Generation of Carcinoembryonic Antigen (CEA)-Specific T-Cell Responses in HLA-A*0201 and HLA-A*2402 Late-Stage Colorectal Cancer Patients after Vaccination with Dendritic Cells Loaded with CEA Peptides

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ABSTRACT

Purpose: We intranodally immunized metastatic colorectal carcinoma patients, who had failed standard chemotherapy, with dendritic cells (DCs) pulsed with HLA-A*0201- or HLA-A*2402-restricted carcinoembryonic antigen (CEA) peptides to evaluate the safety of this treatment and the immune response against CEA peptides before and after the treatment.

Experimental Design: Six patients with the HLA-A*2402 genotype and 4 patients with the HLA-A*0201 genotype were enrolled. A single CEA peptide (YLSGANLNL) or two CEA peptides (QYSWFVNYTF and TYACFVSNL) were used for patients with the HLA-A*0201 or HLA-A*2402 genotype, respectively. Autologous DCs were generated by culturing adherent mononuclear cells with interleukin 4 and granulocyte macrophage colony-stimulating factor for 6 days. Maturation of DCs was then induced with tumor necrosis factor α for 40 h. Mature DCs were pulsed with appropriate CEA peptides for 2 h. After washing, 1 million peptide-pulsed DCs were injected into one inguinal lymph node under sonographic guidance. Each patient received four injections.

Results: No grade II/III toxicity or autoimmunity was observed. An increase in the number of CEA-specific T cells after DC vaccination could be detected in 7 of 10 (70%) patients. Two (20%) patients had stable disease for at least 12 weeks. One of these 2 patients experienced a transient decrease in CEA levels during the treatment period and also had the most significant T-cell response against the immunizing CEA peptides.

Conclusions: These results suggest that our vaccination procedure can generate or boost specific T-cell responses and may provide clinical benefit in certain cancer patients.

INTRODUCTION

The incidence of colorectal cancer has increased in the past decade. Most patients who die of colorectal cancer have distant metastasis. The standard treatment for these patients is chemotherapy with a combination of 5-fluorouracil, CPT-11, and/or oxaliplatin. After failure with these treatments, there are currently no other effective treatment options. Therefore, alternative medical intervention is needed to improve the survival and quality of life of these patients. Immunotherapy has emerged as one of the most promising strategies for providing a clinical benefit to cancer patients. One possible mechanism by which the immune system is involved in tumor evasion occurs when the tumor-associated antigens (TAAs) of tumor cells are not properly presented to CTLs by professional antigen presenting cells during the development of a malignancy, which results in T-cell ignorance or tolerance and consequent outgrowth of tumor cells (1). A proposed strategy to break tolerance to TAAs is to immunize cancer patients with antigen presenting cells pulsed in vitro with appropriate TAAs under optimal activation conditions. It is anticipated that such treatment might generate or reactivate a CTL response against tumor cells and thereby inhibit tumor growth (2, 3).

Dendritic cells (DCs) are the most potent type of antigen presenting cells in the human body, and are involved in the regulation of both innate and adaptive immune responses (4–6). Vaccination with DCs loaded with tumor-specific antigens or TAAs has been shown to induce protective immune responses in several animal models (7, 8). The results of several clinical trials that have investigated the use of tumor antigen-pulsed DC vaccines for the treatment of various advanced malignancies have been reported (9, 10). One important issue in applying DC-based cancer immunotherapy is the choice of the appropriate TAA. For colorectal cancer, several different approaches have been developed for boosting the immune response against carcinoembryonic antigen (CEA; Ref. 11). Elevated expression of CEA is observed in most colorectal carcinomas. Although CEA expression is also observed in normal colon epithelial cells, the expression levels tend to be relatively low (12). Immune responses to CEA have been reported in many cancer
patients (13, 14), indicating that the tolerance to CEA is incomplete. Thus, enhancing the immune response of a patient against CEA may trigger a stronger immune attack against tumor cells that express high levels of CEA. DCs present antigens to T cells in an HLA-restricted manner. Three CTL epitopes on CEA have been identified: one epitope is presented by HLA-A*0201 (15, 16) and two are presented by HLA-A*2402 (17, 18). It has been demonstrated that CTLs specific for these epitopes can be generated from peripheral blood mononuclear cells (PBMCs) of normal donors and that these CTLs are capable of killing tumor cells expressing CEA and the appropriate HLA molecules (19–21).

