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A Frameshift Peptide Neoantigen-Based Vaccine for Mismatch Repair-Deficient Cancers: A Phase I/IIa Clinical Trial

Matthias Kloor[1,2,3], Miriam Reuschenbach[1,2,3], Claudia Pauligk[4], Julia Karbach[5], Mohammad-Reza Rafiyan[5], Salah-Eddin Al-Batran[4], Mirjam Tariverdian[6], Elke Jäger[5], and Magnus von Knebel Doeberitz[1,2,3]

ABSTRACT

Purpose: DNA mismatch repair (MMR) deficiency is a hallmark of Lynch syndrome, the most common inherited cancer syndrome. MMR-deficient cancer cells accumulate numerous insertion/deletion mutations at microsatellites. Mutations of coding microsatellites (cMS) lead to the generation of immunogenic frameshift peptide (FSP) neoantigens. As the evolution of MMR-deficient cancers is triggered by mutations inactivating defined cMS-containing tumor suppressor genes, distinct FSP neoantigens are shared by most MMR-deficient cancers. To evaluate safety and immunogenicity of an FSP-based vaccine, we performed a clinical phase I/IIa trial (Micoryx).

Patients and Methods: The trial comprised three cycles of four subcutaneous vaccinations (FSP neoantigens derived from mutant *AIM2, HT001, TAF1B* genes) mixed with Montanide ISA-51 VG over 6 months. Inclusion criteria were history of MMR-deficient colorectal cancer (UICC stage III or IV) and completion of chemotherapy. Phase I evaluated safety and toxicity as primary endpoint (six patients), phase IIa addressed cellular and humoral immune responses (16 patients).

Results: Vaccine-induced humoral and cellular immune responses were observed in all patients vaccinated per protocol. Three patients developed grade 2 local injection site reactions. No vaccination-induced severe adverse events occurred. One heavily pretreated patient with bulky metastases showed stable disease and stable CEA levels over 7 months.

Conclusions: FSP neoantigen vaccination is systemically well tolerated and consistently induces humoral and cellular immune responses, thus representing a promising novel approach for treatment and even prevention of MMR-deficient cancer.

Introduction

The success of immune therapy in cancer is linked to the presence of appropriate cancer antigens that can be recognized and targeted by the immune system. The number of nonsynonymous mutations determines the load of mutational neoantigens and may represent a valuable biomarker predicting the efficacy of immune intervention (1). Microsatellite-unstable (MSI, in the present article used synonymous with high-level microsatellite-unstable or MSI-H) cancers represent a particularly promising tumor type for immune therapy. They arise as a consequence of DNA mismatch repair (MMR) deficiency (2) and therefore accumulate more than 10-fold higher numbers of somatic mutations than microsatellite-stable tumors (3, 4). MSI occurs in about 15% of colorectal cancers, about 25% of endometrial cancers, and a variety of other tumors.

MSI cancers have a heterogeneous pathogenesis. Notably, tumors developing in the context of the most frequent inherited cancer syndrome, Lynch syndrome, are characterized by MSI (2, 5). Lynch syndrome is caused by monoallelic germline mutations of one of the four DNA MMR genes, *MLH1, MSH2*, and, with lower penetrance, *MSH6* followed by *PMS2* (5). Individuals with Lynch syndrome have a lifetime risk of up to 80% to develop MSI cancer, predominantly in the colorectum and the endometrium (6). Independent from Lynch syndrome, MSI cancers can also develop sporadically, either as a consequence of *MLH1* promoter methylation in frame of the CpG island methylator phenotype (CIMP), or of biallelic MMR gene inactivation caused by two somatic mutations (7, 8). Families with clinical evidence for an inherited risk of developing MSI cancer, but no identifiable MMR gene mutations are commonly referred to as "Lynch-like" (9).

In MSI cancers, MMR deficiency leads to the accumulation of a plethora of insertion or deletion mutations at microsatellite sequence stretches, which, if located in gene-encoding regions, cause translational frameshifts and give rise to the generation of frameshift peptide (FSP) antigens (10–12). Several such MMR deficiency-induced FSP antigens have been characterized in detail (13–15), and patients with MSI cancer spontaneously develop immune responses towards FSP antigens (12, 16).

The evolution of MSI cancers is promoted by mutational inactivation of a limited number of coding microsatellites (cMS)-containing tumor suppressor genes. This concept of recurrent and shared mutational events with functional relevance has first been described in a

[1]Department of Applied Tumor Biology, Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany. [2]Clinical Cooperation Unit Applied Tumor Biology, DKFZ (German Cancer Research Center) Heidelberg, Heidelberg, Germany. [3]Molecular Medicine Partnership Unit (MMPU), EMBL Heidelberg, Heidelberg, Germany. [4]Institute of Clinical Cancer Research (IKF), Krankenhaus Nordwest, UCT University Cancer Center, Frankfurt, Germany. [5]Clinic for Oncology and Hematology, Krankenhaus Nordwest, Frankfurt, Germany. [6]Department of Surgery, University Hospital Heidelberg, Heidelberg, Germany.

