

Online supplementary material, Schuhmacher et al.

Supplementary table S1: Inclusion and exclusion criteria of the open label clinical phase I/II study

Inclusion criteria	Exclusion Criteria
≥ 18 years	Patient is a candidate for relevant therapies that are the current standard of care for their cancer disease
Patients prostatectomised (PT) due to histologically verified adenocarcinoma of the prostate gland who currently are not being treated, or expected within the next 8 months to be treated, with any anti-cancer treatment. Patients may or may not have measurable PSA.	Patient has been treated with Androgen Deprivation Therapy (ADT) or expected to receive such treatment within the next 8 months from enrolment.
Able to understand the study procedures and willing to provide informed consent.	Concurrent chemotherapy or radiotherapy within 12 weeks of 1st vaccination, or expected to receive such treatment within the next 8 months from enrolment.
Able and willing to comply with study requirements and complete all visits.	Patients have undergone major surgery or have had major bleeding within the last month prior to the first vaccination.
Using adequate contraceptive measures. All non-vasectomized patients must use condoms during the study and for one month after the last vaccination with RV001V, or have a female partner who either has been post-menopausal for more than one year or is using a highly effective method of contraception (i.e., a method with less than 1% failure rate).	Patients with brain or leptomeningeal metastasis.
Eastern Cooperative Oncology Group (ECOG) status 0 or 1.	Prior treatment with any therapeutic anti-cancer vaccine(s).
Recovered/stabilized at grade ≤2 from all toxicities related to prior treatment(s) in accordance with Common Terminology Criteria for Adverse Events (CTCAE)	History of second malignancy (except for adequately managed basal cell carcinoma and squamous cell carcinoma of the skin).
Laboratory values obtained ≤30 days prior to the first vaccination, and more than 3 weeks after potential chemotherapy <ul style="list-style-type: none"> <li>• Haemoglobin ≥5.6 mmol/L</li> <li>• Absolute granulocyte count ≥1.5 x 10<sup>9</sup> /L</li> <li>• Platelets ≥100 x 10<sup>9</sup> /L</li> <li>• Total bilirubin ≤1.5 x upper limit of normal (ULN)</li> <li>• Creatinine ≤1.5 x ULN</li> <li>• Alanine aminotransferase/aspartate aminotransferase/ alkaline phosphatase ≤2.5 xULN</li> </ul>	Patients in need of or treated the last 30 days before the first vaccination with systemic steroids or other immune suppressive therapy. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.
	History of alcohol or substance abuse within the last 5 years.
	History of acquired immune deficiency syndrome or positive serological test for human immunodeficiency virus infection.
	History of acquired immune deficiency syndrome or positive serological test for human immunodeficiency virus infection.
	History of viral hepatitis B as determined by positive antibody immunoglobulin M (IgM) to core antigen for hepatitis B or positive for hepatitis B surface antigen, or viral hepatitis C as determined by positive antibody for hepatitis C.

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	Participation in any investigational trial or use of any investigational drug(s) within 30 days prior to inclusion in this trial.
	Any known serious infections, e.g. tuberculosis
	History of significant autoimmune disease such as: Inflammatory bowel disease, Systemic lupus erythematosus, Ankylosing spondylitis, Scleroderma, Multiple sclerosis.
	Severe medical conditions, such as but not limited to severe asthma/chronic obstructive pulmonary disease (COPD), New York Heart Association (NYHA) grading 3 or above, poorly regulated insulin-dependent diabetes, any significant organ damage as judged by the Investigator.
	Other medications or conditions that in the Investigator's opinion would contraindicate study participation for safety reasons or interfere with the interpretation of study results.
	History of drug allergies or known allergy/hypersensitivity to Montanide ISA 51, or intolerance to subcutaneous injection.

Supplementary table S2: Patient's characteristics

PATIENT	AGE	WEIGHT [kg]		PSA LEVEL [ $\mu\text{g/L}$ ]			
		Baseline (visit 1)	13 months post-vaccination (visit 17)	Baseline (visit 1)	4 weeks post-vaccination (visit 13)	13 months post-vaccination (visit 17)	Last PSA measurement (months** from visit 13)
001	64	91,5	83	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (24,0)
002	74	93	92	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (23,6)
003	69	87	87	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (24,0)
004	76	100	100	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (22,4)
005	65	104	108	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (21,2)
006	71	112	112	0,50	0,60	0,80	1,0 (22,5)
007	62	100	101	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (23,2)
009	65	88,5	87	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (22,2)
010	68	79	89	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (19,9)
011	68	110,5	101	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (22,2)
012	74	82	80	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (22,4)
013	65	93	90	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (21,9)
015*	73	95,5	93	$\leq 0,10$	$\leq 0,10$	0,20	0,28 (25,4)
016	69	98,5	93	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (21,5)
017	71	75	75	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (21,5)
018	77	90	96	1,10	1,10	1,30	1,30 (21,7)
019	70	72,5	73	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (21,8)
020	55	110	109	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (21,5)
021	72	96,5	97	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (20,6)
022	54	83,5	91	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (20,9)
023	60	126	131	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (20,5)
024	66	84,5	81	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$	$\leq 0,10$ (21)

\*Patient received only seven vaccinations instead of 11.

\*\* Assumption 30 days per months.

