

Significant Clinical Response of Advanced Colon Cancer to Peptide Vaccine Therapy: A Case Report

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Partial response (PR) was obtained in a patient with advanced colon cancer following peptide vaccine therapy. A 61-year-old woman was referred to our hospital for peptide vaccine therapy. She had undergone sigmoidectomy at a nearby hospital and eventually developed multiple metastases to the lung and pelvic lymph nodes with left hydronephrosis. A ureteral stenting catheter had been inserted for left hydronephrosis, and oral opioids had been administered for relief of pain in the left pelvic region. Three tumor-antigen-derived peptides (RNF43, TOMM34, and KOC1) and two human VEGFR-derived peptides (VEGFR1 and VEGFR2) were used as a cocktail. The peptide cocktail was subcutaneously inoculated on days 1, 8, 15, and 22 and repeated at 14-day intervals. The patient's serum level of carcinoembryonic antigen was 28.9 ng/mL ($N < 5$ ng/mL) before treatment, and it decreased promptly after the initiation of therapy to within a normal range. Evaluation of computed tomography images at week 5 revealed PR as determined by the Response Evaluation Criteria in Solid Tumor criteria. After month 3, the oral opioid was discontinued. The PR lasted for 4 months and was followed by stable disease for another 4 months. No particular adverse effects were observed. A cytotoxic T lymphocyte (CTL) response was evaluated by immunosorbent spot assay, and a positive CTL response was recognized against at least one of five peptides at each end of the six courses. Immunotherapy has been proven to slow tumor growth by inducing an active antitumor immune response; and therefore, significant tumor shrinkage is rarely observed. To our knowledge, this is the first case report of PR presented in a patient with advanced colon cancer.

Key words: Colorectal cancer, immunotherapy, partial response, peptide vaccine, vaccine

INTRODUCTION

Partial response (PR) was obtained in a patient with advanced colon cancer after peptide vaccine therapy. Serum levels of carcinoembryonic antigen (CEA) decreased promptly after the initiation of treatment, and PR was attained at week 5. Small lung metastases less than 1 cm in size and multiple pelvic lymph node metastases were no longer visible on computed tomography (CT). While previous trials of colon cancer vaccines have failed to demonstrate significant clinical response when assessed by the Response Evaluation Criteria in Solid Tumor (RECIST) [1], a marked clinical response was attained in the present case.

CASE REPORT

A 61-year-old woman was referred in July 2009 from a nearby hospital, at which she had undergone sigmoidectomy for sigmoid colon cancer in December 2005. The pathological diagnosis in 2005 was moderately differentiated adenocarcinoma confined to proper muscle without lymph node metastasis (T2 N0 M0). In May 2008, she developed lymph node metastasis at the root of the inferior mesenteric artery and underwent a second anterior resection with lymph node dissection. She underwent adjuvant chemotherapy with oral tegafur/uracil plus leucovorin but

eventually developed multiple metastases to the lung and pelvic lymph nodes with left hydronephrosis. In January 2009, FOLFOX with bevacizumab was started but was discontinued after one cycle at the patient's request. A ureteral stenting catheter was inserted for left hydronephrosis. Oral opioid was administered for relief of pain in the left pelvic region. Otherwise, she had a good performance status.

In July 2009, after confirming her HLA type as HLA-A2402, she was enrolled in the clinical study approved by the institutional review board at Tokai University School of Medicine. The primary end-point of this phase I/II clinical study was the safety, and the second end-point was clinical responses and immunological responses. Patients who had histologically confirmed colorectal cancer, unsuitable for surgical resection, and failed to prior standard chemotherapy were enrolled. Patients were required to have completed prior chemotherapy at least 4 weeks before enrollment. Other inclusion criteria included an Eastern Cooperative Oncology Group performance status of 0 - 2; age between 20 and 80 years; life expectancy > 3 months; and adequate liver function (bilirubin < 1.5 mg/dl and transaminase within 3 times our institution's upper limit of normal), renal function (serum creatinine < 1.4 mg/dl), and bone marrow function (white blood cell count > 3000/mm³, hemoglobin