Colorectal cancer ranks third on the cancer mortality list in Taiwan. Therefore, we conducted a pilot clinical trial of DC-based immunotherapy to treat metastatic colorectal carcinomas that have failed standard chemotherapy. In our study, autologous DCs were pulsed with peptides representing known CTL epitopes on CEA. Phase I trials using CEA peptides in DC-based immunotherapy against malignancies have been reported by other investigators (22, 23). However, our current study significantly differs from these studies in at least three aspects. First, in our study, synthetic CEA peptides that can bind to either HLA-A*0201 or HLA-A*2402, which are both common genotypes in Taiwan, were used in the appropriate patients. Previous clinical trials have primarily used the HLA-A*0201-restricted peptide because of the predominance of HLA-A*0201 expression in Caucasian populations (22). Secondly, peptide-loaded and tumor necrosis factor (TNF)-matured DCs, rather than immature DCs, were used for vaccination. It has been shown that mature DCs are more potent stimulators of T cells and more resistant to immune suppressive factors released by tumor cells. Furthermore, they can maintain their activated phenotypes after withdrawal of cytokines (10). Therefore, immunization with mature DCs pulsed with antigenic peptides is likely to induce a stronger immune response. Finally, the DC vaccine was introduced into patients by intranodal injection instead of i.v. or intradermal injection. Recent reports have suggested that intradermal injection of DCs results in more efficient migration to lymph nodes than s.c. or i.v. injections (24, 25). However, the number of DCs that can migrate to lymph nodes after intradermal injection is still very limited. The direct intranodal injection of DC method used in our study should greatly increase the number of peptide-pulsed DCs in lymph nodes and should, therefore, result in a stronger immune response. Because of these important differences, our study can provide valuable information that was not available in previous trials. In this study, we have demonstrated that intranodal vaccination with DCs pulsed with CEA peptides is feasible and safe for late-stage colorectal cancer patients. Moreover, such treatment can generate CEA-specific T-cell responses in most cancer patients. Additional clinical studies are required to better evaluate the efficacy of similar treatment approaches.

PATIENTS AND METHODS

Patients. This study protocol was approved by the Institutional Review Board of National Taiwan University Hospital and by the Department of Health, Taiwan. Signed informed consent was obtained from each patient. Ten patients were enrolled in this study. All of the patients had experienced metastasis from their primary colorectal cancer and had failed a first-line chemotherapy regimen containing 5-fluorouracil and a second-line chemotherapy regimen containing CPT-11 or oxaliplatin. Six patients with the HLA-A*2402 genotype and 4 patients with the HLA-A*0201 genotype were enrolled. Determination of the HLA-A allele was performed with an HLA-A High Resolution Typing System and analyzed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Serum CEA levels were higher than normal in all of the patients. All of the patients had adequate bone marrow, liver, and renal function defined as WBC ≥3,500/mm³, neutrophil ≥1,500/mm³, lymphocyte ≥1,000/mm³, platelet ≥100,000/mm³, bilirubin ≤1.5 times normal range, and creatinine ≤2.0 times the upper limit of normal. Patient performance status ranged from 0–1 on the ECOG scale. Patients who had central nervous system metastasis, autoimmune disease, or active acute/chronic infection, and patients who received chemotherapy, steroid, or biological treatment within 6 weeks before enrollment were excluded from this study. Patient characteristics are shown in Table I.

