

# Phase I clinical trial of survivin-derived peptide vaccine therapy for patients with advanced or recurrent oral cancer

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Survivin, a member of the inhibitor of apoptosis protein (IAP) family, is abundantly expressed in most malignancies, but is hardly detectable in normal adult tissues. Previously we have identified a human leukocyte antigen (HLA)-A24-restricted antigenic peptide, survivin-2B80-88 (AYACNTSTL), recognized by CD8<sup>+</sup> cytotoxic T lymphocytes (CTL). Survivin-2B80-88-specific CTL were induced efficiently from peripheral blood mononuclear cells (PBMC) of oral cancer patients after stimulation with the peptide *in vitro*. We conducted a phase I clinical study to evaluate the safety and the efficacy of survivin-2B80-88 peptide vaccination in HLA-A24-positive patients with advanced or recurrent oral cancer. The vaccines were given subcutaneously or intratumorally six times at 14-day intervals. Eleven patients were enrolled and 10 patients completed the vaccination protocol. No adverse events were observed in any patients. In two patients, the levels of serum squamous cell carcinoma (SCC) antigen decreased transiently during the period of vaccination. Tumor regression that was compatible with a partial response (PR) was noted in one patient. The remaining nine patients experienced progressive disease (PD). Immunologically, an increase of the peptide-specific CTL frequency was detected in six of the eight patients evaluated by HLA-A24/peptide tetramer analysis. The present clinical trial revealed that survivin-2B peptide vaccination was safe and had therapeutic potential for oral cancer patients. However, subsequent clinical trials in combination with various adjuvant drugs will be required to improve the immunological and therapeutic efficacy. This trial was registered with University Hospital Medical Information Network (UMIN) number UMIN00000976. (*Cancer Sci* 2011; 102: 324–329)

Oral cancer consistently ranks as one of the 10 most frequently diagnosed cancers worldwide.<sup>(1)</sup> It encompasses a range of malignant tumors arising from various diverse and complex structures that have major physiological and aesthetic importance. For most early stage oral cancers, high cure rates are achieved with either surgery or definitive irradiation and both speech and swallowing functions can often be preserved. On the other hand, locally advanced or recurrent oral cancers are usually treated with combination therapy consisting of either surgery followed by postoperative chemoradiation or chemoradiation with surgical salvage if needed. However, most patients remain at high risk for locoregional recurrence and distant metastasis.<sup>(2)</sup> Therefore, advances in new therapeutic modalities such as tumor-specific immunotherapy for patients with locally advanced or recurrent oral cancers are urgently needed.

A large number of tumor-associated antigens have been identified from melanomas and other cancers, and clinical trials of peptide-based immunotherapy have been carried out. Melanoma antigen peptides were the first to be tested in phase I and phase II studies for active immunization of metastatic melanoma

patients.<sup>(3,4)</sup> During the first stage of the studies, clinical responses were observed in Europe and the United States.<sup>(5,6)</sup> However, in 2003, Rosenberg *et al.*<sup>(7)</sup> reported that <5% of patients who received peptide vaccines such as gp100, MART-1 and tyrosinase plus IL-2 showed an overall objective response (complete response [CR] + partial response [PR]). On the other hand, investigational immunotherapy that targeted MAGE-A3 tended to reduce the risk of recurrence by 27% when used as an adjuvant therapy with surgery in stage IB/II non-small-cell lung cancer. Furthermore, enrolment in the global phase III trial of adjuvant MAGE-A3 for non-small-cell lung cancer has already started according to a certain European Union (EU)-based pharmaceutical company. This finding provides hope for current and future immunotherapies and has accelerated a variety of investigations concerned with human tumor immunology.