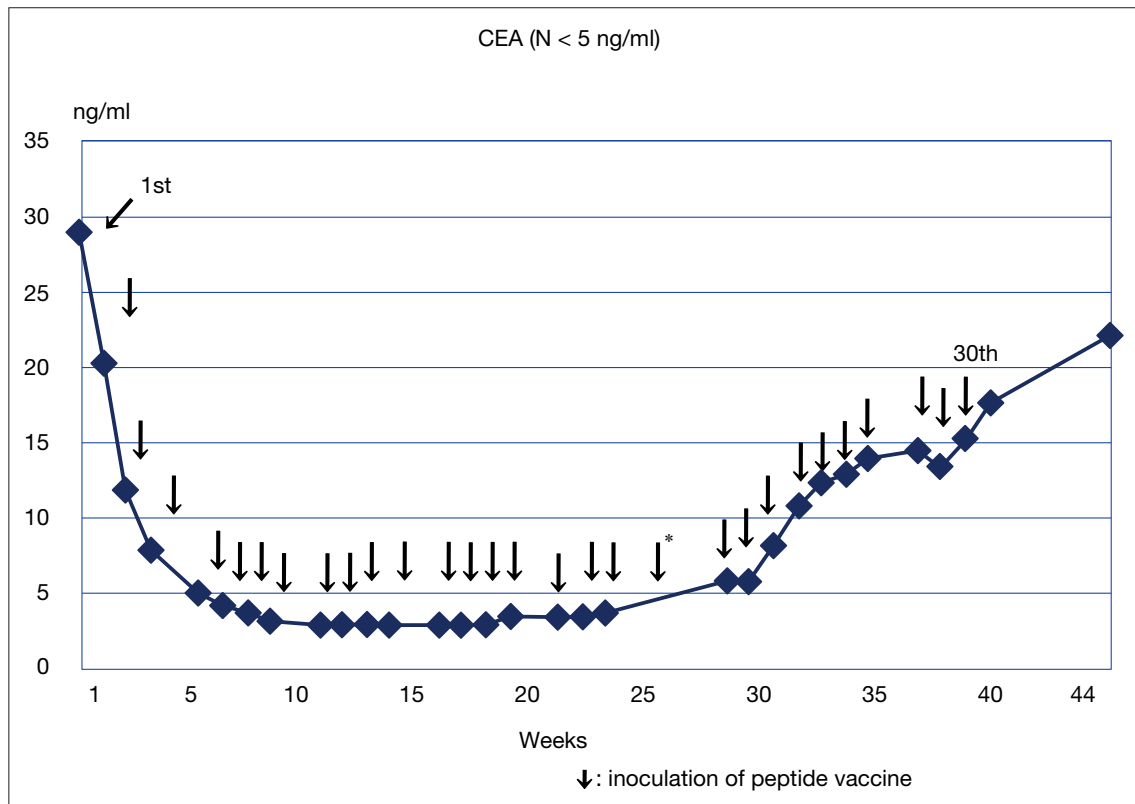


Fig. 1 The serum level of CEA ($N < 5$ ng/mL) decreased promptly after the initiation of therapy. Peptide vaccine was regularly inoculated until the fourth course. Before entering the sixth course, there was a 3-week interval (*) corresponding to the rise of CEA.

> 10 g/dl and platelet count $> 75,000/\text{mm}^3$) were required. The exclusion criteria included pregnancy, active infectious or cardiovascular disease, and concurrent treatment with steroids or immunosuppressive agents. Five peptide vaccines were used as a cocktail: RNF43-721 (NSQPVWLCL), TOMM34-299 (KLRQEVKQNL), KOC1-508 (KTVNELQNL), VEGFR1-1084 (SYGVLLWEI), and VEGFR2-169 (RFVPDGNRI). The peptide cocktail was administered emulsified with incomplete Freund's adjuvant (Montanide ISA-51 VG; SEPPIC, Paris, France) and subcutaneously inoculated in the bilateral axillary or inguinal regions on days 1, 8, 15, and 22 (once a week, 4 inoculations) and repeated at 14-day intervals. This study protocol was registered in the UMIN Clinical Trial Registry as UMIN 000004945 (<http://www.umin.ac.jp/ctr/index.htm>).