Supplementary table S3: HLA-typing of individual patients and cell lines

PATIENT/ CELL LINE	HLA- A (1)	HLA- A (2)	HLA- B (1)	HLA- B (2)	HLA- C (1)	HLA- C (2)	HLA- DRB1 (1)	HLA- DRB1 (2)	HLA- DQB1 (1)	HLA- DQB1 (2)	HLA- DPB1 (1)	HLA- DPB1 (2)
<b>001</b>	01:01	02:01	08:01	40:01	03:04P	07:01P	03:01	13:02	02:01	06:04	01:01	04:02
<b>002</b>	29:01	68:01	07:05	50:01	06:02	15:05	07:01P	10:01	02:02P	05:01	02:01	13:01
<b>003</b>	01:01	11:01	08:01	40:01	03:04P	07:01P	03:01	13:02	02:01	06:04	04:01	04:01
<b>004</b>	02:01	30:02	18:01	27:05	01:02	05:01	01:01	03:01	02:01	05:01	04:01	04:01
<b>005</b>	02:01	na	07:02	51:01	02:02	07:02	04:04	11:01	03:01	03:02P	04:01	16:01
<b>006</b>	02:01	na	08:01	44:02	05:01	07:01	01:01	14:54	05:01	05:03P	03:01P	04:01P
<b>007</b>	01:01	68:02	08:01	14:02	07:01	08:02	03:01	13:03	02:01P	03:01P	01:01P	02:01P
<b>009</b>	11:01	na	35:01	na	04:01	na	01:03	14:54	05:01P	05:03P	04:01P	04:02P
<b>010</b>	02:01	na	08:01	15:01	03:03	07:01	03:01	04:01	02:01	03:02	01:01	04:01
<b>011</b>	01:01	32:01	08:01	15:01	03:03	07:01	07:01P	07:01P	02:01P	02:01P	04:01	17:01
<b>012</b>	02:01	68:01	40:01	44:02	03:04	07:04	11:01	12:01P	03:01	03:01	09:01	03:01P
<b>013</b>	02:01	68:01	27:05	44:02	02:02	07:04	11:01	15:01	03:02	06:02	03:01P	04:01P
<b>015</b>	02:01	na	07:02	40:01	03:04	07:02	04:04	15:01	03:02	06:02	03:01	05:01
<b>016</b>	24:02P	25:01	39:06	44:02	05:01	07:02	04:01	08:01P	03:02	04:02	02:01	04:01
<b>017</b>	02:01	32:01	40:01	na	03:04	na	04:08	15:01	03:01	06:02	02:01	04:01
<b>018</b>	01:01	02:01	40:01	51:01	03:04	15:02	04:04	13:02	03:02P	06:04P	04:01P	222:01
<b>019</b>	03:01	24:02P	07:02	35:03	07:02	12:03	15:01	15:01	06:02	06:02	04:01	04:01
<b>020</b>	02:01	32:01	07:02	40:02	02:02	07:02	15:01	16:02	05:02	06:02	04:01	04:01
<b>021</b>	02:01	03:01	15:39	18:01	04:01	07:01	11:02	11:04	03:01	03:01	04:02	10:01
<b>022</b>	11:01	31:01	27:05	44:02	02:02	05:01	04:01	12:01P	03:01	03:02P	03:01P	04:02P
<b>023</b>	02:01	32:01	15:01	15:07	03:03	na	04:04	04:04	03:02	03:02	02:01	04:01
<b>024</b>	02:01	na	15:01	51:01	03:04	15:02	01:01	04:04	03:02	05:01	04:01	04:01
<b>MGAR</b>	26:01	26:01	08:01	08:01	07:01	07:01	15:01	15:01	06:02	26:02	04:01	04:01
<b>H0301</b>	03:01	03:01	14:02	14:02	08:02	08:02	13:02	13:02	06:09	06:09	05:01	05:01

P: ambiguities; na: not available.

Supplementary table S4: IFN- $\gamma$  ELISpot raw data spot counts per analysis time and patient