Survivin is a recently characterized inhibitor of apoptosis protein (IAP) that is abundantly expressed in most solid and hematological malignancies, but is barely detectable in normal adult tissues.<sup>(8)</sup> It has been shown to increase tumor resistance to apoptotic stimuli such as radiation and chemotherapy.<sup>(9,10)</sup> A number of reports have demonstrated that survivin expression in cancer cells has a prognostic value and is associated with increased tumor recurrence and a lower survival rate,<sup>(11–16)</sup> although the opposite correlation is observed in certain cancers.<sup>(17)</sup> We previously reported that survivin-2B, a splicing variant of survivin, is also expressed abundantly in various tumor cell lines and the survivin-2B80-88 (AYACNTSTL) peptide derived from the exon 2B-encoded region is recognized by CD8<sup>+</sup> cytotoxic T lymphocytes (CTL) in the context of human leukocyte antigen (HLA)-A24 molecules.<sup>(18)</sup> The CTL specific for this peptide were successfully induced from PBMC in six of seven HLA-A24-positive patients (83%) with colorectal cancers and exerted cytotoxicity against HLA-A24-positive/survivin-positive adenocarcinoma cells.<sup>(19)</sup> Furthermore, we recently demonstrated that survivin-2B peptide-specific CTL were induced in four of eight (50%) HLA-A24-positive patients with oral cancer with over stage II progression.<sup>(20)</sup> Based on these observations, a phase I clinical study of survivin-2B peptide vaccination was initiated for patients with locally advanced or recurrent oral cancer. The present clinical trial demonstrated the safety and suggested the marginal clinical effectiveness of the survivin-2B peptide vaccination alone for oral cancer patients.

## Materials and Methods

**Eligibility criteria.** The study protocol was approved by the Clinical Institutional Ethical Review Board of the Medical

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Institute of Bioregulation, Sapporo Medical University, Japan. All patients gave their written informed consent before entry into the study. Patients enrolled in this study were required to conform to the following criteria: (i) to have histologically proven oral cancer; (ii) to be HLA-A\*2402 positive; (iii) to have survivin-positive cancerous lesions by immunohistochemistry; (iv) to have HLA class I-positive cancerous lesions by immunohistochemistry using the anti-pan HLA class I mAb EMR8-5; (v) to be 20–85 years old; (vi) to have an unresectable, locally advanced or recurrent tumor; and (vii) to have an Eastern Cooperative Oncology Group (ECOG) performance status of between 0 and 3. The exclusion criteria included: (i) prior cancer therapy such as chemotherapy, radiation therapy, steroid therapy or other immunotherapy within the previous 4 weeks; (ii) the presence of other cancers that might influence the prognosis; (iii) immunodeficiency or a history of splenectomy; (iv) severe cardiac insufficiency, acute infection or hematopoietic failure; (v) pregnancy or breast-feeding; and (vi) unsuitability for the trial based on clinical judgment. This study was carried out at the Department of Oral Surgery, Sapporo Medical University Primary Hospital from September 2003.

**Peptide preparation.** The survivin-2B80-88 peptide (amino acid sequence AYACNTSTL), which was derived from a splicing variant survivin-2B-specific exon 2B, was prepared under good manufacturing practice conditions by Multiple Peptide Systems (San Diego, CA, USA). The identity of the peptide was confirmed by mass spectral analysis and the purity was shown to be more than 98% as assessed by high-pressure liquid chromatography analysis. The peptide was supplied as a freeze-dried, sterile white powder. It was dissolved in 1.0 mL of physiological saline (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and stored at  $-80^{\circ}\text{C}$  until just before use.

**Treatment protocol.** Vaccinations with survivin-2B peptide were administered subcutaneously (s.c.) into the ipsilateral neck or intratumorally six times at 14-day intervals. Two incremental dose levels were planned for the peptide administration, with a starting dose of 0.1 mg. Six patients received 0.1 mg (group 1) and four patients received 1.0 mg (group 2), while each group was divided into the two different administration sites as stated above. Before proceeding to the next dose level, all previously administered patients had to have completed the trial period. Dose escalation for group 2 was allowed if no patients in group 1 experienced grade 3–4 toxicity.

If patients hoped for continuation of this peptide vaccine therapy, we conducted it in the same manner after the sixth administration.

**Delayed-type hypersensitivity (DTH) skin test.** The DTH skin test was performed at each vaccination. The peptide (10  $\mu\text{g}$ ) solution in physiological saline (0.1 mL) or physiological saline alone (0.1 mL) was separately injected intradermally (i.d.) into the forearm. A positive reaction was defined as area of erythema and induration with a diameter of more than 4 mm, 48 h after the injection.