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Corresponding Author: Matthias Kloor, Heidelberg University, Heidelberg 69120, Germany. Phone: 4962-2156-5210; Fax: 4962-2156-5981; E-mail: matthias.kloor@med.uni-heidelberg.de

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Translational Relevance

Effective cancer-preventive vaccines would mark a revolution in medicine. Demonstration of their feasibility is a major milestone on the way towards tailored prevention approaches. This is only possible in defined high-risk populations. We demonstrate in the setting of Lynch syndrome and history of mismatch repair-deficient colorectal cancer that vaccination with shared and predictable neoantigens is clinically safe and does not induce systemic side effects. Moreover, our data provide evidence that a combinatorial vaccine of three recurrent mutation-induced neoantigens leads to the induction of measurable immune responses in all patients vaccinated per protocol. Our study underlines the feasibility of vaccination with mutational neoantigens for cancer prevention in Lynch syndrome. These results strongly call for clinical trials evaluating possible cancer-preventive effects of this approach.

landmark study identifying *TGFBR2* as a commonly mutated cMS-containing target gene in MSI cancers (17). Consequently, MSI cancer development is associated with the generation of defined FSP neoantigens that are shared by the majority of MSI cancers, rendering them potentially targetable by an FSP vaccine (**Fig. 1A**). In addition, the fact that MSI cancers occur in the context of Lynch syndrome provides a unique opportunity to evaluate cancer-preventive vaccines in a realistic scenario, because Lynch syndrome mutation carriers are at very high risk of developing cancer and develop sufficient numbers of tumors to evaluate the efficacy of a preventive vaccine in a short time span.

Starting from a bioinformatics-based model (18) our group has defined a set of such shared FSP antigens, which occur particularly frequently as a result of functionally relevant "driver" mutations and which elicit T-cell responses in patients with MSI cancer (**Fig. 1**; refs. 16, 19).

Here we present the first-in-human step toward the goal of a cancer-preventive vaccine in Lynch syndrome. We report the results of a vaccine trial with three shared FSP antigens (Micoryx). The results of the trial demonstrate that FSP antigen vaccination is safe and induces cellular and humoral immune responses in all patients vaccinated per protocol.

Patients and Methods

Investigational agents

The following peptides were used for vaccination: AIM2(-1) HSTIKVIKAKKKHREVKRTNSSQLV, HT001(-1) EIFLPKGRSN-SKKKGRRNRIPAVLRTEGEPLHTPSVGMRETTGLGC, TAF1B(-1) NTQIKALNRGLKKKTILKKAGIGMCVKVSSIFFINKQKP. FSPs were synthesized, formulated, packed, and distributed in accordance with current GMP protocols by Bachem. Montanide ISA-51 VG was supplied by SEPPIC GmbH. Before vaccination, the lyophilized peptides (150 μg) were removed from the freezer and dissolved separately in 300-μL water [HT001(-1), TAF1B(-1)] or 300-μL PBS [AIM2(−1)]. Two hundred microliters of each of the peptide solutions (100 μg) was mixed with 200-μL Montanide ISA-51 VG until both liquids were intermixed and a thick, creamy, opaque, and consistent emulsion was generated. Four hundred microliters of the emulsions (100-μg peptide each) was administered separately subcutaneously. Vaccination dosing was selected according to previous peptide vaccination trials (20). For DTH, 90 μL of the dissolved peptides (30-μg peptide) was mixed with an additional 210-μL PBS and injected intradermally at separate sites distant from the vaccination sites to generate a visible and palpable skin depot.

Study endpoints and inclusion criteria

This study was an open-label single-arm phase I/IIa study of immunization with MSI-induced FSPs combined with MONTANIDE ISA-51 VG in patients with advanced MSI colorectal cancer. Primary endpoints were safety (phase I) and immunogenicity (phase IIa). Secondary endpoints were tumor response (both phases) and immunogenicity (phase I) and safety (phase IIa), following a study scheme described previously (21). The study started as phase I with six patients. Interim safety results of phase I were submitted to the regulatory authorities, the Paul-Ehrlich Institute and the responsible IRB (Ethics Committee Landesärztekammer Hessen), after approval the study continued as phase IIa ($n = 16$).

