Short peptide vaccine induces CD4+ T helper cells in patients with different solid cancers

Keywords:
cancer vaccine, CD4+ T helper cell, short peptide vaccine

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Study number / Clinicaltrials.gov reference
EMR 200032-001 / NCT01012102
Abstract

Previous cancer vaccination trials often aimed to activate CD8⁺ cytotoxic T-cell (CTL) responses with short (8-10mer) peptides and targeted CD4⁺ helper T cells (T_H) with HLA class II–binding longer peptides (12-16mers) that were derived from tumor antigens. Accordingly, study immunomonitoring focused on the detection of CTL responses to the short, and T_H-responses to the long, peptides. The possible induction of concurrent T_H-responses to short peptides was widely neglected.

In a recent Phase I vaccination trial, 53 patients with different solid cancers were vaccinated with EMD640744, a cocktail of five survivin-derived short (9 or 10mer) peptides in Montanide® ISA 51VG. We monitored 49 patients, and found strong CD8⁺ T-cell responses in 63% of the patients. In addition, we unexpectedly found CD4⁺ T_H cell responses against at least two of the five short peptides in 61% (23/38) of patients analyzed. The two peptides were recognized by HLA-DP4– and HLA-DR–restricted T_H1 cells. Some short peptide–reactive (sp)CD4 T cells showed high functional avidity.

Here, we show that a short peptide vaccine is able to activate a specific CD4⁺ T cell repertoire in many patients, facilitating a strong combined CD4⁺/CD8⁺ T-cell response.
Introduction

Different forms of antigens for anti-cancer vaccination have been used recently in numerous clinical trials: tumor-associated proteins, DNA or RNA encoding the antigens, or long (12-16mers) or even “overlong” peptides (30 to 100mers), but most trials so far have vaccinated with short (8 to 10mer) peptides (1-5). Short (8 to 10mer) peptides are known to bind to HLA class I and induce CD8⁺ cytotoxic T-cell (CTL) responses, whereas longer peptides (12 or more amino acids) are required to activate CD4⁺ helper T cells (T_h) via presentation on HLA class II (6). Accordingly, immunomonitoring in clinical vaccination trials focused on detecting the expected immune responses - i.e., CD8⁺ CTL responses after vaccination with short peptides.

Here we report the follow-up of a Phase I cancer vaccination trial with EMD640744, a cocktail of five survivin-derived short (9 to 10mer) peptides in Montanide® ISA 51 VG (7). The trial’s primary objective was a comparison of three vaccine doses for immunological efficacy. Its secondary objectives were safety, tolerability, clinical efficacy, and the overall CD8⁺ T-cell responses detected by ELISpot and peptide-HLA (pHLA)-multimer assays. The study was remarkable, because of 49 patients eligible for immune monitoring, 31 (63%) showed vaccine-activated peptide-specific T-cell responses, as determined by IFN-γ ELISpot assays and/or pHLA-multimer analyses: 16 of 49 (33%) ex vivo and up to 28 of 36 (78%) after short-term in vitro stimulation.

To obtain more information on the quality of the induced immune responses, we further analyzed samples of 38 patients by a function-based flow cytometric assay combining intracellular staining for different cytokines and degranulation (multi-functional T-cell assay, MFTC). Using this assay, we confirmed the majority of previously observed immune responses, detected three additional CD8⁺ T-cell responses, and in a subgroup of 23 patients, found CD4⁺ T-cell responses to two of the five short peptides that were originally shown to bind to HLA-A2 and HLA-A3.
Materials and Methods

The reporting of the methods has been aligned with the MIATA guidelines (8,9). A detailed description of this section as a MIATA checklist can be found in the supplemental information.

Study design

The aim of the phase I trial was to determine the immunologic activity, safety and tolerability and clinical activity of EMD640744 in Montanide® ISA 51 VG in subjects with advanced solid tumors, conducted in five centers in Switzerland (Clinical trials.gov identifier nCt01012102). Details of the trial have been published (7).

