Safety and Activity of PolyPEPI1018 Combined with Maintenance Therapy in Metastatic Colorectal Cancer: an Open-Label, Multicenter, Phase Ib Study

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ABSTRACT

Purpose: Although chemotherapy is standard of care for metastatic colorectal cancer (mCRC), immunotherapy has no role in microsatellite stable (MSS) mCRC, a "cold" tumor. PolyPEPI1018 is an off-the-shelf, multi-peptide vaccine derived from 7 tumor-associated antigens (TAA) frequently expressed in mCRC. This study assessed PolyPEPI1018 combined with first-line maintenance therapy in patients with MSS mCRC.

Patients and Methods: Eleven patients with MSS mCRC received PolyPEPI1018 and Montanide ISA51VG adjuvant subcutaneously, combined with fluoropyrimidine/biologic following first-line induction with chemotherapy and a biologic (NCT03391232). In Part A of the study, 5 patients received a single dose; in Part B, 6 patients received up to three doses of PolyPEPI1018 every 12 weeks. The primary objective was safety; secondary objectives were preliminary efficacy, immunogenicity at peripheral and tumor level, and immune correlates.

Results: PolyPEPI1018 vaccination was safe and well tolerated. No vaccine-related serious adverse event occurred. Eighty percent of patients had $CD8^+$ T-cell responses against ≥3 TAAs. Increased density of tumor-infiltrating lymphocytes were detected post-treatment for 3 of 4 patients' liver biopsies, combined with increased expression of immune-related gene signatures. Three patients had objective response according to RECISTv1.1, and 2 patients qualified for curative surgery. Longer median progression-free survival for patients receiving multiple doses compared with a single dose (12.5 vs. 4.6 months; $P = 0.017$) suggested a dose–efficacy correlation. The host HLA genotype predicted multi-antigen–specific T-cell responses ($P = 0.01$) indicative of clinical outcome.

Conclusions: PolyPEPI1018 added to maintenance chemotherapy for patients with unresectable, MSS mCRC was safe and associated with specific immune responses and antitumor activity warranting further confirmation in a randomized, controlled setting.

Introduction

Despite the many advances made in the molecular classification and treatment of colorectal cancer over the past decade, colorectal cancer remains among the leading causes of cancer-related deaths worldwide (1). Although many patients initially present with localized disease, ultimately about 50% progress to advanced, unresectable, or metastatic disease (2). For advanced/unresectable disease, median overall survival ranges from 27 to 30 months (3, 4). A number of considerations impact the choice of first-line therapy of metastatic colorectal cancer (mCRC; refs. 5–8).

The therapeutic mainstay in the management of microsatellite stable (MSS) mCRC remains combination chemotherapy, including infusional or oral fluoropyrimidine/oxaliplatin-based cytotoxic regimens, frequently in combination with biologic therapy. These regimens were traditionally used until the development of disease progression or intolerance. However, given the cumulative side effects associated with such regimens, many patients are not able to tolerate prolonged therapy with full doses. Hence, drug holidays have been used (9) or the proactive use of maintenance therapy with more tolerable regimens, frequently with a subset of the drugs that were used for first-line induction phase (10–14).

Immunotherapy is an attractive strategy for the maintenance setting. Aside from the lack of cross-resistance with typical chemotherapy regimens used for maintenance therapy, the setting of a lower tumor burden following successful induction therapy is also conducive of optimal activity. However, while novel checkpoint inhibitors (CPI) have yielded dramatic improvements in microsatellite instability–high (MSI-H), there has been no activity in MSS colorectal cancer (15, 16). Nevertheless, in carefully selected settings the appropriate form of immunotherapy coupled with other standard agents may well hold promise in MSS colorectal cancer. Immunotherapy may serve to rejuvenate tumor immunity and potentially eradicate micrometastatic or oligometastatic disease. In addition, immunotherapy may be potentially additive or synergistic with chemotherapy and antiangiogenic therapy (17–19). 5-fluorouracil (5-FU) may cause some tumor cells to be more visible to the adaptive immune system, resulting in enhanced eradication of tumor cells by effector T cells (20–22). Both 5-FU and bevacizumab may be cytotoxic to immunosuppressive myeloid-derived suppressor cells and regulatory T cells restoring antitumor immunity (23–26). In addition, bevacizumab normalizes tumor vasculature increasing T-cell infiltration (26, 27). These considerations formed the rationale for the OBERTO-101 study.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

PolyPEPI1018 is a cancer vaccine composed of multiple tumor–associated peptides designed to engage multiple T-cell clones against the patients' tumor. To our knowledge, this is the first study to assess safety and initial efficacy of a peptide vaccine as add-on to fluoropyrimidine/bevacizumab maintenance therapy in patients with microsatellite stable metastatic colorectal cancer. The study showed immunologically and clinically meaningful activity of this treatment.

OBERTO-101 is a first-in-human study evaluating the safety, tolerability, immunogenicity, and efficacy of a subcutaneously injected vaccine, PolyPEPI1018, along with Montanide ISA51VG adjuvant as an add-on immunotherapy to fluoropyrimidine-based maintenance therapy for patients with MSS mCRC following a period of induction treatment with conventional chemotherapy and biologics. PolyPEPI1018 was designed to address the dual challenges of patient and tumor heterogeneity. Specifically, six synthetic long peptides derived from seven tumor-associated antigens (TAA) frequently expressed in colorectal cancer (28–34), altogether contain 12 novel and shared epitopes that can result in the induction of broad antitumor immunity in high proportion of patients (35). It is hypothesized that the activation and proliferation of multiple cytotoxic T lymphocytes will result in the killing of tumor cells in collaboration with the background therapy.

Patients and Methods

Study design

This was a phase IIb, open-label, nonrandomized, multicenter study to evaluate the safety, tolerability, immunogenicity, and efficacy of a single injection (Part A) or multiple subcutaneous injections every 12 weeks for three doses (Part B) of PolyPEPI1018 as an add-on immunotherapy to the maintenance therapy (Supplementary Fig. S1). The study included a 12-week follow-up period after the last vaccination. After completion of the vaccination(s), subjects remained on maintenance therapy until disease progression or intolerance per discretion of the investigator.

The clinical sites conducting the study were Pisa University Clinic, Pisa, Italy (Part A) and Mayo Clinic, Rochester, MN (Part A and B). This study was carried out in accordance with International Conference on Harmonization Good Clinical Practice and the Declaration of Helsinki. The protocol was approved by the institutional review board of each participating site. All patients signed written informed consent.

Male or female patients, 18 to 75 years of age with histologically confirmed stage IV metastatic adenocarcinoma originating from the colon or the rectum were enrolled during their transition to maintenance therapy with a fluoropyrimidine (5-FU or capecitabine) plus the same biologic agent used during induction (**Table 1**). Treatment with PolyPEPI1018 was initiated within 3 weeks of transition to maintenance therapy. Before enrollment, patients experienced partial response (PR) or stable disease (SD) during this first-line treatment. Prior systemic immunotherapy treatment was excluded. Presence of at least one measurable reference lesion according to RECIST v1.1 criteria was needed. Patients with MSI-H tumors, documented *BRAF* mutations, and those with central nervous system metastases were excluded. Patients must have had Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

Study protocol is deposited at ClinicalTrials.gov, NCT03391232.

Procedures

Design of PolyPEPI1018

For the design of PolyPEPI1018, dominant epitopes of the target TAAs were determined using an *in silico* model cohort as described earlier (36). Briefly, overlapping 9 mers of the 7 protein antigens were scanned to predict their binding to the 152 HLA class I alleles of 433 individuals with mixed ethnicity comprising the *in silico* cohort representative of the global population, as previously described (37). As a next step, the predicted epitopes restricted to multiple (≥3) autologous HLA alleles (PEPI) were determined for each of the 433 subjects and selected those that are most frequently shared among them. After considering also physico-chemical properties of the peptides, twelve 15 mers were selected containing the dominant (core) 9 mers to maximize the predicted HLA class II bindings, as well (Supplementary Table S1). To gain long peptides (30 mers; ref. 38), the twelve 15-mer peptides were paired creating six 30-mer peptides which do not contain neoepitopes (8 mers) at the joining region homologous with the human proteosome (Basic Local Alignment Search Tool analysis). As a result, PolyPEPI1018 vaccine contains six 30-mer peptides, consisting of immunodominant epitopes from seven colorectal cancer–specific TAAs (EPCAM, SURVIVIN, TSP50, FBXO39, SPAG9, CAGE1, MAGE-A8). When considering all six 30 mers of PolyPEPI1018, 98% of subjects in the *in silico* cohort were predicted to have at least one vaccine-specific HLA class I PEPI, 91% have 2 PEPIs, and 100% of them have HLA class II PEPIs, potentially covering large fraction of the study population, as well (Supplementary Table S1; ref. 37).