For both study phases, patients were eligible for inclusion if they fulfilled the following criteria: (i) history of histologically confirmed Lynch syndrome-related or sporadic MSI colorectal cancer of advanced-stage (UICC stage III/UICC stage IV), comprising patients with lymph node metastases (UICC III) and patients with metastasis to distant organs or peritoneal carcinomatosis (UICC IV); (ii) prior adjuvant standard therapy or prior palliative standard therapy in the first-, second-, and third-line or patient has refused adjuvant or palliative standard therapy; (iii) expected survival of at least 6 months, (iv) full recovery from surgery, chemotherapy or radiation therapy; (v) ECOG performance status 0, 1, or 2; (vi) adequate laboratory values (neutrophil count $\geq 1.5 \times 10^9$/L, lymphocyte count $\geq 0.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L, serum bilirubin <2 mg/dL). In certain cases ($n = 3$), patients affected by Lynch syndrome and being at a high risk of disease relapse or secondary tumors were included if they developed recurrent cancers of stages lower than UICC III.

MSI status was determined by PCR amplification of the Bethesda marker panel recommended by the NCI (BAT25, BAT26, D2S123, D5S346, D17S250; ref. 22) complemented by the mononucleotide marker CAT25 (22). Fluorescently labeled oligonucleotide primers were used for amplification, and amplified fragments were visualized on an ABI3100 sequencer as described previously (23). When two or more of the markers displayed novel alleles, a tumor was classified as MSI.

Patients were excluded if they had chemotherapy, radiation therapy, or immunotherapy within 4 weeks before study entry, untreated brain metastases, immunodeficiency syndromes or autoimmune disease, or did not give written informed consent. The study was conducted in accordance with the Declaration of Helsinki. The study was performed after approval by regulatory authorities and an institutional review board (Paul-Ehrlich institute and ethics committee of the Landesärztekammer Hessen) and registered (ClinicalTrials.gov Identifier: NCT01461148, EudraCT No. 2011-000765-12). All study patients gave written informed consent. The study was sponsored by Oryx GmbH und Co KG. All authors had access to the study data and reviewed and approved the final manuscript.

Statistical considerations

Assuming a likelihood of 10% for a vaccine-induced immune response (defined as positive response against one vaccine peptide for at least one assay modality) and a binomial distribution for the number of responses, a total of 22 patients (six patients from phase I, 16 patients from phase IIa) was required to obtain 80% power. Therefore, a sample size of 16 patients was set for phase IIa. For a likelihood of

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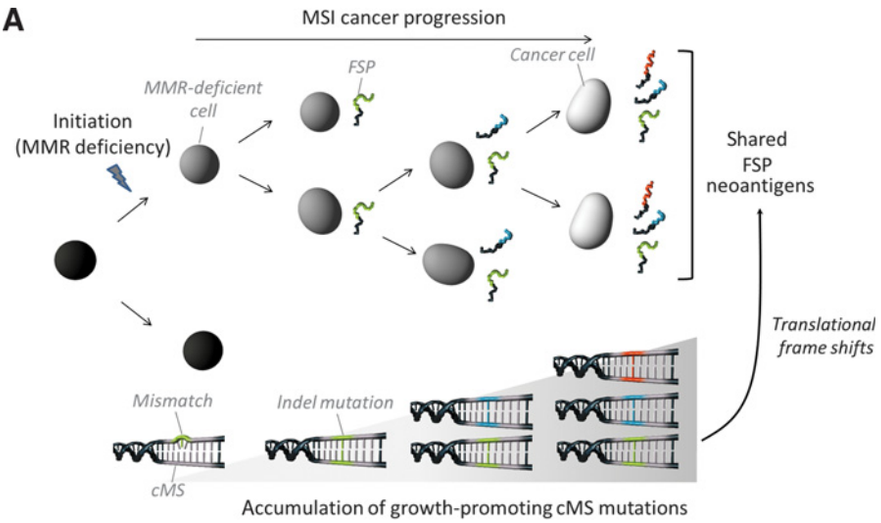

	cMS type	Mutation frequency (CRC)§	Mutation frequency (EC)§
TAF1B(−1)	A11	74.6%	50.0%
HT001(−1)	A11	86.2%	92.9%
AIM2(−1)	A10	81.6%	71.4%

TAFB(−1): H-NTQIKALNRGLKKKTILKKAGIGMCVKVSSIFFINKQKP-OH
AIM2(−1): H-HSTIKVIKAKKKHREVKRTNSSQLV-OH
HT001(−1): H-EIFLPKGRSNSKKKGRRNRIPAVLRTEGEPLHTPSVGMRETTGLGC-OH

Figure 1.

