Supplementary Materials

Cancer Immunology, Immunotherapy (submitted in 2013) – Volker Lennerz et al.

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Supplementary MIATA information

Information on….		
….the sample	Patients	• Demographic data: Supplementary Table 4 • Disposition: Supplementary Figure 1 • HLA types: HLA-A1, HLA-A2, HLA-A3, HLA-A24, HLA-B7 • Disease: patients had a variety of different solid survivin expressing cancers • Treatment: vaccination with EMD640744 in Montanide ISA 51 VG (see Figure 1 for treatment schedule) • Co-medication: none
	Techniques	• Whole blood collection and PBMC processing was done in the five Swiss study centers by trained personnel; • 95 ml whole blood was obtained by venipuncture using Li-Heparin blood collection tubes at time points indicated in Figure 1 (if applicable: leukaphereses at baseline and week 12); • PBMC were isolated using Ficoll densitiy gradient centrifugation with Leukosep separation tubes (Greiner Bio-one, #227290); whole blood was not diluted; leukaphereses were diluted 1:2 with PBS/EDTA; • PBMC were frozen in FCS/10% DMSO at $1x10^7$/vial using 2-propanol-filled cryobox freezing containers (Nalgene, #5100-0001) at -80°C overnight and afterwards stored in liquid nitrogen until transport to the experimental laboratories in Mainz and Erlangen • PBMC were transported from study centers to experimental labs on dry ice within 24 hours • Upon arrival, all shipped vials were controlled for completeness and proper condition, and the vials were rapidly transferred to liquid nitrogen for storage until use; (every step of the shipment was monitored, respective data loggers were signed and sent to the study sponsor and a monitoring agency) • After thawing, before applied to the respective assays, PBMC were counted by trypan-blue staining, adjusted to $2x10^6$/ml in AIM and rested overnight in an incubator to remove dead as well as apoptotic cells; • Number and quality of PBMC was determined after ON-incubation again by trypan-blue staining and flow cytometry analyses; results are shown in Supplementary Figure 2A;

ELISpot		
….the assay	evELISpot	• *ex vivo* (ev)ELISpot assays are described in the Methods section; controls were PHA controls and a CMV/EBV peptide (CE-) mix (11 peptides binding to the relevant HLA alleles); responses are summarized in Supplementary Figure 2C; all spot counts were calculated as spot forming cells (SFC) per 10^5 CD8+ T cells, CD3/CD8 stains of all samples are shown in Supplementary Figure 2B;
	ivsELISpot	• *in vitro stimulated* (ivs)ELISpot assays (described in Methods)
	Reagents	• Reagents: ELISpot antibodies were Mab1-D1K and Mab7-B6-1-Biotin (Mabtech), assay medium was AIM V (Invitrogen) w/o serum; culture medium for short term T cell cultures was AIM V supplemented with 10% pooled human serum from healthy donors; Phytohaemagglutinin (PHA, Murex Biotech); CE peptide mix was received from JPT peptide technologies; EMD640744 and single peptides were supplied by the sponsor; antiCD3- and antiCD8 FACS antibodies were from BD Biosciences; media and sera were pretested for performance;
	Materials/ Equipment	• Materials/equipment: assay plates were MultiScreen Filterplates MSIP S4510 (Millipore); cell incubators, centrifuges and laminar flow cabinets were state-of-the-art devices for the sterile handling of human cell material;
….data acquisition	Machines/ Software	• ELISpot assays were analyzed using the KS Elispot Automated Reader System (Zeiss) running analysis software KS Elispot 4.9; Flow Cytometry analysis was done using the FACS Canto II Flow Cytometer (BD Biosciences) equipped with FACS Diva software; for Flow Cytometry analyses, FlowJo 7.5 was used;
	Calibration	machines were calibrated according to the manufacturers recommendations on a regular basis (FACS Canto once per week, KS Elispot approx. once per month);
	ELISpot analyses	• Spot analysis was performed exclusively by well trained and experienced persons; all well pictures were saved at high resolution to enable re-analysis of all data; back-ups of all analyzed data were saved on separate hard drives;
….results	Response criteria	• For evELISpot assays, a response was scored positive when the spot number was ≥10 and two-fold higher than background (PBMC only) and two-fold higher than the standard deviation of all combined negative values; for significance testing the student's T Test was applied to the calculation of p-values;
		• For ivsELISpot assays, responses of microcultures were scored positive when spot

