Supplementary Materials

Materials and methods

Ex vivo IFN-γ ELISpot on CD4+ and CD8+ T cell depleted PBMCs

Cryopreserved PBMCs from selected patients were rapidly thawed and incubated at 37 °C for at least 1 hour in RPMI medium (Sigma) and benzonase (Merck Chemicals Ltd) prior to the test. Cells were counted and divided for CD4+ and CD8+ T cell depletion protocols (Miltenyi Biotec Ltd), as well as for the whole PBMCs fraction test. Following the depletion procedure as per manufacturer instructions, both unfractionated and depleted PBMCs were plated at 2.5 x 10^5 cells per well as per ex vivo IFN-γ ELISpot assay described in Materials and Methods. Stimulation of all fractions was performed with patient-specific pools of 5T4 peptides which elicited a response in freshly isolated cells, with the exception of the non-responder patient, whose cell fractions were stimulated with the total pool of 5T4 peptides.

Semi-quantitative ELISA

5T4- and MVA-specific antibody responses were measured using a validated semi-quantitative ELISA. Polyclonal plasma, known to be positive for both 5T4 and MVA antibodies, was used as a standard curve for each assay. The standard curves for each ELISA were assigned a nominal value of 5T4 or MVA antibody relative units (RU) and were titrated from 200 to 1.56 RU. A cut-point was established for each assay by analysing 5T4- and MVA-specific antibody levels in plasma recovered from 50 healthy donors. Cut-points of 11.17 RU and 5.00 RU were established for 5T4 and MVA, respectively. Variation in the level of 5T4 and MVA antibody levels was assessed in cancer patients who had not received any 5T4- or MVA-targeted therapies. A 1.54-fold increase in 5T4 antibody and a 1.76-fold

increase in MVA antibody were established as the level at which a 1% false positive rate could be expected. A positive response was reported if the post-vaccination antibody levels exceeded the cut-point and the increase, relative to the baseline, exceeded the predetermined fold increase for each antigen.

Figures

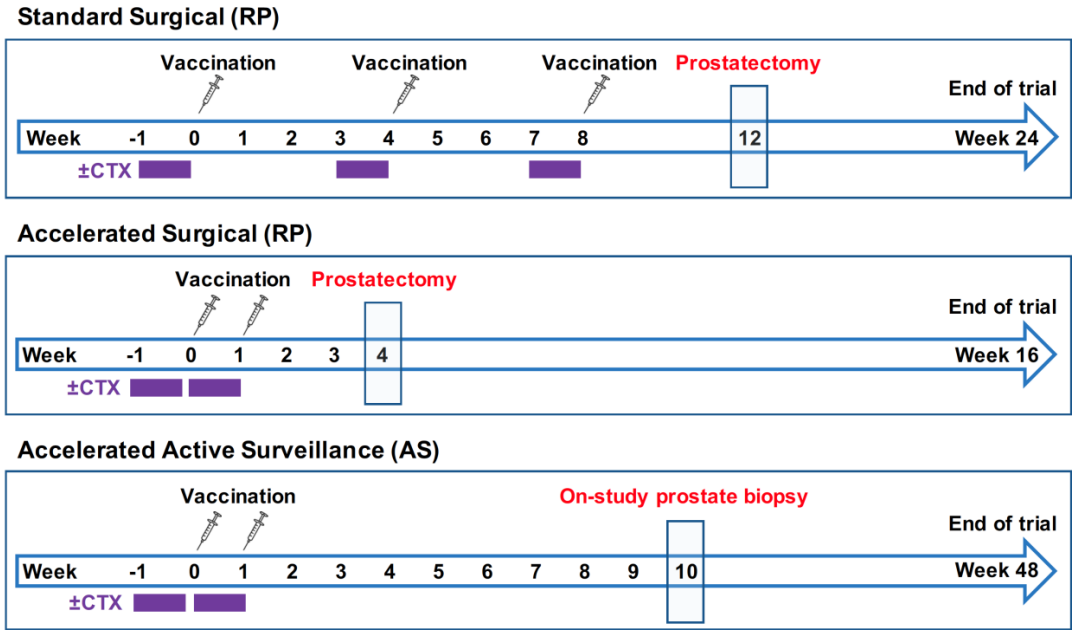

Figure S1. VANCE study design. Study treatment schedule in the three vaccination regimens. RP: radical prostatectomy: AS: active surveillance; CTX: cyclophosphamide.