Vaccine peptides were manufactured using solid-phase peptide synthesis (Ambiopharm Inc). DMSO/Water solution of peptide mixtures were filled (Bioserv Corp) into two vials (0.2 mg/mL, each peptide). Before vaccination, each mixture was separately emulsified with equal volume of Montanide ISA51VG adjuvant (Seppic) using the standard two-syringe method. Each mixture was injected subcutaneously into two anatomic sites: the two arms and two thighs.

HLA Genotyping

HLA genotyping was performed by LabCorp from saliva sample (buccal swab) using next-generation sequencing. HLA-class I and II allele bindings were predicted using the Immune Epitope Database and NetMHCII (IC_{50} < 500 nmol/L) prediction tools, respectively.

In vitro stimulated ELISpot assay

Every 3 weeks, 45 mL peripheral blood was drawn into EDTA tubes and peripheral blood mononuclear cells (PBMC) were isolated using Ficoll gradient centrifugation for each subject. PBMCs were thawed in RPMI1640 with 10% FBS and incubated for 7 days at 37°C, 5% CO_2. On day 1 and 4, the medium was refreshed and supplemented with 5 ng/mL IL7 or 5 ng/mL IL7 and 4 ng/mL IL2 (R&D Systems), respectively. After 7 days, PBMCs were harvested and rested for 16 hours, then seeded in 2 or 3 replicates (depending on sample availability) on IFNγ/Granzyme-B/TNFα FluoroSpot plates (Mabtech) at 100,000 to 150,000 cells/well in RPMI-10% FBS. Cells were incubated either with the RPMI-10% FBS medium, or the peptides (5 μg/mL), or peptide pools (5 μg/mL per peptide) overnight at 37°C, 5% CO_2. Development was performed according to the manufacturer's instructions. Results were acquired with a Mabtech

Table 1. Patients' baseline characteristics and major outcomes.

Patient ID[a]	Diagnosis	Metastasis	Genetic profile	Induction therapy	Response on induction (RECIST v1.1)	Vaccine Dose(s)	Maintenance therapy	ECOG score	KPS	Age at enrollment	Sex	Post vaccine response (RECIST v1.1)	Post vaccine response (irRECIST)	PFS (weeks)	Additional anticancer treatment
01-0001[A]	Adenocarcinoma of the left colon	Liver	*KRAS* mut, MSS, *HER2* pos	FOLFOX + bevacizumab	SD	1	5-FU, leucovorin, bevacizumab	1	90	62	M	PD	NE	13	NA
02-0001[A]	Adenocarcinoma of the hepatic flexure	Synchronous liver	*KRAS* mut, MSS	FOLFOXIRI + bevacizumab	PR	1	5-FU, leucovorin, bevacizumab	0	100	64	F	PD	irPD	10	NA
02-0002[A]	Adenocarcinoma of the left colon	Synchronous liver	*RAS/BRAF* wt, *HER2* neg, MSS	FOLFOX + panitumumab	SD	1	5-FU, leucovorin, panitumumab	0	100	59	M	SD	NE	31	NA
02-0003[A]	Rectal adenocarcinoma	Synchronous liver and lung	*KRAS* mut, MSS	FOLFOX + bevacizumab	PR	1	5-FU, leucovorin, bevacizumab	0	100	73	M	SD	NE	26[b]	Radio-therapy
02-0004[A]	Adenocarcinoma of the right colon	Synchronous liver	*KRAS* mut, MSS	FOLFOXIRI + bevacizumab	PR	1	5-FU, leucovorin, bevacizumab	1	100	66	M	PR	NE	27	NA
01-0008[B]	Rectal adenocarcinoma	Liver	*KRAS* mut, MSS, *HER2* neg	FOLFOX + bevacizumab	SD	1	Capecitabine, bevacizumab	0	90	48	M	PD	irPD	10	NA
01-0003[B]	Rectosigmoid adenocarcinoma	Liver and lung	*RAS* wt, *BRAF* wt, MSS	FOLFOX + bevacizumab	SD	2	5-FU, leucovorin, bevacizumab	0	90	49	M	SD	NE	25[b]	NA
01-0002[B]	Rectal adenocarcinoma	Liver	*KRAS* mut, MSS, *HER2* neg	FOLFOXIRI + bevacizumab	PR	3	5-FU, leucovorin bevacizumab	0	100	54	M	SD	NE	54	NA
01-0004[B]	Adenocarcinoma of the cecum	Liver and omentum, mesentery	*KRAS* mut, MSS, *HER2* neg	FOLFOX + bevacizumab	SD	3	Capecitabine, bevacizumab	0	90	45	M	PR	irPR	25[b]	Surgery
01-0005[B]	Rectosigmoid adenocarcinoma	Liver and peritoneal	*KRAS* mut, MSS, *HER2* neg	FOLFIRI + bevacizumab	SD	3	Capecitabine, bevacizumab	0	100	37	M	PD	irPD	32	NA
01-0007[B]	Rectosigmoid adenocarcinoma	Liver, with lymphadenopathy – supraclavicular, axilla, mediastinum, hilum	*RAS/BRAF* wt, MSS, *HER2* neg	FOLFOX + cetuximab	PR	3	5-FU, leucovorin, cetuximab	0	90	52	M	PR	irPR	56[b]	Surgery

Abbreviations: FOLFOX, 5-fluorouracil (Adrucil, Fluorouracile Teva), leucovorin calcium, oxaliplatin (Eloxatin, Oxaliplatino Sun); FOLFOXIRI, 5-fluorouracil (Adrucil, Fluorouracile Teva), leucovorin calcium, oxaliplatin (Eloxatin, Oxaliplatino Sun), irinotecan (Camptosar, Irinto), bevacizumab (Avastin), capecitabine (Xeloda, Capecitabina Accord), panitumumab (Vectibix), cetuximab (Erbitux); KPS, Karnofsky Performance Score; NE, not evaluated.
[a]Superscripts in patient ID indicate the study part (A or B).
[b]Censored data.

IRIS reader and analyzed using the Mabtech Apex software. *In vitro* stimulated (IVS) ELISpot results were considered positive when after subtracting the corresponding nonstimulated control (ΔSFU), the result was >2.5-fold higher than the DMSO negative control. A response was considered "boosted" compared with pre-vaccination when at least two-fold increase in ΔSFU was achieved.

Ex vivo ELISPOT assay

The IFNγ/Granzyme-B/TNFα FluoroSpot plates were blocked with RPMI-10% FBS and cryopreserved PBMCs (100,000 cells/well) plated in 2 or 3 replicates depending on sample availability. The cells were incubated either with DMSO as negative control, the individual peptides (5 μg/mL) or peptide pools (5 μg/mL per peptide) overnight at 37°C, 5% CO_2. Plates were developed according to the manufacturer's instructions and data measured as described above. *Ex vivo* ELISPOT results were considered positive when after subtracting the corresponding nonstimulated control (ΔSFU), the result was higher than the DMSO negative control. A response was considered "boosted" (vaccine-specific) when at least two-fold increase in ΔSFU was achieved compared with pre-vaccination.

Ex vivo intracellular cytokine staining

Two hundred thousand cryopreserved PBMCs/well were seeded in 2 or 3 replicates on sterile round-bottom 96-well plates (Thermo Fisher) in RPMI1640 supplemented with 10% Human Serum HI. After 2-hour incubation either with DMSO, or the peptides (5 μg/mL) or peptide pools (5 μg/mL per peptide), BD GolgiPlug (BD Biosciences) was added to the wells at 1 μL/mL. After 4 hours incubation, cells were treated with dissociation buffer (Thermo Fisher) and incubated with Zombie NIR Viability Dye (BioLegend) at room temperature for 15 minutes. Cells were incubated in Fc Blocking reagent for 5 minutes and stained with anti-CD3 (Miltenyi Biotec; catalog no. 130–113–141, RRID: AB_ 2725966), anti-CD4 (BD Biosciences; catalog no. 564724, RRID:AB_2738917), and anti-CD8 (BD Biosciences; catalog no. 612754, RRID:AB_2870085) antibodies for 30 minutes at 4°C, then washed, permeabilized and fixed according to the manufacturer's recommendations (BD Biosciences). Intracellular cytokines were labeled using a staining mixture of anti-IFNγ (BioLegend; catalog no. 502515, RRID:AB_493029), anti-IL2 (BioLegend; catalog no. 500315, RRID:AB_493370), and anti-TNFα (BioLegend; catalog no. 502909, RRID:AB_315261) antibodies at 4°C for 30 minutes. Cells were washed twice before acquisition. All flow cytometry data was acquired with a LSRFortessa X-20 and analyzed using the FlowJo V10 software (RRID: SCR_008520). Results were considered positive if higher than the DMSO negative control that was determined for each time point.

ELISpot and ICS assays were performed by ImmunXperts SA.

