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Immunization with mutant p53- and K-ras-derived peptides in cancer patients: Immune response and clinical outcome — Source link

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Abstract

PURPOSE: To determine the ability to induce tumor-specific immunity with individual mutant K-ras-or p53-derived peptides and to monitor clinical outcome. PATIENTS AND METHODS: Patients in varying stages of disease underwent genetic analysis for mutations in K-ras and p53. Thirty-nine patients were enrolled. Seventeen-mer peptides were custom synthesized to the corresponding mutation. Baseline immunity was assessed for cytotoxic T-lymphocyte (CTL) response and interferon gamma (IFN-gamma) release from mutant peptide-primed lymphocytes. Patients' peripheral-blood mononuclear cells were pulsed with the corresponding peptide, irradiated, and applied intravenously. Patients were observed for CTL, IFN-gamma, interleukin (IL) -2, IL-5, and granulocyte-macrophage colony-stimulating factor responses, for treatment-related toxicity, and for tumor response. RESULTS: No toxicity was observed. Ten (26%) of 38 patients had detectable CTL against mutant p53 or K-ras, and two patients were positive for CTL at baseline. Positive IFN-gamma responses occurred in 16 patients (42%) after vaccination, whereas four patients had positive IFN-gamma reaction before vaccination. Of 29 patients with evident disease, five experienced a period of stable disease. Favorable prognostic markers were detectable CTL activity and a positive IFN-gamma reaction but not IL-5 release. Median survival times of 393 v 98 days for a positive versus negative CTL response ($P = .04$), respectively, and of 470 v 88 days for a positive versus negative IFN-gamma response ($P = .02$), respectively, were detected. CONCLUSION: Custom-made peptide vaccination is feasible without any toxicity. CTL and cytokine responses specific to a given mutation can be induced or enhanced with peptide vaccines. Cellular immunity to mutant p53 and K-ras oncopeptides is associated with longer survival.

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ORIGINAL REPORT

Immunization With Mutant *p53*- and *K-ras*–Derived Peptides in Cancer Patients: Immune Response and Clinical Outcome

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose
To determine the ability to induce tumor-specific immunity with individual mutant *K-ras*– or *p53*-derived peptides and to monitor clinical outcome.

Patients and Methods
Patients in varying stages of disease underwent genetic analysis for mutations in *K-ras* and *p53*. Thirty-nine patients were enrolled. Seventeen-mer peptides were custom synthesized to the corresponding mutation. Baseline immunity was assessed for cytotoxic T-lymphocyte (CTL) response and interferon gamma (IFN-γ) release from mutant peptide-primed lymphocytes. Patients' peripheral-blood mononuclear cells were pulsed with the corresponding peptide, irradiated, and applied intravenously. Patients were observed for CTL, IFN-γ, interleukin (IL) -2, IL-5, and granulocyte-macrophage colony-stimulating factor responses, for treatment-related toxicity, and for tumor response.

Results
No toxicity was observed. Ten (26%) of 38 patients had detectable CTL against mutant *p53* or *K-ras*, and two patients were positive for CTL at baseline. Positive IFN-γ responses occurred in 16 patients (42%) after vaccination, whereas four patients had positive IFN-γ reaction before vaccination. Of 29 patients with evident disease, five experienced a period of stable disease. Favorable prognostic markers were detectable CTL activity and a positive IFN-γ reaction but not IL-5 release. Median survival times of 393 *v* 98 days for a positive versus negative CTL response ($P = .04$), respectively, and of 470 *v* 88 days for a positive versus negative IFN-γ response ($P = .02$), respectively, were detected.

Conclusion
Custom-made peptide vaccination is feasible without any toxicity. CTL and cytokine responses specific to a given mutation can be induced or enhanced with peptide vaccines. Cellular immunity to mutant *p53* and *K-ras* oncopeptides is associated with longer survival.

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INTRODUCTION

Immunologic targeting of cancer with vaccines against the host's tumors bears the potential for nontoxic and specific therapy. Molecular characterization of activated proto-oncogenes and inactivated tumor suppressor genes has linked these genes to malignant progression.[1] Mutations of *p53* and *K-ras* are frequent and often result in the generation of novel protein sequences that are overexpressed in tumor cells bearing these mutations.[2-4] Novel protein sequences generated by point mutations expressed intracellularly can be processed and presented on the cell surface in the

context of major histocompatibility complex (MHC) class I and, thus, become accessible to cytotoxic T cells.[5-8] Thus, the overexpressed mutant p53 and K-ras oncoproteins provide novel potential T-cell epitopes for targeting by cellular immunity.