Patient	Visit	Antigen	R1	R2	R3	R4	R5	R6	Patient	Visit	Antigen	R1	R2	R3	R4	R5	R6		
001	Visit 2	RV001	na	na	na				006	Visit 2	RV001	8	16	18					
		neg	na	na	na	na	na	na			na	neg	5	14	17	5	4	9	
	Visit 6	RV001	1203	853	1148					Visit 6	RV001	975	956	1102					
		neg	44	71	55	29	28	32			neg	12	15	12	15	15	20		
	Visit 8	RV001	1334	1292	1232					Visit 8	RV001	1026	807	882					
		neg	0	2	0	4	2	1			neg	54	56	68	67	39	45		
	Visit 13	RV001	1252	1276	1163					Visit 13	RV001	352	261	296					
		neg	0	0	0	0	0	0			neg	69	217	47	49	42	42		
	Visit 14	RV001	1260	1371	1328					Visit 14	RV001	438	499	653					
		neg	0	0	0	0	0	0			neg	9	8	7	6	4	2		
	Visit 15	RV001	1348	1341	1342					Visit 15	RV001	11	4	12					
		neg	6	0	1	2	0	0			neg	1	1	1	0	2	3		
	Visit 16	RV001	140	128	135					Visit 16	RV001	55	81	58					
		neg	0	0	0	0	0	0			neg	5	6	6	5	3	4		
	Visit 17	RV001	1044	1031	1013					Visit 17	RV001	220	213	226					
		neg	0	0	0	0	0	0			neg	29	47	37	31	32	28		
	003	Visit 2	RV001	189	118	171					007	Visit 2	RV001	59	69	67			
neg			137	115	145	137	131	142	neg	41			43	27	40	27	33		
Visit 6		RV001	1527	1560	1522				Visit 6	RV001		127	106	132					
		neg	20	16	20	18	18	20		neg		71	77	69	96	107	59		
Visit 8		RV001	1345	1423	1399				Visit 8	RV001		98	98	93					
		neg	96	149	108	118	99	100		neg		13	8	16	8	11	15		
Visit 13		RV001	1477	1416	1627				Visit 13	RV001		100	94	97					
		neg	22	33	40	32	19	35		neg		14	23	14	17	5	16		
Visit 14		RV001	2000	2000	2000				Visit 14	RV001		27	33	37					
		neg	3	2	4	3	2	0		neg		6	5	6	3	12	5		
Visit 15		RV001	1571	1665	1605				Visit 15	RV001		415	500	433					
		neg	23	25	25	14	15	20		neg		21	33	27	24	22	28		
Visit 16		RV001	1080	1064	1040				Visit 16	RV001		188	216	208					
		neg	0	0	0	0	0	0		neg		36	21	27	33	34	23		
Visit 17		RV001	2000	2000	2000				Visit 17	RV001		307	312	269					
		neg	138	106	127	82	99	104		neg		155	131	159	179	158	154		
004		Visit 2	RV001	54	11	22				009		Visit 2	RV001	13	1	9			
	neg		11	18	7	12	40	43	neg		3		1	7	7	9	7		
	Visit 6	RV001	105	93	117				Visit 6		RV001	335	399	152					
		neg	391	39	433	40	56	28			neg	5	12	8	8	6	12		
	Visit 8	RV001	1133	1088	1104				Visit 8		RV001	1208	1065	1081					
		neg	28	4	9	9	5	3			neg	27	26	19	16	17	19		
	Visit 13	RV001	1194	1091	1140				Visit 13		RV001	1567	1592	1538					
		neg	28	49	25	22	24	41			neg	46	183	141	53	43	43		
	Visit 14	RV001	375	400	306				Visit 14		RV001	1094	1162	1279					
		neg	33	35	21	19	20	27			neg	4	1	1	0	0	4		
	Visit 15	RV001	382	379	343				Visit 15		RV001	1258	1206	1046					
		neg	5	4	4	0	2	2			neg	1	0	0	1	1	1		
	Visit 16	RV001	449	225	326				Visit 16		RV001	1324	1347	1263					
		neg	6	0	0	1	2	2			neg	6	2	5	3	1	0		
	Visit 17	RV001	883	884	807				Visit 17		RV001	805	827	874					
		neg	13	6	9	5	10	6			neg	2	1	3	4	3	5		
	005	Visit 2	RV001	8	3	3					010	Visit 2	RV001	31	19	17			
neg			4	2	4	3	4	2	neg	23			22	25	34	19	24		
Visit 6		RV001	894	836	962				Visit 6	RV001		82	84	96					
		neg	17	13	5	26	11	16		neg		74	65	58	85	76	77		
Visit 8		RV001	991	1002	1516				Visit 8	RV001		71	61	96					
		neg	12	7	9	9	15	13		neg		74	72	73	52	79	45		
Visit 13		RV001	1495	1439	1482				Visit 13	RV001		53	59	38					
		neg	1	5	3	5	4	11		neg		26	35	21	9	24	15		
Visit 14		RV001	1408	1388	1317				Visit 14	RV001		170	130	128					
		neg	0	0	0	0	0	0		neg		134	101	90	76	82	81		
Visit 15		RV001	1104	1100	1125				Visit 15	RV001		98	100	103					
		neg	0	0	2	2	0	0		neg		17	16	17	14	20	22		
Visit 16		RV001	1444	1413	1424				Visit 16	RV001		101	143	112					
		neg	0	1	1	3	2	2		neg		2	7	5	5	2	2		
Visit 17		RV001	1456	1432	1436				Visit 17	RV001		496	457	450					
		neg	0	3	3	2	1	2		neg		5	2	3	4	1	0		