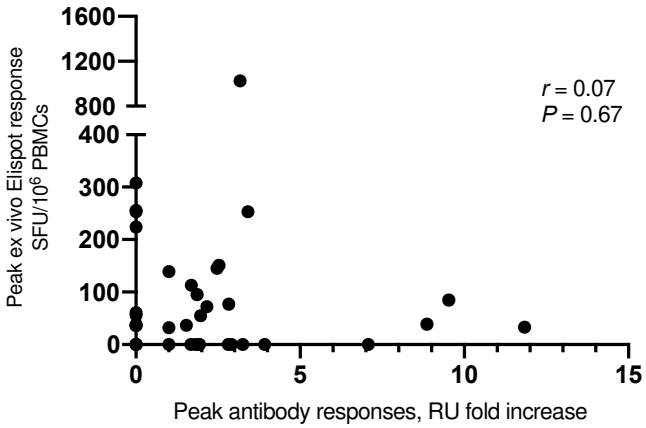

Figure S2. Correlation analysis of vaccination induced T cell and antibody responses. Two-tailed Spearman's correlation of peak T cell and antibody responses.

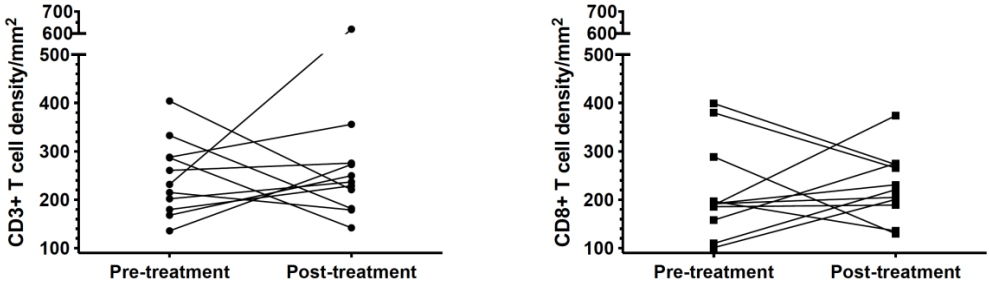

Figure S3. T cell infiltration in the prostate gland of vaccinated active surveillance patients. Archived diagnostic prostate biopsies and matched on study biopsies collected at week 10 from patients in the active surveillance arms were tested for T cell infiltration by immunohistochemistry. Formalin-fixed paraffin-embedded sections were stained with anti-CD3 and anti-CD8 antibodies and cell densities were calculated. Comparison of CD3+ (left) and CD8+ (right) T cell densities, expressed as number of cells per mm^2.

Tables

Table S1. Antibody panel for flow cytometry staining of stimulated cultured PBMCs and TILs.

Antibody	**Fluorophore**	**Clone**	**Supplier**	**Cat Nr**
Zombie NIR Dye	Near IR		Life Technologies Ltd	L10119
CD3	AF700	UCHT1	eBioscience	56-0038-82
CD4	APC	RPA-T4	eBioscience	17-0049-42
CD8a	eFluor450	RPA-T8	eBioscience	48-0088-42
IFN-γ	FITC	4S.B3	eBioscience	11-7319-82
IFN-γ	PE	4S.B3	Life Technologies Ltd	12-7319-82
TNF-α	FITC	MAb11	Life Technologies Ltd	11-7349-82
TNF-α	PE	MAb11	eBioscience	12-7349-82

Table S2. Baseline demographics and disease characteristics of VANCE study.