**Evaluation of toxicity and response.** Patients were examined closely for signs of toxicity during and after the vaccination. The US National Cancer Institute Common Toxicity Criteria (NCI-CTC Version 2.0, Jan.30, 1998) were used to classify the toxicity grades.

Physical examinations and hematological examinations were conducted before and after each vaccination. The serum level of squamous cell carcinoma (SCC) antigen, which is the current standard tumor marker for head and neck cancer, was examined at 14-day intervals. A SCC antigen level of 1.5 ng/mL was generally taken as the upper limit of the normal range. The tumor size was evaluated by visual inspection, computed tomography (CT) and magnetic resonance imaging (MRI) before treatment, after three vaccinations and at the end of the study period. The tumor response was evaluated according to the Response Evalu-

ation Criteria in Solid Tumors (RECIST) guidelines:<sup>(21)</sup> a complete response (CR) was defined as the disappearance of all target lesions; and a partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameters of the target lesions for at least 4 weeks without the appearance of new lesions. Progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameters of the target lesions or the appearance of one or more new lesions. Stable disease (SD) was defined as neither sufficient shrinkage to qualify for a PR nor a sufficient increase to qualify for PD.

**In vitro stimulation of PBMC.** The PBMC were isolated from blood samples by Ficoll–Conray density gradient centrifugation and then frozen and stored at  $-80^{\circ}\text{C}$ . As needed, frozen PBMC were thawed and incubated in the presence of 30  $\mu\text{L}/\text{mL}$  survivin-2B peptide in AIM-V medium containing 10% human serum at room temperature. Interleukin-2 (IL-2) was added at a final concentration of 50 U/mL for 1 h on days 0, 2, 4 and 6 of culture. On day 7, the PBMC were analyzed by tetramer staining.

**Tetramer staining.** HLA-A24/peptide tetramers were constructed according to the procedure described by Altman *et al.*<sup>(22)</sup> Briefly, recombinant HLA-A24 heavy chain<sup>(23)</sup> and human  $\beta$ -2-microglobulin were refolded with the survivin-2B80-88 peptide as described previously.<sup>(24)</sup> The resulting HLA-A24-peptide monomer was biotinylated by incubation with the enzyme BirA (Avidity, Denver, CO, USA) for 17 h at room temperature and purified using fast protein liquid chromatography. A tetrameric HLA-peptide complex was produced by incubating streptavidin-PE (Vector Laboratories, Burlingame, CA, USA) with the biotinylated monomer at a 1:4 molar ratio. For flow cytometric analysis, the PBMC, which were stimulated *in vitro* as above, were stained with the phycoerythrin (PE)-labeled tetramer at  $37^{\circ}\text{C}$  for 20 min, followed by staining with an FITC-conjugated anti-CD8 mAb (Becton Dickinson Biosciences, San Jose, CA, USA) at  $4^{\circ}\text{C}$  for 30 min. The cells were washed twice with PBS before fixation in 1% formaldehyde. Flow cytometric analysis was performed using a FACSCalibur and the CellQuest software program (Becton Dickinson Biosciences). The frequency of the CTL precursors was calculated as the number of tetramer-positive cells over the number of CD8-positive cells. Moreover, the PBMC were stained with an FITC-labeled HLA-A\*2402-restricted human immunodeficiency virus (HIV) peptide (RYLRDQQLL) tetramer and PE-labeled HLA-A\*2402-survivin-2B80-88 peptide tetramer, which were purchased from MBL Co., Ltd. (Nagoya, Japan), at  $37^{\circ}\text{C}$  for 20 min, followed by staining with an FITC- or PerCP-conjugated anti-CD8 mAb (Becton Dickinson Biosciences) at  $4^{\circ}\text{C}$  for 30 min. The frequency of the CTL precursors was calculated in the same manner.

