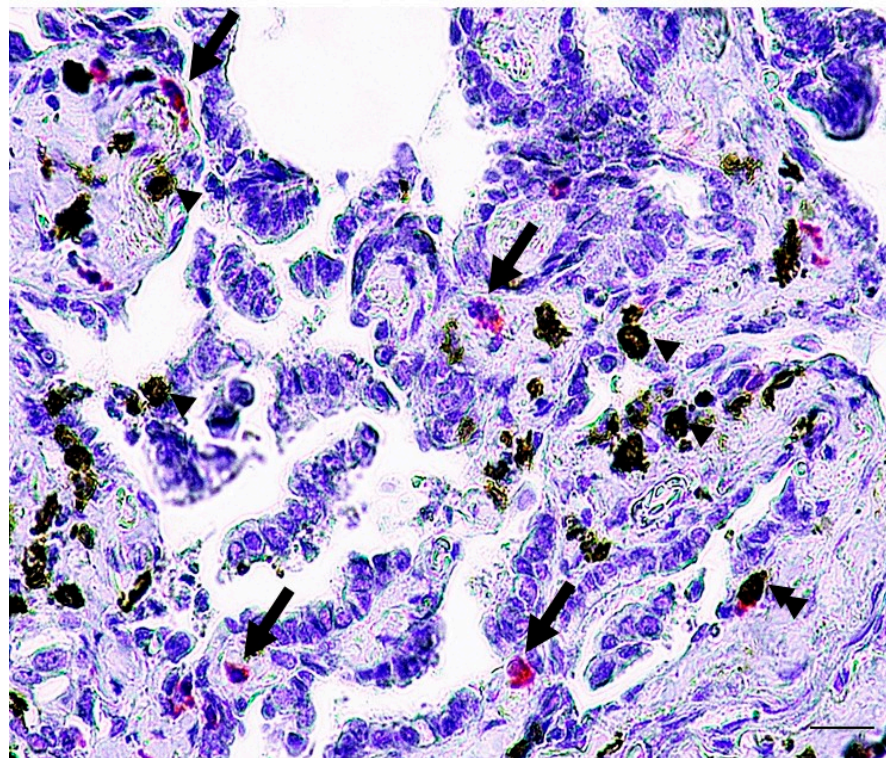
Figure S1. CD8-GZMB double staining. CD8 (brown, single arrowheads) – GZMB (red, single arrows). Double arrowhead depict CD8+GZMB+ cells. Scale bar: 20μm.
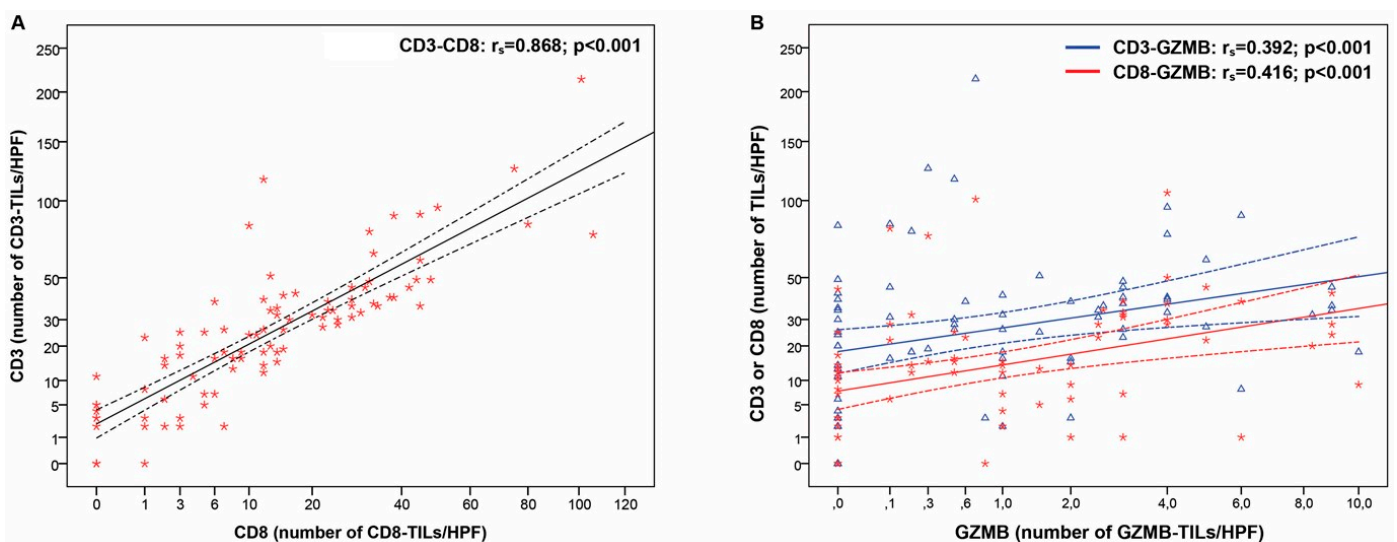
Figure S2. Correlation of CD3-TILs, CD8-TILs and GZMB-TILs. Scatter plots in power scale of (A) CD3-TILs and CD8-TILs and (B) GZMB-TILs with CD3-TILs (blue) or CD8-TILs (red). The *p* values are calculated by Spearman correlation analysis. The solid lines represent linear regression trendlines and dashed lines indicate 95% confidence intervals. r_s: Spearman correlation coefficient.