The host immune system involves a multitude of cell types and mediators interacting with tumor cells. Two important populations of effector cells responsible for tumor lysis are the cytotoxic T cell and the natural killer cells. The cytotoxic lymphocyte recognizes endogenous gene products in the context of MHC class I by the T-cell receptor of $CD8^+$ cells. Typically, short peptide fragments of endogenous proteins are found to be eight or nine residues long and are presented on the cell surface.[9] These presented peptides are generated by endogenous antigen processing of either intracellular or membrane-bound proteins. Oncoprotein-derived peptides from both p53 and K-ras have been shown to be processed and presented in the context of MHC class I in tumor cells in mice[8,10-12] and humans.[13-17]

In the present trial, we investigated the potential of oncopeptide immunization for the purpose of immunotherapy in cancer patients. Immunization included a cellular vaccine with a single peptide of either p53 or K-ras surrounding the tumor-specific point mutation characteristic of the tumor cells. Peptides were chosen to encompass the mutation. The peptide was loaded on peripheral-blood mononuclear cells (PBMC) from the patient, which functioned as antigen-presenting cells (APCs). The present protocol represents a unique immunization approach and establishes the ability of peptide-loaded nonprofessional APCs to function as cellular vaccines after irradiation in humans.

PATIENTS AND METHODS

Patient Inclusion Criteria

Patients with several types of cancers (lung, colon, breast, ovarian, head and neck, pancreatic, esophageal, gastric, and others) were eligible (National Cancer Institute [NCI] trial T93-0148). Approval of the local human investigation committee and by the Department of Health and Human Services was ascertained. Patients with either evident disease and no curative option or with no evident disease but a greater than 50% probability of recurrence were eligible. Genetic analysis for suitable mutations in *K-ras* and *p53* was performed. One of the following was required: a point mutation altering the protein sequence, a frame shift mutation, or an insertion or deletion internal to the coding sequence. Eastern Cooperative Oncology Group performance status of 0 or 1 and adequate organ functions were required. Cytotoxic chemotherapy or corticosteroids were not allowed within 4 weeks of the vaccination. It was not allowed to have curative option delayed. To avoid treatment of anergic individuals, one positive skin test for delayed hypersensitivity to a common antigen injected intradermally (*Candida*, mumps, and *Trichophyton*) had to be present. To make this criterion more relevant to the immunologic end points, a positive interferon gamma (IFN-γ) response to whole influenza virus infection of autologous cells was added as a requirement for the protocol after the first seven patients. An IFN-γ response of at least two-fold greater than the response in the absence of influenza virus or a positive cytotoxic T-lymphocyte (CTL) response to influenza was required.

Mutation Analysis of p53 and K-ras

RNA and DNA extraction was as described elsewhere.[18] Mutations were screened by using single-strand conformational polymorphism analysis on polymerase chain reaction–amplified DNA, and the exact mutation was determined by DNA sequencing.

Peptide Synthesis and Verification

Peptides were synthesized under good laboratory practices conditions by Peninsula Laboratories (Belmont, CA), Bachem California (San Diego, CA), or Multiple Peptide Systems (San Diego, CA) before shipment to the NCI for vialing. An automated synthesizer with standard solid-phase chemistry to produce peptides of 17 residues was used.

Immunization

All peptides were subjected to high-performance liquid chromatography analysis and sterility, pyrogen, and animal toxicity tests. PBMC were drawn from a patient's peripheral vein, and cells were made devoid of RBCs by gradient centrifuging. Alternatively, pheresis was performed to obtain 10^9 PBMC, and 5×10^8 PBMC were pulsed with peptide; after aliquots were removed for testing, the recovered cells were infused (usually 3 to 4×10^8 per dose). Cells were pulsed with peptide at the appropriate concentration and irradiated with 25 Gy before intravenous injection. Immunization was repeated 21 days later. Repeat immunizations were administered every 2 months for a total of four immunizations or longer.

Assessment of Cellular Immune Response

To test for immunity against *p53*- and *K-ras*–derived mutant oncopeptides, patients' PBMC were obtained and tested for reactivity in vitro. The plasma supernatant of restimulated PBMC was collected and saved for antibody studies. For evaluating CTL and cytokine activity in peripheral blood, PBMC were cultured in the presence of 1 to 10 μmol/L of peptide and 50 U/mL of recombinant interleukin (IL) -2 (Cetus Corporation, Emoryville, CA). Cytotoxicity against a specific peptide was assessed using autologous Epstein-Barr virus (EBV) –transformed lymphoblastoid cell lines as target cells.[19] The percent specific lysis was determined, on triplicate samples, by the following formula: percent specific lysis = (release − spontaneous release) ÷ (maximum release − spontaneous release) × 100.