## Results

**Patient characteristics.** Eleven patients (six males, five females) were eligible and agreed to participate in this phase I study. The patients' characteristics are summarized in Table 1. The patients' median age at enrolment was 66.5 years, with a range 38–84 years. Based on the ECOG classification, five patients were PS1, five were PS2, and one was PS3. The patients' primary tumor sites were: buccal mucosa, three; palate, two; upper or lower alveolus and gingiva, two; mandible, one; floor of mouth, one; submandibular gland, one; and tongue, one. The histological type was SCC in seven patients, adenoid cystic carcinoma (ACC) in three and alveolar soft part sarcoma (ASPS) in one. Table 2 summarize the clinical and immunological outcomes for the 11 patients. One patient discontinued the regimen after four vaccinations. She (case 8) had a growing locoregional recurrence and her general condition deteriorated. Subsequently she was removed from the study after four vaccinations because she refused to continue the protocol. None of

**Table 1. Summary of the characteristics of patients enrolled in the present study**

| Patient no. | Histology | Age/Sex | PS | Primary tumor site         | Recurrent or metastatic sites |
|-------------|-----------|---------|----|----------------------------|-------------------------------|
| 1           | ASPA      | 38/M    | 1  | Mandible                   | Local, brain, lung            |
| 2           | ACC       | 60/M    | 1  | Hard palate                | Local, lung                   |
| 3           | SCC       | 84/F    | 3  | Floor of mouth             | Locoregional                  |
| 4           | ACC       | 50/F    | 2  | Submandibular gland        | Lung                          |
| 5           | SCC       | 83/F    | 2  | Upper alveolus and gingiva | Locoregional                  |
| 6           | SCC       | 72/M    | 2  | Buccal mucosa              | Local                         |
| 7           | SCC       | 55/M    | 2  | Tongue                     | Locoregional                  |
| 8           | SCC       | 82/F    | 2  | Lower alveolus and gingiva | Neck                          |
| 9           | SCC       | 73/M    | 1  | Hard palate                | Lung, liver                   |
| 10          | SCC       | 82/F    | 1  | Buccal mucosa              | Neck                          |
| 11          | SCC       | 68/M    | 1  | Buccal mucosa              | Locoregional                  |

ACC, adenoid cystic carcinoma; ASPA, alveolar soft part sarcoma; SCC, squamous cell carcinoma.

**Table 2. Profiles of the enrolled patients and clinical responses to the survivin-2B peptide vaccination**

| Patient no. | Dose of peptide (mg) | Injection route | HLA class I expression | Prior therapy (washout time)  | Adverse events | Tetramer staining† (pre-/post-) | Tumor marker | Clinical response | Follow up (months) | Progress |
|-------------|----------------------|-----------------|------------------------|-------------------------------|----------------|---------------------------------|--------------|-------------------|--------------------|----------|
| 1           | 0.1                  | Intratumoral    | +                      | S + C (1 month)               | -              | ND                              | ND           | PD                | 43                 | AWD      |
| 2           |                      | Intratumoral    | +                      | S + C (1 month)               | -              | 121/103                         | ND           | PD                | 25                 | DOD      |
| 3           |                      | Intratumoral    | +                      | C + R (1 month)               | -              | ND                              | ND           | PD                | 3                  | DOD      |
| 4           |                      | s.c.            | +                      | S + C + R (6 years, 4 months) | -              | 1/100                           | ND           | PD                | 15                 | DOD      |
| 5           |                      | s.c.            | +                      | C (1 month)                   | -              | 6/16                            | INC          | PD                | 6                  | DOD      |
| 6           |                      | s.c.            | +                      | S + R + C (1 months)          | -              | 65/244                          | INC          | PD                | 3                  | DOD      |
| 7           | 1.0                  | Intratumoral    | +                      | S + R + C (1 month)           | -              | 96/528                          | ND           | PD                | 6                  | DOD      |
| 8‡          |                      | Intratumoral    | +                      | S + R (1 month)               | -              | ND                              | ND           | ND                | 2                  | DOD      |
| 9           |                      | s.c.            | +                      | S + C (2 months)              | -              | 77/204                          | DEC          | PD                | 5                  | DOD      |
| 10          |                      | Intratumoral    | +                      | S + C (1 month)               | -              | 5/20                            | DEC          | PR                | 5                  | DOD      |
| 11          |                      | s.c.            | +                      | S + R + C (5 months)          | -              | 5/1                             | ND           | PD                | 8                  | DOD      |