	Auditing Correlation between data sets	numbers were at least two times higher than the average values of the background control plates (COS-7/HLA-transfectants); • Analyzed plates were checked for unexpected results (artifacts, high reactivity to negative control wells etc.) or discrepancies between spot counts and the visual impression of the respective wells; wells with very high spot numbers showing confluent spots were set arbitrarily to 500 or 1000 (only in cases where wells were completely coated with stain); • Since objective clinical response rates are generally low after cancer vaccination, clinical efficacy can only be reasonably assessed in an appropriately designed trial with a time-to-event endpoint. Therefore, a formal analysis to correlate clinical efficacy with immunologic efficacy was not planned.
….the lab environment	SOPs Qualification/ Training of personnel	• Standardized and validated *working practices* were in place for ELISpot assays, data evaluation and data storage • Only well trained personnel with more than five years' experience with all methods and technologies performed the experiments and data analyses as well as interpreted and reported results;
pHLA Multimer staining		
….the assay	evMultimer ivsMultimer Reagents Materials/ Equipment	• *ex vivo* (ev) peptide/HLA-multimer staining procedure is described in the Methods section; between 3.4 and 9 million cells (median: 6.8 million cells) were used; responses are summarized in Figure 2A; representative staining examples are shown in Supplementary Figure 3B • *in vitro stimulated* (ivs) peptide/HLA-multimer staining is also described in Methods • Reagents: peptide-HLA-tetramers for HLA-A1, A2 or A3 (Beckman Coulter, Krefeld, Germany) or PE-labeled peptide-HLA-dextramers for HLA-A24 and B7 (Immudex, Copenhagen, Denmark), surface-staining antibodies (CD8-PerCP, CD45RA-PE-Cy7, CD3-APC-H7, CD4-V450 and CCR7-AlexaFluor647 from BD and CD14-PacificOrange from Invitrogen), fix/perm solution and perm/wash (both eBioscience, Frankfurt a.M., Germany), intracellular staining antibody Granzyme B-FITC (BD), assay medium was RPMI1640 with 10% pooled human serum (both from Lonza), gentamicin, pyruvate and non-essential amino acids (Biochrome). EMD640744 and single peptides were supplied by the sponsor; media and sera were pretested for performance; • Cell incubators, centrifuges and laminar flow

		cabinets were state-of-the-art devices for the sterile handling of human cell material
….data acquisition	Machines/ Software Calibration Gating	• All Flow Cytometry assays were acquired on a FACS Canto II Flow Cytometer (BD Biosciences) equipped with FACS Diva software; for Flow Cytometry analyses, FlowJo 9.5 was used; • the FACS Canto II Flow Cytometer was calibrated according to the manufacturers recommendations on a regular basis (CS&T Performance Check on every day of usage); • For basic fitting of gates FMO (fluorescence minus one) controls were used; in addition, control cells (stimulated PBMC from healthy donors frozen in aliquots) were used as external references. The gating strategy is shown in Supplementary Figure 3A
….results	Response criteria Auditing Correlation between data sets	• Standard cut-off criteria for a positive response by pHLA-multimer staining were a minimum of 50 cells detected in the multimer-gate and a minimum percentage of 0.03% of the CD8+ T cells. In 8 patients smaller numbers of cells, just below the cut-off criteria were detected in the multimer gate in the evMultimer anaylsis but formed clear and well separated populations and were in 7 of 8 patients confirmed by ivsMultimer assays. Therefore after auditing those results were also considered positive. • All dotplots were audited and cells within the multimer gate were checked by backgating • Since objective clinical response rates are generally low after cancer vaccination, clinical efficacy can only be reasonably assessed in an appropriately designed trial with a time-to-event endpoint. Therefore, a formal analysis to correlate clinical efficacy with immunologic efficacy was not planned.
….the lab environment	SOPs Qualification/ Training of personnel	• Standardized and validated *working practices* were in place for evMultimer and ivsMultimer assays, data aquisition and analysis • Only well trained personnel with more than five years' experience with all methods and technologies performed the experiments and data analyses as well as interpreted and reported results;

Raw data are not accessible in general; however, all raw data have been reported to health authorities according to established regulations.