A, Concept of FSP vaccines against MSI cancer. Tumor initiation and progression of MSI cancers are triggered by DNA MMR deficiency. MMR deficiency leaves errors in DNA, which result from polymerase slippage events during DNA replication, unrepaired. Therefore, MSI cells accumulate a high number of somatic mutations. Most mutations are incompatible with cell survival; however, if the right combination of mutations occurs in an MMR-deficient cell, the respective clone can progress to manifest cancer. Selection of cell clones capable of progressing into cancer results in the occurrence of identical mutations across individual MSI cancers. Most mutations in MMR-deficient cells result from polymerase slippage and represent insertion/deletion (indel) mutations at repetitive sequence stretches. Indels affecting coding microsatellites can give rise to predictable neoantigens, termed FSP neoantigens. Vaccination with such FSP neoantigens holds the potential of sensitizing the immune system specifically against neoantigens associated with cancer initiation and progression. **B,** The FSP vaccine encompasses peptides derived from the (−1) frameshift sequence of the cMS-bearing genes *TAF1B, HT001, AIM2*. Type of the coding microsatellite and mutation frequency in MSI colorectal cancer and MSI endometrial cancer are provided in the table. Amino acid sequences of the peptides are provided in the bottom panel. §, Mutation frequencies derived from Ballhausen, et al (24).

13.5% for a patient to display a response, the power in this scenario is 90%.

Vaccine scheme

Patients were vaccinated once a week for 4 weeks, followed by a 4-week rest period (one cycle). This schedule was repeated up to a total of three cycles (12 vaccinations; **Fig. 2**). Vaccinations were performed alternately on the right and left upper arm. Study treatment was discontinued in case of progression or intolerable toxicity.

Toxicity assessment

Patients were considered evaluable for toxicity if they received at least one vaccination. Patients were seen at baseline/screening and at every vaccination visit for safety assessment until disease progression or discontinuation for other reasons. Patients were assessed for adverse events by nondirective questioning. Adverse events were also recorded when reported by the patient during or between visits or through physical examination, laboratory test, or other assessments. Toxicity assessment was performed according to the protocols of previous peptide vaccination studies (21). Laboratory tests included autoantibody status (ANA, ANCA, AMA, microsomal antibodies, TSH receptor antibodies), regular complete blood cell counts, and chemistry. All adverse events were documented according to the Common Toxicity Criteria for Adverse Events (CTCAE, version 4.0, https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Potential relationships of adverse events to the investigational agents were determined by the investigator.

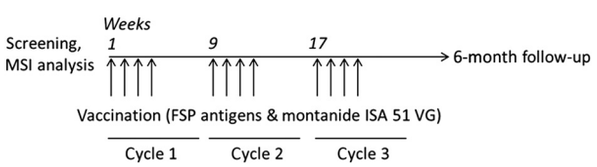

Figure 2.

Vaccination scheme. A total of 12 applications of the three study FSP antigens were administered together with Montanide ISA 51 VG in three cycles over a 6-month period.

Immunogenicity assessment

Patients were considered evaluable for immunologic efficacy if they completed at least one study cycle with subsequent immune assessment (week 9). Antigen-specific efficacy of the vaccine was defined as the induction of immune responses against each FSP. Immunologic responses were evaluated in weeks 9, 17, and 25 (T cells by DTH and ELISpot) and in weeks 3, 9, 11, 17, 19, and 25 (antibodies by ELISA) and were compared with the antigen-specific immune response measured prior to vaccination (week 1). Immune responses were reassessed 2 months after end of the study. For the assessment of DTH, 30 μg of each FSP was administered intradermally without adjuvant, and reactions were documented after 48 hours. DTH was considered positive if an area of redness and induration of >4 mm in diameter (+) or an enlarged area of redness and palpable induration of >8 mm in

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diameter and/or central necrosis (++) were observed, following criteria described previously (21).

To determine the presence of FSP-specific T cells in the peripheral blood, CD4-positive and CD8-positive T cells were separated from peripheral blood lymphocytes by antibody-coated magnetic beads (Minimacs; Miltenyi Biotec). IFNγ ELISpot was performed as reported previously (20). ELISpot responses were considered positive if the number of spots in peptide-exposed wells was at least two times higher than the number of spots in unstimulated wells, with a minimum of 10 peptide-specific spots per 25,000 T cells. FSP-specific antibodies were determined by peptide ELISA from serum samples as described previously (25). Briefly, peptides were coated to 96-well polystyrol microtiter plates "Maxisorp" (Nunc) at a concentration of 40 µg/mL in PBS. Peptide binding and saturating peptide concentrations were determined by alkaline phosphatase peptide competition. Sera were diluted 1:100 in PBS and 0.5% casein. Antibodies were detected using an HRP-labeled rabbit anti–human-IgG antibody (Jackson Immunoresearch; 1:10,000 in PBS, 0.5% casein) and TMB as a substrate (Sigma). Optical density (450 nm) was measured after adding 1 N H_2SO_4.

Positive immune responses were defined as positive DTH response and/or humoral immune response and/or CD8 and/or CD4 cellular immune response against at least one of the FSPs exceeding the assay-specific cut-off values.