	Radical prostatectomy arms		**Active surveillance arms**
	Standard vaccination regimen	**Accelerated vaccination regimen**	**Accelerated vaccination regimen**
Number of patients (n)	11	16	13
Median age / range (years)	63 / 53-73	58 / 50-75	69 / 53-77
Gleason score (n)			
>6	0	1	10
High volume 6	0	1	1
3+4	8	11	2
4+3	3	3	0
Clinical stage (n)			
≥T1c	3	2	13
T2a	2	7	0
T2b	6	7	0

Table S3. 5T4- and MVA-specific antibody responses in plasma of vaccinated patients.

Patient ID	Group	Timepoint (week)	5T4 antibody (RU)	Response	MVA antibody (RU)	Response
VAN-001	**4**	**0**	27.23	N/A	7.39	N/A
VAN-001		**2**	29.05	Negative	10.15	Negative
VAN-001		**4**	25.01	Negative	13.03	Positive
VAN-001		**5**	51.88	Positive	125.99	Positive
VAN-001		**8**	44.90	Positive	116.84	Positive
VAN-001		**9**	48.14	Positive	125.77	Positive
VAN-001		**12**	47.41	Positive	108.15	Positive
VAN-001		**24**	35.21	Negative	52.70	Positive
VAN-002	**3**	**0**	20.33	N/A	8.56	N/A
VAN-002		**2**	17.91	Negative	65.44	Positive
VAN-002		**4**	25.12	Negative	57.50	Positive
VAN-002		**5**	37.28	Positive	70.88	Positive
VAN-002		**8**	36.18	Positive	64.30	Positive
VAN-002		**9**	30.35	Negative	50.63	Positive
VAN-002		**12**	29.31	Negative	43.20	Positive
VAN-002		**24**	14.09	Negative	34.55	Positive
VAN-003	**2**	**0**	19.22	N/A	5.22	N/A
VAN-003		**2**	30.90	Positive	8.23	Negative
VAN-003		**4**	31.77	Positive	8.53	Negative
VAN-003		**5**	32.10	Positive	42.79	Positive
VAN-003		**8**	25.89	Negative	72.23	Positive
VAN-003		**9**	30.14	Positive	79.45	Positive
VAN-003		**12**	47.49	Positive	79.91	Positive
VAN-003		**24**	24.22	Negative	55.76	Positive
VAN-004	**1**	**0**	43.21	N/A	6.48	N/A
VAN-004		**2**	44.39	Negative	6.23	Negative
VAN-004		**4**	48.02	Negative	9.23	Negative
VAN-004		**5**	46.49	Negative	27.11	Positive
VAN-004		**8**	48.14	Negative	62.50	Positive
VAN-004		**9**	85.00	Positive	98.95	Positive
VAN-004		**12**	81.46	Positive	71.63	Positive
VAN-004		**24**	35.25	Negative	40.24	Positive
VAN-005	**6**	**0**	28.52	N/A	11.70	N/A
VAN-005		**1**	28.94	Negative	10.23	Negative
VAN-005		**2**	40.82	Negative	70.87	Positive