Restricting HLA allele	Peptide designation / amino acid substitution	Amino acid sequence*	References
A1	Sur93-101/T2	F<u>T</u>ELTLGEF	[1]
A2	Sur96-104/M2	L<u>M</u>LGEFLKL	[2, 3]
A3	Sur18-27/K10	RISTFKNWP<u>K</u>	[1]
A24	Sur20-28	STFKNWPFL	[4]
B7	Sur6-14	LPPAWQPFL	[5]

**The underlined amino acids are mutated and do not correspond to naturally-occurring survivin*

Supplementary Table 1: Peptides in EMD640744 and the respective restricting HLA alleles in the human population

References

1. Reker S, Meier A, Holten-Andersen L, Svane IM, Becker JC, thor Straten P, Andersen MH (2004). Identification of novel survivin derived CTL epiptopes. Cancer Biol Ther 3:173-179.
2. Andersen MH, Pedersen LO, Becker JC, Straten PT (2001) Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. Cancer Res 61:869-72.
3. Andersen MH, Pedersen LO, Capeller B, Bröcker EB, Becker JC, thor Straten P (2001) Spontaneous cytotoxic T-cell responses against survivin-derived MHC class I-restricted T-cell epitopes in situ as well as ex vivo in cancer patients. Cancer Res 61:5964-8.
4. Andersen MH, Soerensen RB, Becker JC, thor Straten P (2006) HLA-A24 and survivin: possibilities in therapeutic vaccination against cancer. J Trans Med 4:38-41.
5. Survivin-derived peptides and use thereof. US 20070148184 A1 http://www.google.co.in/patents/US8318174

Inclusion criteria

- Histologically or cytologically documented metastatic or locally advanced survivin-expressing solid tumor for which no established therapy exists
- Disease measurable by RECIST criteria or evaluable by clinical, radiographic, or laboratory criteria established for the given tumor entity
- Expressing ≥1 of the following HLA alleles: HLA-A1, -A2, -A3, -A24, and -B7
- ECOG performance status ≤1 and estimated life expectancy of ≥3 months
- Adequate:
  - hematologic function (WBC ≥3 × 109/L, lymphocyte count ≥0.5 × 10^9/L, hemoglobin ≥10 g/dL, and platelet count ≥100 × 10^9/L)
  - blood coagulation parameters (aPTT and INR ≤1.5 × ULN)
  - renal function (serum creatinine ≤2 × ULN)
  - hepatic function (total bilirubin ≤2 × ULN, and AST and ALT levels ≤2.5 × ULN [or ≤5 × ULN in subjects with liver metastases])
- Effective contraception for female and male subjects if the risk of conception existed
- ≥18 years of age

Exclusion criteria

- Treatment in another clinical trial within the past 30 days prior to the first administration of trial treatment
- Previous treatment with an investigational anticancer vaccine
- Requirement for concurrent treatment with a non-permitted drug*
- Receipt of allogeneic stem cell transplantation
- Active significant autoimmune disease (with the exception of vitiligo)
- Radiotherapy, chemotherapy, surgery (excluding prior diagnostic biopsy), immunotherapy, or any investigational drug within 30 days before the start of trial treatment
- Primary brain tumors and brain metastases**
- Rapidly progressive disease (e.g. tumor lysis syndrome)
- Significant acute or chronic infections (e.g. viral hepatitis, HIV)
- Pregnancy or lactation
- Active drug or alcohol abuse
- Known hypersensitivity to the trial treatment or any of its components
- Any significant disease requiring exclusion in the investigator's opinion
- Persisting toxicity to prior therapy at Grade ≥2 (NCI-CTCAE version 3.0)

*Including immunotherapy, immunosuppressive drugs, growth factors, oral anticoagulants. **Except brain metastases that are stable after irradiation or surgically-resected brain metastases if subjects had been asymptomatic for ≥6 months.
ALT: alanine aminotransferase; aPTT: activated partial thromboplastin time; AST: aspartate aminotransferase; ECOG: Eastern Cooperative Oncology Group; INR: international normalized ratio; NCI-CTCAE: Common Terminology Criteria for Adverse Events; RECIST: Response Evaluation Criteria In Solid Tumors; ULN: upper limit of normal; WBC: white blood cell count.

Supplementary Table 2: Eligibility criteria

Analysis set	30 µg		100 µg		300 µg		Total	
	N	(%)	N	(%)	N	(%)	N	(%)
Randomized subjects	17		17		17		51	
Safety/ITT analysis set	19	(100.0)	17	(100.0)	17	(100.0)	53	(100.0)
ID analysis set	12	(63.2)	13	(76.5)	13	(76.5)	38	(71.7)
ID-ITT analysis set	15	(78.9)	17	(100.0)	17	(100.0)	49	(92.5)