Briefly, EMD640744 is a cocktail of Montanide® ISA 51 VG with five short peptides based on the amino acid sequence of different regions of the survivin protein previously shown to bind to HLA-A1 (FTELTLGEF, Sur93-101/T2), HLA-A2 (LMLGEFLKL, Sur96-104/M2), HLA-A3 (RISTFKNWPK, Sur18-27/K10), HLA-A24 (STFKNWPFL, Sur20-28), or HLA-B7 (LPPAWQPFL, Sur6-14), respectively.

Planned treatment was to last for 11 weeks of initiation therapy (8 treatments), followed by 13 weeks maintenance therapy (treatment every 4 weeks). Patients expressed at least one of the HLA-A1, -A2, -A3, -A24, -B7 alleles and were suffering from metastatic or locally advanced survivin-expressing solid tumors for which no established therapy exists. The primary objective of this trial was to compare three doses of EMD640744 administered by subcutaneous injection in combination with Montanide® ISA 51 VG with regard to immunologic efficacy. Secondary objectives of this trial comprised the assessment of the safety and tolerability and clinical efficacy of the three doses of study treatment.

Cells, peptides, and blocking antibodies

Peripheral blood mononuclear cell (PBMC) samples before and after vaccination were available for immunomonitoring from 49 of the 53 patients who started treatment. The monitoring with ELISpot and pHLA multimers, including all information about peptides, sample collection, and preparation have been described (7).

In brief: PBMC samples were prepared at the five study sites by Ficoll density gradient centrifugation. Cells were frozen in aliquots in freezing medium (90% FCS with 10% DMSO) and shipped to the immunomonitoring laboratory under dry ice and upon arrival rapidly transferred to liquid nitrogen for storage until use.

EBV-transformed lymphoblastic cell lines (EBV-LCLs) were generated as described elsewhere (10) and cultured in RPMI1640 supplemented with 20% FCS (PAA) and gentamycin. For peptide loading, EBV-LCLs were washed twice in PBS and incubated in
RPMI1640 together with the corresponding peptide at 5 μg/ml for one hour at room temperature. Then cells were washed in MLPC-Medium and used for the assay at a ratio of 1:1.

Survivin-peptides (Bachem) had a purity of >95%.

For blocking of pHLA–T-cell receptor (TCR) interactions the following antibodies were used at a concentration of 10 μg/ml: anti-HLA-ABC (BD, DX17), anti-HLA-DR/DP/DQ (BD, Tu39), anti-HLA-DR (Biolegend, L243), anti-HLA-DQ (Beckman Coulter, SPVL3) and anti-HLA-DP (abcam, B7/21).

**Multi-functional T cell assay (MFTC)**

Thawed cells were seeded in MLPC-Medium (RPMI1640 with 10% pretested human pooled serum (Lonza), gentamycin, pyruvate and nonessential amino acids) and stimulated with the corresponding peptide or the EMD640744 peptide mix (5-10 μg/ml). The next day, IL2 (5 U/ml, Roche) and IL-7 (10 ng/ml, TEBU) were added. Half of the medium was replaced every 3–4 days with fresh MLPC-medium containing IL2 (5 U/ml). No IL2 was given to the in vitro stimulated PBMCs in the last two days before the assay (day 12 – 15). On day 12 – 15 cells were restimulated overnight (37°C, 5% CO2) in MLPC medium with or without the corresponding peptide and blocking antibodies (as indicated in Fig. 2) in the presence of BrefeldinA, Monensin, CD107a- and CD154- antibodies. The next day, cells were washed, stained with dead-cell stain Live/Dead aqua (Invitrogen) according to the manufacturer’s instructions and subsequently with surface-staining antibodies (CD8, CD4, CD14). After washing, cells were fixed and permeabilized with fix/perm solution and perm/wash (both eBioscience) according to the manufacturer’s instructions. Intracellular staining was performed in perm/wash with IL2-, TNFα- and IFNγ- antibodies, for 30 minutes. Cells were then washed and resuspended in PBS.