VAN-005		**4**	42.11	Negative	223.65	Positive
VAN-005		**17**	61.56	Positive	122.79	Positive
VAN-006	**8**	**0**	22.20	N/A	<5	N/A
VAN-006		**1**	18.85	Negative	<5	Negative
VAN-006		**2**	20.27	Negative	<5	Negative
VAN-006		**4**	23.84	Negative	11.77	Positive
VAN-006		**8**	22.89	Negative	9.25	Positive
VAN-006		**10**	22.61	Negative	8.62	Negative
VAN-006		**22**	19.99	Negative	5.06	Negative
VAN-006		**34**	19.03	Negative	<5	Negative
VAN-006		**48**	34.31	Negative	<5	Negative
VAN-007	**7**	**0**	12.80	N/A	<5	N/A
VAN-007		**1**	12.80	Negative	<5	Negative
VAN-007		**2**	14.14	Negative	10.67	Positive
VAN-007		**4**	38.19	Positive	24.90	Positive
VAN-007		**8**	40.57	Positive	18.39	Positive
VAN-007		**10**	38.46	Positive	14.81	Positive
VAN-007		**22**	19.95	Positive	12.53	Positive
VAN-007		**34**	13.98	Negative	8.75	Negative
VAN-007		**48**	14.21	Negative	7.26	Negative
VAN-008	**2**	**0**	30.16	N/A	8.13	N/A
VAN-008		**2**	27.19	Negative	9.94	Negative
VAN-008		**4**	27.63	Negative	9.48	Negative
VAN-008		**5**	30.81	Negative	8.51	Negative
VAN-008		**8**	91.41	Positive	31.50	Positive
VAN-008		**9**	357.00	Positive	127.25	Positive
VAN-008		**12**	273.81	Positive	98.64	Positive
VAN-008		**24**	79.14	Positive	27.22	Positive
VAN-010	**6**	**0**	18.39	N/A	9.10	N/A
VAN-010		**1**	19.16	Negative	5.58	Negative
VAN-010		**2**	19.59	Negative	17.62	Positive
VAN-010		**4**	20.26	Negative	28.82	Positive
VAN-010		**16**	31.07	Positive	47.25	Positive
VAN-011	**3**	**0**	26.85	N/A	<5	N/A
VAN-011		**2**	27.39	Negative	70.36	Positive
VAN-011		**4**	36.81	Negative	61.80	Positive
VAN-011		**5**	143.44	Positive	46.99	Positive
VAN-011		**8**	136.85	Positive	89.78	Positive

VAN-011		**9**	175.45	Positive	95.62	Positive
VAN-011		**12**	164.23	Positive	133.75	Positive
VAN-011		**24**	64.25	Positive	76.67	Positive
VAN-012	**5**	**0**	14.61	N/A	<5	N/A
VAN-012		**1**	14.91	Negative	<5	Negative
VAN-012		**2**	16.23	Negative	9.32	Positive
VAN-012		**4**	18.09	Negative	105.44	Positive
VAN-012		**16**	22.49	Positive	35.44	Positive
VAN-013	**7**	**0**	47.48	N/A	<5	Negative
VAN-013		**1**	45.04	Negative	<5	N/A
VAN-013		**2**	44.96	Negative	<5	Negative
VAN-013		**4**	45.70	Negative	10.09	Positive
VAN-013		**8**	48.87	Negative	10.99	Positive
VAN-013		**22**	60.93	Negative	7.35	Negative
VAN-013		**34**	47.65	Negative	5.49	Negative
VAN-013		**48**	52.92	Negative	5.60	Negative
VAN-015	**8**	**0**	14.84	N/A	7.01	Negative
VAN-015		**1**	14.76	Negative	7.86	Negative
VAN-015		**2**	14.65	Negative	35.01	Positive
VAN-015		**4**	19.88	Negative	161.63	Positive
VAN-015		**8**	28.73	Positive	103.58	Positive
VAN-015		**22**	18.86	Negative	78.50	Positive
VAN-015		**34**	16.08	Negative	68.74	Positive
VAN-015		**48**	18.36	Negative	53.43	Positive
VAN-016	**8**	**0**	5.78	N/A	<5	N/A
VAN-016		**1**	5.78	Negative	<5	Negative
VAN-016		**2**	6.38	Negative	28.77	Positive
VAN-016		**4**	11.74	Positive	171.66	Positive
VAN-016		**8**	19.72	Positive	194.35	Positive
VAN-016		**22**	12.68	Positive	135.41	Positive
VAN-016		**34**	8.81	Negative	107.05	Positive
VAN-016		**48**	8.09	Negative	83.54	Positive
VAN-018	**8**	**0**	10.67	Negative	<5	Negative
VAN-018		**1**	11.36	Negative	<5	Negative
VAN-018		**2**	12.81	Negative	<5	Negative
VAN-018		**4**	11.17	Negative	<5	Negative
VAN-018		**8**	13.40	Negative	<5	Negative
VAN-018		**22**	20.73	Positive	<5	Negative