Supplementary Table 3: Analysis sets

Characteristic	30 µg (N=19)		100 µg (N=17)		300 µg (N=17)		Total (N=53)	
	N	(%)	N	(%)	N	(%)	N	(%)
Sex								
Male	11	(57.9)	10	(58.8)	10	(58.8)	31	(58.5)
Female	8	(42.1)	7	(41.2)	7	(41.2)	22	(41.5)
Age (years)								
Mean (SD)	55.9 (9.92)		58.7 (11.07)		53.8 (14.67)		56.1 (11.92)	
Median (Q1–Q3)	56.0 (49.3–63.2)		60.0 (53.0–64.9)		58.2 (50.0–61.6)		57.7 (50.9–63.4)	
Range	38–71		36–80		20–79		20–80	
Age category								
<65 years	15	(78.9)	13	(76.5)	15	(88.2)	43	(81.1)
≥65 years	4	(21.1)	4	(23.5)	2	(11.8)	10	(18.9)
Height (CM)								
Mean (SD)	172.5 (10.02)		170.5 (9.43)		170.2 (9.89)		171.2 (9.63)	
Median (Q1–Q3)	171.5 (167.0–179.0)		170.0 (167.0–180.0)		165.5 (164.0–179.0)		170.0 (165.0–179.0)	
Weight (kg)								
Mean (SD)	68.16 (16.085)		71.31 (12.055)		71.72 (12.535)		70.31 (13.612)	
Median (Q1–Q3)	65.00 (58.00–76.00)		71.00 (60.00–80.00)		74.00 (59.50–82.00)		70.00 (59.00–80.00)	
Body surface area								
Mean (SD)	1.814 (0.2285)		1.848 (0.1677)		1.789 (0.2117)		1.819 (0.2022)	
Median (Q1–Q3)	1.805 (1.678–1.899)		1.883 (1.716–1.980)		1.699 (1.626–1.988)		1.805 (1.671–1.959)	
ECOG PS								
0	16	(84.2)	11	(64.7)	15	(88.2)	42	(79.2)
1	3	(15.8)	6	(35.3)	2	(11.8)	11	(20.8)

Supplementary Table 4: Demographic characteristics (Safety / ITT analysis set)

HLA allele	30 µg (N=19)		100 µg (N=17)		300 µg (N=17)		Total (N=53)	
	N	(%)	N	(%)	N	(%)	N	(%)
A1	6	(31.6)	6	(35.3)	6	(35.3)	18	(34.0)
A2	13	(68.4)	11	(64.7)	10	(58.8)	34	(64.2)
A3	5	(26.3)	5	(29.4)	3	(17.6)	13	(24.5)
A24	2	(10.5)	3	(17.6)	3	(17.6)	8	(15.1)
B7	6	(31.6)	3	(17.6)	2	(11.8)	11	(20.8)

Supplementary Table 5: Presence of survivin peptide-presenting HLA class I alleles in the different dose groups (Safety / ITT analysis set)

Patient	Gender	Age	HLA type	Dose group	Tumor
C01P002	Male	56	A01, A03, B07	30 µg	Urothelial cancer
C01P007	Female	67	A03	100 µg	Colorectal cancer
C01P008	Male	57	A02	300 µg	Colorectal cancer
C01P009	Female	67	A01, A02	30 µg	Colorectal cancer
C01P012	Male	62	A02	300 µg	Colorectal cancer
C01P013	Male	36	A02	100 µg	Sarcoma
C01P015	Male	51	B07	30 µg	Renal cancer
C01P016	Female	28	A02	300 µg	Ovarian cancer
C01P017	Female	63	A01	30 µg	Adenoid cystic cancer
C01P018	Female	55	A01, A02	100 µg	Breast cancer
C01P024	Male	79	A01	300 µg	Colorectal cancer
C01P027	Male	60	A03	300 µg	Mesothelioma
C01P028	Female	44	A01, A03, B07	30 µg	Ovarian cancer
C01P029	Female	67	A02, A03	100 µg	Ovarian cancer
C01P032	Female	69	A02	30 µg	Ovarian cancer
C01P033	Female	51	A02	100 µg	Melanoma
C01P035	Male	58	A02, A24	30 µg	Head and neck cancer
C01P037	Female	58	A02	300 µg	Ovarian cancer
C01P040	Male	45	A02	30 µg	Mesothelioma
C02P003	Male	62	A01, A02	30 µg	Melanoma
C02P004	Male	63	A03	30 µg	Colorectal cancer
C02P007	Female	53	A03	300 µg	Melanoma
C02P010	Female	43	A02	30 µg	Melanoma
C03P001	Male	65	A24, B07	100 µg	Sarcoma
C03P002	Female	49	A03	30 µg	NSCLC
C03P005	Male	51	A02, B07	30 µg	NSCLC
C03P009	Female	20	A02	300 µg	Melanoma
C03P012	Male	71	A01, A24, B07	100 µg	Colorectal cancer
C03P014	Female	53	A02	100 µg	Breast cancer
C03P015	Male	65	A02	100 µg	Colorectal cancer
C03P016	Male	71	A01, A02	30 µg	Colorectal cancer
C03P017	Male	38	A01, A02	100 µg	Testicular cancer
C03P019	Male	34	A02	300 µg	Sarcoma
C03P020	Male	55	A01, A02	100 µg	Melanoma
C03P021	Male	52	A01	100 µg	Head and neck cancer
C04P004	Female	68	A02, B07	300 µg	Mesothelioma
C04P005	Male	60	A02, A03, B07	100 µg	NSCLC
C04P007	Male	58	A03	100 µg	Mesothelioma
C04P012	Female	64	A02, A24	100 µg	NSCLC
C04P013	Male	52	A01, A03	300 µg	Melanoma
C04P014	Male	68	A02, A03, B07	30 µg	Thymoma
C04P015	Male	80	A02	100 µg	Adenoid cystic cancer
C04P016	Female	59	A02	300 µg	Colorectal cancer
C05P002	Female	61	A01, A03	100 µg	Ovarian cancer
C05P005	Female	63	A01	300 µg	Ovarian cancer
C05P011	Male	51	A24	300 µg	Neuroendocrine tumor
C05P012	Male	61	A01, A24, B07	300 µg	Melanoma
C05P014	Male	47	A01, A02	300 µg	Melanoma
C05P017	Male	63	A01, A02	300 µg	Neuroendocrine tumor