For each sample a negative buffer control (without peptide) was assessed. The values obtained from those negative controls were subtracted from the corresponding test samples. Criteria for a positive response were: results of individual cytokine-producing subsets at least two-times higher than the corresponding subsets in the negative control (background), and the sum of all cytokine-producing subsets greater than 0.03%.

Representative example data for an MFTC assay, including gating strategy, can be seen in Supplemental Fig. S1.

Samples were acquired on a FACS CANTO II flow cytometer with FACSDiVa software. The performance status of the FACS Canto II flow cytometer was checked with cytometer setup and tracking beads (BD) each day right before the sample acquisition. For Data analysis FlowJo version 9.7.5 was used.
Ethical considerations

This trial was conducted in accordance with the protocol and protocol amendments, the International Conference on Harmonization (ICH) guideline for Good Clinical Practice (GCP), applicable local regulations and the Declaration of Helsinki, and was approved by independent ethics committees and by Swiss Medic. Written informed consent was received from participants prior to inclusion in the study.
Results

Induction of CD4+ and CD8+ T cell responses after vaccination

From the 53 patients vaccinated and 49 eligible for primary response analysis, a subgroup of 38 patients had sufficient PBMCs for a more detailed analysis of immune responses by a function-based flow cytometric assay (multi-functional T-cell assay, MFTC). Compared to the ELISpot and pHLA-multimer analyses, the MFTC assay could characterize the cytokine profiles and polyfunctionality of the responding T cells, analyzing CD8+ and CD4+ T-cell subsets separately. Presuming activation of CD8+ T-cell responses by vaccination, at first only the peptides matching the patients class I HLA types were tested. After discovering CD4+ T-cell reactivity (Fig. 1A), the complete cocktail of the five peptides contained in EMD640744, and single peptides not matching patients HLA, were tested for the induction of CD4+ T-cell responses (if sufficient PBMC material was available).

We detected three additional CD8+ T-cell responses by MFTC, which raised the number of patients with a CD8+ T-cell response to the vaccine to 34 out of 49 patients (69%, summarized in Table 1). In addition to the CD8+ T-cell responses, the MFTC assay detected CD4+ T cells responding to the short peptides contained in EMD640744 in 23 of the 38 patients (61%) after vaccination. Similar to the published CD8+ T-cell responses (7), no differences in the CD4+ T-cell responses were observed among the three different vaccine-dose groups (primary objective of the trial).

In 15 of the 38 patients PBMC material collected before vaccination was available for analysis by MFTC. In only one of the 15 pre-vaccination samples a CD4+ T-cell response was detected. However, in this patient (C03P015) the amount of responding CD4+ T cells increased 30-fold from 0.1% at baseline to 3% analyzed 17 weeks after start of vaccination. Of the remaining 14 patients, seven developed a CD4+ T-cell response after vaccination, indicating a de novo induction of spCD4 T-cell responses in at least those seven patients.

In 17 of the 23 responding patients we identified either the HLA-A2 or the -A3 binding peptides Sur96-104/M2 and Sur18-27/K10 as inducers of short peptide CD4+ (spCD4) T-cell responses (e.g., in Fig. 1A). The remaining six patients had too little PBMC material available to test the single peptides, so only the cocktail EMD640744 was tested. In general, patients with a spCD4 response, tested with the single peptides and EMD640744, had a response to EMD640744 and either Sur96-104/M2 or Sur18-27/K10, indicating that only those two of the five peptides were capable of inducing spCD4 responses.

The spCD4 T-cell responses were characterized by a pronounced production of TNFα alone or in combination with IFNγ and/or IL2, a clear Th1-cytokine profile (Fig. 1B).
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**HLA restriction of short peptide (sp)CD4 T-cell responses**

To further characterize the newly detected spCD4 responses, MFTC assays were repeated with or without pan-HLA class I- or class II-blocking antibodies. In all cases investigated, the HLA class II antibodies completely blocked the spCD4 T-cell responses, whereas HLA class I-specific antibody had no effect, indicating that spCD4 T cells recognize the survivin peptides in the HLA class II context (Fig. 2A).