Patient	Visit	Antigen	R1	R2	R3	R4	R5	R6	Patient	Visit	Antigen	R1	R2	R3	R4	R5	R6	
011	Visit 2	RV001	25	40	47				016	Visit 2	RV001	33	31	39				
		neg	34	28	37	39	34	39			neg	11	18	31	20	21	21	
	Visit 6	RV001	955	1028	1016					Visit 6	RV001	133	113	111				
		neg	89	98	112	127	101	102			neg	15	23	22	21	20	36	
	Visit 8	RV001	1357	1342	1374					Visit 8	RV001	408	371	328				
		neg	24	13	16	15	20	22			neg	58	57	38	79	76	55	
	Visit 13	RV001	1325	1368	1313					Visit 13	RV001	257	269	346				
		neg	22	46	20	9	14	18			neg	22	15	9	9	16	8	
	Visit 14	RV001	1551	1539	1556					Visit 14	RV001	361	436	374				
		neg	32	33	48	21	27	37			neg	53	68	52	78	50	54	
	Visit 15	RV001	1737	1724	1736					Visit 15	RV001	673	739	761				
		neg	10	16	11	6	10	8			neg	3	2	0	1	1	2	
	Visit 16	RV001	1279	1228	1272					Visit 16	RV001	24	35	19				
		neg	6	7	5	2	1	3			neg	3	4	2	5	3	2	
Visit 17	RV001	1244	1229	1360				Visit 17	RV001	13	12	15						
	neg	2	5	5	4	2	3		neg	2	0	0	0	0	1			
012	Visit 2	RV001	132	113	97			017	Visit 2	RV001	3	2	5					
		neg	7	8	6	7	5			6	neg	3	2	1	1	1	3	
	Visit 6	RV001	715	699	875				Visit 6	RV001	8	6	14					
		neg	11	8	11	9	6			7	neg	8	4	2	2	13	3	
	Visit 8	RV001	1674	1676	1680				Visit 8	RV001	27	14	17					
		neg	4	14	26	19	5			11	neg	3	4	1	6	2	2	
	Visit 13	RV001	2000	2000	2000				Visit 13	RV001	113	88	57					
		neg	7	11	2	12	6			7	neg	0	0	0	3	3	1	
	Visit 14	RV001	2000	2000	2000				Visit 14	RV001	146	134	159					
		neg	14	15	13	20	12			12	neg	19	28	18	18	19	17	
	Visit 15	RV001	1167	1214	1315				Visit 15	RV001	603	545	562					
		neg	1	0	0	0	0			1	neg	22	14	15	5	6	6	
	Visit 16	RV001	222	283	323				Visit 16	RV001	23	29	35					
		neg	2	0	1	2	0			1	neg	0	0	1	2	2	0	
Visit 17	RV001	1228	1166	1176			Visit 17	RV001	38	36	45							
	neg	0	0	0	0	1		0	neg	4	5	2	3	1	2			
013	Visit 2	RV001	9	13	8			018	Visit 2	RV001	19	39	27					
		neg	13	9	14	12	14			7	neg	28	17	20	18	20	26	
	Visit 6	RV001	1226	1218	1243				Visit 6	RV001	1061	1091	1096					
		neg	13	12	8	11	7			7	neg	86	124	106	107	83	103	
	Visit 8	RV001	1534	1543	1532				Visit 8	RV001	832	829	799					
		neg	14	6	5	9	8			7	neg	16	17	19	21	13	21	
	Visit 13	RV001	1639	1635	1618				Visit 13	RV001	1272	1295	1308					
		neg	17	4	6	11	12			7	neg	36	34	25	54	24	34	
	Visit 14	RV001	1505	1502	1603				Visit 14	RV001	1154	1187	1255					
		neg	14	15	23	2	16			11	neg	2	4	4	5	5	5	
	Visit 15	RV001	1583	1519	1469				Visit 15	RV001	1363	1353	1308					
		neg	16	14	17	8	7			8	neg	8	17	22	9	11	11	
	Visit 16	RV001	2000	2000	2000				Visit 16	RV001	1242	1215	1272					
		neg	6	5	5	3	4			6	neg	36	45	41	35	29	32	
Visit 17	RV001	2000	2000	2000			Visit 17	RV001	1221	1203	1206							
	neg	3	7	2	6	4		1	neg	17	19	14	12	16	8			
015	Visit 2	RV001	4	2	3			019	Visit 2	RV001	13	17	10					
		neg	4	1	4	2	6			1	neg	11	4	1	3	7	16	
	Visit 6	RV001	8	3	7				Visit 6	RV001	374	510	399					
		neg	52	2	1	1	0			4	neg	3	3	3	4	4	3	
	Visit 8	RV001	427	440	362				Visit 8	RV001	1324	1029	990					
		neg	49	24	23	24	31			26	neg	6	12	8	7	2	291	
	Visit 13	RV001	na	na	na				Visit 13	RV001	1199	1114	1246					
		neg	na	na	na	na	na			na	neg	6	3	7	0	10	6	
	Visit 14	RV001	962	873	864				Visit 14	RV001	52	53	81					
		neg	11	4	15	4	4			4	neg	0	0	0	0	1	0	
	Visit 15	RV001	1381	1413	1657				Visit 15	RV001	940	939	999					
		neg	4	2	2	1	4			1	neg	0	1	1	0	0	0	
	Visit 16	RV001	1121	1164	1121				Visit 16	RV001	123	251	90					
		neg	5	1	4	8	5			4	neg	0	0	0	0	0	0	
Visit 17	RV001	1126	1178	1073			Visit 17	RV001	78	26	44							
	neg	45	33	24	59	36		55	neg	0	0	0	0	0	0			

Patient	Visit	Antigen	R1	R2	R3	R4	R5	R6	Patient	Visit	Antigen	R1	R2	R3	R4	R5	R6	
020	Visit 2	RV001	72	25	48				024	Visit 2	RV001	0	0	0				
		neg	29	42	71	68	67	53			neg	0	0	1	0	0	0	
	Visit 6	RV001	167	117	166					Visit 6	RV001	21	20	24				
		neg	93	126	171	171	172	125			neg	24	16	20	13	34	19	
	Visit 8	RV001	465	575	557					Visit 8	RV001	5	5	6				
		neg	107	62	86	24	49	58			neg	7	5	13	5	8	14	
	Visit 13	RV001	1194	1115	1176					Visit 13	RV001	3	2	8				
		neg	132	102	141	80	82	115			neg	5	4	5	1	3	2	
	Visit 14	RV001	1391	1423	1538					Visit 14	RV001	1	2	3				
		neg	0	0	0	0	0	1			neg	2	0	1	2	4	3	
	Visit 15	RV001	1551	1369	1445					Visit 15	RV001	9	3	4				
		neg	1	1	2	1	1	2			neg	6	5	7	5	16	5	
	Visit 16	RV001	826	804	788					Visit 16	RV001	2	3	5				
		neg	3	1	0	1	0	1			neg	5	4	2	9	6	2	
Visit 17	RV001	2000	2000	2000				Visit 17	RV001	9	3	5						
	neg	2	1	0	4	3	1		neg	11	11	8	9	9	8			
021	Visit 2	RV001	3	3	4				022	Visit 2	RV001	15	14	4				
		neg	0	1	1	1	1	1			neg	9	2	8	12	9	7	
	Visit 6	RV001	17	24	17					Visit 6	RV001	961	966	1017				
		neg	12	12	7	17	12	12			neg	18	14	14	27	18	21	
	Visit 8	RV001	488	539	496					Visit 8	RV001	335	334	320				
		neg	3	1	2	0	1	2			neg	42	57	44	35	28	41	
	Visit 13	RV001	1292	1298	1321					Visit 13	RV001	945	878	805				
		neg	0	0	1	1	2	0			neg	83	100	107	112	106	76	
	Visit 14	RV001	1425	1442	1397					Visit 14	RV001	946	925	963				
		neg	5	1	6	3	6	2			neg	7	16	3	8	3	6	
	Visit 15	RV001	1465	1602	1497					Visit 15	RV001	623	642	649				
		neg	2	0	1	2	2	3			neg	3	5	0	0	1	3	
	Visit 16	RV001	1321	1325	1377					Visit 16	RV001	424	406	461				
		neg	2	2	2	3	2	2			neg	77	96	106	66	63	94	
Visit 17	RV001	1428	1414	1435				Visit 17	RV001	480	421	432						
	neg	2	4	5	2	4	3		neg	3	6	5	4	2	5			
023	Visit 2	RV001	358	433	475				023	Visit 2	RV001	16	19	16				
		neg	293	288	314	218	267	323			neg	13	14	8	11	12	9	
	Visit 6	RV001	39	35	26					Visit 6	RV001	39	35	26				
		neg	26	28	21	17	23	21			neg	26	28	21	17	23	21	
	Visit 8	RV001	287	160	209					Visit 8	RV001	287	160	209				
		neg	222	228	191	149	122	137			neg	222	228	191	149	122	137	
	Visit 13	RV001	3	1	0					Visit 13	RV001	3	1	0				
		neg	0	2	1	1	2	1			neg	0	2	1	1	2	1	
	Visit 14	RV001	39	38	51					Visit 14	RV001	39	38	51				
		neg	44	64	51	76	59	49			neg	44	64	51	76	59	49	
	Visit 15	RV001	24	28	44					Visit 15	RV001	24	28	44				
		neg	20	25	38	39	48	27			neg	20	25	38	39	48	27	
	Visit 16	RV001	29	24	38					Visit 16	RV001	29	24	38				
		neg	26	16	33	21	22	19			neg	26	16	33	21	22	19	