Generation of DCs from PBMCs. PBMCs obtained from leukapheresis of 1000 ml of blood were additionally enriched by density gradient centrifugation with Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden). The PBMCs were incubated for 2 h at 37 °C in AIM-V medium (Invitrogen, Carlsbad, CA), and adherent cells were cultured in X-VIVO15 medium (BioWhittaker, Walkersville, MD) containing 2% heat-inactivated human plasma containing 750 units/ml granulocyte macrophage colony-stimulating factor (Leukomax; Novartis International AG, Basel, Switzerland). On day 6, loosely attached or floating immature DCs were collected and matured by culturing in X-VIVO15 medium containing 2% heat-inactivated autologous plasma and 1000 units/ml human interleukin 4 (GMP-grade; Strathmann Biotec AG, Hannover, Germany), and 500 units/ml granulocyte macrophage colony-stimulating factor (Leukomax; Novartis International AG, Basel, Switzerland). On day 8, floating and loosely adherent cells were collected as mature DCs.

Vaccine Preparation and the Vaccination Protocol. Mature DCs (1.5 × 10⁶) were suspended in 1 ml of AIM-V medium and pulsed with appropriate CEA peptides (50 µg/ml each) for 2 h according to the HLA-typing of the patient. One peptide with the sequence YLSGANLNL was used for patients with the HLA-A*0201 genotype (15, 16). Two peptides with the sequence ASLG...
sequences QYSWFVNGTF (peptide-1) and TYACFVSNL (peptide-2) were used for patients with the HLA-A*2402 genotype (17, 18). All of the peptides were synthesized by AnaSpec Inc. (San Jose, CA) under GMP conditions. After extensive washing, $1 \times 10^6$ DCs were suspended in 0.3 ml PBS containing 1% autologous plasma and injected into one inguinal lymph node under sonographic guidance. Patients were vaccinated once a week for 3 weeks followed by a single booster 2 weeks later. Whole blood (30 ml) was collected from patients 2 weeks after the first and the last injections. PBMCs were purified and cryopreserved for evaluation of the immune responses against the CEA peptides.

**Flow Cytometry Analysis.** Cells to be analyzed for the expression of surface markers were stained with different fluorescence-labeled monoclonal antibodies (mAbs) and then analyzed using a flow cytometer (EPICS XL-MCL; Beckman Coulter). The mAbs used in this study were: FITC-anti-HLA-DR, phycoerythrin-anti-CD86, FITC-anti-CD80, phycoerythrin-anti-CD83, phycoerythrin-anti-CD14 (Immunotech, Marseille Cedex, France), and FITC-anti-CD40 (Serotec, Oxford, United Kingdom). Isotype-matched control mAbs were obtained from Immunotech and Caltag (Burlingame, CA).

**IFN-γ Enzyme-Linked Immunospot (ELISpot) Assay.** The ELISpot assay was performed as described previously (2, 26, 27). Briefly, PBMCs ($2 \times 10^5$/well $\times 5$ wells) obtained before, during, and after vaccination were cultured individually with 50 μg/ml of the immunizing CEA peptide (one peptide for HLA-A*0201 and two separate peptides for HLA-A*2402 patients) or a control peptide in 96-well filtration plates (Multi-screen-IP; Millipore, Bedford, MA) precoated with an anti-IFN-γ mAb (Mabtech AB, Nacka, Sweden). After a 24-h incubation, the wells were extensively washed and then stained with a biotin-labeled anti-IFN-γ mAb (Mabtech) followed by avidin-conjugated alkaline phosphatase. The alkaline phosphatase substrate was then added to allow development of a blue color, and the number of blue spots (representing the numbers of IFN-γ secreting cells) was counted. Counting was done in a blinded manner to prevent undesired bias. The HLA-A*0201-restricted CEA peptide was used as a negative control for samples from HLA-A*2402 patients and vice versa. Phorbol 12-myristate-13-acetate plus ionomycin (Sigma, St. Louis, MO) was used as a positive control (27). The number of spots in the negative control normally ranges from 0 to 26, and the number of spots in the positive control normally ranges from 124 to 195. The average number of spots in the negative control group was subtracted from that of the test group. The final number of CEA-specific spots was adjusted with a normalizing factor subtracted from that of the test group. The percentage of the control group was always $<0.05$ and was subtracted from that of the test group.