Cytokine Assays

The ability of patient PBMC to produce IL-2 to mutant oncogene peptide was tested.[20] IFN-γ, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were measured on the same culture supernatants using commercial enzyme-linked immunosorbent assay kits (Endogen, Boston, MA for IFN-γ; R&D Systems, Minneapolis, MN; and Biosource, Camarillo, CA). For IL-2 response, PBMC were cultured for 7 days in the presence or absence of patient-specific mutant oncogene peptide and antihuman IL-2–receptor antibody, which blocks IL-2 consumption (Anti-Tac, a gift of T.A. Waldmann, Bethesda, MD). Positive control wells were stimulated with influenza A, phytohaemagglutinin, or allogeneic PBMC. Culture supernatants were harvested and stored frozen until assayed for IL-2 content. The

IL-2 assay consisted of culturing an IL-2–dependent cytotoxic T-cell line in the presence of titrated amounts of the culture supernatants for 42 hours with the addition of [^{3}H]thymidine for the last 18 hours. The wells were harvested, and the amount of ^{3}H incorporation was measured with a liquid scintillation counter. After subtraction of background counts (counts from supernatants of unstimulated PBMCs), the amount of IL-2 produced by patient PBMC was compared with a standard curve generated with a known amount of recombinant human IL-2. PBMC were also used to determine immunity against influenza. Patients were eligible for vaccination if the IFN levels were ≥ two-fold compared with similar cultures without influenza.

Definition of Immunologic Response

Patients were considered to have had a positive immune response if either of the following two conditions were met: cytotoxic T-cell response was positive and there was detectable preimmunization-specific killing or a 1.5-fold increase compared with the preimmunization percent killing was observed. If there was no detectable preimmunization-specific lysis, a postimmunization-specific lysis of ≥ 10% compared with background (peptide *v* no peptide; a widely used threshold for positivity) was considered a positive response only if specific lysis of targets pulsed with peptide was also statistically different from the lysis of cells without peptide using the Student's *t* test. A cytokine response was scored positive (IFN-γ, IL-2, and IL-5) if there was detectable (≥ 50 pg/mL) preimmunization peptide-specific cytokine response and a ≥ two-fold increase over the preimmunization response was detected. If there was no detectable preimmunization response, a postimmunization response was considered a positive response if this value was at least two-fold greater than the no-peptide control, greater than 50 pg/mL, and significantly different from the no-peptide control using the Student's *t* test.

Statistical Analysis

In case of CTL detection, a statistical difference test (paired Student's *t* test) was used. Fisher's exact test was used where appropriate. Survival data analysis was performed using the Kaplan-Meier method, and a log-rank test was performed. Statistical significance was considered as $P < .05$. Stepwise multiple Cox regression analysis was performed for multivariate analysis. For survival analysis, patients with baseline or positive results after vaccination were scored.

RESULTS

Two hundred seventy-six patients were enrolled onto the screening. Reasons for not receiving vaccination were lack of a suitable mutation and progression of disease. Of the screened patients, 39 patients (14%) were eligible and entered onto the study and received at least one immunization. Of the 38 immunologically valuable patients (Table 1), 20 (56%) received fewer than the four baseline vaccinations planned. This was a result of progression of disease among the patients with advanced malignancies. Six patients without progressive or recurrent disease and with positive immune responses to vaccinations were elected to receive additional maintenance vaccinations. No toxicity, acute or delayed, was attributed to vaccination. Follow-up was until death, with a mean follow-up time of 280 days (ranging until 580 days) for patients vaccinated with evident disease and a mean of 380 days (ranging until 5 years) after adjuvant immunization. Patient symptoms and/or deterioration were attributed to the underlying cancer.

Immunologic Responses

Immunologic responses are listed in Table 2. Of 39 patients who received one or more immunizations, 38 patients underwent analysis. One patient progressed rapidly and died soon after one vaccination.

Detectable cellular immunity against mutant *p53* or *K-ras*, as determined by either peptide-specific cytokine release of IFN-γ (chosen because it is the cytokine most widely used as an indication of a CD8$^+$ T-cell response) or peptide-specific cellular cytotoxicity, was more likely to be found in patients treated adjuvantly than in patients with evident disease. Detection of positive CTL occurred in four of nine patients in the adjuvant setting and in six of 28 patients with evident disease ($P = .2$). A positive IFN-γ reaction was observed in eight of nine patients treated adjuvantly and in eight of 28 patients treated with evident disease ($P = .002$). Of the 35 patients undergoing immunologic assessment for influenza, 25 had responses, seven were not tested, and three (placed on the study early) tested negative. Immunity against a recall antigen from influenza was comparable in patients immunized adjuvantly (eight of eight patients) or with evident disease (17 of 20 patients; $P = .5$).