Supplementary Table 6: Patient demographics, HLA type and tumor entity

Best response	**30 µg (N=19)**		**100 µg (N=17)**		**300 µg (N=17)**		**Total (N=53)**	
	N	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**
Complete response	0	–	0	–	0	–	0	–
Partial response	0	–	0	–	0	–	0	–
Stable disease	4	(21.1)	5	(29.4)	6	(35.3)	15	(28.3)
Progressive disease	11	(57.9)	9	(52.9)	9	(52.9)	29	(54.7)
Not evaluable	4	(21.1)	3	(17.6)	2	(11.8)	9	(17.0)

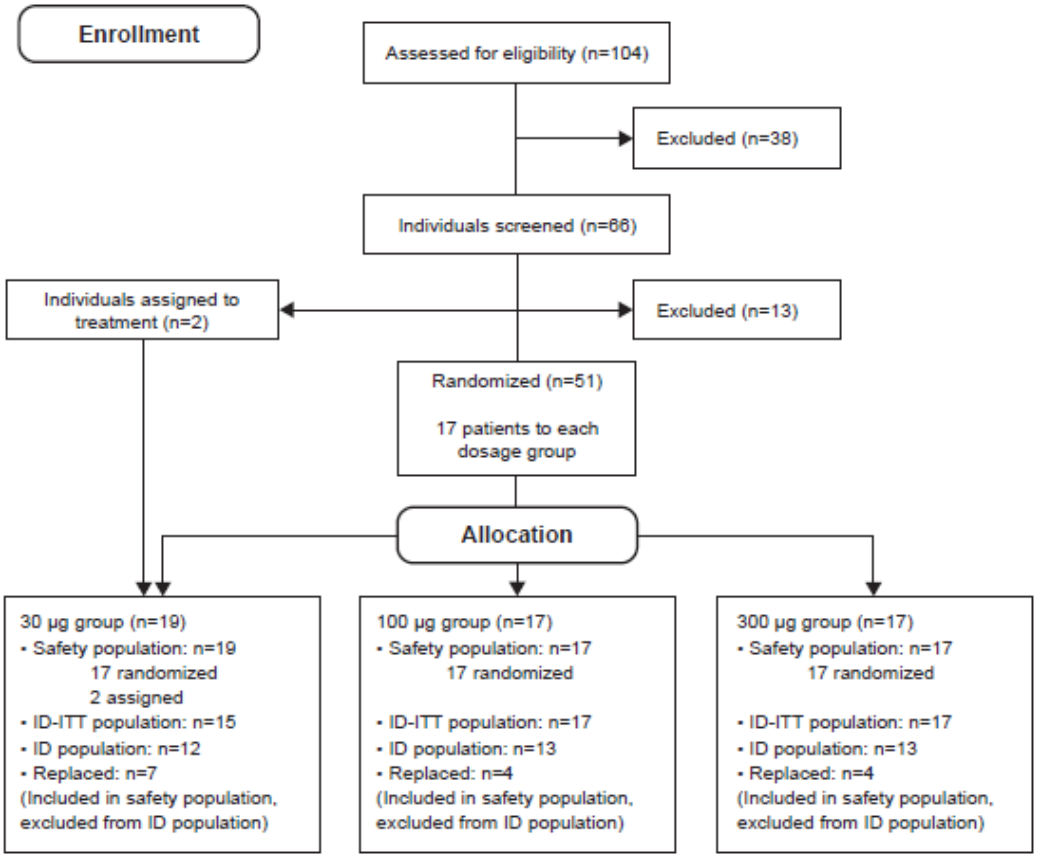
Supplementary Table 7: Best overall response within distinct dose groups (Safety / ITT analysis set)