**Toxicity and Clinical Evaluation.** Toxicity grading was done and recorded according to National Cancer Institute Common Toxicity Criteria Scale, version 2.0. The clinical responses were defined as follows: (a) stable disease, $<25\%$ change in size with no new lesions developing for 6 weeks; and (b) disease progression, the appearance of new lesions or $>25\%$ increase in the area of existing lesions.

**RESULTS**

**Characteristics of DCs Generated for Vaccination and Evaluation of Adverse Effects.** The primary end point of this study was to evaluate the safety of immunizing colorectal cancer patients with peptide-pulsed DCs generated in our laboratory. Thus, it was critical to monitor the quality of DCs obtained from different preparations from participating patients. For each DC preparation, the culture supernatant on day 6 and the supernatant after the last washing on day 8 were examined for Mycoplasma, fungal, bacterial, or endotoxin contamination. All of the samples were found to be negative for microorganism contamination and to have endotoxin levels $<0.15$ endotoxin unit/ml. The average yield of DCs obtained on day 8 was $\sim7\%$ of input PBMCs. DCs obtained on days 6 and 8 (after incubation with TNF-α for 40 h) were stained for several surface markers to monitor their characteristics. Day 6 DCs displayed a typical immature DC staining profile: HLA-DRdim, CD86dim, CD80dim, CD14dim, and CD83dim (Table 2; Fig. 1). However, substantial variations did exist among different patients, which may reflect their different previous chemotherapy protocols. Due to the limited patient number, the influence of such variations on the generation of immune responses or clinical responses is not entirely clear at present.

**Evaluation of Adverse Effects.** No grade II-IV toxicity was observed in any patient after intranodal injection of the DC vaccine. Patient 2 (HLA-A*2402) developed a mild fever ($<37.4^\circ C$) that lasted $\leq 24$ h after the second and third injections. No obvious swelling was observed at the injection site.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Day 6 (%)</th>
<th>Day 8 (%)</th>
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<tbody>
<tr>
<td>DR</td>
<td>96 ± 4.9</td>
<td>95.7 ± 5.3</td>
</tr>
<tr>
<td>CD86</td>
<td>98 ± 1.7</td>
<td>98.2 ± 3.0</td>
</tr>
<tr>
<td>CD80</td>
<td>26.8 ± 11.6</td>
<td>57.7 ± 19.6</td>
</tr>
<tr>
<td>CD83</td>
<td>16 ± 7.5</td>
<td>58.9 ± 17.0</td>
</tr>
<tr>
<td>CD40</td>
<td>99.4 ± 0.4</td>
<td>97.2 ± 4.3</td>
</tr>
<tr>
<td>CD14</td>
<td>20.2 ± 16.0</td>
<td>7.8 ± 9.9</td>
</tr>
</tbody>
</table>
There was no evidence of treatment-related autoimmune response as determined by the absence of antinuclear antibodies, rheumatoid factors, and antithyroid antibodies (data not shown). These results confirm that injection of peptide-pulsed DCs into the inguinal lymph nodes of cancer patients is both feasible and safe.

**Evaluation of Immune Responses against CEA.** The secondary end point of this study was to evaluate the immune response against CEA before and after DC vaccination. Cryopreserved peripheral blood mononuclear cells (PBMCs) obtained before vaccination (day 0, d0), 1 week after the second (day 22, d22), and 2 weeks after the fourth injection (day 50, d50) were thawed on the same day for analysis. Additional PBMCs were obtained on day 120 from patients 5 and 7, who both had stable disease. PBMCs were stimulated with the immunizing CEA peptide(s) or a control peptide, and the number of peptide-specific T cells was determined by IFN-γ ELISPOT assay and intracellular IFN-γ flow cytometry analysis. Minor to moderate increases in the number of CEA peptide-specific, IFN-γ-producing T cells were observed in all 4 of the injection (day 50) PBMCs from patients who were HLA-A*0201 or HLA-A*2402, respectively. The number of IFN-γ-secreting cells was detected by a standard enzyme-linked immunospot assay. Counting of spots was done in a blinded fashion to prevent undesired bias. The average number of spots in the negative control group was subtracted from that of the test group. The final number of spots was adjusted with a normalizing factor obtained from the positive control group containing ionomycin and phorbol 12-myristate-13-acetate.