This provided an explanation for the observation in patients C03P005, C04P012 and C04P015 where a spCD4 T-cell response against the A3-binding peptide was detected despite the fact that these patients were HLA-A3 negative.

To define the HLA class II-restriction, T cells were stimulated with the respective peptides in the presence of HLA class II subtype-specific antibodies to specifically block TCR-interactions with HLA-DR, HLA-DQ, or HLA-DP, respectively. spCD4 responses to Sur96-104/M2 could be blocked with the HLA-DP-binding antibody in the four patients analyzed (Fig. 2B), whereas responses to Sur18-27/K10 were blocked by the HLA-DR-specific antibody in three patients analyzed. In one of those patients (C04P012) a very high background (without peptide) reactivity was observed, however, peptide-reactivity was clearly stronger and blocking with HLA-DR antibody reduced the response far below the observed background. Unfortunately there were no cells left to repeat the experiment. (Fig. 2C).

HLA-typing of all responding patients for HLA-DR, HLA-DQ, and HLA-DP revealed that all patients reacting to Sur96-104/M2 were positive for HLA-DP4, and spCD4 cells only recognized peptide-loaded HLA-DP4, but not HLA-DP1/DP2-expressing EBV-transformed lymphoblastic cell lines (EBV-LCLs) (Fig. 2D), demonstrating that peptide Sur96-104/M2 was recognized in the HLA-DP4 context.

Patients reacting to the Sur18-27/K10 peptide were positive for HLA-DR4, -DR7, -DR11, or -DR16. Loading of the peptide onto different EBV-LCLs expressing only one of these alleles showed strong cross-reactivity to these closely related alleles (data not shown), indicating that Sur18-27/K10 exhibits a rather promiscuous binding to different HLA-DR alleles and that there are T cells recognizing the peptide in the context of various HLA alleles.

**Functional avidity of Sur96-104/M2- and Sur18-27/K10-specific spCD4+ T cells**

In six patients tested with different concentrations of the respective peptides, spCD4 T-cell responses were readily detectable at concentrations of 1-5 μg/ml. spCD4 T cells of...
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patients C02P010 and C01P007 still showed weak responses at 0.1 μg/ml and spCD4 T cells of patient 0004-0015 even reacted to peptide Sur18-27/K10 at a concentration as low as 0.01 μg/ml (Fig. 3A).

Recognition of native peptides by modified peptide-activated spCD4 T cells

Three peptides in EMD640744 (Sur96-104/M2 and Sur18-27/K10 among them) contained optimized anchor residue, i.e., are modified in one position compared to the native sequence. We and others have shown, at least for CD8+ T cells in the HLA class I context, that such modified peptides can induce T cells that recognize their native counterparts, as well (7,11,12).

Here, we demonstrate that the spCD4 T cells induced by vaccination with the modified peptides contained in EMD640744 also could recognize the native counterparts of Sur96-104/M2 (Sur96-104) and Sur18-27/K10 (Sur18-27), though responses to the native forms were weaker or almost absent in some cases (Fig. 3B). In Patient C04P015 recognition was tested of longer variants of the native peptide (Fig. 3C). Despite the fact that spCD4 T cells induced by Sur18-27/K10 barely recognize the native sequence of Sur18-27, those cells do respond to the slightly longer peptide Sur17-28 almost as well as to Sur18-27/K10. Further lengthening of the peptide increases the reactivity only slightly, up to the maximum response seen with Sur18-27/K10.
Discussion

In this report we show that the majority of patients undergoing active cancer immunotherapy with EMD640744 had combined CD4+/CD8+ T-cell responses to short peptides. In addition to the CD8+ T-cell responses generated by EMD640744-vaccination and revealed by ELISpot- and pHLA-multimer staining assays (7), the MFTC assay detected prominent CD4+ T-cell responses to EMD640744, in particular against two of the five short peptides. Thus, the EMD640744 vaccine can activate both CD8+ and CD4+ T-cell responses. In several patients CD4+ T-cell responses were even stronger (i.e., of higher frequency) than CD8+ T-cell responses (representative example in Fig. 1A). In eight of the 23 patients with spCD4 T cell reactivity the spCD4-response was tested but not detectable in pre-vaccination samples, implying a vaccine-mediated de novo induction of responses. In only one patient was a pre-vaccination spCD4 T-cell response detected (C03P0015), which increased up to 30-fold after vaccination. This pre-existing spCD4 T-cell response also points towards a role in vivo.