Tumor status assessment and clinical response

Assessment of tumor status and clinical response was performed according to previously described study protocols (21). Patients were considered evaluable for tumor response if they had measurable tumors with radiological tumor imaging (CT, MRI) at baseline. For those patients, imaging was performed every 8 weeks until end or discontinuation of study. Post-study follow-up was performed after 6 months. Tumor status was classified according to RECIST whenever applicable. According to the protocol, signs of progressive disease, and death events were assessed and recorded during the trial and at study follow-up. Progression-free survival (PFS) was defined as the time period between the day of first study treatment and the detection of progressive disease, or death from any cause, whichever occurred first. Overall survival (OS) was defined as the time period between the day of first study treatment and death from any cause. Patients without the respective event being observed at the time of maximum follow-up were censored at this time point.

Results

Patients

Patients with MSI colorectal cancer ($n = 22$) were included in the study (phase I: $n = 6$, phase II: $n = 16$; consort diagram is provided in **Fig. 3**). Patients' baseline characteristics are provided in **Table 1**. The majority of patients had UICC stage III cancer ($n = 15$), three patients had UICC stage IV cancer, two patients had UICC stage II cancer, one patient had UICC stage I cancer at first cancer diagnosis. In 20 patients (90.9%), the primary tumor was localized in the colon, and two patients had a history of rectal cancer. Nineteen patients were tumor-free at study inclusion, thus three patients were evaluable for assessing tumor response. All patients had received at least one of the three common modalities of solid tumor treatment, that is, surgery, irradiation, and systemic therapy prior to study inclusion. The majority of patients was male ($n = 15$), median age was 54.8 years with only five (23%) patients being older than 60 years at study inclusion. Most patients had a normal or only slightly impaired ECOG performance status (ECOG 0, $n = 15$; ECOG 1, $n = 6$). Only one patient was included into the study with ECOG 2. In 18 patients, systemic chemotherapy had been applied prior to study inclusion (for details see Supplementary Data S2). For 16 patients, information about MMR gene germline sequencing was available; 13 patients harbored a pathogenic germline variant in one of the MMR genes, in the remaining three patients, no germline variant had been identified in the four MMR genes.

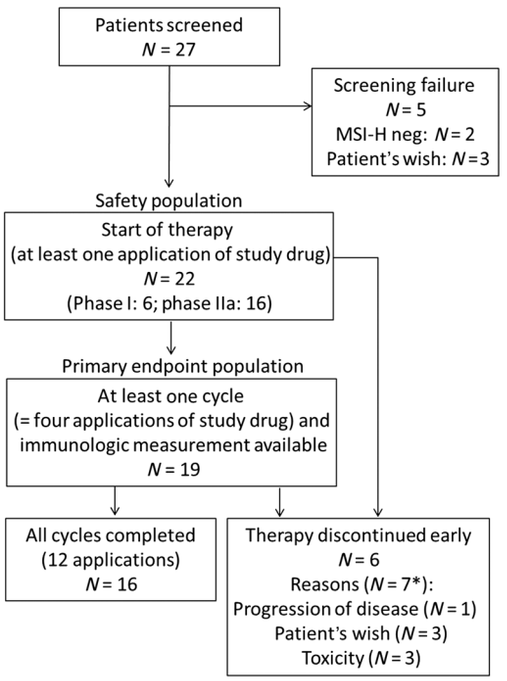

Figure 3.
Trial profile. *, One patient had two reasons for study discontinuation: Phase I: progressive disease, $n = 1$; toxicity, $n = 1$; patient's wish, $n = 1$; toxicity and patient's wish, phase II: toxicity, $n = 1$; patient's wish, $n = 1$.

All 22 patients received at least one application of the FSP vaccine, 19 completed the first treatment cycle (four applications) and were available for first analysis of immunogenicity. Sixteen patients completed the entire vaccination scheme of three cycles (12 applications). Premature discontinuation occurred in six patients and was due to progressive disease ($n = 1$), toxicity ($n = 3$), or patient's wish ($n = 3$) with two simultaneous reasons for discontinuation in one patient.

Toxicity

During phase I of the trial, seven SAEs occurred in two patients; however, all SAEs were restricted to patients with UICC stage IV cancer, and none of the SAEs was considered as possibly related to the study treatment. In none of the vaccinated patients, any evidence of autoimmunity was detectable. Therefore, the safety profile of the vaccine was considered favorable, and continuation of the study with phase IIa was approved.

In the combined analysis of phase I and phase IIa, all 22 patients were evaluable for toxicity. No SAR or SUSAR were documented, and no new or unexpected risks appeared in the study. No deaths with possible relation to study therapy occurred. Reported death events during the study (two patients) were related to progression of the underlying tumor disease and not connected to the study medication. All reported SAEs were unrelated to the study treatment. Adverse drug reactions, that is, events reported to have an at least possible

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Table 1. Patient baseline characteristics.