Gene expression profiling

NanoString Technologies' nCounter PanCancer Immune Profiling Panel and protocol was used. RNA was purified from formalin-fixed, paraffin-embedded (FFPE) slices using an optimized protocol based on "RNeasy FFPE Kit" from QIAGEN. To evaluate the quality of extracted RNA each sample was tested on Agilent Bioanalyzer (RRID: SCR_019389) using RNA 6000 Nano Kit. The process included internal controls automatically analyzed by the system. RNA hybridization was performed for 16 hours using capture and reporter probes. The samples were then immobilized into a cartridge and loaded onto the nCounterTM Prep Station. Digital images were processed within the nCounterTM Digital Analyser instrument. The quality control, normalization, and differential expression analyses were performed according to the manufacturer's instructions.

IHC

IHC of FFPE slices were obtained from liver tumor biopsy taken at pre-vaccination and at every 12-week intervals. First hematoxylin and eosin staining was applied to assess the proportion of tumor area in the whole tissue and the percentage of tumor cells in the tumor area. Then Immunoscore CR TL assay was done that consists of immunostaining on two consecutive slides for quantification of positive $CD3^+$ and $CD8^+$ cells in the core tumor (CT) and invasive margin (IM). All immunostainings were performed on the automated system Ventana BenchMark autostainer (Roche Diagnostics). Image acquisition was done on the Hamamatsu, NanoZoomer XR scanner.

IHC and gene expression measurements were performed by HalioDx SA.

Statistical analysis

All patients enrolled in the study (Part A and Part B combined) were included in the safety, immunology, and the efficacy analysis datasets. No patients were excluded from immune analyses for other reasons than sample availability. For tumor-infiltrating lymphocyte (TIL) analysis only pairs of biopsies were used. No imputation was carried out on missing values. Clinical response assessment was performed at Week 12 in Part A and Week 36 in Part B of the study. Progression-free survival (PFS) was calculated as the time between the date of initiating maintenance therapy and the date of either radiologic or clinical/symptomatic disease progression. Significance was compared between and among groups using *t* tests; $P < 0.05$ was considered significant. Kaplan–Meier method was used to estimate PFS, and average HR statistics to estimate HRs. Significance was assessed by log-rank test. Pearson test was performed to assess correlations. The correlation was considered strong if $R > 0.7$, moderate if $0.5 < R \leq 0.7$, and weak if $0.3 < R \leq 0.5$. Dependent variables were determined using Fisher exact test for a 2×2 contingency table.

Data availability

Raw data for this study were generated at the facilities identified in the Patients and Methods section. Derived data supporting the findings of this study are available from the corresponding author upon reasonable request.

Results

Between April 2018 and December 2018, 11 patients were enrolled in the study. All patients had MSS mCRC. Five patients received a single dose of vaccine (Part A). Six patients entered Part B of the study for multiple-dose vaccination. However, 1 patient had progressive disease (PD) before second vaccination and was thus not eligible for further vaccination, 1 patient voluntarily withdrew from the study after two doses of vaccine (due to signs of progression not confirmed by the investigator), and 1 patient withdrew consent after three doses received due to progression (Supplementary Fig. S1).

The median age of participants was 54 years [interquartile range (IQR), 14.5 years], 10 (91%) participants were male, and all participants were White, non-Hispanic, or Latino (**Table 1**). During the first-line induction therapy, 6 of 11 (55%) patients achieved SD and 5 of 11 (45%) PR as best overall response (BOR). Individual patient characteristics along with the maintenance therapy received in combination with PolyPEPI1018 during the study are reported in **Table 1**.

Three grade 1 or 2 treatment-related adverse events (TEAE) were considered definitely related to PolyPEPI1018; the most common was administration site reactions (45.5%), followed by muscle disorders (9.0%) and neurologic disorders (9.0%). One grade 3 serious adverse

Table 2. Treatment-related adverse events ($n = 11$).

Adverse event	Grade 1–2 *n* (%)	Grade 3 *n* (%)
Anemia	1 (9%)	—
Arthralgia	1 (9%)	—
Constipation	1 (9%)	—
Erythema	1 (9%)	—
Fatigue	1 (9%)	—
Injection site reactions[a]	5 (45.4%)	—
Myalgia	1 (9%)	—
Noninfectious acute encephalitis	—	1 (9%)[b]
Burning feeling	1 (9%)	—
Superficial thrombophlebitis	1 (9%)	—
Vomiting	1 (9%)	—

Note: Data show all treatment-emergent adverse events of Grade 1 or 2 and 3 reported as possibly or definitely related to treatment. Relatedness to treatment was determined by the investigator.
[a]Raised erythematous patches, subcutaneous nodules, swelling, burning/pain.
[b]Possibly related.

event (SAE; noninfective encephalitis) occurred 49 days after a single dose, recorded by the investigator as possibly related to the treatment (**Table 2**). There were neither treatment-related deaths nor study discontinuations due to AEs.

Vaccination to multiple anatomic sites was well tolerated according to patient diaries. Redness, itchiness, and swelling were reported by patients for 85 of a total of 380 injections (Supplementary Fig. S2). The severity of these complaints was mild ("some"). There were no "very" recorded for swelling, while "very" was recorded for itchiness by one patient for all four injection sites, which lasted for 2 days. Patients generally felt "some" fatigue; 1 patient recorded it as "very". Redness ("very") was recorded by 1 patient for one injection site of the four. The number of records tended to increase after the second or third dose and in most of the cases resolved in 8 to 12 days after vaccination (Supplementary Fig. S2).

By the end of the study, of the 11 vaccinated patients on maintenance therapy, 3 (27.3%) patients achieved confirmed PR, 4 (36.3%) had SD, and 4 (36.3%) had PD. Response evaluation between RECIST and irRECIST was concordant (**Table 1**). Maximum changes from baseline in target lesion size are summarized in **Fig. 1A**. One of the patients with PR received a single dose (Part A) and two of them received three doses (Part B), despite higher percentage of patients (3/5) with PR on induction treatment being included in Part A of the study than in Part B (2/6; **Fig. 2A**). Longitudinal changes in tumor burden during the study are shown in **Fig. 1B–E** and post-study follow-up data are represented in **Fig. 2**.

Tumor burden continuously decreased compared with baseline for the 3 patients achieving objective response and additionally for 2 patients with SD not achieving the RECIST criteria for objective response. Remarkably, for patient 01–0002, the size of the target lesion in the liver decreased slowly from 72 to 58 mm by week 41, representing 19% tumor shrinkage (**Fig. 1B**). Recurrence was experienced post-study, 21 weeks after the last vaccine dose (**Fig. 2**).

Patient 02–0004 achieved PR during the first-line chemotherapy, as BOR. Conversely, at screening, the last two radiologic assessments indicated one increasing and one decreasing target lesion (**Fig. 1C**). After receiving a single dose of vaccine, both lesions decreased, and by week 6 of the study achieved PR. Recurrence was experienced post-study, 22 weeks after the vaccination (**Fig. 2**).

Patient 01–0004 achieved SD during the first-line chemotherapy, as BOR. He was admitted to the study with one target lesion in the liver and two target lesions in the omentum (**Fig. 1D**). Radiologic assessments showed continuous decrease of the patient's target lesions to the point where the patient qualified for curative surgery (week 26). By the time of the surgery, two lesions out of three resolved to complete response (CR; **Fig. 1D**). Post-surgical pathology showed that peritoneum was positive, while liver and mesentery were negative for tumor cells and that 8 of 29 lymph nodes were positive for cancer cells. Recurrence was experienced post-study, 27 weeks after the last vaccine dose/surgery (**Fig. 2**).

For Patient 01–0007, the first-line treatment produced PR as BOR. Patient 01–0007 transitioned to maintenance therapy with a target lesion in the liver of 29 mm and two nontarget lesions in the colon and lung. Radiologic assessments showed continuous decrease of the patient's target lesion (achieving PR in the study at week 36) to the point where the patient qualified for curative surgery post-study, at week 56 after entering the study (**Fig. 1B** and **E**). Post-surgical pathology showed neither residual nor metastatic adenocarcinoma at multiple resected sites, and 27 of 27 lymph nodes were negative for cancer cells. It was concluded that the patient achieved a CR (score 0) with no viable cancer cells. At the last contact (35 months after entering the study), the patient had no signs of progression (**Fig. 2**).

Durability of responses were assessed *post hoc* by collecting post-study PFS data from the investigators, for all subjects (**Fig. 2A**). The median PFS (mPFS) on maintenance therapy was 31 weeks. Patients obtaining multiple doses controlled their disease for significantly longer period than patients with a single dose [mPFS 54 weeks vs. 20 weeks, respectively; HR = 0.17; 95% confidence interval (CI), 0.04–0.87; $P = 0.017$; **Fig. 2B**]. These data should be treated with some caution, however, because patient numbers are small.

PolyPEPI1018 vaccination induced $CD8^+$ T-cell responses in 9 of 10 (90%), and $CD4^+$ T-cell responses in 10 of 10 (100%) patients as determined using IVS ELISpot (**Fig. 3A** and Supplementary Table S2). $CD8^+$ T-cell responses were assessed by stimulation with twelve individual 9-mer peptides (Supplementary Table S1) specific for the six 30-mer vaccine peptides, while $CD4^+$ T-cell responses were detected using the pool of 30-mer peptides in the IVS ELISpot (**Fig. 3A** and **B**; Supplementary Fig. S3A and S3B).