Ten patients (26%) had a CTL response to mutant peptide on at least one occasion; an example is shown in Figure 1A. Two patients (6%) had CTL responses to mutant peptide at baseline before immunization (patients UT [The University of Texas] 33 and NCI 89; Table 1). Both patients had a further positive CTL response to vaccination as indicated by an increase in CTL compared with the initial positive response. Six of 10 patients had positive CTL responses on more than one occasion. Concanavalin A blasts were used as autologous targets for patients NCI 90, NCI 94, UT 26, UT 116, UT 132, and UT 143 (Table 1) because no EBV blasts could be generated. Three of these six patients had at least one positive CTL assay. The remainder of the patients had EBV-transformed lymphocytes used as autologous targets.

Positive responses to IFN- γ by stimulation index criteria occurred in a total of 17 patients (45%) before and after vaccination and 16 patients (42%) after vaccination. An example is show in Figure 1B. Five patients (13%) had positive IFN-γ assays before vaccination (patients NCI 35, UT 33, NCI 37, UT 117, and NCI 90). Two of these five patients (UT 33 and NCI 90) had significant increases over their baseline positive responses, indicating a positive response to vaccination. Three patients had decreases from baseline positive responses (NCI 35, NCI 37, and UT 117). Assays for IL-2, IL-5, and GM-CSF were also performed,

Table 1. Patient Characteristics

Patient No.	Age (years)	Sex	Disease	AJCC Stage	No. of Vaccinations	Point Mutation
K-ras evident						
NCI 2	48	M	Lung	IV	3	K-ras 12 Gly to Val
NCI 4	60	M	Colon	IV	4	K-ras 12 Gly to Ser
NCI 18	61	F	Colon	IV	2	K-ras 12 Gly to Asp
NCI 35	44	M	Lung	IV	3	K-ras 12 Gly to Val
UT 1	66	M	Pancreas	IV	2	K-ras 12 Gly to Val
UT 14	63	F	Pancreas	IV	2	K-ras 12 Gly to Arg
UT 23	70	F	Lung	IV	4	K-ras 12 Gly to Asp
UT 33	65	M	Pancreas	IV	2	K-ras 12 Gly to Val
UT 39	73	M	Pancreas	IV	1	K-ras 12 Gly to Arg
UT 46	69	M	Pancreas	IV	2	K-ras 12 Gly to Asp
UT 54	66	F	Pancreas	IV	2	K-ras 12 Gly to Asp
UT 58	49	M	Pancreas	IV	2	K-ras 12 Gly to Asp
UT 63	68	M	Pancreas	IV	2	K-ras 12 Gly to Asp
VU 1	54	F	Lung	IV	4	K-ras 12 Gly to Asp
VU 3	62	F	Lung	IV	4	K-ras 12 Gly to Val
UT 71	48	M	Colon	IV	4	K-ras 12 Gly to Asp
NCI 46	63	M	Colon	IV	1	K-ras 12 Gly to Cys
NCI 63	68	M	Lung	IV	9	K-ras 12 Gly to Cys
K-ras adjuvant						
UT 22	64	M	Colon	III	5	K-ras 12 Gly to Asp
NCI 94	65	M	Pancreas	II	3	K-ras 12 Gly to Asp
UT 89	57	F	Colon	II	24	K-ras 13 Gly to Asp
p53 evident						
NCI 7	56	M	Colon	IV	1	p53 152 Pro to Leu
NCI 16	69	M	Colon	IV	1	p53 273 Arg to Cys
UT 40	66	M	Lung	IV	4	p53 220 Tyr to Cys
UT 78	57	F	Breast	IV	4	p53 273 Arg to Cys
NCI 37	56	M	Lung	IV	2	p53 246 Met to Val
NCI 48	41	F	Lung	IV	4	p53 254 Ile to Asn
NCI 95	53	M	Colon	IV	4	p53 245 Gly to Ser
NCI 116	57	M	Colon	IV	2	p53 282 Arg to Trp
UT 117	61	M	Colon	IV	3	p53 249 Arg to Met
UT 156	59	F	Ovarian	IV	2	p53 248 Arg to Gln
VU 6	65	M	Colon	IV	1	p53 228 del2bp
p53 adjuvant						
UT 26	55	F	Colon	III	4	p53 157 Val to Phe
NCI 55	47	M	Head and neck	II	8	p53 143 Val to Glu
NCI 90	55	F	Lung	II	6	p53 159 Ala to Pro
UT 132	52	F	Breast	II	4	p53 176 Cys to Ser
UT 143	61	F	Colon	II	5	p53 245 Gly to Ser
VU 7	63	F	Breast	II	4	p53 237 Met to Ile