The patient's serum level of CEA was 28.9 ng/mL ($N < 5$ ng/mL) before treatment, and it decreased promptly after the initiation of therapy (Fig. 1). Evaluation of CT images at week 5 revealed PR as determined by the RECIST for cancer chemotherapy (Fig. 1). The PR lasted for 4 months and was followed by stable disease (SD) for another 4 months.

Metastases to the pelvic lymph nodes were no longer visible on CT images at month 5. Similarly, all five metastatic lung nodules less than 1 cm in size had completely disappeared (Fig. 2) including the largest node of 28 mm in diameter. Multiple lung metastases were recognized on CT images before treatment. Among them, five lesions were less than 10 mm in diameter and all five lesions were also no longer vis-

ible on CT images until her last visit in May 2010. In contrast, larger lung metastatic nodules of 28 mm and 14 mm in diameter did not disappear; they decreased to 11.9 mm and 5 mm, respectively, and regrowth was observed at month 8.

After month 3, the oral opioid was discontinued. Peptide vaccine was administered a total of 30 times. No particular adverse effects were observed other than slight indurations with no erythema at the inoculation sites. Peptide vaccine therapy had to be discontinued after the patient was unable to visit our hospital because of psychiatric disease (adjustment disorder) in May 2010. Thereafter, she received palliative care at another hospital until February 2011 when she expired due to progression of metastases of colon cancer.

DISCUSSION

Merika *et al.* [1] noted in their comprehensive review that a variety of cancer vaccines have been used to treat patients with colorectal cancer. In our study protocol, three tumor-antigen-derived peptides and two human VEGFR-derived peptides were used in a cocktail.

RNF43 (ring finger protein 43) is upregulated in more than 80% of colorectal cancer tissues and is not detectable in human adult noncancerous tissue. RNF43 function is associated with the proliferation of tumor cells [2, 3]. TOMM34 is a translocase of the outer membrane of mitochondria, and enhanced expression is observed in 80% (16/20) of colorectal cancers. Enhanced expression is associated with growth of cancer cells [3, 4]. KOC1 (K homology domain-containing