VAN-018		**34**	19.22	Positive	<5	Negative
VAN-018		**48**	34.63	Positive	<5	Negative
VAN-019	**8**	**0**	31.63	N/A	18.54	N/A
VAN-019		**1**	30.82	Negative	18.51	Negative
VAN-019		**2**	32.06	Negative	50.55	Positive
VAN-019		**4**	31.12	Negative	278.87	Positive
VAN-019		**8**	37.88	Negative	273.53	Positive
VAN-019		**22**	39.16	Negative	148.04	Positive
VAN-019		**34**	33.41	Negative	101.59	Positive
VAN-019		**48**	24.70	Negative	104.30	Positive
VAN-020	**7**	**0**	19.56	N/A	<5	Negative
VAN-020		**1**	21.48	Negative	<5	Negative
VAN-020		**2**	22.33	Negative	24.82	Positive
VAN-020		**4**	22.52	Negative	148.16	Positive
VAN-020		**8**	26.59	Negative	87.96	Positive
VAN-020		**22**	28.17	Negative	41.68	Positive
VAN-020		**34**	29.76	Negative	34.89	Positive
VAN-020		**48**	29.10	Negative	30.19	Positive
VAN-021	**7**	**0**	23.26	N/A	5.18	N/A
VAN-021		**1**	25.60	Negative	5.02	Negative
VAN-021		**2**	21.05	Negative	6.53	Negative
VAN-021		**4**	24.79	Negative	9.14	Positive
VAN-021		**8**	26.19	Negative	7.01	Negative
VAN-021		**22**	26.41	Negative	7.23	Negative
VAN-021		**34**	25.84	Negative	7.09	Negative
VAN-021		**48**	30.55	Negative	<5	Negative
VAN-022	**8**	**0**	60.50	N/A	25.25	N/A
VAN-022		**1**	56.15	Negative	22.57	Negative
VAN-022		**2**	60.83	Negative	115.22	Positive
VAN-022		**4**	63.04	Negative	481.99	Positive
VAN-022		**8**	66.52	Negative	367.45	Positive
VAN-022		**22**	59.50	Negative	229.51	Positive
VAN-022		**34**	58.68	Negative	198.55	Positive
VAN-022		**48**	81.67	Negative	155.49	Positive
VAN-023	**2**	**0**	16.15	N/A	<5	N/A
VAN-023		**2**	16.41	Negative	<5	Negative
VAN-023		**4**	15.68	Negative	<5	Negative
VAN-023		**5**	18.16	Negative	<5	Negative