Abbreviations: AJCC, American Joint Comittee on Cancer; NCI, National Cancer Institute; UT, The University of Texas; VU, Vanderbilt University; M, male; F, female; Gly, glycine; Val, valine; Ser, serine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Pro, proline; Leu, leucine; Tyr, tyrosine; Met, methionine; Ile, isoleucine; Asn, asparagine; Trp, tryptophan; Gln, glutamine; Phe, phenylalanine; Glu, glutamic acid; Ala, alanine.

with intermittent positive results using stimulation index criteria, both before and after vaccination. Six (18%) of the 34 patients who were tested had a positive IL-5 assay at baseline, with 11 (32%) of 34 patients having positive post-vaccination assays. Assays for IL-2 were performed on only six patients, with two patients having positive assays before vaccination and two different patients having positive assays after vaccination. Assays for GM-CSF release were performed on only 13 patients, with one patient having a positive assay at baseline with no subsequent positive response and five patients (38%) having new positive responses after vaccination.

One patient who had evidence of cytokine response to mutant peptides was also tested for response to corresponding wild-type peptides. Figure 1C shows this patient's PBMCs with significantly increased IFN-γ production when exposed to influenza and to mutant p53 peptide but not when exposed to wild-type p53. Comparison assays between mutant and wild-type controls are not available for other patients.

Clinical Outcome

The median survival was 115 days (range, 26 to 685 days). Of 29 patients with evident disease, 24 progressed, and five had periods of stable disease (range, 4 to 40

Table 2. Immunologic Responses

Patient No.	CTL Response After Vaccination	Effector to Target Ratio	CTL Activity in Presence of Mutant Peptide (%)	CTL Activity Without Peptide (%)	IFN-γ Response After Vaccination (SI)
K-ras evident					
NCI 2	Neg		Neg		Neg
NCI 4	Neg		Neg		3.9, week 22
NCI 18	Neg		Neg		Neg
NCI 35	Pos week 3	42:1	20	−3	3.9, week 11
UT 1	Neg		Neg		Neg
UT 14	Neg		Neg		Neg
UT 23	Neg		Neg		Neg
UT 33	Pos week 3	55:1	24	0	4.9, week 3
UT 39	Neg		Neg		Neg
UT 46	Neg		Neg		Neg
UT 54	Neg		Neg		Neg
UT 58	Neg		Neg		Neg
UT 63	Neg		Neg		Neg
VU 1	Neg		Neg		Neg
VI 3	Neg		Neg		Neg
UT 71	Pos week 30	25:1	19	7	6.0, week 35
NCI 46	Neg		Neg		Neg
NCI 63	Pos week 3	120:1	67	6	4.8, week 3
	12	100:1	51	4	
	68	244:1	19	9	
	70	9:1	19	8	
K-ras adjuvant					
UT 22	Neg		Neg		2.7, week 22
NCI 94	Neg		Neg		2.3, week 3
UT 89	Pos week 14	400:1	25	5	10.2, week 91
	23	400:1	15	−1	
	58	6:1	57	38	
	59	22:1	22	0	
	64	51:1	66	40	
	67	69:1	28	1	
	82	9:1	36	18	
	84	71:1	33	7	
p53 evident					
NCI 7	Neg		Neg		Neg
NCI 16	Neg		Neg		Neg
UT 40	Neg		Neg		Neg
UT 78	Neg		Neg		Neg
NCI 37	Neg		Neg		Neg
NCI 48	Pos week 3	44:1	27	11	
	11	29:1	24	9	3.4, week 11
NCI 95	Neg		Neg		2.4, week 22
NCI 116	Neg		Neg		Neg
UT 117	Pos week 6	76:1	23	3	
	11	55:1	32	16	2.7, week 11
UT 156	Neg		Neg		Neg
VU 6	NA		NA		NA
p53 adjuvant					
UT 26	Pos week 19	136:1	14	3	67.0, week 47
	56	170:1	20	5	
NCI 55	Neg		Neg		6.6, week 11
					3.6, week 22
					4.3, week 46
NCI 90	Pos week 3	24:1	18	2	2.6, week 3
	12	204:1	16	6	5.1, week 12
	14	200:1	20	5	2.0, week 14
	30	13:1	15	5	2.6, week 22
					2.0, week 38
					5.6, week 55
UT 132	Neg		Neg		2.0, week 2
					3.3, week 11
UT 143	Neg		Neg		2.2, week 31
VU 7	Pos week 3	20:1	30	19	Neg

Abbreviations: CTL, cytotoxic T lymphocyte; IFN-γ, interferon gamma; SI, stimulation index; NCI, National Cancer Institute; UT, The University of Texas; VU, Vanderbilt University; Neg, negative; Pos, positive; NA, not applicable.