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**Fig. 1** Surface marker expression of dendritic cells from two separate peripheral blood mononuclear cell preparations from 1 representative patient. Dendritic cells obtained on day 6 (dark areas) and after incubation with tumor necrosis factor-α for 40 h on day 8 (white areas) were examined for several surface markers to monitor their characteristics. Cells were stained with monoclonal antibodies against HLA-DR, CD86, CD80, CD83, CD14, and CD40. ---, isotype-matched monoclonal antibody controls. Results generated from two separate peripheral blood mononuclear cell preparations (V2 and V3) from 1 representative patient are shown.

**Fig. 2** Results of IFN-γ enzyme-linked immunospot assay from 10 patients before and after dendritic cell vaccination. Cryopreserved peripheral blood mononuclear cells (PBMC) obtained before vaccination (day 0, d0), 1 week after the second (day 22, d22), and 2 weeks after the fourth injection (day 50, d50) were thawed on the same day for analysis. Additional PBMCs were obtained on day 120 (d120) from patients 5 and 7, who both had stable disease. PBMCs (2 × 10⁶/well × 5 wells) from 4 HLA-A*0201 (A) and 6 HLA-A*2402 (B and C) patients were cultured with 50 µg/ml immunizing carcinoembryonic antigen (CEA) peptide(s) or a negative control peptide in 96-well filtration plates precoated with an anti-IFN-γ monoclonal antibody. One CEA peptide YLSGANLNL (A), and two CEA peptides QYSWFVNGTF (B) and TYACFVSNL (C), were used for PBMCs from patients who were HLA-A*0201 or HLA-A*2402, respectively. The number of IFN-γ-secreting cells was detected by a standard enzyme-linked immunospot assay. Counting of spots was done in a blinded fashion to prevent undesired bias. The average number of spots in the negative control group was subtracted from that of the test group. The final number of spots was adjusted with a normalizing factor obtained from the positive control group containing ionomycin and phorbol 12-myristate-13-acetate.
HLA-A*0201 patients and in 3 of the 6 HLA-A*2402 patients by ELISPOT assay (Fig. 2). These 3 HLA-A*2402 patients responded to both HLA-A*2402-restricted CEA peptides used in the DC vaccine. Using intracellular IFN-γ flow cytometry analysis, a substantial increase in the percentage of CEA-specific T cells was detected in patients 4 and 6, whereas a minor increase was observed in patients 1 and 2 (Fig. 3; Table 3). Although 7 patients had an enhanced CEA-specific T-cell response with the ELISPOT assay, only patients 1 and 6 also experienced an increased T-cell response as detected by intracellular cytokine flow cytometry analysis. The source of the discrepancy between these results is not known. However, it has been reported that the sensitivity of ELISPOT assay is higher than intracellular cytokine flow cytometry analysis (29). In addition, because of the limitation of our clinical blood samples, we can only use 25,000 cells from each setting for flow cytometry analysis. Thus, the results of our intracellular cytokine analysis are less informative. Nevertheless, these results suggest that our vaccination procedure can boost or generate specific T-cell responses in some cancer patients.