PolyPEPI1018 vaccination generated $CD8^+$ T-cell responses against average 4.8 (range 2–7) TAAs in the responder patients (**Fig. 3A**). Eight of 10 (80%) patients had $CD8^+$ T-cells induced against at least three TAAs and three against all seven (**Fig. 3A** and **B**). Spontaneous $CD8^+$ T-cell responses covered each of the seven vaccine TAAs across the 7 of 10 patients, with average three targets detected per patient, indicating the presence of real colorectal cancer targets (**Fig. 3A** and **B**). Preexisting responses were augmented (boosted) upon vaccinations in all 7 patients against 1–5 TAAs. For 8 of 10 patients (80%), *de novo* immune responses were induced against 1 to 7 TAAs (**Fig. 3A** and **B**). $CD4^+$ T-cell responses measured using the pool of 30-mer peptides in the IVS ELISpot assays were detected for all 10 analyzed subjects both pre- and post-vaccination (**Fig. 3A**). The breadth and the magnitude (**Fig. 3B** and **C**) of $CD8^+$ T-cell responses significantly increased with second or third dose compared with a single dose ($P = 0.021$ and 0.026, respectively). The $CD4^+$ T-cell responses were also boosted ($P = 0.045$; **Fig. 3A**). The kinetics of immune responses after the first dose suggests a peak response at week 3+6 combined time point for 5 out of 8 patients (Supplementary Fig. S3B).

Ex vivo ELISpot detected effector-type $CD8^+$ T cells were present for 5 of 9 (56%) and $CD4^+$ T cells in 9 of 9 (100%) patients as a result of

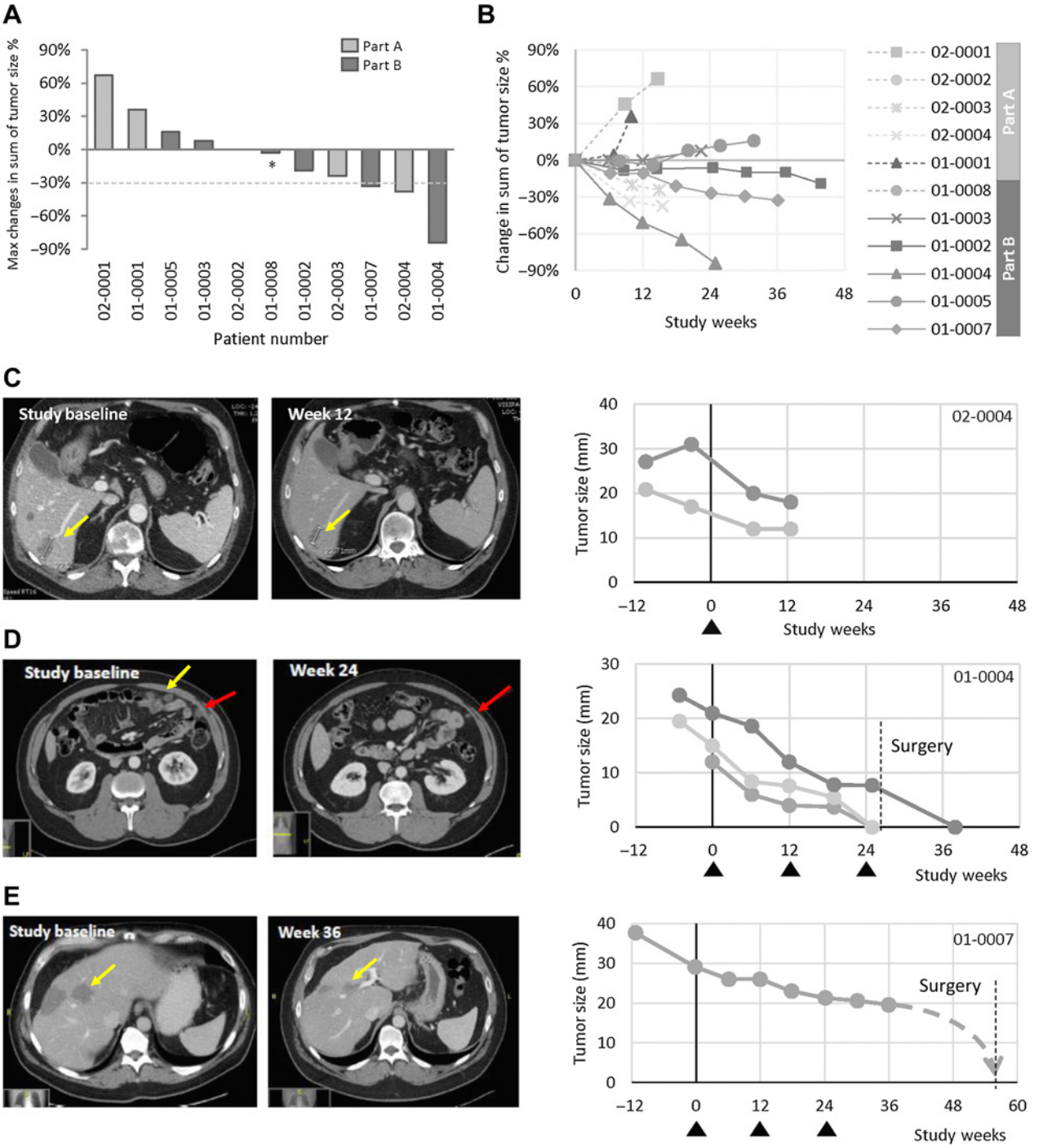

Figure 1.

Antitumor activity. **A,** Maximum changes from baseline in sum of target lesion size. **B,** Spider plot showing the changes of the target lesion sizes at each radiologic assessment (6-weekly). Each data point was compared with the lesion size measured at baseline. Dotted lines indicate patients receiving a single dose. CT scans and individual target lesion size changes for Patient 02–0004 (**C**), Patient 01–0004 (**D**), and Patient 01–0007 (**E**). Dotted lines denote post-study data for Patient 01–0007. Triangles indicate vaccination dates. Different gray shades on the line graphs and different colored arrows on the scans indicate different target lesions of the patients. *, This patient was considered with PD due to clinical progression by the investigator.

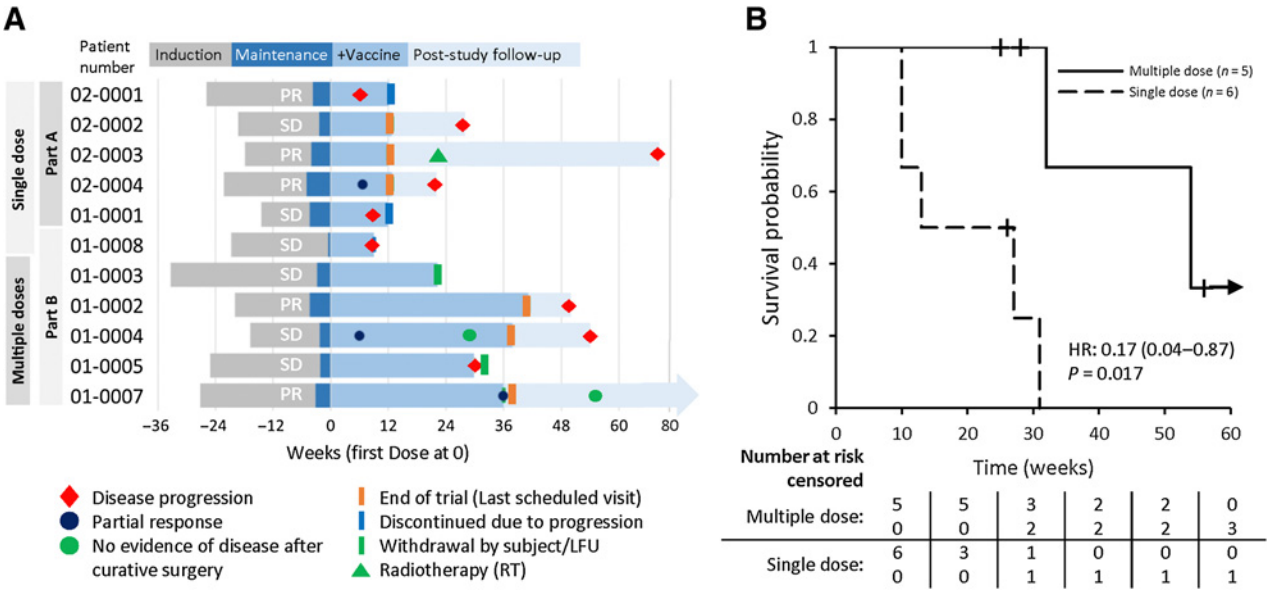

Figure 2.