R: Replicate; neg = negative control (ddH<sub>2</sub>O); na= not available. For all patients and tests, 0.2 x 10<sup>6</sup> cells were plated/ well, except for Patient 021 visit 2 to visit 13 and Patient 012 visits 16 and 17, here 0.17 x 10<sup>6</sup> cells were plated/ well. Patient 002 dropped out of the immunological analysis due to inconsistent results (data not shown). Visit 2 for Patient 001 and visit 13 for Patient 015 were not tested as no PBMC samples were available. Spots counts above 2000/well or TNTC (too numerous to count) are set to 2000 and marked in red. For details on the ELISpot, see Material and Methods.

Supplementary table S5: Monoclonal antibody panels for flow cytometry analysis

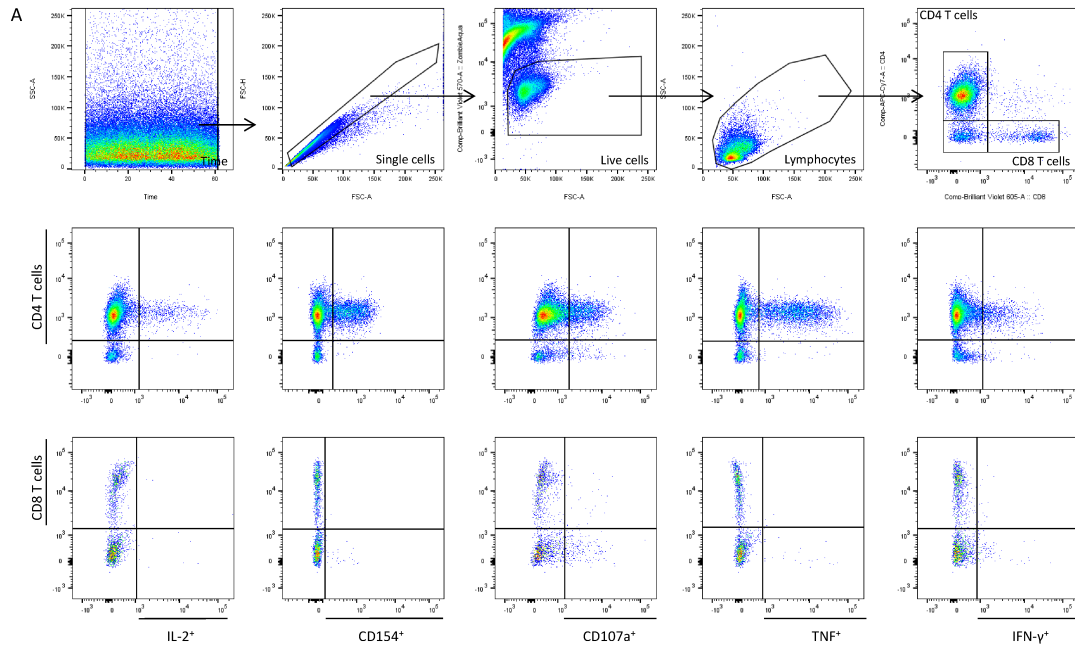
		Marker	Fluorophore	Clone	Manufacturer	Cat. No.
Panel 1	Extracellular staining	LD	Zombie Aqua	-	BioLegend	423102
		CD4	APC-Cy7	RPA-T4	BD	557871
		CD8	BV605	RPA-T8	BioLegend	301040
		CD107a	FITC	H4A3	BD	555800
	Intracellular staining	TNF	Pacific Blue	MAB11	BioLegend	502920
		IFN- $\gamma$	PE-Cy7	4S.B3	BD	557844
		IL-2	PE	MQ1-17H12	BD	554566
CD154		APC	24-31	BioLegend	310810	
Panel 2	Extracellular staining	LD	Zombie Aqua	-	BioLegend	423102
		CD4	APC-Cy7	RPA-T4	BD	557871
		CD8	AF700	SK1	BioLegend	344723
		CD107a	FITC	H4A3	BD	555800
		CD45RA	BV711	HI100	BioLegend	304138
		CCR7	BV605	G043H7	BioLegend	353224
		LAG-3	BV650	1C3C65	BioLegend	369316
		OX-40	APC	Ber-ACT35	BioLegend	350008
		PD-1	BV421	EH12.1	BD	565935
	Intracellular staining	TNF	PE	MAB11	BioLegend	502908
	Panel 3	Extracellular staining	LD	Zombie Aqua	-	BioLegend
CD127			PE-Dazzle 594	A019D5	BioLegend	351335
CD8			APC	RPA-T8	BioLegend	301014
CD3			PE-Cy5.5	SK7	eBioscience	35-0036-42
CD4			APC-Cy7	RPA-T4	BD	557871
CD25			PE-Cy7	BC96	BioLegend	302611
Intracellular staining		FoxP3	FITC	PCH101	Invitrogen	11-4776-42
Extracellular staining		Isotype control	PE-Cy7	MOPC-21	BioLegend	400125
Intracellular staining		Isotype control	FITC	eBR2a	eBioscience	11-4321-42