VAN-023		**8**	29.90	Positive	73.92	Positive
VAN-023		**9**	153.87	Positive	84.42	Positive
VAN-023		**12**	89.75	Positive	80.31	Positive
VAN-023		**24**	27.82	Positive	51.90	Positive
VAN-024	**7**	**0**	12.59	N/A	5.58	N/A
VAN-024		**1**	12.96	Negative	5.60	Negative
VAN-024		**2**	19.30	Negative	7.93	Negative
VAN-024		**4**	24.94	Positive	52.00	Positive
VAN-024		**8**	22.15	Positive	40.49	Positive
VAN-024		**22**	17.08	Negative	24.59	Positive
VAN-024		**34**	27.41	Positive	21.69	Positive
VAN-024		**48**	35.62	Positive	17.68	Positive
VAN-026	**7**	**0**	15.22	N/A	<5	N/A
VAN-026		**1**	16.86	Negative	<5	Negative
VAN-026		**2**	17.69	Negative	12.81	Positive
VAN-026		**4**	20.62	Negative	89.02	Positive
VAN-026		**8**	22.80	Negative	61.33	Positive
VAN-026		**22**	16.04	Negative	29.47	Positive
VAN-026		**34**	19.13	Negative	27.18	Positive
VAN-026		**48**	22.12	Negative	22.00	Positive
VAN-029	**5**	**0**	47.84	N/A	<5	N/A
0011029		**1**	43.48	Negative	<5	Negative
0011029		**2**	44.78	Negative	<5	Negative
0011029		**4**	45.29	Negative	6.77	Negative
0011029		**16**	50.59	Negative	<5	Negative
VAN-031	**4**	**0**	18.18	N/A	9.57	N/A
VAN-031		**2**	18.69	Negative	48.68	Positive
VAN-031		**4**	22.37	Negative	40.62	Positive
VAN-031		**5**	129.69	Positive	99.15	Positive
VAN-031		**8**	135.30	Positive	168.39	Positive
VAN-031		**9**	139.82	Positive	175.58	Positive
VAN-031		**12**	164.86	Positive	182.18	Positive
VAN-031		**24**	190.61	Positive	128.02	Positive
VAN-034	**6**	**0**	12.92	N/A	18.21	Negative
VAN-034		**1**	12.37	Negative	19.42	Negative
VAN-034		**2**	14.52	Negative	33.10	Positive
VAN-034		**4**	14.45	Negative	99.26	Positive
VAN-034		**16**	17.09	Negative	67.78	Positive

VAN-801	**5**	**0**	20.14	N/A	<5	N/A
VAN-801		**1**	20.88	Negative	<5	Negative
VAN-801		**2**	21.07	Negative	<5	Negative
VAN-801		**4**	26.59	Negative	24.94	Positive
VAN-801		**16**	27.37	Negative	11.68	Positive
VAN-802	**5**	**0**	10.56	N/A	<5	N/A
VAN-802		**1**	10.88	Negative	<5	Negative
VAN-802		**2**	12.56	Negative	<5	Negative
VAN-802		**4**	15.93	Negative	43.80	Positive
VAN-802		**16**	13.62	Negative	9.77	Positive
VAN-803	**3**	**0**	16.22	N/A	8.65	N/A
VAN-803		**1**	17.27	Negative	8.81	Negative
VAN-803		**2**	47.15	Positive	15.63	Positive
VAN-803		**4**	38.14	Positive	178.03	Positive
VAN-803		**16**	35.89	Positive	159.01	Positive
VAN-804	**3**	**0**	19.52	N/A	<5	N/A
VAN-804		**2**	17.66	Negative	<5	Negative
VAN-804		**4**	19.39	Negative	<5	Negative
VAN-804		**5**	172.93	Positive	114.02	Positive
VAN-804		**8**	101.07	Positive	67.67	Positive
VAN-804		**9**	129.44	Positive	60.26	Positive
VAN-804		**12**	79.42	Positive	60.00	Positive
VAN-804		**16**	28.90	Negative	15.35	Positive
VAN-805	**6**	**0**	20.38	N/A	6.10	N/A
VAN-805		**1**	54.37	Positive	6.25	Negative
VAN-805		**2**	79.83	Positive	7.62	Negative
VAN-805		**4**	52.94	Positive	45.27	Positive
VAN-805		**16**	48.85	Positive	17.45	Positive
VAN-806	**5**	**0**	28.86	N/A	23.80	N/A
VAN-806		**1**	27.11	Negative	21.90	Negative
VAN-806		**2**	26.30	Negative	26.20	Negative
VAN-806		**4**	27.70	Negative	43.41	Positive
VAN-806		**16**	38.27	Negative	28.14	Negative
VAN-807	**3**	**0**	21.23	N/A	14.61	N/A
VAN-807		**2**	25.78	Negative	566.83	Positive
VAN-807		**4**	29.56	Negative	371.77	Positive
VAN-807		**5**	53.98	Positive	323.16	Positive
VAN-807		**8**	49.21	Positive	228.06	Positive