MedDRA SOC	30 µg (N=19)		100 µg (N=17)		300 µg (N=17)		Total (N=53)	
	N	(%)	N	(%)	N	(%)	N	(%)
General disorders and administration site conditions	15	(78.9)	13	(76.5)	15	(88.2)	43	(81.1)
Gastrointestinal disorders	11	(57.9)	9	(52.9)	10	(58.8)	30	(56.6)
Musculoskeletal and connective tissue disorders	7	(36.8)	6	(35.3)	6	(35.3)	19	(35.8)
Respiratory, thoracic and mediastinal disorders	10	(52.6)	3	(17.6)	4	(23.5)	17	(32.1)
Skin and subcutaneous tissue disorders	7	(36.8)	3	(17.6)	4	(23.5)	14	(26.4)
Metabolism and nutrition disorders	4	(21.1)	4	(23.5)	4	(23.5)	12	(22.6)
Nervous system disorders	3	(15.8)	5	(29.4)	4	(23.5)	12	(22.6)
Infections and infestations	6	(31.6)	2	(11.8)	3	(17.6)	11	(20.8)
Investigations	3	(15.8)	4	(23.5)	4	(23.5)	11	(20.8)
Blood and lymphatic system disorders	3	(15.8)	1	(5.9)	2	(11.8)	6	(11.3)
Neoplasms benign, malignant and unspecified	1	(5.3)	3	(17.6)	1	(5.9)	5	(9.4)
Psychiatric disorders	1	(5.3)	2	(11.8)	2	(11.8)	5	(9.4)
Vascular disorders	2	(10.5)	1	(5.9)	1	(5.9)	4	(7.5)
Hepatobiliary disorders	0	-	0	-	2	(11.8)	2	(3.8)

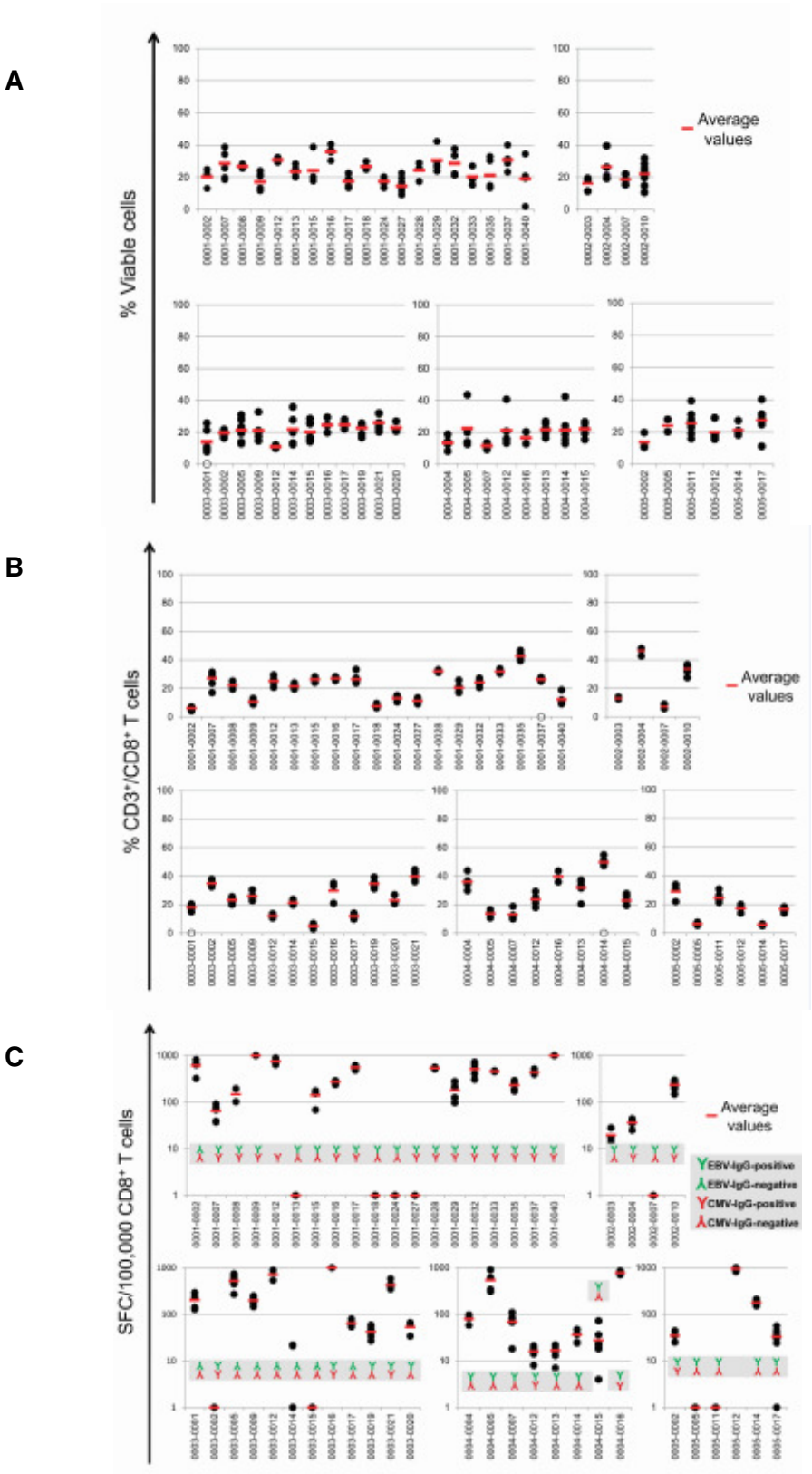
Supplementary Table 8: Incidence of TEAEs by MedDRA system organ class
(Safety / ITT analysis set; ≥10% incidence in any group)

