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Phase I clinical trial of a five-peptide cancer vaccine combined with cyclophosphamide in advanced solid tumors

Mutsunori Murahashi [a,1], Yasuki Hijikata [a,1], Kazunari Yamada [a,1], Yoshihiro Tanaka [b], Junji Kishimoto [c], Hiroyuki Inoue [b], Tomotoshi Marumoto [b], Atsushi Takahashi [b], Toshihiko Okazaki [a], Kazuyoshi Takeda [d], Masakazu Hirakawa [e], Hiroshi Fujii [f], Shinji Okano [f], Masaru Morita [g], Eishi Baba [h], Kazuhiro Mizumoto [i], Yoshihiko Maehara [g], Masao Tanaka [i], Koichi Akashi [h], Yoichi Nakanishi [j], Koji Yoshida [k], Takuya Tsunoda [k], Kazuo Tamura [l], Yusuke Nakamura [k], Kenzaburo Tani [a,b,*]

[a] Department of Advanced Cell and Molecular Therapy, Kyushu University Hospital, Fukuoka, Japan
[b] Division of Molecular and Clinical Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan
[c] Digital Medicine Initiative, Kyushu University, Fukuoka, Japan
[d] Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan
[e] Department of Radiology, Kyushu University Hospital, Fukuoka, Japan
[f] Department of Pathology, Kyushu University, Fukuoka, Japan
[g] Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[h] Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan
[i] Department of Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[j] Institute of Diseases of Chest, Kyushu University, Fukuoka, Japan
[k] Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan
[l] Division of Oncology, Hematology and Infectious Diseases, Department of Internal Medicine, Fukuoka University, Fukuoka, Japan

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ABSTRACT

We designed a phase I trial to investigate the safety, immune responses and clinical benefits of a five-peptide cancer vaccine in combination with chemotherapy. Study subjects were patients positive for HLA-A2402 with locally advanced, metastatic, and/or recurrent gastrointestinal, lung or cervical cancer. Eighteen patients including nine cases of colorectal cancer were treated with escalating doses of cyclophosphamide 4 days before vaccination. Five HLA-A2402-restricted, tumor-associated antigen (TAA) epitope peptides from KOC1, TTK, URLC10, DEPDC1 and MPHOSPH1 were injected weekly for 4 weeks. Treatment was well tolerated without any adverse events above grade 3. Analysis of peripheral blood lymphocytes showed that the number of regulatory T cells dropped from baseline after administration of cyclophosphamide and confirmed that TAA-specific T cell responses were associated significantly with longer overall survival. This phase I clinical trial demonstrated safety and promising immune responses that correlated with vaccine-induced T-cell responses. Therefore, this approach warrants further clinical studies.

1. Introduction

Although many studies have demonstrated the effectiveness of cancer vaccines, no vaccine has shown survival benefits in randomized phase III clinical trials [1,2]. Cancer vaccines alone appear to be unable to outperform conventional therapies; however, a combination of agents aimed at controlling immune tolerance to cancer vaccines might improve outcomes.

Recently, progress has been made in the development of immunological therapies aimed at inhibiting immune tolerance. For example, anti-PD-1 antibody alone improves clinical outcome in malignant melanoma and non-small lung cell cancer [3,4]. Although immune cell therapies, which represent one class of approach to targeting tumor-associated antigens (TAAs), have shown promise [5,6], the requirement of apheresis is burdensome for end-stage cancer patients. The greatest advantage of cancer vaccines is that they are safe and well tolerated in most cases, and injection-site reaction is the only major adverse event revealed by previous studies [2]. In addition, multiple-peptide vaccines appear more promising than single-peptide vaccines, because such

Abbreviations: CPM, cyclophosphamide; Treg cells, regulatory T cells; CTLs, cytotoxic T lymphocytes; TAA, tumor-associated antigen; PFS, progression free survival; OS, overall survival.

* Corresponding author at: Division of Molecular and Clinical Genetics, Medical Institute of Bioregulation, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Project division of ALA advanced medical research, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail address: k-tani@ims.u-tokyo.ac.jp (K. Tani).

[1] These authors contributed equally to this work.

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vaccines are theoretically more likely to prevent escape by cancer cells with many genetic mutations [7,8,9]. The use of cancer vaccines in conjunction with agents intended to control immune tolerance in end-stage cancer patients still represents a reasonable approach, and should be carefully evaluated in clinical trials. However, confirmation of expression of TAAs in target lesions to justify the immunological efficacy of cancer vaccine therapy is not easy in end-stage cancer patients. Moreover, the safety and cost-effectiveness of using a combination of agents to control immune tolerance such as anti-PD-1 antibody have not yet been resolved in practical medicine.

In light of the situation described above, we carefully selected five peptides derived from TAAs, KOC1, DEPDC1, MPHOSPH1, TTK and URLC10, which are highly expressed in solid tumors (esophageal, gastric, colon cancer, cholangiocellular carcinoma, pancreatic cancer, small cell and non-small cell lung cancer, and cervical cancer), and designed a phase I clinical trial using this multiple-peptide cancer vaccine in combination with cyclophosphamide (CPM), without prior pathological confirmation of TAA expression. CPM selectively depletes CD4 + CD25 + regulatory T (Treg) cells and restores T and NK effector function in patients with end-stage cancer [10]. Additionally, in a randomized trial of patients with metastatic renal cell carcinoma, pretreatment with CPM before multiple-peptide cancer vaccination conferred a survival benefit, associated with the immune response, in comparison to patients who did not receive CPM [11]. Because CPM has a long history of use in patients with various cancers, and is available at a much lower price than antibody preparations, CPM is worthy of further study as an agent for controlling immune tolerance. The expression of TAAs in the primary lesion was retrospectively examined as much as possible to pathologically confirm the actual expression of these target antigens in the vaccinated patients. These findings make it possible to discuss the relationships between antigen expression and the induction of immune responses by vaccination. Finally, based on one case of a long-term survivor who received radiation therapy before and after this trial, we discuss the possible usefulness of radiation therapy as a modality that could be combined with cancer vaccines [12].