VAN-807		**9**	59.70	Positive	234.70	Positive
VAN-807		**12**	56.67	Positive	194.89	Positive
VAN-807		**24**	30.49	Negative	119.19	Positive
VAN-808	**6**	**0**	13.35	N/A	<5	N/A
VAN-808		**1**	13.78	Negative	<5	Negative
VAN-808		**2**	16.92	Negative	146.12	Positive
VAN-808		**4**	18.51	Negative	176.13	Positive
VAN-808		**16**	15.70	Negative	70.89	Positive
VAN-809	**5**	**0**	12.51	N/A	<5	N/A
VAN-809		**1**	12.51	Negative	<5	Negative
VAN-809		**2**	15.01	Negative	45.41	Positive
VAN-809		**16**	15.26	Negative	152.17	Positive
VAN-810	**5**	**0**	17.89	N/A	13.71	N/A
VAN-810		**1**	75.86	Positive	14.13	Negative
VAN-810		**2**	126.60	Positive	45.36	Positive
VAN-810		**4**	114.48	Positive	166.36	Positive
VAN-811	**1**	**0**	6.26	N/A	<5	N/A
VAN-811		**2**	6.53	Negative	<5	Negative
VAN-811		**4**	6.71	Negative	<5	Negative
VAN-811		**5**	6.93	Negative	<5	Negative
VAN-811		**8**	6.79	Negative	<5	Negative
VAN-811		**9**	8.30	Negative	6.64	Negative
VAN-811		**12**	7.64	Negative	5.02	Negative
VAN-811		**24**	10.47	Positive	<5	Negative
VAN-812	**5**	**0**	16.65	N/A	5.48	N/A
VAN-812		**1**	17.37	Negative	5.76	Negative
VAN-812		**2**	19.16	Negative	24.14	Positive
VAN-812		**4**	17.27	Negative	56.34	Positive
VAN-812		**16**	42.10	Positive	28.76	Positive
VAN-813	**6**	**0**	34.15	N/A	<5	N/A
VAN-813		**1**	34.33	Negative	<5	Negative
VAN-813		**2**	37.25	Negative	<5	Negative
VAN-813		**4**	63.52	Positive	28.08	Positive
VAN-813		**16**	54.13	Positive	9.78	Positive
VAN-814	**6**	**0**	24.24	N/A	<5	N/A
VAN-814		**1**	29.41	Negative	<5	Negative
VAN-814		**2**	29.78	Negative	<5	Negative
VAN-814		**4**	29.22	Negative	11.03	Positive

VAN-814		**16**	29.42	Negative	6.18	Negative

Table S4. *Ex vivo* IFN-γ ELISpot results on frozen PBMCs depleted of CD4+ or CD8+ T cells.

		Frozen PBMCs			Fresh PBMCs
Group	Patient ID	Whole PBMCs ($SFCx10^6$)	CD4 depleted PBMCs ($SFCx10^6$)	CD8 depleted PBMCs ($SFCx10^6$)	Whole PBMCs ($SFCx10^6$)
2	VAN-003	0	0	0	88
2	VAN-802	166	43	127	253
3	VAN-011	0	0	0	152
4	VAN-031	0	0	0	132
5	VAN-029	59	0	91	308
5	VAN-801	0	0	0	0
5	VAN-812	101	97	0	151
6	VAN-808	75	100	0	224
7	VAN-007	0	0	0	181

Table S5. T cell density in the prostate gland of vaccinated surgical patients measured by IHC. SD = standard deviation.

Patient ID	**Immune cell infiltration in surgical specimens (number of positive cells per 1 mm^2)**	
	CD3+	**CD8+**
VAN-001	89	46
VAN-003	291	170
VAN-004	241	164
VAN-005	174	138
VAN-008	232	163
VAN-010	203	155
VAN-011	187	161

VAN-012	317	138
VAN-023	210	82
VAN-029	186	137
VAN-031	61	50
VAN-034	157	116
Mean	196	127
SD	73	44