Durability of responses (*post hoc* analysis). **A,** Swimmer plot analysis for the individual disease courses prior-, during-, and post-study. **B,** Kaplan–Meier curve for the PFS of single- and multiple-dose groups. One patient (Patient 01–0008) from Part B who progressed after one dose and left the trial was included in the single dose group. Number of patients at risk is shown below the Kaplan–Meier curve. The Kaplan–Meier analysis contains censored data for curative surgery, RT, and LFU. PFS was calculated as the time from the date of initiating maintenance therapy to the date of first progression or censored. Post-study, the patients continued on maintenance treatment; for the 3 patients with curative surgery/RT, therapy was discontinued after surgery/RT. LFU, lost to follow-up; RT, radiotherapy.

vaccination (Supplementary Fig. S3C and S3D). Because it is challenging to detect robust effector T-cell responses by *ex vivo* ELISpot assay, supporting flow cytometry analysis was performed. The frequency of *ex vivo* ICS detected vaccine peptide-specific $CD8^+$ T cells increased in 7 of 10 (70%) and $CD4^+$ T cells in 9 of 10 (90%) subjects post-vaccination, confirming the *ex vivo* ELISpot results (Supplementary Fig. S4A and S4B). The $CD8^+$ T cells primarily produced IFNγ, TNFα, and IL2 while $CD4^+$ T cells were positive for mainly IL2 and IFNγ (Supplementary Fig. S4C). Increase in proportion of fully functional IFNγ/TNFα positive cells could be observed upon multiple vaccinations (Supplementary Fig. S4D and S4E; representative IFNγ – CD8/CD4 ICS dot plots are presented in Supplementary Fig. S4F).

Pre- and post-vaccination metastatic liver biopsy pairs were analyzed using the Immunoscore assay to determine immune cell infiltration ($CD3^+$ and $CD8^+$ TILs) in the tumor area (CT and IM; **Fig. 3D**). At baseline, each tumor had "Low" Immunoscore (I = 2) characteristic for liver tumors with bad prognosis in mCRC (Supplementary Fig. S5A; ref. 39). Vaccination induced recruitment of TILs to tumor area for three of the four tested pairs.

Interestingly, for Patient 02–0004, after a single dose of PolyPEPI1018, the number of $CD3^+$ T cells increased by 450% in the IM area of the tumor, and no increase in TILs were observed in the CT. For Patients 01–0002 and 01–0007, increased number of TILs were observed also in the CT, after three doses of PolyPEPI1018 received. The number of $CD8^+$ TILs in the CT continuously increased with subsequent doses such that the ratio of $CD8^+/CD3^+$ T cells achieved 0.66 for Patient 01–0002 and 0.86 for Patient 01–0007, suggesting that PolyPEPI1018 induced accumulation of cytotoxic T cells capable to enter the CT (Supplementary Fig. S5B). Post-vaccination, "High" Immunoscore (I = 3) was obtained for Patient 01–0002 (could not be assessed for Patient 01–0007 because of lack of IM data; Supplementary Fig. S5A).

To evaluate the effect of vaccination on immune-related gene expression in the tumor, we analyzed the CD8 effector T-cell gene signature, IFNγ gene signature and programmed death ligand 1 and 2 (PD-L1, PD-L2) gene signature, previously described as related to the efficacy of immunotherapies in "hot" tumors (40). The median expression level of all the three sets of genes increased with at least two-fold in post-treatment samples of responding tumors ($n = 3$), but not in the nonresponder tumor ($n = 1$; **Fig. 3E**).

Further studies indicated that patients who experienced durable clinical benefit (DCB) had preexisting T-cell responses boosted by the vaccine against higher number of TAAs than others had ($n = 10$, $P = 0.018$; **Fig. 3F**). Correlative studies confirmed that the breadth of vaccine-boosted $CD8^+$ T-cell responses influenced both tumor shrinkage (R = –0.510, $P = 0.132$) and PFS (R = 0.689, $P = 0.030$; Supplementary Fig. S6A and B). Interestingly, no significant difference was observed between the two groups for the preexisting T-cell responses (boosted or not), the *de novo* generated T-cell responses, or their sum, suggesting that restored spontaneous immunity is the main factor for antitumor activity (Supplementary Fig. S6C).

Next, we investigated the predicted HLA-binding capacity of the immunogenic peptides detected for each subject. Using each patients' complete HLA class I genotype, the number of HLA class I alleles predicted to bind each of the 9-mer core peptides were determined (Supplementary Table S3) and results compared with the *in vitro* measured immune responses measured for the same peptides (Supplementary Fig. S7A). Analysis revealed association of detected $CD8^+$ T-cell responses with epitopes restricted to at least three autologous HLA alleles (Personal Epitopes, PEPIs; $P = 0.013$) but not with single HLA allele-restricted epitopes ($P = 1.00$), which highly overestimated detected T-cell responses (no negatives; Supplementary Fig. S7B). In addition, the magnitude of $CD8^+$ T-cell responses generated by PEPIs was significantly higher than those generated by

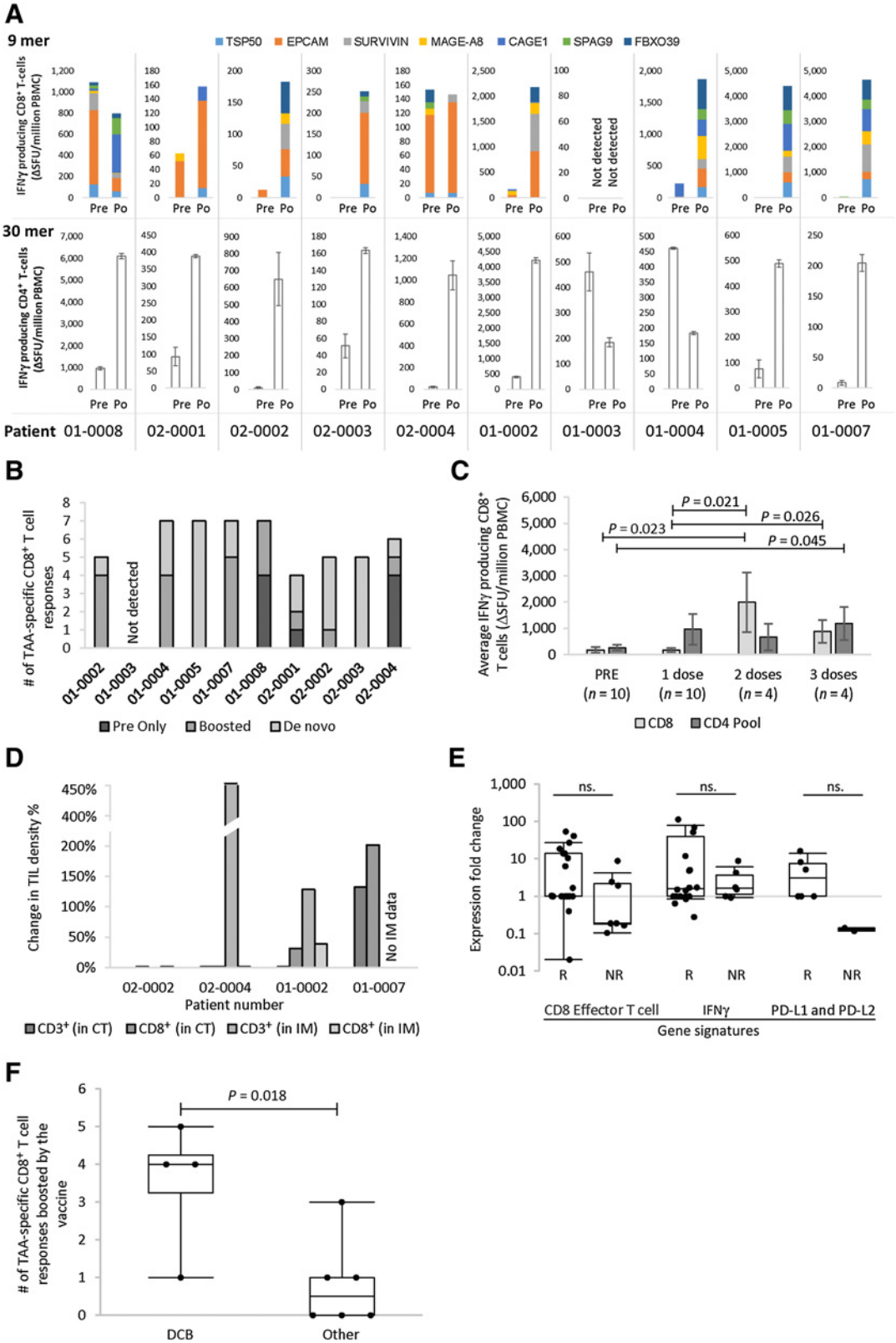
A
9 mer
TSP50
EPCAM
SURVIVIN
MAGE-A8
CAGE1
SPAG9
FBXO39
IFNγ producing CD8+ T-cells (ΔSFU/million PBMC)
Not detected
Not detected
Pre Po
30 mer
IFNγ producing CD4+ T-cells (ΔSFU/million PBMC)
Patient 01-0008 02-0001 02-0002 02-0003 02-0004 01-0002 01-0003 01-0004 01-0005 01-0007
B
# of TAA-specific CD8+ T cell responses
Not detected
01-0002 01-0003 01-0004 01-0005 01-0007 01-0008 02-0001 02-0002 02-0003 02-0004
Pre Only
Boosted
De novo
C
Average IFNγ producing CD8+ T cells (ΔSFU/million PBMC)
P = 0.021
P = 0.026
P = 0.023
P = 0.045
PRE (n = 10)
1 dose (n = 10)
2 doses (n = 4)
3 doses (n = 4)
CD8
CD4 Pool
D
Change in TIL density %
No IM data
02-0002 02-0004 01-0002 01-0007
Patient number
CD3+ (in CT)
CD8+ (in CT)
CD3+ (in IM)
CD8+ (in IM)
E
Expression fold change
ns.
ns.
ns.
R NR R NR R NR
CD8 Effector T cell
IFNγ
PD-L1 and PD-L2
Gene signatures
F
# of TAA-specific CD8+ T cell responses boosted by the vaccine
P = 0.018
DCB
Other

Table 3. Correlation between patients' HLA class I genotype predicted TAA-specific PEPIs and TAA-specific CD8$^+$ T-cell responses detected by IVS ELISpot.