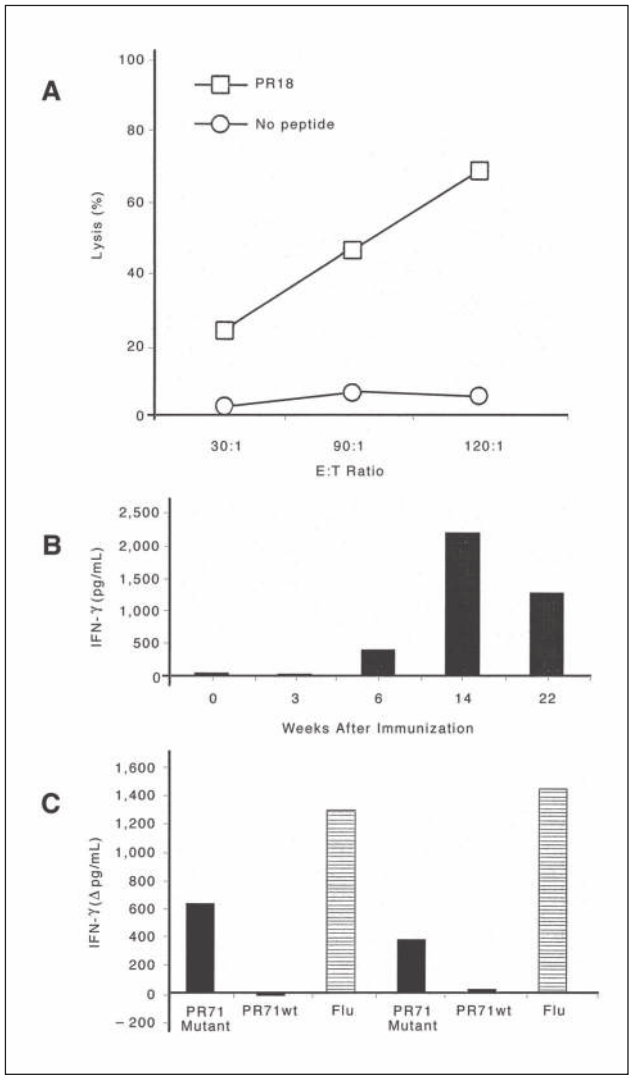

Fig 1. Peptide-specific immune responses. (A) Cytotoxic T-lymphocyte (CTL) response in patient NCI 63 to K-ras 12-Cys peptide PR18. (B) Interferon gamma (INF-γ) response to mutant K-ras by autologous mononuclear cells in patient NCI 4 over time after repeated immunization. (C) Postvaccination INF-γ production responses to mutant but not wild-type p53-derived peptide in patient NCI 55. E:T ratio, effector (CTL) to target (Epstein-Barr virus lymphoblastoid cells); PR17, p53 peptide.

months). Of the nine patients treated adjuvantly, three patients relapsed (with one death), whereas the other six patients remain without evidence of disease. Overall, there was no statistically significant association between successful induction of immunity against K-ras or p53 peptides by means of strength or quality of the immune response and clinical outcome. Notably, one patient (UT 89) with metastatic colon cancer who was without evidence of disease after resection of a metastatic lesion in the lung continued to be in remission nearly 5 years after resection. This patient has had multiple positive CTL responses. Only one of the five patients having periods of stable disease after vaccination had more than one positive CTL response.

Univariate Analysis

The median survival of patients with evident disease was 115 days (range, 26 to 685 days). Survival was similar for male and female patients. There was no association of survival with age. Patients immunized with mutant K-ras or p53 peptide had similar outcomes (Fig 2A). No survival benefit was detected in patients with positive CTL or IFN-γ responses to influenza virus. Prolonged survival was associated with positive CTL detected against mutant p53 or mutant K-ras. The median survival increased from 98 to 393 days ($P = .038$; Fig 2B). A positive IFN-γ release of PBMC to stimulation with mutant peptide was associated with an increased median survival from 88 to 470 days