**DISCUSSION**

The primary end point of this pilot study was to evaluate the feasibility and safety of the proposed vaccination procedure on late-stage colorectal cancer patients. The *in vitro* preparation of the DC vaccine was performed in our laboratory according to Good Laboratory Practice regulations. No grade II or III toxicity, or side effects associated directly with the DC vaccination procedure were observed. We also conclude that intranodal injection of inguinal lymph node for late-stage colorectal cancer patients is feasible and safe. However, physical variations between patients did exist. For most patients, several lymph nodes on either side were visible under sonography; therefore, the DC vaccine was injected into lymph nodes on alternate sides for each injection. However, in patients in whom only one lymph

**Evaluation of Toxicity and Clinical Responses.** Two of the 10 patients (patients 5 and 7) had stable disease for at least 12 weeks (Table 3). There was a transient decrease (from 496 to 189.2 ng/ml) in the CEA level in patient 5 during the treatment period. Interestingly, this patient, who was HLA-A*2402, also had the most significant T-cell responses against both CEA peptides as determined by ELISPOT. Unfortunately, concurrent T-cell responses analyzed by intracellular cytokine flow cytometry were not available for this patient. Although patients 1 and 6 had an increased T-cell response that was detected by both methods, they had progressive disease when evaluated 6 weeks after the first vaccination. Stabilization of CEA level in patients 4 and 10 was observed during the treatment period (up to day 50). These 2 patients also displayed an increase in T cells against the immunizing CEA peptides with intracellular cytokine flow cytometry or ELISPOT analysis.

**Fig. 3** A representative result of intracellular IFN-γ flow cytometry analysis from patient 4 before and after dendritic cell vaccination. Cryopreserved peripheral blood mononuclear cells from patient 4 (HLA-A*2402), obtained before vaccination (day 0), 1 week after the second (day 22), and 2 weeks after the fourth injection (day 50), were thawed on the same day for analysis. Peripheral blood mononuclear cells were cultured with either one of the two HLA-A*2402-restricted carcinoembryonic antigen peptides (peptide-1: QYSWFVNGTF and peptide-2: TYACFVSNL) or a control HLA-A*0201-restricted carcinoembryonic antigen peptide for 18 h. The GolgiStop reagent was added during the last 4 h of culture to block protein secretion. Cells were harvested, washed, and stained with a phycoerythrin-labeled anti-CD8 monoclonal antibody. After washing, cells were fixed, permeabilized, and stained with a FITC-labeled anti-IFN-γ monoclonal antibody. Cells (25,000 cells) were subjected to flow cytometry analysis to determine the percentage of CD8⁺, IFN-γ-producing cells.
node was clearly visible, all four of the injections were targeted to the same lymph node. The impact of such differences is not known.

The secondary end point of this study was to evaluate the immune response against the CEA peptide before and after DC vaccination. We observed a substantial increase in the number of CEA-specific T cells by ELISPOT or intracellular cytokine flow cytometry in most patients (70%) after DC vaccinations, which indicates that our vaccination procedure may generate or boost a CEA-specific T-cell response in certain patients. Although not a primary goal of this trial, we followed the clinical outcomes of these patients. Two of the 10 (20%) patients experienced disease stabilization for at least 12 weeks, which is comparable with or slightly better than the outcome of other similar studies (20, 21). However, most patients had progressive disease, which was expected because they tended to have a heavy tumor burdens at study entry, which was reflected by high CEA levels (day 0; Table 3).

Our study design differed from previous DC vaccination studies in three important ways. First, both HLA-A*0201 and HLA-A*2402-restricted CEA peptides were used. This allowed us to include a greater proportion of Taiwanese patients. Enhanced CEA-specific T-cell responses were observed in both HLA-A*0201 and HLA-A*2402 patients. In the 3 HLA-A*2402 patients who had increased CEA-specific T-cell responses, both HLA-A*2402-restricted CEA peptides included in the DC vaccine were recognized. One HLA-A*0201 patient and 1 HLA-A*2402 patient had disease stabilization. The sample number is too small to draw any firm conclusions, but these results suggest that our DC vaccination protocol may be applicable to both HLA-A*0201 and HLA-A*2402 patients.