	PEPIs and *in vitro* measured responses									
	Patients									
Vaccine TAA	01–0002	01–0003	01–0004	01–0005	01–0007	01–0008	02–0001	02–0002	02–0003	02–0004
TSP50	TN	TN	FP	TN	TP	TP	TP	FP	TP	TP
EPCAM	TP	TN	TP	TP	FP	TP	TP	FP	FP	TP
SURVIVIN	TP	TN	TP	TN	FP	FP	TN	FP	FP	FP
MAGE-A8	FP	TN	TN	TN	FP	TP	FP	FP	TN	TP
CAGE1	TP	TN	TP	FP	FP	TP	TP	FP	TN	FP
SPAG9	TN	FP	TN	TN	FP	TP	TN	FP	FP	TP
FBXO39	FP	FP	TN	FP	TP	TP	TN	TP	TP	TP

Note: A predicted positive response means the patient was able to make a PEPI derived from the vaccine TAA, based on his/her HLA-genotype. FN, false negative; FP, false positive; PEPI, personal epitope (predicted epitope binding to ≥3 autologous HLA class I allele); TN, true negative; TP, true positive. PEPI-predicted and ELISpot measured matching results (TP and TN) are highlighted in gray ($P = 0.01$, Fisher exact). FP – PEPI was predicted but negative by ELISpot; FN – no PEPI was predicted but positive by ELISpot. Table related to Supplementary Table S3 and Supplementary Fig. S7A.

non-PEPIs ($P = 0.011$; Supplementary Fig. S7C). We found that 79% of predicted TAA-specific PEPIs (for 7 TAAs × 10 patients) were confirmed by IFNγ$^+$ CD8$^+$ T-cell responses detected *in vitro* (**Table 3**). PEPIs in general underestimated the subjects' overall T-cell repertoire, however they precisely predicted subjects' PEPI-specific CD8$^+$ T-cell responses.

Consequently, although not statistically significant, patients with DCB had predicted PEPIs for higher number of TAAs than Others (mean 2.68 vs. 1.65, $P = 0.432$), in conformance with the breadth of measured CD8$^+$ T-cell responses in this group (Supplementary Fig. S7D).

Discussion

The OBERTO-101 trial showed that vaccination with PolyPEPI1018 combined with maintenance treatment was safe and well tolerated and appears to induce durable antitumor responses in patients with previously unresectable MSS mCRC following induction therapy. The most common TEAEs were Grade 1/2 injection site reactions likely associated with the use of Montanide ISA 51VG adjuvant (41). No severe (Grade 3 or higher) local skin reactions were observed by the investigators, or reported by the patients, despite each dose being administered to four different anatomic sites. One Grade 3 SAE (noninfectious acute encephalitis) was observed and defined as possibly related to the treatment by the investigator. After careful review of the published scientific data and the data generated in the study, causality could not be confirmed taking into consideration lack of temporary association (49 days post-dose), as well. Specifically, no data were found on the elevated expression of the vaccine-targeted TAAs in normal brain tissue, except for SPAG9. Multiple datasets within the Human Protein Atlas, including the GTEx Portal (https://gtexportal.org; dbGaP Accession phs000424.v8.p2) providing RNA transcript data, documented the overexpression of SPAG9 in healthy tissues (including brain; ref. 42), not found by others in earlier studies using IHC or RT PCR methods (31, 43). These observations indicate that the newer methods may shed new light on TAA selection. Nevertheless, the patient (02–0004) had no SPAG9-specific T-cell responses boosted by the vaccine, but many other patients had, without any SAE observed. Overall, the immunologic responses mounted by the vaccine were very similar to other patients' responses in terms of breadth and magnitude. Nevertheless, each 30-mer vaccine peptide was inspected for sequence homology with human proteome (during design), without any cross-reactive neoepitopes identified.

The de-intensified chemo backbone adopted in the setting of maintenance therapy with the addition of PolyPEPI1018 further reduced the tumor burden achieved by the induction chemotherapy for 3 patients (ORR, 27.3%) and maintained tumor responses for another 3 patients. Response to induction therapy (PR) did not seem to

Figure 3.

Colorectal cancer–specific immune responses induced by PolyPEPI1018 at peripheral and tumor level indicate treatment benefit. **A,** The breadth of TAA-specific CD8$^+$ T-cell responses and magnitude of CD4$^+$ T-cell responses in each patient measured by IVS ELISpot. Post-vaccination (Po) results are the maximum responses measured for each subject during the study. CD8 T-cell responses were measured using the individual 9-mer test peptides listed in Supplementary Table S1, and summed for obtaining TAA-specific responses. CD4 T-cell responses were measured with the pooled 30-mer peptides. **B,** Number of responsive vaccine TAAs plotted by patient ($n = 10$). Dark gray bars: Pre-only: No change or <2-fold increase in responses compared with pre-vaccination; mid gray bars: response boosted compared with pre-vaccination (at least 2-fold increase by IVS ELISpot); light gray: *de novo* induced vaccine-specific immune responses (no pre-vaccination response measured). **C,** Magnitude of PolyPEPI1018 vaccine-specific CD8$^+$ and CD4$^+$ T-cell responses detected at baseline and after multiple doses. **D,** T-cell (TIL) infiltration to the CT and IM area of the tumors post-vaccination assessed by HalioDx's Immunoscore CR TL assay. Changes in TIL density were calculated from IHC data obtained for pre/post-vaccination biopsy pairs; pre: baseline sample (except for Patient 01–0002, it was week 12 sample); Post: tumor biopsy at week 12 (for Patients 02–0002 and 02–0004) or week 38 (Patients 01–0002 and 01–0007). **E,** Whisker plot of upregulated gene-expression signatures upon treatment with PolyPEPI1018 for responder (R) tumors (Patients 02–0004, 01–0002, and 01–0007) and a nonresponder (NR) tumor (Patient 02–0002) assessed by NanoString's PanCancer gene-expression panel. Each dot represents the fold change of a gene in a sample. Boxes on the plot represents the quartiles (1 to 3). CD8$^+$ effector T-cell gene signature: [CD8A molecule (*CD8A*), CD8B molecule (*CD8B*), eomesodermin (*EOMES*), granzyme A (*GZMA*), granzyme B (*GZMB*), IFNγ (*IFNG*), and perforin 1 (*PRF1*)]; IFNγ gene signature: [indoleamine 2,3-dioxygenase 1 (*IDO1*), C-X-C motif chemokine ligand (*CXCL*) 9 and 10, MHC class II DR α (*HLA-DRA*), signal transducer and activator of transcription 1 (*STAT1*) and *IFNG*] and PD-L1 and PD-L2 gene signatures. **F,** Impact of TAA-specific CD8$^+$ T-cell responses boosted by the vaccine (measured by IVS ELISpot) on treatment benefit; DCB, durable clinical benefit = patients with objective tumor response (PR) and/or stabilized disease (SD) for at least 50 weeks on maintenance treatment; Others: patients with no DCB. ΔSFU, background corrected spot-forming units; #, number. ND, not detected; ns., not significant. Error bars represent SEM.

be a prerequisite for the tumor responses obtained in the study, as the five PRs entering the study become 2 PR, 2 SD, and 1 PD on maintenance plus vaccine treatment, respectively. However, of the three PRs on maintenance plus vaccine treatment, two were PR on induction therapy and one transitioned from SD. Radiologic assessments of the tumors showed slow but continuous decrease of the tumor size, characteristic for the delayed effect seen for immunotherapies (44).