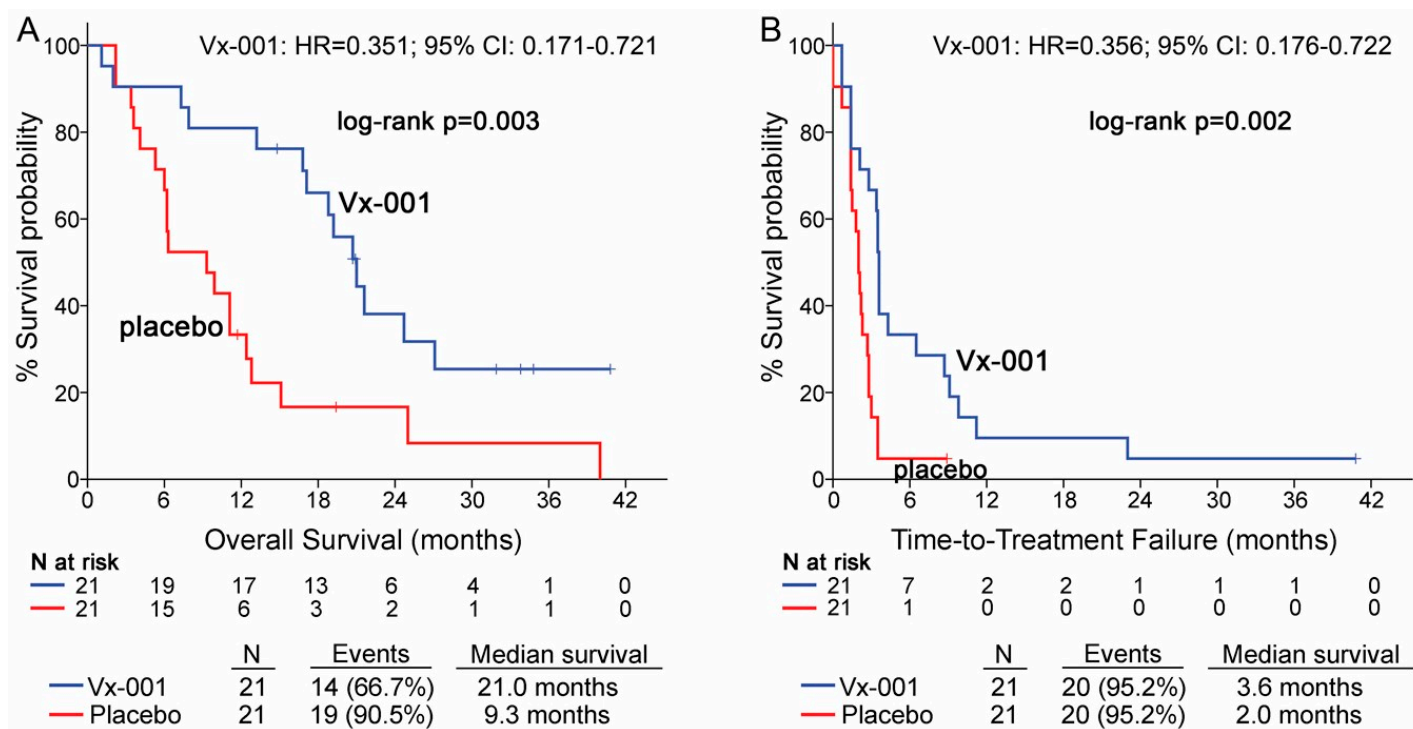
Figure S3. OS (A) and TTF (B) in patients with tumors lacking CD8-TIL/GZMB double positive cells.