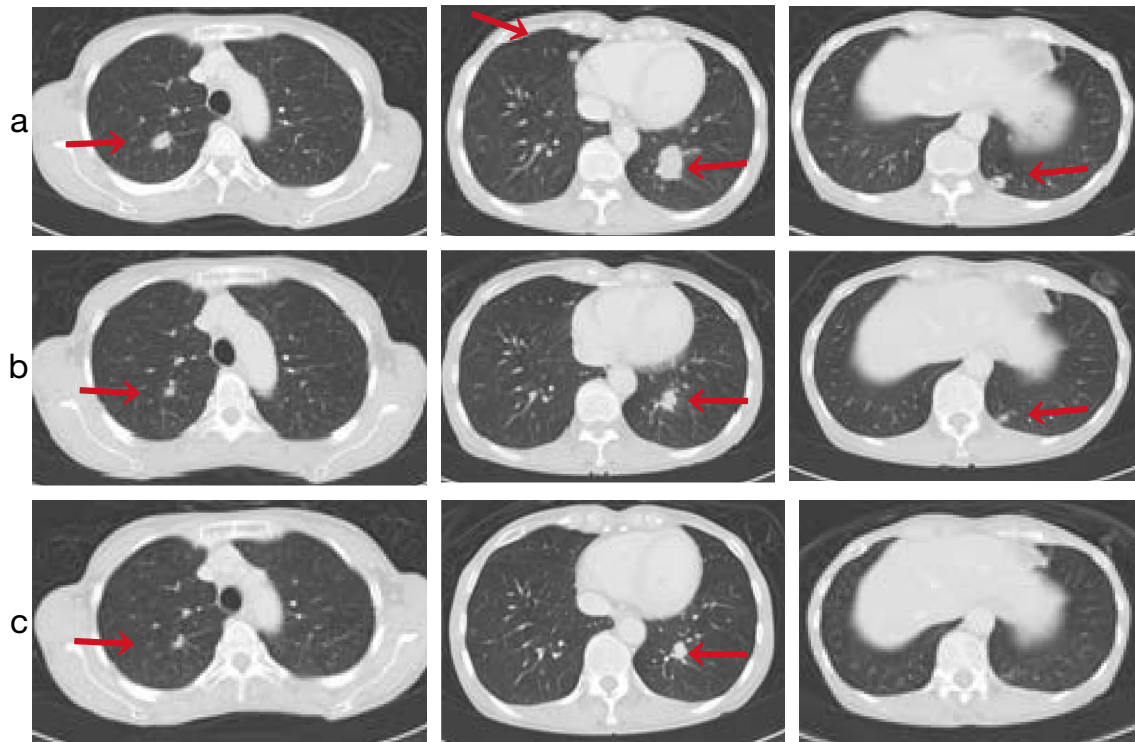


Fig. 2 Chest CT images showing lung metastases (arrow) before treatment (a), at week 5 (b), and at week 10 (c). Significant tumor shrinkage is recognized at week 5, and small metastases were no longer visible at week 10.

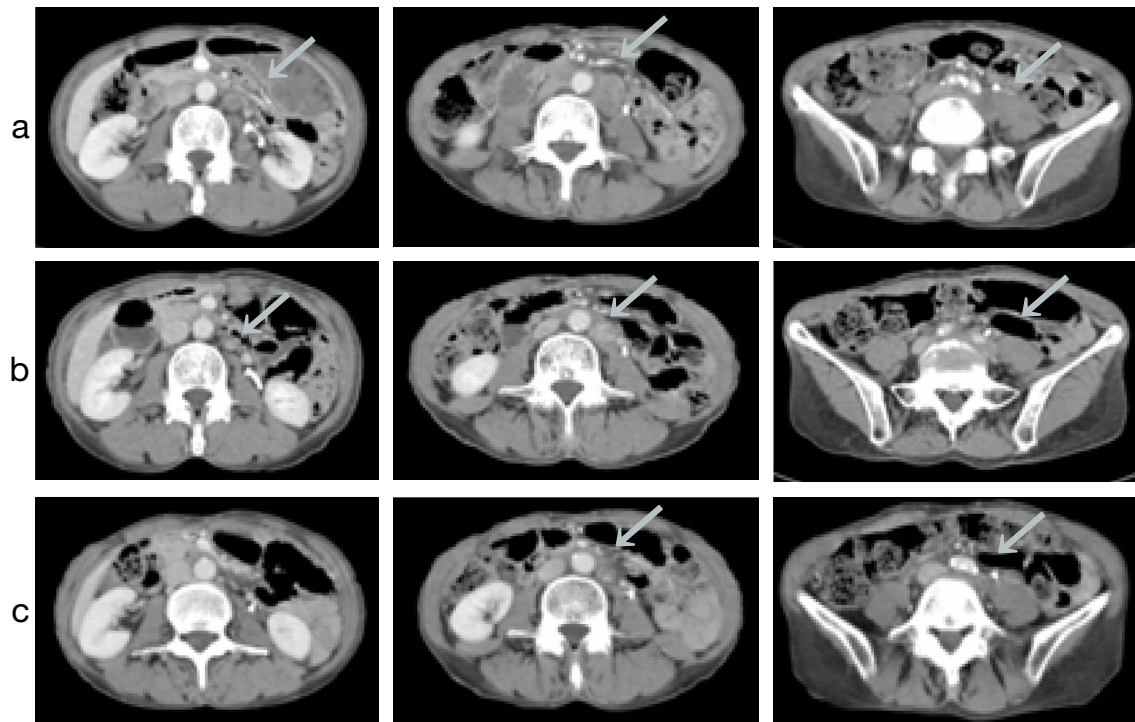


Fig. 3 Abdominal CT images showing lymph node metastases (arrow) before treatment (a), at week 5 (b), and at week 10 (c). Significant tumor regression is observable at week 5, and further tumor shrinkage is evident at week 10.