The mPFS for the patients who received multiple vaccinations in Part B was 12.5 months. This compares favorably with mPFS of 7.39 months obtained in the large MODUL trial applying same maintenance treatment with/without CPI following induction therapy in MSS-mCRC subjects, as well as to the mPFS obtained in two other large studies using 5-FU/capecitabine and bevacizumab maintenance treatment for mCRC (10, 13, 16). Importantly, in the MODUL trial, CPI combined with the maintenance therapy did not improve mPFS and no objective tumor responses were recorded for MSS-mCRC subjects. While there are significant limitations in comparing these results with data from large phase III studies, objective tumor responses obtained in our study support the increased mPFS compared with historical data and may show promise this regimen could extend time on maintenance therapy and delay the initiation of second-line therapy. However, given that most of the patients achieving PR in the study (2/3) also progressed within 22 to 27 weeks after the last vaccine dose, suggests optimization of the dosing regimen may be needed in order to maintain high frequency of circulating effector-memory T cells. Every 3 months vaccination regime was clearly suboptimal as $CD8^+$ T-cell responses, while still detectable, declined by Week 12 post-vaccination compared with peak response at Week 3 to 6.

Another unexpected finding of the study was that 2 patients had disease regression to the point that they were considered for curative intent surgery, despite being deemed unresectable at diagnosis and after induction therapy. One of these patients has been off systemic therapy since surgical resection two years ago, and as of the time of this publication has not had disease recurrence. For a third patient, radiotherapy of the lung nodule was also made feasible. These results provide a signal that PolyPEPI1018 may improve the chances of conversion from unresectable to resectable disease in mCRC.

The breadth of immune responses elicited by the vaccine was remarkably high. Multi-antigenic $CD8^+$ T-cell responses detected for 80% of patients exceed immune response rates reported for other multi-epitope off-the-shelf or personalized neoantigen-based cancer vaccines (45–47) and confirm our *in silico* model population-based vaccine design approach (36, 37). Immune responses against multiple epitopes were previously described as related to overall survival, also shown in our study for PFS and decrease in tumor burden (45). Subjects' complete HLA genotype influenced their $CD8^+$ T-cell responses and promiscuous autologous allele-binding capacity (PEPI) was a key feature of immunogenic epitopes. Correlation between predicted PEPIs and *in vitro* measured T-cell responses were already observed in our previous studies conducted with COVID-19 convalescent subjects and patients with cancer, also in our meta-analysis conducted with 94 cancer vaccine studies (36, 37, 48). HLA-genotype, through multiple TAA-derived PEPIs tended to predict treatment benefit, too. This is in good agreement with the recent finding where patients' HLA class I genotype (HLA heterozygosity) influenced their response to CPI therapy presumably due to efficient HLA presentation of TAAs triggering efficient $CD8^+$ T-cell responses (49). In our study, this hypothesis is supported by the dramatic increase of the density of $CD3^+/CD8^+$ TILs, and by upregulation of the genes related to fully functional, cytotoxic, $CD8^+$ T cells in the responder tumors, post-treatment. Of note, both the examined $CD8^+$ T-cell gene signatures and the "High" Immunoscore are known to describe a "hot" tumor, associated with response to immunotherapies (7, 39, 40). Interestingly, we found that preexisting, multi-antigenic $CD8^+$ T-cell responses successfully boosted by the vaccine were the main factor for antitumor activity and subsequent clinical benefit. Thus, PolyPEPI1018 vaccination restored and boosted spontaneous, HLA-genotype–dependent antitumor immunity and increased the frequency of cytotoxic TILs (turning "cold" tumor into "hot" tumor) which could explain how PolyPEPI1018 vaccination showed clinical activity in combination with 5-FU/bevacizumab. Given that CPIs are known to be effective in "hot" tumors, our results suggest that studies conducted in "cold" tumors (like the MODUL trial or OBERTO-101) could benefit from the use of PolyPEPI1018 in combination with CPIs.

A limitation of our study is the nonrandomized design of the trial, which precludes definitive conclusions on the components of the study therapy. Although this study was not designed to be definitive, nevertheless we attempted to mitigate this risk by testing two schedules of the vaccine and noticed an initial correlation between longer dosing and PFS. In addition, as the background chemotherapy consisted of a subset of the induction therapy, it is unlikely that the tumor responses would have deepened with the chemotherapy alone. Another limitation is that the immune responses that were noted in the vast majority of the patients treated with the vaccine resulted in clinical benefit of a lesser magnitude/frequency. This could have two reasons: one is the sub-optimal dosing (single dose for half of the study population) and dosing regimen applied in the study, as discussed above. The other reason is the "typical" critical issue for the field in its quest to develop immune biomarkers with predictive potential, on which however we present new insight and unmatched data in the literature. We did not evaluate circulating tumor cells or circulating tumor DNA in our studies. Perhaps these additional assessments would have provided additional insights on antitumor activity and potential correlations with immune responses. In addition, the majority of patients enrolled in the study were males of White race and 73% of them harbored a *KRAS* mutation while *BRAF* mutations were excluded. Future investigations will need to study safety, immunogenicity, and efficacy in patients with molecular alterations more representative of the general mCRC population.

In conclusion, the correlating clinical and immune responses warrant further investigation of PolyPEPI1018 combined with maintenance therapy in patients with MSS mCRC, in a randomized controlled setting. Patients receiving multiple doses of PolyPEPI1018 had longer disease control and augmented immune responses compared with those patients with a single vaccination. Therefore, further studies with PolyPEPI1018 will involve multiple vaccinations and more frequent dosing in attempt to improve response rates and disease control. In addition, HLA-genotype dependent PEPI biomarker may be developed to select likely responders in order to "personalize" the therapy while maintaining its "off-the-shelf" nature. PolyPEPI1018 may provide a new opportunity for immune-based therapy for patients with MSS mCRC.

Authors' Disclosures

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work. E.R. Tőke reports personal fees from Treos Bio Zrt outside the submitted work; in addition, E.R. Tőke has a patent for PolyPEPI1018 vaccine composition pending and issued, and holds shares of Treos Bio Ltd. R.P. Graham reports grants from Bristol-Myers Squibb; and other support from Incyte outside the submitted work. H. Youssoufian reports personal fees from Treos Bio, Verastem Oncology, Agenus Bio, and OncXerna Therapeutics outside the submitted work. O. Lőrincz reports personal fees from Treos Bio Zrt outside the submitted work; in addition, O. Lőrincz has a patent for PolyPEPI1018 vaccine composition (co-inventor) pending, and holds shares at Treos Bio Ltd. L. Molnár reports personal fees from Treos Bio Zrt outside the submitted work; in addition, L. Molnár has a patent for PolyPEPI1018 vaccine composition pending, and holds shares at Treos Bio Ltd. Z. Csiszovszki reports personal fees from Treos Bio Zrt outside the submitted work; in addition, Z. Csiszovszki has a patent for PolyPEPI1018 vaccine composition pending, and holds shares at Treos Bio Ltd. J. Tóth reports personal fees from Treos Bio Zrt outside the submitted work; in addition, J. Tóth has a patent for PolyPEPI1018 vaccine composition pending, and holds shares at Treos Bio Ltd. C. Cremolini reports grants and personal fees from Merck, Servier, and Bayer, as well as personal fees from Roche, Amgen, Nordic Pharma, Merck Sharp & Dohme, and Pierre Fabre outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