Characteristics	Total $n = 22$
Age, median (range), years	54.8 (31.0–80.7)
<45, *n (%)*	4 (18)
45 to ≤50	3 (14)
50 to ≤55	3 (14)
55 to ≤60	7 (32)
>60	5 (23)
Gender, *n* (%)	
Female	7 (32)
Male	15 (68)
Tumor location, *n* (%)	
Colon	20 (91)
Rectum	2 (9)
Initial UICC stage, *n* (%)	
I	1 (5)
II	2 (9)
III	15 (68)
IV	3 (14)
Unknown	1 (5)
Tumor status at study inclusion, *n* (%)	
Tumor-free	19 (86)
Not tumor-free	3 (14)
Time since initial diagnosis, *n* (%)	
<5 years	8 (36)
5 to ≤10 years	4 (18)
10 to ≤15 years	4 (18)
15 to ≤20 years	2 (9)
>20 years	4 (18)
MMR gene germline mutation, *n* (%)	
MLH1	6 (27)
MSH2	6 (27)
MSH6	1 (5)
PMS2	0
None in the genes above	3 (14)
No germline data available	6 (27)
Performance status (ECOG), *n* (%)	
0	15 (68)
1	6 (27)
2	1 (5)

relationship to the administration of the study drug, had a maximum of grade 2 (**Table 2**). Apart from injection site reactions in 17 (78%) of patients (grade 1 or 2), adverse drug reactions were rare. Three patients discontinued trial participation because of toxicity, one of these was dose limiting (allergic reaction, NCI grade 2). Two patients showed prolonged redness, induration, and swelling at the injection sites, which persisted more than 1 year after the last vaccine administration.

Table 2. Adverse drug reactions, maximum CTC severity grade by patient and by category (CTCAE 4.0).

Event	Total $n = 22$	Grade 1	2	3	4
Allergic reaction, *n* (%)	1 (5)	—	1	—	—
Dizziness	1 (5)	1 (5)	—	—	—
Dyspnea	1 (5)	—	1	—	—
Injection site reaction	17 (78)	14 (64)	3 (14)	—	—
Peripheral sensory neuropathy	1 (5)	1 (5)	—	—	—
Rash	3 (14)	3 (14)	—	—	—
Skin hyperpigmentation	1 (5)	1 (5)	—	—	—

Immunogenicity

In total, 19 patients were evaluable for immunologic efficacy of the FSP vaccine, because they completed at least one treatment cycle followed by at least one subsequent assessment of immune responses (week 9 or later). FSP-specific immune responses were monitored by delayed-type hypersensitivity reaction (DTH), IFNγ ELISpot (CD4 and CD8 T cells separately), and ELISA (humoral immune responses). Although baseline immune responses (week 1) against the vaccine peptides were detected in 11 of 22 patients (50%), all evaluable patients showed an immune response against at least one of the vaccine peptides during and after vaccination (**Fig. 4**). All 16 patients who were vaccinated per protocol and completed the entire vaccination scheme developed an immune response against at least one of the vaccine peptides. In most patients, positive immune reactions emerged at week 9 and remained detectable until the end of treatment. The most frequent type of detectable immune response was of antibody nature, followed by CD4 T cells, and CD8 cell responses (**Fig. 4**).

A subset of patients ($n = 5$) showed a delayed time hypersensitivity (DTH) response upon vaccination. In detail, two (10.5%) of the evaluable patients showed induction of a DTH reaction upon vaccination for the peptides AIM2 (patients 8 and 10) and HT001 (patients 10 and 12), 3 (15.7%) for the peptide TAF1B (patients 3, 10, and 11). Strong baseline DTH reactions were seen in one patient (patient 5) for all three peptides.

Full response data for the intention-to-treat population are provided as Supplementary Data S1.

Tumor response

Tumor response according to RECIST criteria could be assessed in three patients. Two (66.6%) of these patients showed stable disease as best overall response. One heavily pretreated patient with bulky metastases (patient 12) showed a stable disease and stable CEA levels over 7 months under the study treatment. In one patient, study participation was discontinued because of toxicity, and no further restaging was performed. No secondary tumor events were recorded during the study period.

Discussion

Of 19 analyzed patients (per protocol set), all patients (100%; 95% confidence interval, 82.35%–100%) showed FSP-specific immune responses after vaccination, demonstrating that vaccination with FSP antigens induces significant humoral and cellular immune responses. Notably, also the majority of patients with baseline immunity against at least one of the FSP antigens (11 patients) developed additional immune responses in another category after vaccination (10 patients, 91%).