CD4+ T-cell responses to short peptides have recently been reported in the context of influenza A- and mycobacterium tuberculosis–derived peptides (13,14). The first such report in the context of tumors concerned a Melan-A/MART-1–derived decamer peptide resembling a minimal CD4+ T cell epitope, as presented by P. Romero´s group (15). The same group also found CD4+ T cells specific for the short Melan-A/MART-1 peptide in patients after vaccination with the peptide (16). In addition, the group of K. Itoh reported a single case of spCD4 T cells that were induced by vaccination with a nonamer peptide derived from the ubiquitin-conjugated enzyme variant Kua (UBE2V) (17).

Precisely how the short peptides are presented on HLA class II still remains unclear. It is likely that they represent minimal core peptides, having the correct anchor amino acid residues for binding to the respective class II alleles. Recognition of the core peptide by the TCRs of spCD4 T cells is sufficient to cause activation. The binding motif of the DP4 allele displays two main hydrophobic/aromatic anchors at position P1 (preferentially phenylalanine or leucine) and P6 (preference for phenylalanine) and an additional anchor at P9 favoring leucine (18). The DP4-binding Sur96-104/M2, with its sequence LMLGEFLKLL, corresponds well to the DP4 binding motif. In contrast to this, the Sur18-27/K10 sequence RISTFKNWPK does not fit to the described (19) peptide binding repertoires of common HLA-DR types. Only the isoleucine in position 2 might resemble a P1 anchor for HLA-DR4. Therefore, one could speculate that in the case of Sur18-27/K10, the presence of classical anchor residues might not be the primary reason for HLA class II binding properties, which may rely on other, yet unknown, features of the sequence. This is in line with our finding of promiscuous binding of this peptide to several HLA-DR alleles.
and associated crossreactivity of spCD4 T cells. Of note is that the native variant of Sur18-27 is not, or is only very weakly, recognized by Sur18-27/K10-specific spCD4 T cells. However, Sur17-28 is merely two amino acids longer, and is recognized almost equally well as Sur18-27/K10 (Fig. 3C). This indicates an important role for HLA-binding of the lysine at position 10 in the short peptide; however the presence of the leucine at the neighboring position in the longer variant seems to compensate for the presence of the native phenylalanine at P10.

spCD4 responses were thought to be rare cases and rather anecdotal, because they have not been observed in other trials utilizing short nonamer peptides. A likely explanation for why spCD4 T-cell responses to short peptides are not observed more often is that standard immunomonitoring technologies like ELISpot assays with PBMC cannot discriminate between CD8+ and CD4+ T cells and pHLA class I-multimer staining only detects T-cell responses in the HLA class I context. It was the implementation of the MFTC assay that enabled us to separate CD4+ from CD8+ T-cell responses and characterize the T\textsubscript{H}-subtype responding to the short target peptides. By MFTC we also detected three additional CD8+ T-cell responses, indicating that in some cases the MFTC assay might be even more sensitive than other assays, though also consuming more cells.