J.M. Hubbard: Formal analysis, investigation, methodology, writing–original draft. **E.R. Tőke:** Data curation, formal analysis, methodology, writing–original draft, writing–review and editing. **R. Moretto:** Investigation, writing–review and editing. **R.P. Graham:** Investigation, methodology. **H. Youssoufian:** Formal analysis, writing–review and editing. **O. Lőrincz:** Formal analysis. **L. Molnár:** Formal analysis, visualization, statistical analysis. **Z. Csiszovszki:** Methodology. **J.L. Mitchell:** Project administration. **J. Wessling:** Project administration. **J. Tóth:** Formal analysis, methodology. **C. Cremolini:** Formal analysis, investigation, writing–original draft, writing–review and editing.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019;69:7–34.
2. Saunders M, Iveson T. Management of advanced colorectal cancer: state of the art. Br J Cancer 2006;95:131–8.
3. Cremolini C, Antoniotti C, Rossini D, Lonardi S, Loupakis F, Pietrantonio F, et al. Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): a multicenter, open-label, phase III, randomized, controlled trial. Lancet Oncol 2020;21:497–507.
4. Venook AP, Niedzwiecki D, Lenz HJ, Innocenti F, Fruth B, Meyerhardt JA, et al. Effect of first-line chemotherapy combined with cetuximab or bevacizumab on overall survival in patients with KRAS wild-type advanced or metastatic colorectal cancer: a randomized clinical trial. JAMA 2017;317:2392–401.
5. Moretto R, Cremolini C, Rossini D, Pietrantonio F, Battaglin F, Mennitto A, et al. Location of primary tumor and benefit from anti-epidermal growth factor receptor monoclonal antibodies in patients with RAS and BRAF wild-type metastatic colorectal cancer. Oncologist 2016;21:988–94.
6. Tejpar S, Stintzing S, Ciardiello F, Tabernero J, Van Cutsem E, Beier F, et al. Prognostic and predictive relevance of primary tumor location in patients with RAS wild-type metastatic colorectal cancer: retrospective analyses of the CRYSTAL and FIRE-3 trials. JAMA Oncol 2017;3:194–201.
7. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumors with combination immunotherapies. Nat Rev Drug Discov 2019;18:197–218.
8. Boisen MK, Johansen JS, Dehlendorff C, Larsen JS, Østerlind K, Hansen J, et al. Primary tumor location and bevacizumab effectiveness in patients with metastatic colorectal cancer. Ann Oncol 2013;24:2554–9.
9. Tournigand C, Cervantes A, Figer A, Lledo G, Flesch M, Buyse M, et al. OPTIMOX1: a randomized study of FOLFOX4 or FOLFOX7 with oxaliplatin in a stop-and-go fashion in advanced colorectal cancer–a GERCOR study. J Clin Oncol 2006;24:394–400.
10. Hegewisch-Becker S, Graeven U, Lerchenmuller CA, Killing B, Depenbusch R, Steffens CC, et al. Maintenance strategies after first-line oxaliplatin plus fluoropyrimidine plus bevacizumab for patients with metastatic colorectal cancer (AIO 0207): a randomized, noninferiority, open-label, phase III trial. Lancet Oncol 2015;16:1355–69.
11. Hubbard JM, Grothey A. When less is more: maintenance therapy in colorectal cancer. Lancet 2015;385:1808–10.
12. Koeberle D, Betticher DC, von Moos R, Dietrich D, Brauchli P, Baertschi D, et al. Bevacizumab continuation versus no continuation after first-line chemotherapy plus bevacizumab in patients with metastatic colorectal cancer: a randomized phase III noninferiority trial (SAKK 41/06). Ann Oncol 2015;26:709–14.
13. Simkens LH, van Tinteren H, May A, ten Tije AJ, Creemers GJ, Loosveld OJ, et al. Maintenance treatment with capecitabine and bevacizumab in metastatic colorectal cancer (CAIRO3): a phase III randomized controlled trial of the Dutch colorectal cancer group. Lancet 2015;385:1843–52.
14. Tournigand C, Chibaudel B, Samson B, Scheithauer W, Vernerey D, Mésange P, et al. Bevacizumab with or without erlotinib as maintenance therapy in patients with metastatic colorectal cancer (GERCOR DREAM; OPTIMOX3): a randomized, open-label, phase III trial. Lancet Oncol 2015;16:1493–505.
15. Kreidieh M, Mukherji D, Temraz S, Shamseddine A. Expanding the scope of immunotherapy in colorectal cancer: current clinical approaches and future directions. Biomed Res Int 2020;2020:9037217.
16. Grothey A, Tabernero J, Arnold D, De Gramont A, Ducreux MP, O'Dwyer PJ, et al. Fluoropyrimidine (FP) + bevacizumab (BEV) + atezolizumab vs FP/BEV in BRAFwt metastatic colorectal cancer (mCRC): findings from cohort 2 of MODUL – a multicenter, randomized trial of biomarker-driven maintenance treatment following first-line induction therapy. Ann Oncol 2018;29:viii714–5.
17. Mansfield AS, Nevala WK, Lieser EA, Leontovich AA, Markovic SN. The immunomodulatory effects of bevacizumab on systemic immunity in patients with metastatic melanoma. Oncoimmunology 2013;2:e24436.
18. Emens LA, Middleton G. The interplay of immunotherapy and chemotherapy: harnessing potential synergies. Cancer Immunol Res 2015;3:436–43.
19. Galluzzi L, Humeau J, Buqué A, Zitvogel L, Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. Nat Rev Clin Oncol 2020;17:725–41.
20. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunologic effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 2015;28:690–714.
21. Gmeiner WH. Fluoropyrimidine modulation of the antitumor immune response-prospects for improved colorectal cancer treatment. Cancers 2020;12:1641.
22. Fang H, Ang B, Xu X, Huang X, Wu Y, Sun Y, et al. TLR4 is essential for dendritic cell activation and antitumor T-cell response enhancement by DAMPs released from chemically stressed cancer cells. Cell Mol Immunol 2014;11:150–9.
23. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell–dependent antitumor immunity. Cancer Res 2010;70:3052–61.
24. Roland CL, Lynn KD, Toombs JE, Dineen SP, Udugamasooriya DG, Brekken RA. Cytokine levels correlate with immune cell infiltration after anti-VEGF therapy in preclinical mouse models of breast cancer. PLoS One 2009;4:e7669.

25. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet A-L, et al. VEGF-A modulates expression of inhibitory checkpoints on $CD8^+$ T cells in tumors. J Exp Med 2015;212:139–48.
26. Wallin JJ, Bendell JC, Funke R, Sznol M, Korski K, Jones S, et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. Nat Commun 2016;7:12624.
27. Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, et al. Normalization of the vasculature for treatment of cancer and other diseases. Physiol Rev 2011; 91:1071–121.
28. Zheng L, Xie G, Duan G, Yan X, Li Q. High expression of testes-specific protease 50 is associated with poor prognosis in colorectal carcinoma. PLoS One 2011;6: e22203.
29. Goossens-Beumer IJ, Zeestraten EC, Benard A, Christen T, Reimers MS, Keijzer R, et al. Clinical prognostic value of combined analysis of Aldh1, Survivin, and EpCAM expression in colorectal cancer. Br J Cancer 2014;110: 2935–44.
30. Choi J, Chang H. The expression of MAGE and SSX, and correlation of COX2, VEGF, and survivin in colorectal cancer. Anticancer Res 2012;32:559–64.
31. Kanojia D, Garg M, Gupta S, Gupta A, Suri A. Sperm-associated antigen 9 is a novel biomarker for colorectal cancer and is involved in tumor growth and tumorigenicity. Am J Pathol 2011;178:1009–20.
32. Dakshinamurthy AG, Ramesar R, Goldberg P, Blackburn JM. Infrequent and low expression of cancer-testis antigens located on the X chromosome in colorectal cancer: implications for immunotherapy in South African populations. Biotechnol J 2008;3:1417–23.
33. Song MH, Ha JC, Lee SM, Park YM, Lee SY. Identification of BCP-20 (FBXO39) as a cancer/testis antigen from colon cancer patients by SEREX. Biochem Biophys Res Commun 2011;408:195–201.
34. Hasegawa H, Mori M, Haraguchi M, Ueo H, Sugimachi K, Akiyoshi T. Expression spectrum of melanoma antigen-encoding gene family members in colorectal carcinoma. Arch Pathol Lab Med 1998;122:551–4.
35. Hubbard JM, Cremolini C, Graham RP, Moretto R, Mitchell JL, Wessling J, et al. A phase I study of PolyPEPI1018 vaccine plus maintenance therapy in patients with metastatic colorectal cancer with a predictive biomarker (OBERTO). J Clin Oncol 2019;37:3557.
36. Somogyi E, Csiszovszki Z, Molnar L, Lorincz O, Toth J, Pattijn S, et al. A peptide vaccine candidate tailored to individuals' genetics mimics the multi-targeted T-cell immunity of COVID-19 convalescent subjects. Front Genet 2021;12:684152.
37. Lorincz O, Toth J, Molnar L, Miklos I, Pantya K, Megyesi M, et al. *In silico* model estimates the clinical trial outcome of cancer vaccines. Cells 2021;10: 3048.
38. Saxena M, van der Burg SH, Melief CJM, Bhardwaj N. Therapeutic cancer vaccines. Nat Rev Cancer 2021;21:360–78.
39. Wang Y, Lin HC, Huang MY, Shao Q, Wang ZQ, Wang FH, et al. The Immunoscore system predicts prognosis after liver metastasectomy in colorectal cancer liver metastases. Cancer Immunol Immunother 2018;67:435–44.
40. Ayers M, Lunceford J, Nebozhyn M, Murphy E, Loboda A, Kaufman DR, et al. IFNγ-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest 2017;127:2930–40.
41. van Doorn E, Liu H, Huckriede A, Hak E. Safety and tolerability evaluation of the use of Montanide ISA51 as vaccine adjuvant: a systematic review. Hum Vaccin Immunother 2016;12:159–69.
42. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. Science 2015;23; 347:1260419.
43. Yi F, Ni W, Liu W, Pan X, Han X, Yang L, et al. SPAG9 is overexpressed in human astrocytoma and promotes cell proliferation and invasion. Tumour Biol 2013;34: 2849–55.
44. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412–20.
45. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, et al. Multi-peptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 2012;18: 1254–61.
46. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature 2017;547:217–21.
47. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Lower M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature 2017;547:222–6.
48. Toke ER, Megyesi M, Molnar L, Tóth J, Lőrincz O, van der Burg SH, et al. Prediction the clinical outcomes of cancer patients after peptide vaccination. J Clin Oncol 2019;37(15_suppl):e14295.
49. Chowell D, Morris LGT, Grigg CM, Weber JK, Samstein RM, Makarov V, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. Science 2018;359:582–7.