Secondly, TNF-α-matured DCs rather than immature DCs were used for our vaccine preparation. It has been shown that mature DCs are more potent stimulators of T cells, are more resistant to immune-suppressive factors released by tumor cells, and can maintain an activated phenotype after cytokine withdrawal. Therefore, immunization with mature DCs pulsed with antigenic peptides is expected to induce a stronger immune response. The expression of both CD80 and CD83 on DCs was enhanced after stimulation with TNF-α. Recent studies by other investigators have suggested that the use of both TNF-α and IFN-γ can impart DCs with an improved capacity for promoting T-helper 1 and CTL responses (30). Therefore, we will also adapt to this approach in future vaccination regimens.

Thirdly, the peptide-pulsed DCs were introduced into patients by intranodal injection instead of i.v. or intradermal injection. The direct intranodal injection of DCs in our study was expected to increase the number of peptide-pulsed DCs in lymph nodes and, therefore, elicit a better immune response. However, it is not known whether injected DCs localize to the correct compartment in the lymph node or whether the disturbance of lymph nodes by liquid injection interferes with their function. An alternative approach is to s.c. inject the DC vaccine together with an inflammation-inducible agent nearby lymph nodes. The resulting inflammatory microenvironment at the injection site might additionally modulate the coinjected DCs and promote their migration to the lymph nodes through the pathway of DCs exposed to invading pathogens.

Taken together, results from this pilot clinical study are very encouraging. We have proposed a Phase II study to better evaluate and improve the efficacy of such DC-based immunotherapy. The valuable information obtained from this study and our next phase II study should help us in the design and execution of future clinical trials of DC-based cancer immunotherapy.

**REFERENCES**


**Table 3** Summary of immunological and clinical responses

<table>
<thead>
<tr>
<th>Patient</th>
<th>ELISPOTa assayb</th>
<th>Intracellular stainingc</th>
<th>CEA (ng/ml)</th>
<th>Clinical response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0/22/50(120)</td>
<td>Day 0/22/50(120)</td>
<td>Day 0/22/50</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23/40/87</td>
<td>0/0.01/0.08</td>
<td>63.6/128.7/224</td>
<td>PD</td>
</tr>
<tr>
<td>2</td>
<td>13/04</td>
<td>0.01/0.01/0.01</td>
<td>&gt;500/&gt;500/&gt;500</td>
<td>PD</td>
</tr>
<tr>
<td>3</td>
<td>15/046</td>
<td>0.003/0.01/0.15</td>
<td>62.3/167.8/344.2</td>
<td>PD</td>
</tr>
<tr>
<td>4</td>
<td>0/0/52</td>
<td>0.02/0.02/0.02</td>
<td>48.9/55.5/561.</td>
<td>PD</td>
</tr>
<tr>
<td>5</td>
<td>0/66/100(49)</td>
<td>0.02/0.01/0.06</td>
<td>389.6/496/189.2</td>
<td>SD, 12 weeks</td>
</tr>
<tr>
<td>6</td>
<td>500/500/100</td>
<td>0.02/0.01/0.01</td>
<td>120.3/286/457.1</td>
<td>PD</td>
</tr>
<tr>
<td>7</td>
<td>20/20/50(40)</td>
<td>0.02/0.00/0.01</td>
<td>106.4/193/239.9</td>
<td>SD, 12 weeks</td>
</tr>
<tr>
<td>8</td>
<td>51/78/47</td>
<td>0.003/0.06/0.00</td>
<td>273/315.4/397.8</td>
<td>PD</td>
</tr>
<tr>
<td>9</td>
<td>6/19/1</td>
<td>0.01/0.01/0.00</td>
<td>&gt;500/&gt;500/&gt;500</td>
<td>PD</td>
</tr>
<tr>
<td>10</td>
<td>3/15/2</td>
<td>0/0/0.02/0.00/0.00</td>
<td>123/254/227</td>
<td>PD</td>
</tr>
</tbody>
</table>

a ELISPOT, enzyme-linked immunospot; CEA, carcinoembryonic antigen; PD, progressive disease; NA, not available; SD, stable disease.

b spots/2 x 106 cells.

c Percentage of positive cells, the percentage of the control group was subtracted from that of the test group.


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