The relevance of CD4+ T cells for the promotion of productive CD8+ T-cell responses has been shown by Schoenberger et al. (20) and other groups. Similarly, a long CD4+ T cell epitope derived from survivin, among several short CD8+ epitopes, proved to be beneficial in a vaccination trial by the Rammensee group (21). The promotion of specific CD4+ T-cell responses to EMD640744 that we see in this trial may explain the high number of CD8+ immune responses since activation of CD8+ T cells is much more efficient if adequate CD4+ T cell help is available. In fact, of the 29 patients presenting a T-cell response in the MFTC assay, 16 (55 %) showed a combined response of CD4+ and CD8+ T cells, whereas we found a “spCD4 only” response in only in six patients. In the remaining eight patients with a “CD8+ only” response, some spCD4 responses might have been missed, since some patients were only tested with the single peptides matching patients HLA, but not with the full cocktail. In addition, CD4+T cell help may not only benefit the induction of CD8+ T cells. Matsueda et al. showed that antibody responses to short CTL-epitopes are widely detectable and correlate with better overall survival (22). Since the induction of humoral responses is thought to depend on CD4+ T-cell responses, it is likely that also some of the 31 different CTL epitopes used in their study served as spCD4 targets. Unfortunately no serum samples were available in our trial to check for antibody responses to the short peptides from EMD640744. Since we only looked for classical T\textsubscript{H}1 cytokines
such as TNF α, IFN γ, and IL2 we cannot rule out a (maybe even broader) T_{H2} spCD4 response.

Within this small phase I trial it was not possible, and is not planned, to correlate the exceptionally high number of survivin-specific CD8+ T-cell responses together with the unexpected spCD4 T-cell responses induced by EMD640744 with progression-free or overall survival, since the study population was too small and too heterogenous, including a variety of different tumor types.

The physiological relevance of the detected spCD4 T-cell responses still remains unclear. However, a phase II study using two of the five survivin peptides contained in EMD640744, with one of them being an inducer of spCD4 T-cell responses, showed a correlation of prolonged survival with the induction of CD8+ T-cell responses as monitored by pHLA-multimer staining (23). In addition, Hunder et al. reported on the direct antitumor efficacy of CD4+ T cells. They present a case study where a single infusion of NY-ESO-1 specific CD4+ T cell clones led to complete tumor regression and durable clinical remission (24). Hunder et al. primarily discuss the extensive cytokine production of the infused cells and subsequent activation of the patient’s immune system as the mode of action of the infused CD4+ T cells. However, it has also been reported that CD4+ T cells can efficiently destroy tumor cells independently (25,26) and moreover, in a more recent study, that perforin and granzyme B secreting cytotoxic NY-ESO-1 specific CD4+ T cells developed in melanoma patients after treatment with ipilimumab (27).

In aggregate CD4+ T cells specific for small tumor peptides may play an important and hitherto unrecognized role in the immune concert fighting cancer cells and thus should be studied more extensively in future clinical trials.

Acknowledgements

The authors would like to thank the patients, the investigators, co-investigators, and study teams at each of the participating centers and at Merck KGaA, Darmstadt, Germany. Especially we thank Cristiana Sessa (Study center Belinzona) and Juergen Zieschang (Merck KGaA). The contributions are also gratefully acknowledged of Annett Hamann and the team of the cell sorting and immunomonitoring core unit, Erlangen, for their excellent technical assistance.
Disclosures and conflict of interest statement

The study was sponsored by Merck KGaA Darmstadt, Germany. Ulrike Gnad-Vogt was employee of Merck Serono from 2005-2009 and medical consultant for Merck Serono from 2009-2011. Ulf Forssmann was employee of Merck Serono from 2006-2013. The authors take full responsibility for the content of this publication.
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References


Table 1

Results – Summary of all detected T cell responses to EMD640744 (cocktail of five peptides) or the single peptides.

Responses detected by ELIspot and/or peptide HLA multimer staining have been published previously (7) and are summarized together for better comparison in one column. The pre-vaccination responses were derived from one time-point, just before the first vaccination. The post-vaccination responses were measured in available samples (one to eight samples per patient) from different time-points after vaccination (weeks 4, 8, 12, 16, 17, 24, 36, or end-of-study (EOS)). Patients received at least four, usually more, vaccinations according to the vaccination schedule (7). A patient was scored positive if a T cell response could be detected in at least one sample. MFTC assay for detection of spCD4 responses was performed in patients with available samples from pre-vaccination (n = 15) and post-vaccination (n = 38) time-points.