Compared with other vaccine trials (26) with cancer antigens, the frequency of induced immune responses was high. Although quantification of ELISpot data after *in vitro* stimulation with peptides should be performed with caution, substantially elevated spot numbers were observed in patients after vaccination compared with baseline (Supplementary Data S3). This might reflect the fact that mutational FSP neoantigens rarely induce immune tolerance (27), which is a common problem in cancer vaccines based on self-antigens (28). In addition, the FSP neoantigens evaluated in the Micoryx study encompass long neopeptide stretches ranging between 13 [AIM2(−1)] and 32 [HT001(−1)] amino acids, potentially encompassing several immune-relevant epitopes. Although no HLA typing was performed in this study due to limited sample availability, we speculate that the lack of CD8-positive T-cell responses in nine of 16 patients vaccinated per

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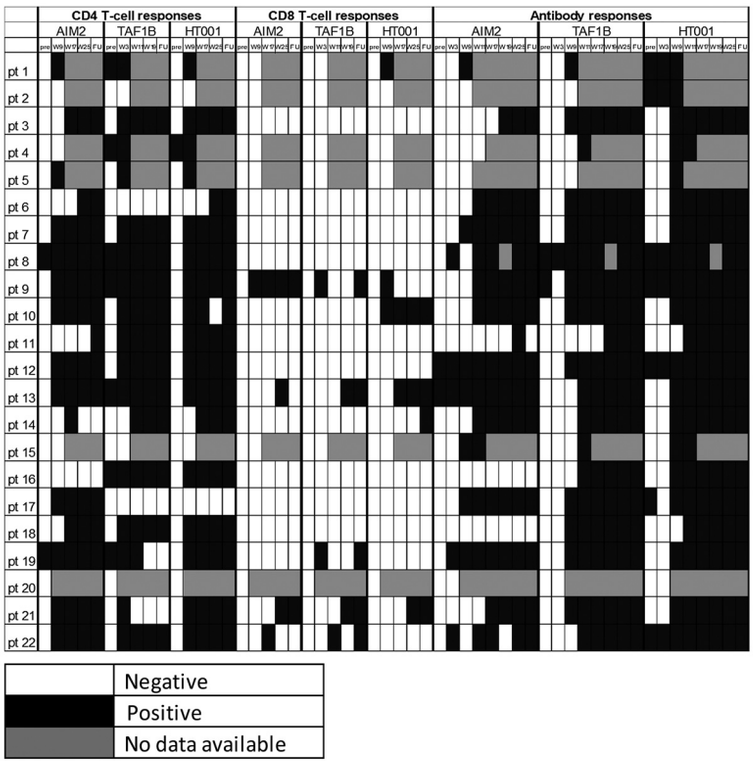

Figure 4.
Immune responses against FSP antigens. FSP-specific immune responses (CD4, CD8, and antibody responses) of all 22 study patients (pt) at baseline (week 1, w1), during vaccination (week 3 to 25, w3 to w25) and 2 months after last vaccination (follow-up, FU). White boxes indicate negative, black cells indicate positive immune responses. Gray boxes, not assessable due to study discontinuation.

protocol may reflect the fact that no HLA class I-binding peptide derived from the FSPs in the vaccine was processed and presented in these patients. Additional studies are required to specifically determine relevant epitopes contained in FSP neoantigens and to compare the efficacy of different vaccination approaches, accounting for the prevalence of different HLA types in target populations (29, 30).

Clinical efficacy of the vaccine could not be examined in frame of this study, because 19 patients were tumor-free at study inclusion. Future clinical studies are currently planned to examine the efficacy of the FSP vaccine in a larger set of patients in a therapeutic and preventive setting.

Potential future applications of the FSP vaccine in a therapeutic setting comprise combinations with novel immune-modulatory agents such as anti–PD-1 or PD-L1 antibodies that have shown high efficacy in a subset of patients with MSI cancer (31, 32). In contrast to immune checkpoint modulators, FSP vaccination also holds high potential for tumor prevention in Lynch syndrome (33), due to the much less pronounced side effect profile. The high-risk population of Lynch syndrome mutation carriers represents a unique scenario to evaluate the concept of tumor antigen-specific vaccines for cancer prevention. Such a tumor-preventive vaccine may be applied alone or in combination with chemopreventive agents such as aspirin (34).

Important from the perspective of prevention, the vaccine specifically targets FSP neoantigens derived from mutations with a potential role as functionally relevant drivers. A driver function of mutations inactivating the genes *AIM2, HT001 (ASTE1), TAF1B* would be compatible with the fact that they occur significantly more frequently in MSI colorectal cancer than expected by chance (www.seltarbase.org; **Fig. 1**). Targeting neoantigens derived from driver mutations ensures that the vaccine should preferentially target clinically relevant outgrowing tumor cell clones, which are at risk of developing into manifest cancer. At the same time, this strategy limits the likelihood of immune evasion through antigen loss. Although frameshift mutations in cMS can result in two possible novel reading frames, mutation data of MSI cancers demonstrate that the reading frame resulting from a single nucleotide deletion (−1) is clearly the predominant type of mutation (www.seltarbase.org), enhancing the potential coverage of the FSP vaccine. Importantly, mutations giving rise to the FSP antigens in the vaccine are also present in extracolonic tumors such as endometrial cancer (35); therefore, these tumor types may also be preventable by the FSP vaccine.