Note: All fluorophore-coupled antibodies were pre-titrated.

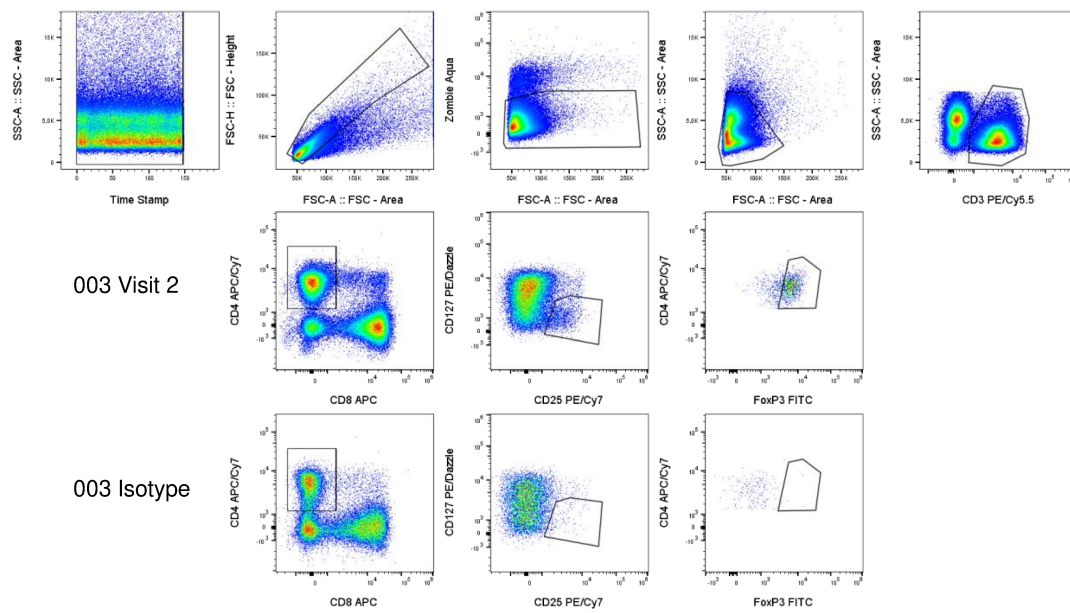
Staining protocol for Ab panels 1 and 2: For staining, cells were washed in FACS-buffer (PBS without Ca/Mg (Lonza), 0,02% NaN<sub>3</sub>, 2 mM EDTA (both Sigma-Aldrich) and 2% hi FBS (Capricorn Scientific) and stained extracellularly for 20 min at 4°C. After fixation and permeabilization for 20 min at 4°C (Cytotfix/Cytoperm, BD), cells were washed with permeabilization buffer (PBS 1X, 0,02% NaN<sub>3</sub>, 0,5% BSA and 0,1% Saponin (Sigma-Aldrich)) and stained intracellularly for 20 min at 4°C. Cells were finally washed with permeabilization buffer and resuspended in FACS-buffer before acquisition.

Staining protocol for Ab panel 3: Fc receptors were blocked by adding 10  $\mu$ l Fc block (BD, final concentration 0.25 mg/ml) for 10 min at room temperature (RT). Without a washing step, cells were extracellularly stained for 20 min at 4°C. Cells were thereafter washed twice, fixed, and permeabilized with 100  $\mu$ l fixation/permeabilization working solution (eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set; Invitrogen) for 30 min at RT. Cells were washed twice with freshly prepared 1x permeabilization buffer (eBioscience Foxp3 / Transcription Factor Staining Buffer Set; Invitrogen). 200  $\mu$ l of permeabilization buffer was added for 15 min at RT. Cells were intracellularly stained with FoxP3 or isotype control antibodies for 30 min at RT. After washing (twice) with permeabilization buffer, cells were resuspended in FACS-buffer before acquisition. The isotype controls were used at the same concentration as the corresponding antibodies, one staining was performed per patient.