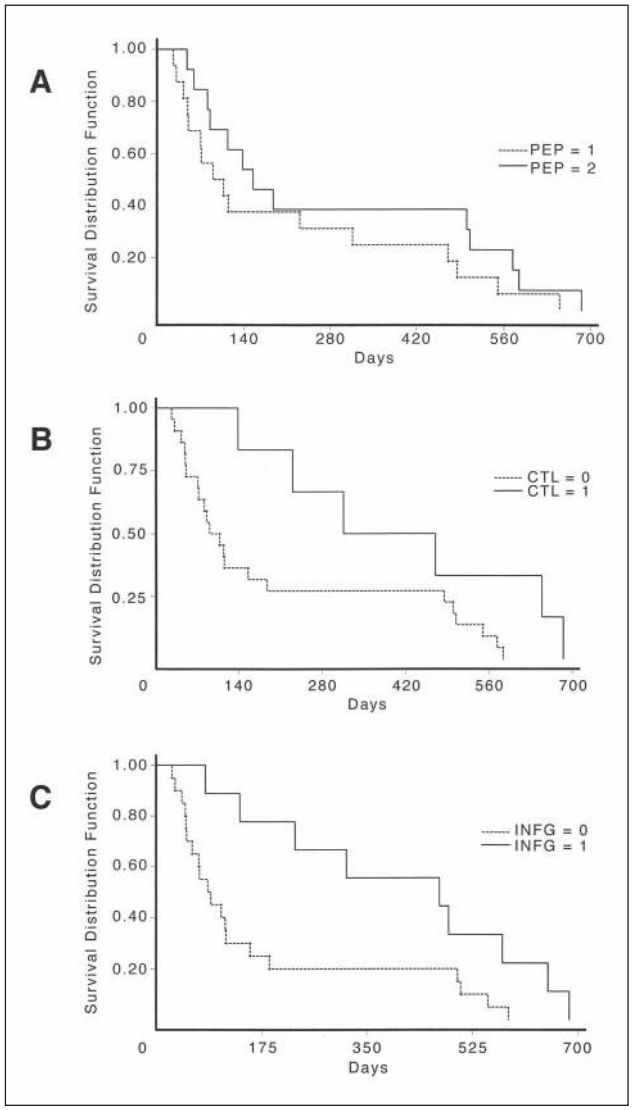

Fig 2. Survival of patients with evident disease. (A) PEP 1 indicates immunization with K-ras; PEP 2 indicates immunization with mutant p53 peptides. (B) Survival and cytotoxic T-lymphocyte (CTL) activity. CTL = 0, no cellular immunity; CTL = 1, positive cellular toxicity ($P = .038$). (C) Survival and interferon gamma (IFN-γ) response. INFG = 0, no IFN-γ release after in vitro stimulation; INFG = 1, positive IFN-γ release ($P = .017$).

($P = .017$; Fig 2C). However, there was no association of a positive IL-5 stimulation response to mutant peptide with survival ($P = .2$).

A total of 19 vaccine doses were administered to the nine patients with evident pancreatic carcinoma (an average of 2.1 vaccines per patient). One patient mounted a positive cellular immune response. Nine patients presented with non–small-cell lung cancer and evident disease and received a total of 35 vaccine doses (an average of 3.9 vaccines per patient). Colorectal adenocarcinoma was diagnosed in 10 patients with evident disease, and a total of 24 vaccines were applied (an average of 2.4 vaccines per patient). The study protocol requiring four vaccines was not feasible for the majority of the patients with evident disease. In terms of the diagnosis, the median survival ranged from 60 days for pancreatic cancer to 207 days for colorectal cancer and 230 days for non–small-cell lung cancer; but overall, the differences were not significant ($P = .15$).

To investigate an association between number of immunizations with survival, patients who were immunized only once were compared with patients who received at least two vaccinations. There was no association between amount of immunization and survival. To exclude patients with bad prognosis independent of vaccine doses administered, we looked for a possible association between the number of immunizations and the immunologic response rate. Analysis was repeated on only those patients who survived at least 45 days after the first immunization. Again, a survival benefit was visible for patients with a positive IFN-γ response to mutant peptide.

Multivariate Analysis

Stepwise regression was performed to avoid more than two factors being tested simultaneously. Sex, age, type of cancer, and positive or negative immunity to influenza, number of vaccine doses administered, a positive CTL reaction, and IL-5 and IFN- γ responses were inspected in the multivariate analysis. A positive CTL reaction was associated with a positive IFN-γ response to mutant peptide. Sex became a prognostic marker mainly because male patients who did not show any positive IFN-γ response to mutant peptide revealed a reduced median survival ($P = .002$). A positive IFN-γ response to mutant peptide was a prognostically independent favorable parameter for survival ($P = .004$).