MedDRA PT	30 µg (N=19)		100 µg (N=17)		300 µg (N=17)		Total (N=53)	
	N	(%)	N	(%)	N	(%)	N	(%)
Gastrointestinal disorders	0	-	1	(5.9)	0	-	1	(1.9)
Nausea	0	-	1	(5.9)	0	-	1	(1.9)
General disorders and administration site conditions	11	(57.9)	10	(58.8)	11	(64.7)	32	(60.4)
Asthenia	0	-	1	(5.9)	0	-	1	(1.9)
Chills	0	-	0	-	1	(5.9)	1	(1.9)
Fatigue	1	(5.3)	1	(5.9)	2	(11.8)	4	(7.5)
Granuloma	0	-	0	-	1	(5.9)	1	(1.9)
Induration	1	(5.3)	1	(5.9)	0	-	2	(3.8)
Injection site erythema	0	-	0	-	1	(5.9)	1	(1.9)
Injection site hematoma	1	(5.3)	0	-	0	-	1	(1.9)
Injection site induration	2	(10.5)	3	(17.6)	5	(29.4)	10	(18.9)
Injection site nodule	1	(5.3)	0	-	1	(5.9)	2	(3.8)
Injection site pain	3	(15.8)	1	(5.9)	2	(11.8)	6	(11.3)
Injection site pruritus	1	(5.3)	0	-	2	(11.8)	3	(5.7)
Injection site reaction	4	(21.1)	5	(29.4)	2	(11.8)	11	(20.8)
Injection site swelling	0	-	0	-	1	(5.9)	1	(1.9)
Pyrexia	0	-	1	(5.9)	0	-	1	(1.9)
Vaccination site induration	0	-	0	-	1	(5.9)	1	(1.9)
Hepatobiliary disorders	0	-	0	-	1	(5.9)	1	(1.9)
Hepatic pain	0	-	0	-	1	(5.9)	1	(1.9)
Musculoskeletal and connective tissue disorders	2	(10.5)	2	(11.8)	2	(11.8)	6	(11.3)
Muscular weakness	0	-	1	(5.9)	0	-	1	(1.9)
Musculoskeletal chest pain	0	-	0	-	1	(5.9)	1	(1.9)
Nodule on extremity	0	-	0	-	1	(5.9)	1	(1.9)
Pain in extremity	2	(10.5)	2	(11.8)	0	-	4	(7.5)
Tendonitis	0	-	1	(5.9)	0	-	1	(1.9)
Renal and urinary disorders	0	-	0	-	1	(5.9)	1	(1.9)
Polyuria	0	-	0	-	1	(5.9)	1	(1.9)
Renal failure	0	-	0	-	1	(5.9)	1	(1.9)

Supplementary Table 9: Incidence of treatment-related TEAEs by MedDRA PT (Safety / ITT analysis set)