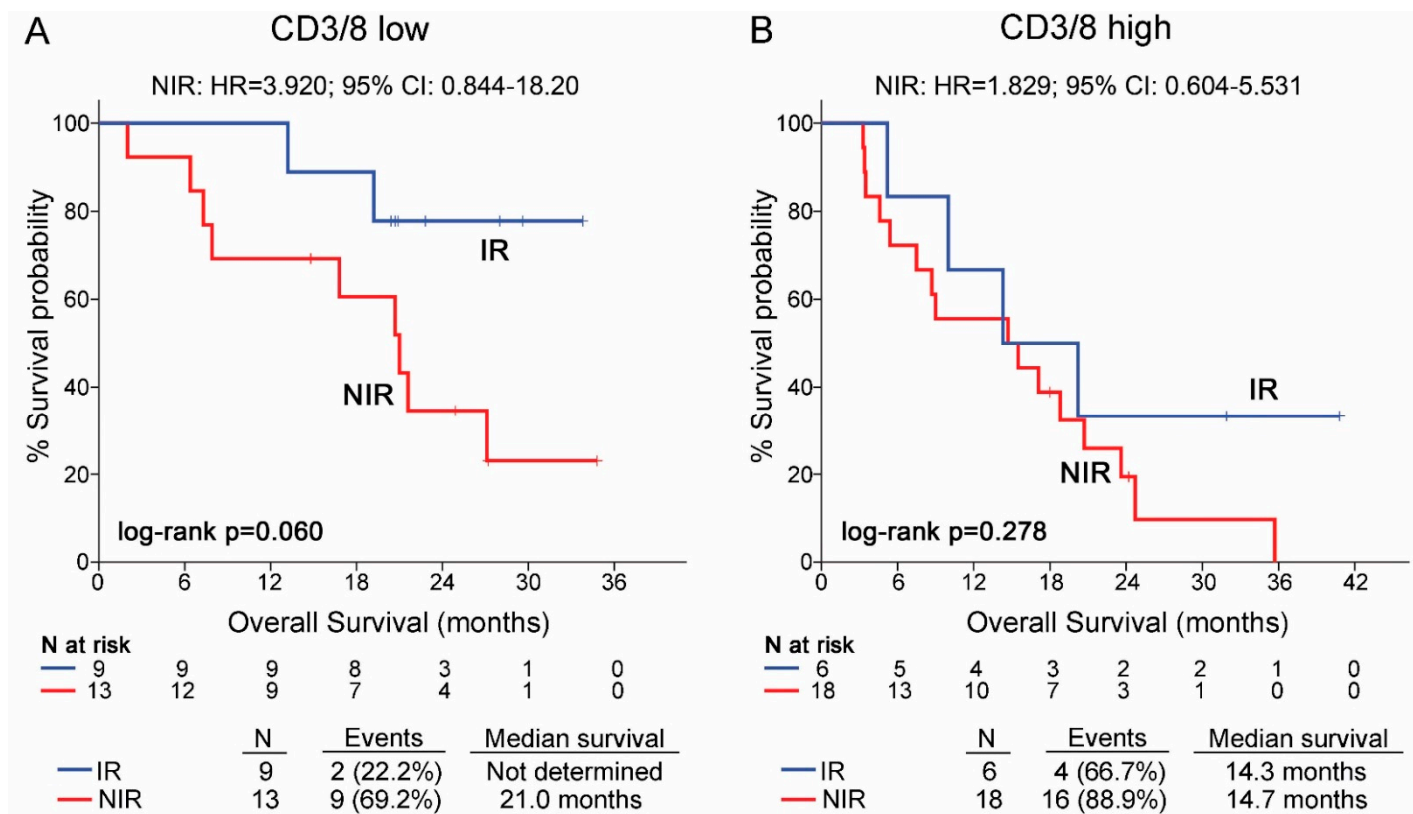

Figure S4. : Correlation of Vx-001 induced immune response and OS in patients with CD3/8-TIL low (A) and CD3/8-TIL high (B) tumors. IR: immune responders, NIR: Non immune responders.