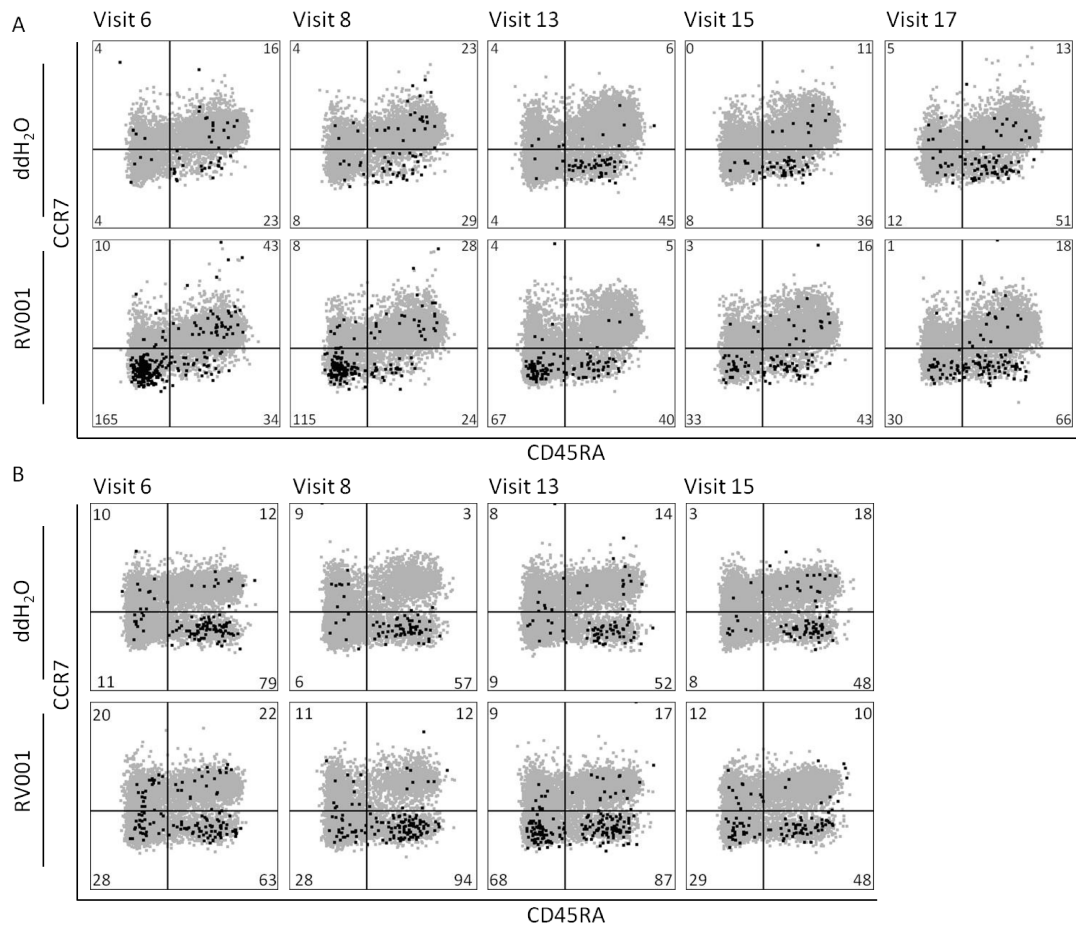




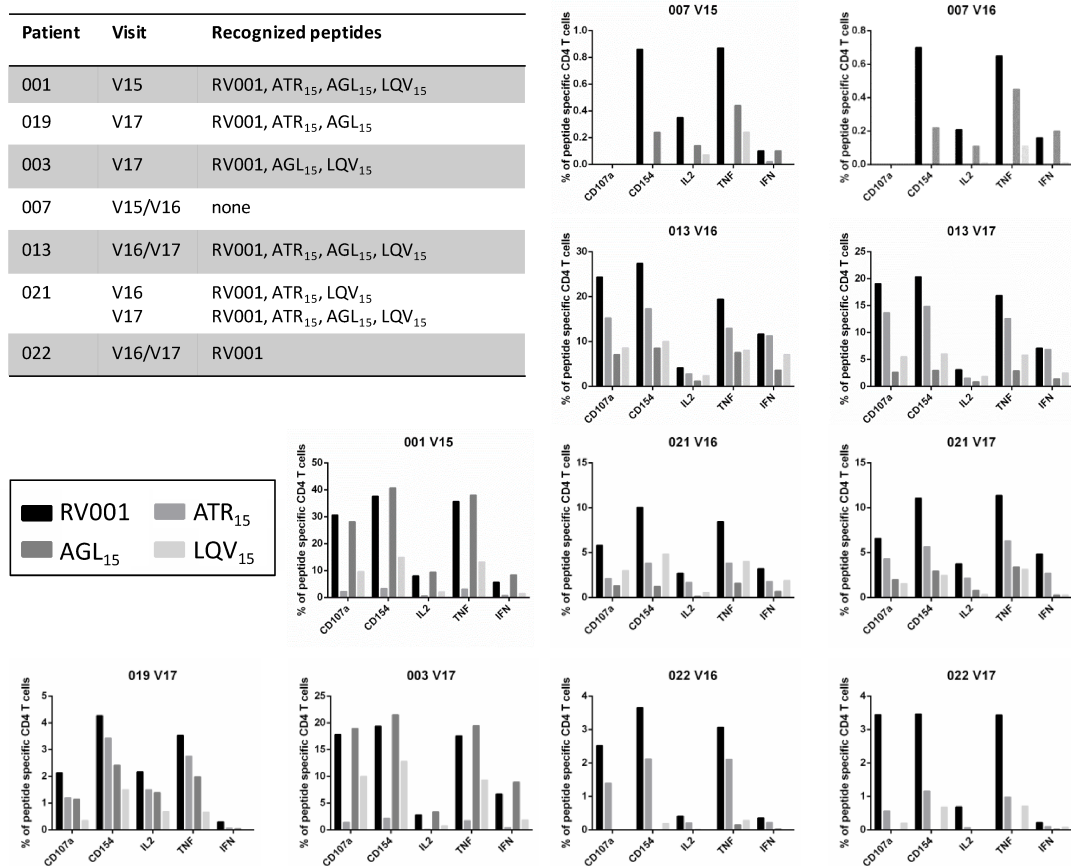




Supplementary figure S2: Gating strategy for the identification of Tregs.  $1.5 \times 10^6$  cells per test were treated with Fc block to avoid unspecific binding and stained (see online Supplementary table S5). Tregs were characterized as: sample flow over time, duplet exclusion (FSC-A/FSC-H), gating on live cells (FSC-A/Zombie Aqua dye), lymphocytes (FSC-A/SSC-A),  $CD3^+$  cells (upper row),  $CD8^-CD4^+$  T cells,  $CD127^-CD25^+$  cells,  $CD4^+FoxP3^+$  cells. Shown is one representative donor (003, middle row) at visit 2 and the respective isotype control (visit 17, bottom row).



Supplementary figure S3: Additional results of the *ex vivo* analysis of RV001 specific CD4 T cells (see also Fig. 3). PBMCs from Patient 018 visit 6 to visit 18 (A) and Patient 005 visit 6 to visit 15 (B) were incubated with the RV001 peptide (lower row) or ddH<sub>2</sub>O (upper row) for 12 h. TNF<sup>+</sup> CD4 T cells were identified (black) and overlaid on the whole CD4 T cell population (grey). Numbers indicate the number the CD4<sup>+</sup>TNF<sup>+</sup> cell counts in each quadrant. For gating, see Figure S1A.