†Tetramer staining: Tetramer(+)CD8(+) in 10 000 CD8(+) cells. ‡Patient refused to continue the protocol (case 8). AWD, alive with disease; C, chemotherapy; DEC, decreased; DOD, dead of disease; HLA, human leukocyte antigen; INC, increased; ND, not determined; PD, progressive disease; post-, after the fourth vaccination; PR, partial response; pre-, before the first vaccination; R, radiotherapy; S, surgery.

the treatment interruptions were due to any adverse reactions to the vaccination. Ten patients received the complete regimen including six vaccinations and thereafter were evaluated.

**Safety.** The peptide vaccination was well tolerated in all 10 patients. No hematological, cardiovascular, hepatic or renal toxicity was observed during or after vaccination. Skin reactions such as induration, pain or rash were not observed in any case.

**DTH skin test.** A DTH skin test was performed at each vaccination and assessed 48 h later. No positive DTH reaction was observed in any patient.

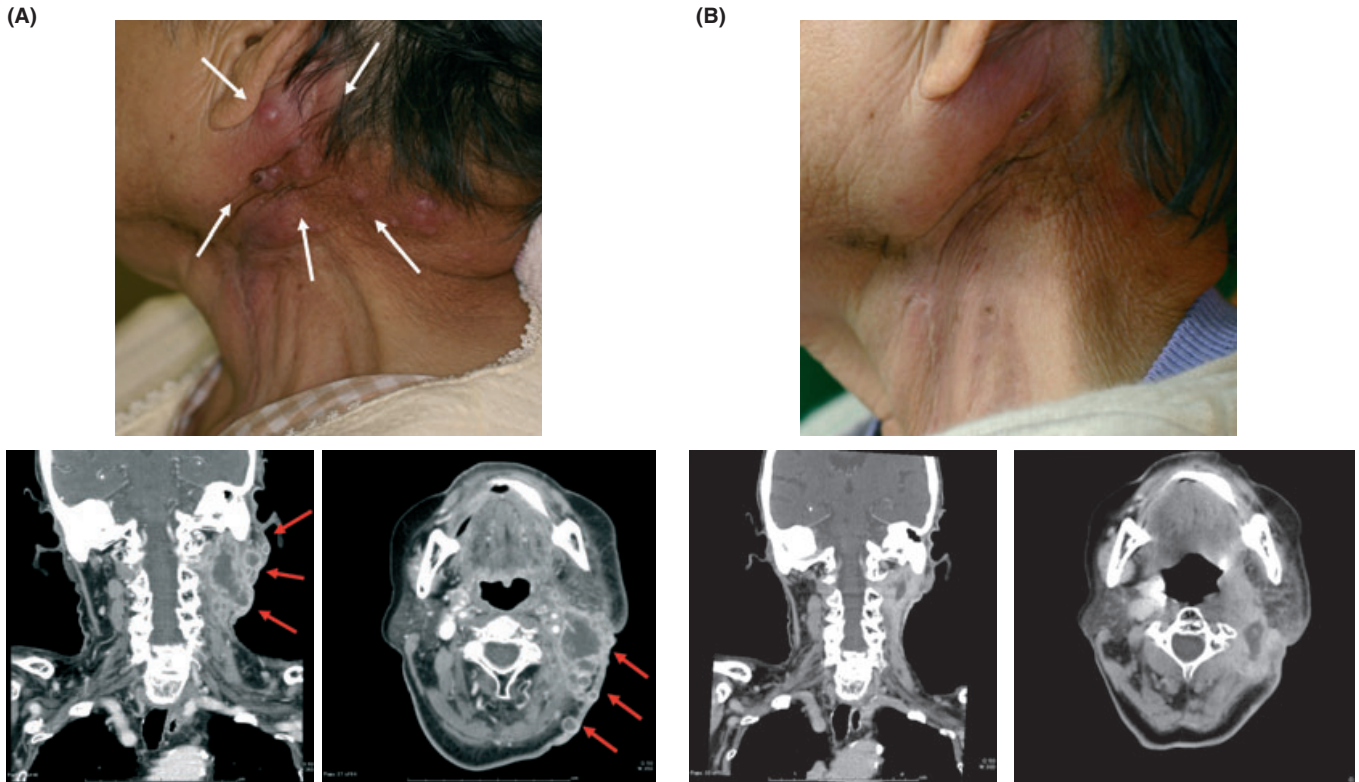
**Clinical responses.** In two patients (cases 9 and 10) the tumor marker level (SCC antigen) transiently decreased. In two patients (cases 5 and 6) it increased and in the remainder it was not useful for monitoring. A PR was observed in one patient (case 10), who also demonstrated a remarkable decrease in the SCC antigen level (6.0 ng/mL → 0.7 ng/mL). The remaining nine patients experienced PD.