Immune evasion mediated through loss of the tumor cells' capacity to present antigens to the immune system has been reported previously in MSI cancers (36, 37). It is conceivable that a potent FSP vaccine may enhance the immunoselective pressure and thereby increase the probability of immune evasion through *Beta2-microglobulin* (*B2M*) mutations (38). Interestingly, patients with *B2M*-mutant cancers apparently have an excellent prognosis (39–42) and do not develop tumor relapses or distant metastases. Hence, FSP vaccination may not only reduce the cancer incidence in Lynch syndrome individuals directly by elimination of emerging MSI cell clones, but also help to make MSI cancer a surgically curable disease.

The study results demonstrate that FSP vaccination has low toxicity, as systemic adverse effects were rare and most likely unrelated to the vaccine agents. However, local reactions at the injection site were commonly observed (grade 2 in three patients). This most likely results from one or more of the following factors: (i) Injection site reactions were most likely triggered or potentiated by Montanide ISA 51 VG, as redness, swelling, and granuloma formation have previously been described as typical side effects (43). In contrast to the present trial, in which 19 of 22 participants were tumor-free at the time point of inclusion, most cancer vaccine trials using Montanide were performed in patients with manifest advanced-stage cancer and therefore potentially a compromised immune status. (ii) It seems plausible that

preexisting FSP-specific immune responses, which have previously been detected in Lynch syndrome mutation carriers and patients with MSI cancer (16), have enhanced the local immune responses upon vaccination. This hypothesis is supported by the fact that the patient who developed most pronounced injection site reactions had strong preexisting DTH responses (patient 5) against all vaccine peptides. (3) In comparison to most tumor antigens previously used in Montanide-containing formulations, FSP antigens are long neoantigens capable of inducing more pronounced immune responses (1). Thus, the observed side effects underline the immunogenicity of FSP neoantigens and their great potential as vaccine agents. The relevance of FSP neoantigens for antitumor immune responses is underlined by the recent observation that the extent of responses to PD-1 immune checkpoint blockade is related to the insertion/deletion (indel) mutation load in MSI tumors (44). For future FSP-based vaccine applications, DTH status should be considered as a potential predictor of local adverse effects, and alternative adjuvant combinations should be considered.

In summary, we demonstrate in a first-in-man study that vaccination with MSI-induced FSP neoantigens is well tolerated and induces T-cell-based and humoral immune responses. This study marks an important step towards the establishment of a first neoantigen-based nonviral vaccine for the prevention of human cancers. Lynch syndrome is a unique scenario to achieve this goal, because the success of a vaccine can be monitored in a defined high-risk population.

Disclosure of Potential Conflicts of Interest

M. Kloor reports grants from Oryx GmbH und Co KG during the conduct of the study. M. Reuschenbach reports grants from Oryx GmbH und Co KG (sponsor of the study) during the conduct of the study, patent for content related to this study pending (as inventor), and reports employment with MSD Sharp & Dohme GmbH. M. Reuschenbach's contribution to the submitted work fully originated from the time she was a full-time employee of Heidelberg University Hospital. M. Tariverdian reports grants from Oryx GmbH und Co KG during the conduct of the study. M. von Knebel Doeberitz reports grants from Oryx GmbH und CoKG during the conduct of the study. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

M. Kloor: Conceptualization, data curation, supervision, investigation, visualization, writing-original draft, writing-review and editing. **M. Reuschenbach:** Conceptualization, data curation, investigation, methodology, writing-review and editing. **C. Pauligk:** Data curation, formal analysis, validation, visualization, writing-review and editing. **J. Karbach:** Data curation, formal analysis, validation, investigation, writing-review and editing. **M.-R. Rafiyan:** Data curation, investigation, writing-review and editing. **S.-E. Al-Batran:** Supervision, investigation, project administration, writing-review and editing. **M. Tariverdian:** Validation, investigation, writing-review and editing. **E. Jaeger:** Conceptualization, resources, supervision, funding acquisition, validation, investigation, project administration, writing-review and editing. **M. von Knebel Doeberitz:** Conceptualization, resources, supervision, funding acquisition, validation, investigation, project administration, writing-review and editing.

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Matthias Kloor, Miriam Reuschenbach, Claudia Pauligk, et al.

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