Supplementary figure S4: Additional ICS results for the identification of RV001-derived HLA-class II epitopes (see also Fig 4). Table: tested patients and visits, as well as the peptides recognized by patients' CD4 T cells (see Material and Methods). Bars indicate percentages of peptide-specific CD4 T cells expressing CD107a, CD154, IL-2, TNF, IFN- $\gamma$  (IFN) when re-stimulated for 12 h with the peptides RV001 (black), ATR<sub>15</sub> (middle grey), AGL<sub>15</sub> (dark grey), LQV<sub>15</sub> (light grey). For gating, see Figure S1B.

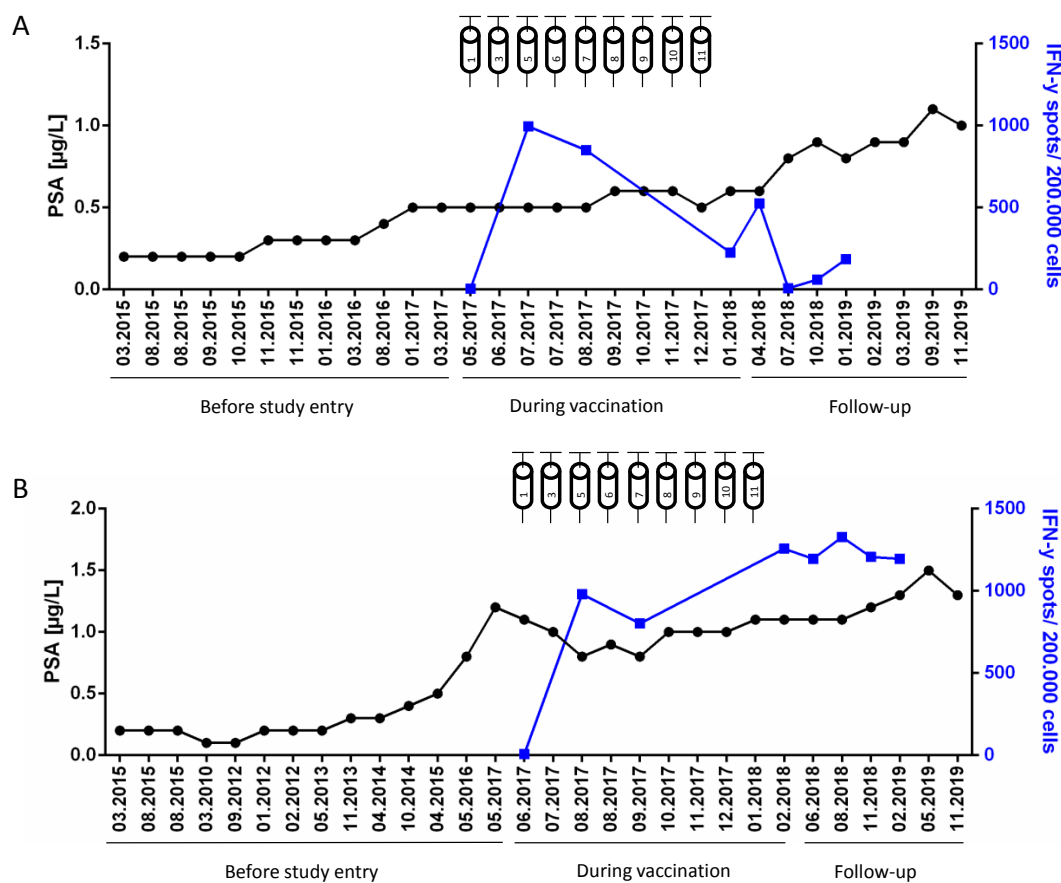


Figure S5: PSA level course before, during, and after vaccination for BCR patients at study entry ((A) Patient 006. (B) Patient 018). PSA levels (left y-axis) were measured by routine clinical testing at every patient visit before, during, and after vaccination (follow up) as indicated (x-axis). Corresponding IFN- $\gamma$  ELISpot counts per 200.000 cells (right y-axis) (see also results section and figure 1) are shown in blue. Syringes indicate times and number of vaccinations.