protein overexpressed in cancer) is also known as insulin-like growth factor-II mRNA-binding protein 3 (IMP3). IMP3 is upregulated in 65.0% (132/203) of primary colon cancers and in 90.9% (60/66) of metastatic lymph nodes [5]. Both vascular endothelial

growth factor receptor (VEGFR) 1 [6] and VEGFR2 [7, 8] have been shown to be overexpressed on the endothelial cells of vessels in various types of primary tumors and metastases. Tumor angiogenesis plays an essential role in tumor progression and tumor metas-

tasis. The peptide vaccines were obtained from the Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo. Expression of these tumor-associated antigens was not examined for the tumor tissue of our patient.

This patient was the tenth patient enrolled in the study and the first to show an objective clinical response. Nagorsen *et al.* [9] noted in their review of colorectal cancer vaccine therapy that one complete remission (CR) and four PRs were reported among a total population of 527 patients, corresponding to an overall response rate of 0.9%. One case involved a patient with complete resolution of lung metastasis and malignant pleural effusion [10], but we were unable to confirm whether other patients experienced CR or PR because no images were presented in the quoted paper. At any rate, there are few cases of CR and PR; and to our knowledge, this is the first case report of PR for colorectal cancer.

Colorectal cancer vaccine therapy has been tested mostly in patients with advanced or end-stage disease or after failure of chemotherapy. In such cases, the immune system might be compromised and significant tumor regression cannot be expected [1, 9, 11]. In our patient, systemic chemotherapy had been conducted for only one cycle before vaccine therapy, and the patient had good performance status.

After initiation of peptide vaccine therapy, the serum CEA level decreased immediately. PR lasted for at least 4 months. Enlarged pelvic lymph nodes disappeared on CT images as did five lung metastases. In contrast, the larger lung metastases decreased but did not disappear. Lymph node metastasis might be more susceptible than lung metastasis to peptide vaccine therapy if tumor size is equal on CT images.

Reported toxicity in the vast majority of studies is mild and manifests mainly as local redness, pain, and swelling at the injection site [9]. In our study group also, no particular adverse effects were observed in any of the preceding nine patients. In the present patient, only slight indurations (< 1 cm) were observed at the injection sites.

After PR had been sustained for about 4 months, tumor regrowth was observed. One possible reason for this regrowth is immune-escape mechanisms. The antitumor effect of cytotoxic T lymphocytes (CTLs) induced by peptide vaccine is suspected to be reduced because of tumor cell heterogeneity and also the downregulation or loss of human leukocyte antigen (HLA) or antigen protein [6, 8]. To counteract these effects, however, our peptide cocktail contains two human VEGFR-derived peptides (VEGFR1 and VEGFR2) in addition to the three tumor-antigen-derived peptides. Another possible explanation is the prolonged interval between two inoculations. Peptide vaccine was regularly inoculated according to the protocol until the fourth course. Before entering the sixth course, however, there was a 3-week interval because of inconvenience by the patient. The serum level of CEA rose from 3.7 ng/mL to 5.7 ng/mL during the corresponding interval.

Peripheral blood mononuclear cells were collected at the end of each course for a total of six consecutive courses, and T cell response was evaluated by immu-

nosorbent spot (ELISPOT) assay by the same methods reported previously [3]. The CTL response was considered to be positive when more than 10 specific spots were detected or the percentage of specific spots was greater than 5%. As a result, a positive CTL response was recognized against at least one of five peptides at the end of each course. To our regret, however, no CTL response was evaluated in blood samples before treatment. Although a positive CTL response was observed in our patient, no assays to measure the antitumor immune response including ELISPOT assays have been validated in prospective clinical trials [11]. CTL-positive results from ELISPOT assays against the TOMM34 antigen are demonstrated in Fig. 4.