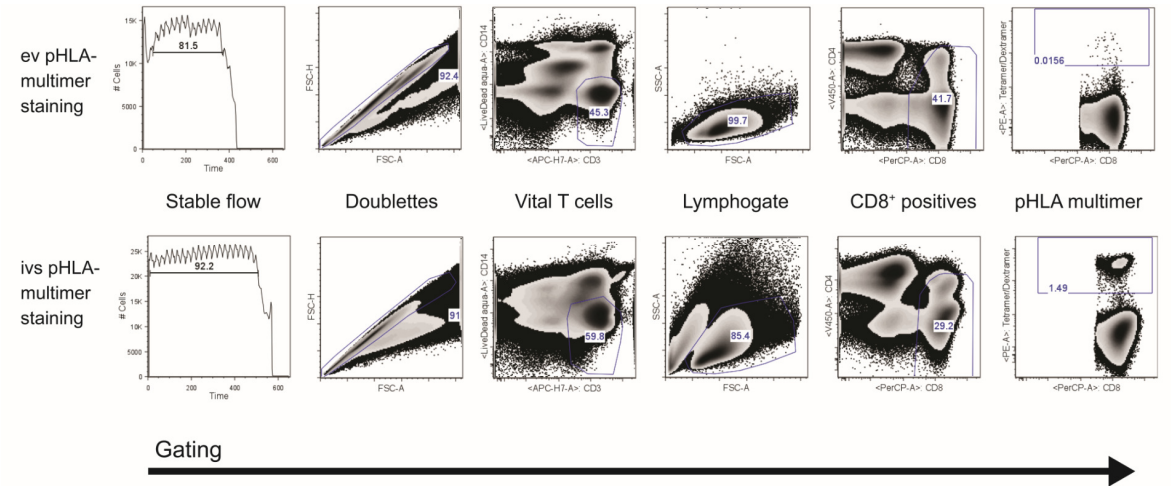


Supplementary Figure 1: Patient disposition

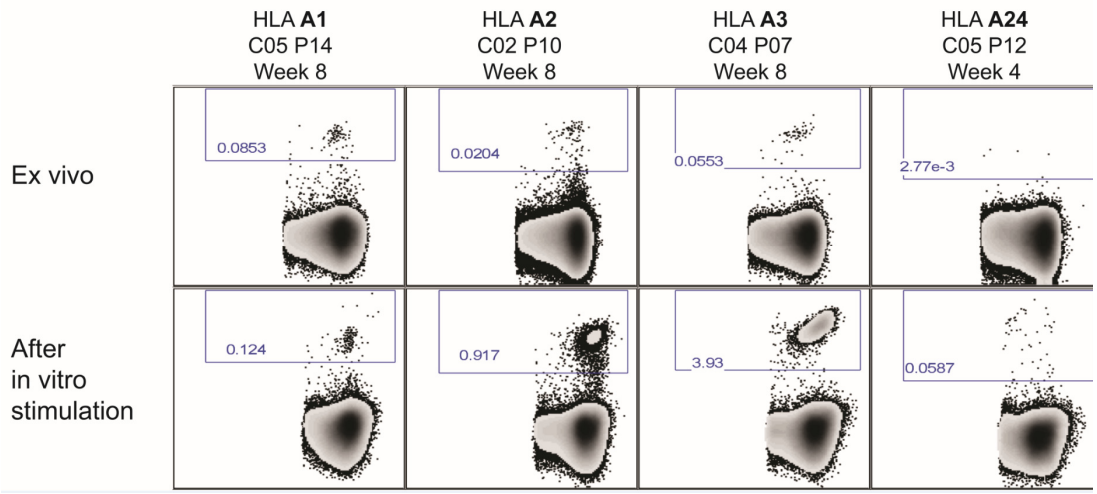


Supplementary Figure 2: Representative example data for controls of ELISpot assays. (A) After thawing of PBMC samples and overnight incubation for the setting up of ELISpot assays, cell yield and viability were determined by microscopy with trypan blue staining. (B) All PBMC samples tested by evELISpots were analyzed by flow cytometry to determine the proportion of $CD3^+/CD8^+$ T cells per sample. (C) In all evELISpot assays, T cell memory responses to a cocktail of CMV- and EBV-peptides (CEmix) were tested as a positive control. Antibody symbols depict the results of the CMV-/EBV-serology that was done for all patients in the centers where the patients were treated. Missing symbols mean that the serology was not done. SFC, spot-forming cells.

A



B



Supplementary Figure 3: Examples of staining and the gating strategy.