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**Figure 1**

**spCD4 T cells.** As shown by MFTC, vaccination with EMD640744 induces not only CD8+ T cell responses but also specific CD4+ T cell responses.

(A) Representative example showing T cell responses to peptide Sur18-27/K10 and the corresponding negative controls (without peptide) before and after vaccination in patient C03P002. (B) Cytokine expression pattern of all patients exhibiting a spCD4 T cell response (different cytokine subsets as % of all cytokine-producing CD4+ T cells, background subtracted). For each patient only one time-point, the one with the highest frequency of spCD4 T cells, is shown. (C) Cumulated results from all patients/ time-points as shown in Fig. 1B; mean percentages of cytokine-producing subsets.
**Figure 2**

**HLA restriction of spCD4 T cell responses.** (A) EMD640744-induced spCD4 responses were blocked with a pan HLA class II blocking (shown in six patients) but not a pan HLA class I blocking antibody (shown in four patients). (B and C) HLA-restriction of the two spCD4 T cell response-inducing peptides, Sur96-104/M2 (B) and Sur18-27/K12 (C), was tested by stimulating T with the respective peptides in the presence of antibodies specifically blocking TCR-interaction with HLA-DR, HLA-DQ or HLA-DP. Each bar in a group resembles one patient. Relevant HLA-alleles of the corresponding patient are given below, behind the patient IDs. Values were normalized with the positive control (pos = stimulation with the respective peptide but without blocking antibodies) set to 100%. This was also done for the negative control (neg = stimulation without peptide and without antibodies). (D) Cells from patient C02P010 were exemplarily tested for reactivity to Sur96-104/M2 loaded either onto DP4 positive or onto DP1/DP2 expressing EBV-B transformed lymphoblastic cell lines (EBV-LCL).
Figure 3

Recognition of Sur96-104/M2 and Sur18-27/K10 and their native variants by spCD4 T cells

(A) Recognition of different concentrations of Sur96-104/M2 and Sur18-27/K10 by spCD4 T cells in six patients. Shown as sum of all cytokine-producing spCD4 cells in % of total CD4 T cells. Corresponding background of each sample (stimulation without peptide) is marked on the axis as peptide dose of 0 μg/ml. Small marks represent values that are below the two-fold background, whereas large marks represent values that are considered positive and are bigger than the two-fold background.

(B) Recognition of the native peptide variants Sur96-104 and Sur18-27 in comparison to the modified peptides Sur96-104/M2 and Sur18-27/K10 contained in EMD640744 was tested in five patients.

(C) In patient C04P015, who strongly reacts to the modified Sur18-27/K10 but only very weakly to the native Sur18-27, also reactivity was tested against three longer peptides containing the native sequence of Sur18-27: Sur13-27 (FLKDHRISTFKNWPF), Sur17-28 (HRISTFKNWPFL) and Sur18-32 (RISTFKNWPFLEGCA).
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No of patients tested 49 15 15 49 38 38
No of responders 8 1 3 31 23 23
% positive of Samples analyzed 16.3 6.7 20.0 63.3 60.5 60.5
Induction of CD4+ T helper cells by a short peptide vaccine in patients with different solid cancers – Figure 1

A

B

C

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Induction of CD4⁺ T helper cells by a short peptide vaccine in patients with different solid cancers – Figure 2

**A**

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<th>Peptide</th>
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**B**

- **Figure 2**

- **A**

**C**

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<th>Peptide</th>
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**D**

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<td>Sur96-104/M2 loaded on HLA DP4</td>
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Induction of CD4+ T helper cells by a short peptide vaccine in patients with different solid cancers – Figure 3

A

B

C

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Cancer Immunology Research

Short peptide vaccine induces CD4+ T helper cells in patients with different solid cancers

Stefanie Gross, Volker Lennerz, Elisa Gallerani, et al.

Cancer Immunol Res  Published OnlineFirst November 12, 2015.

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