2. Materials and methods

2.1. Study design

This was a phase I, open-label study of CPM. Eighteen patients were treated in cohorts of six with escalating CPM doses (150, 300 and 600 mg/m^2). CPM was administered over 2 h as an intravenous infusion once per course. Five peptides derived from TAAs of KOC1, DEPDC1, MPHOSPH1, TTK and URLC10, which are highly expressed in esophageal, gastric, or colon cancers, cholangiocellular carcinoma, pancreatic cancer, small cell and non-small cell lung cancer, and cervical cancer, as described below in detail, were administered subcutaneously as vaccines, once per week for 4 weeks (Suppl. Fig. 1). Our study was primarily aimed at determining the feasibility and safety of these vaccinations, and secondarily at determining whether these vaccines could induce antitumor immune responses without prior confirmation of the expression of these TAAs in patient tumor specimens, because patients' tumors were considered to express at least one of these TAAs (Suppl. Table 1).

Additionally, patients without gastrointestinal bleeding, pleural effusion, and ascites received 350,000 IU of Proleukin (IL-2; Chiron, Amsterdam, The Netherlands) subcutaneously for 3 days after each vaccination. After the first course, all patients were observed closely for 1 week. If patients agreed to continue and they were able to tolerate vaccination, a new course was delivered, followed by 3 weeks of observation.

Toxicity and clinical outcomes were evaluated for all patients who received more than four vaccinations. Blood samples for immune response tests were obtained every week during each course and 4 weeks after the final injection. Computed tomography (CT) assessed clinical responses before and after vaccination. Every measurable lesion was evaluated by the Response Evaluation Criteria in Solid Tumor (RECIST) criteria.

2.2. Patient eligibility

The disease inclusion criterion was locally advanced, metastatic, and/or recurrent esophageal, gastric or colon cancer, cholangiocellular carcinoma, pancreatic cancer, small cell or non-small cell lung cancer, or cervical cancer with measurable disease. Other inclusion criteria was as follows: age, 20–80 years; *HLA-A*2402* positivity, as determined by DNA typing of HLA-A genetic variations using a WAKFlowHLAtyping Kit on a Luminex Multi-Analyte Profiling system (Wakunaga, Hiroshima, Japan), as described elsewhere [13]; Eastern Cooperative Oncology Group performance status of 0–1; no active brain metastases; life expectancy ≥ 3 months; and adequate hematological (2000/μL < WBC count < 15,000/μL; platelet count ≥ 75,000/μL), renal (serum creatinine < 2.0 mg/dL), and hepatic (AST, ALT < 3X ~ ULN value) function. Patients must have recovered from toxic effects of any previous therapy at least 4 weeks before entering the trial, and also had to be negative for syphilis sero-diagnosis, hepatitis B antigen, and antibodies against hepatitis C, HIV, and HTLV-1. Exclusion criteria are described elsewhere [14]. The study was approved by the Institutional Ethical Review Board of Kyushu University (#19-40) and is registered with ClinicalTrials.gov (NCT00676949). Written informed consent was obtained from all patients.

2.3. Dose limiting toxicity (DLT) and maximum tolerated dose (MTD)

The DLT of CPM administered with peptide vaccines was determined during the first course and defined by the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 as grade 4 (leukopenia and neutropenia), grade 3–4 (thrombocytopenia), or grade 3–4 (non-hematological). At least six patients were enrolled at each dose level. If DLT was observed after the first course, three additional patients were enrolled at the same dose. If no patients experienced DLT, the dose was escalated. Dose was never escalated for individual patients. MTD was the dose that produced DLT in two of six patients or all three initial patients.

2.4. Peptides and vaccination

Patients positive for HLA-A2402 were vaccinated with five peptides derived from KOC1 (KTVNELQNL), DEPDC1 (EYYELFVNI), MPHOSPH1 (IYNEYIYDL), TTK (SYRNEIAYL) and URLC10 (RYCNLEGPPI), all of which bind the HLA-A24 molecule. Profiles of the five TAAs targeted in this trial are shown in Table I. These novel TAAs were identified from 32,000 human genes using cDNA microarray analysis coupled with laser microdissection [14,15,16]. All of the proteins from which the TAAs were derived are involved in transcription and cell proliferation. These TAAs were expressed at high levels in lung, cervical, and cholangiocellular carcinoma (CCC), and moderately in esophageal, gastric, colon, and pancreatic cancer, as determined by microarray analyses performed by ourselves or reported elsewhere [14,17,18]. According to these results, with lower 95% confidence bound of probabilities, at least one of the five TAAs was considered to be expressed in NSCLC (prob ≥ 0.9929), SCLC (prob ≥ 0.9735), esophageal cancer (prob ≥ 0.9845), stomach cancer (prob ≥ 0.8290), colon cancer (prob ≥ 0.6433), cervical cancer (prob ≥ 0.9960), cholangiocellular carcinoma (prob ≥ 0.9875), and pancreatic cancer (prob ≥ 0.8035) (Suppl. Table 1). The establishment of CTL clones with specific cytotoxic activities against target tumor cells positive for HLA-A24 and expressing these five peptides and were able to induce TAA-specific T cell responses in cancer patients, as reported previously [19,20,21]. The purity (>97%) and identity of the peptides were determined by analytical high-performance liquid chromatography and mass spectrometry, respectively. Endotoxin levels and bioburden of the peptides were