DISCUSSION

This trial was performed to assess the feasibility of individualized oncopeptide vaccines in patients. Immunization with a cellular vaccine consisting of mutant p53- and K-ras–derived peptide-pulsed irradiated PBMC is immunogenic in cancer patients without any evidence for autoimmune reactions. Several aspects of the trial design are worth noticing. First, only a single T-cell epitope is used for immunization purposes. Second, the peptides that were used have a low intrinsic immunogenicity. Third, several patients with evident disease were able to mount a measurable cellular immune response to mutant oncopeptides. No attempts were made to analyze the peptides for potential matching of binding motifs of the mutant peptides to MHC class I, and their ability to induce T help is unknown.[21]

In the present trial, the vaccine consisted of irradiated PBMC pulsed with the epitope of interest. Whether irradiated PBMC in humans can act as APCs by themselves is unknown. Previous studies in murine systems established the need for irradiation unless a pure population of professional APCs is used.[22] Irradiated APCs survive for a shorter time after intravenous injection and, thus, get cleared more readily.[23] Peptides presented in the context of MHC class I are processed by the host APCs and become accessible for immune response induction.[24,25] To what extent this immunization protocol could be improved (for example, by using professional APCs such as dendritic cells) remains debatable.[26] To achieve better immunization efficacy, various strategies have been proposed.[27-31] However, any efficient immune induction works through optimal antigen presentation to effector cells, which is always accomplished by dendritic or other efficient professional APCs. Direct manipulation of professional APCs to ensure optimal presentation or indirect antigen presentation through molecules, dendritic cell–derived exosomes, or cellular vaccines may equally lead to sufficient immune reaction. Injection of the vaccine into lymph nodes could potentially be more efficient because the antigens become quickly available to professional APC. Because the aim is a strong cellular immune response that will assure successful immunologic targeting, the use of a highly immunogenic antigen may be more important than the method of immunization. Cancer antigens should preferentially have a high intrinsic immunogenicity for both T-help and cytotoxic T-cell epitopes and be easily accessible to effector cells. A potentially more appealing circumstance for cancer vaccines would be immunization against non–host-derived viral epitopes associated with human cancer, such as in cervical cancer.[32] A peptide vaccine of the E7 viral protein showed no evidence for toxicity and some response in two of 19 patients.[33]

The present data confirm reports of pre-existing cellular immunity in cancer patients against defined oncopeptides.[34] In the present trial, positive cellular immunity was induced in all nine patients treated adjuvantly. Evident cancer may impact on the immune function of a host, and thus, it is not surprising to observe less efficient immune induction in patients with a significant tumor load.[35] There are many strategies tumor cells use to evade the immune response. However, most tumors that are clinically relevant remain ignored by the immune system or have evolved strategies to suppress effective immune responses.[36-40]

Strategies improving sustained accessibility of the tumor cells to the immune system may be at least as important as the target epitope.

The present study confirms the immunogenicity of K-ras–derived peptides in cancer patients. Other researchers have shown successful immune induction against mutant K-ras peptide when treating pancreatic carcinoma patients, suggesting prolonged survival of vaccine responders.[41] Compared with our trial, the immunization protocol differed, and intradermal injections of peptides with GM-CSF as an adjuvant were used. The immunologic testing for immune response was based on the T-cell [^{3}H]thymidine uptake, a readout that does not determine cytotoxicity, that we measured in the present trial. In this study, patients with pancreatic cancer, all with *K-ras* mutations, did not react to the immunization, except for one patient. The impaired median survival and the inability to apply more than two vaccines per pancreas cancer patient might have biased the survival analysis, and the worse prognosis of pancreatic cancer patients could partly be responsible for the worse outcome and not the absence of immune response per se.

Whether the ability to mount a cellular immune response against a mutant oncopeptide is directly responsible or indirectly linked to better outcome in vaccine-responding patients in the present trial is not clear. Excluding patients with rapidly progressive disease who died within 45 days after the first immunization did not alter the results. An association of antitumor immunity with reduced progression of metastasis has been reported for melanoma.[42] In melanoma patients, successful induction of immunity has been shown to improve outcome in a randomized adjuvant setting.[43-46] In patients with colorectal cancer, vaccine trials have been conducted but have been mostly limited to the adjuvant setting.[47-49] In patients with metastatic disease, vaccination has not been shown to improve outcome,[50] although one trial suggested that positive immunity was associated with slower disease progression.[51]

One of the remarkable features of the present study is that a minority of 14% of patients screened for mutant *p53* and *K-ras* were eligible for specific immunization. This situation makes a broad clinical approach difficult, especially in the context of custom-made peptide immunization. The effort to screen many patients to obtain a selected population is only cost effective if there is a high probability of successful intervention outcome or if the patient selection result is a spin-off of other screening. The fraction of eligible patients could be increased with automatic sequencing and peptide synthesis processes if additional mutant proteins were used or if patients with earlier stage disease were eligible. To date, none of the tumor vaccines for cancer have yielded high response rates in any disease to justify screening for rare defined epitopes.

Both significant immunity to tumor-specific peptide antigens in human cancer patients and an association between positive immune responses and prognosis were observed. To what extent the association between immunity and survival is a direct consequence of the vaccinations remains unclear. Additional studies in defined sets of patients are underway.

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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