As opposed to chemotherapy with cytoreductive agents, significant tumor shrinkage is rarely observed in immunotherapy. However, an overall survival benefit without a longer progression-free survival has been demonstrated [11, 12]. To account for this finding, Madan *et al* [12] presented the intriguing hypothesis that immunotherapy leads to slower tumor growth by inducing an active antitumor immune response and may lead to substantially longer overall survival. Conventional volumetric response criteria, such as RECIST have proven inadequate for evaluating the efficacy of immunotherapy [11, 12]. Although significant tumor shrinkage was observed in our case, the true efficacy of immunotherapy should be evaluated in terms of survival benefit.

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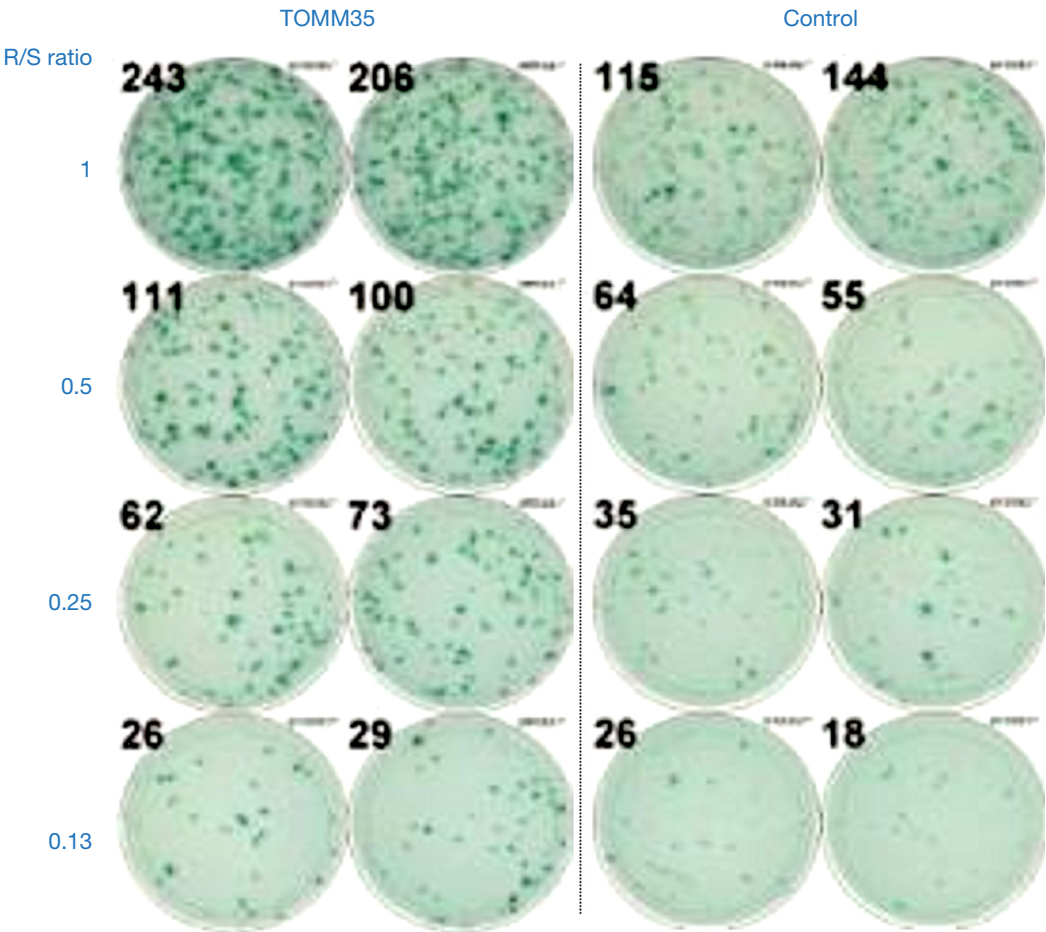


Fig. 4 Results of ELISPOT assay showing the TOMM34-specific CTL response at the end of the second course. The number of spot-forming cells is higher in the well with TOMM34 peptides than in the well with control.
R/S; responder/stimulator ratio

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