Case 9, who had multiple lung metastases, transiently showed a positive level of SCC antigen of 2.1 ng/mL that decreased after the second vaccination and was within the normal range just after the third vaccination. However, after the fourth vaccination it increased abruptly, which closely corresponded to his clinical progress. Until the fourth vaccination, CT imaging of the lung revealed virtually dormant disease, however, it revealed progressive disease after the sixth vaccination.

One responder (case 10) with PR developed multiple neck metastases and skin metastases in the left side of her neck at 3 months after surgery followed by treatment with tegafur/

uracil (UFT) at a daily dose of 400 mg as oral adjuvant chemotherapy. She was judged to be impossible to treat radically because CT imaging showed that the recurrent tumor had metastasized to lymph nodes and the skin, including the parotid gland, submandibular region, posterior cervical region, occipital region of the head, posterior skull base and lower cervical region (Fig. 1A). The metastatic progressive tumor samples from her neck obtained by neck dissection previously were confirmed by immunohistochemical staining to markedly express survivin and HLA class I molecules. Survivin-2B peptide vaccine was administered intratumorally to the left side of her neck nine times at biweekly intervals. The SCC antigen level was 6.0 ng/mL before vaccination. Her skin metastatic tumor and pain disappeared transiently after the fifth vaccination, thus resulting in an improvement in her quality of life. A tumor regression rate of 70% was observed by CT imaging (Fig. 1B). The SCC antigen level decreased to 0.7 ng/mL after the sixth vaccination (Fig. 2). Nevertheless, these effects were maintained for 2 months only.

**Tetramer staining assay.** Peptide-specific immunological responses were evaluated in eight patients by HLA-A24/survivin-2B80-88 peptide tetramer analysis. The change of the tetramer-positive CTL frequency was evaluated by comparison with that before the first vaccination and that after each vaccination. The frequency of tetramer-positive CTL tended to increase after the vaccination in six patients (cases 4, 5, 6, 7, 9 and 10) (Table 2). In Figure 3, the peptide-specific CTL frequencies in cases 9 and 10 are indicated as the percentages of



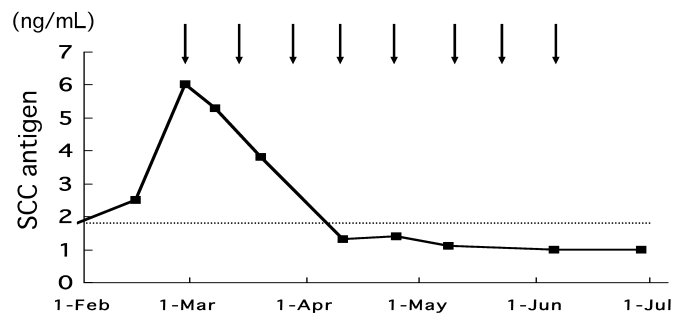
**Fig. 1.** Photograph of skin on the neck and computed tomography (CT) scan image of the neck showing metastatic tumors of case 10. (A) Photograph of skin on the neck and CT scan image of the neck before vaccination. Axial contrast-enhanced CT image shows multiple metastatic tumors (arrows). (B) Photograph of skin on the neck and CT scan image of the neck after the fifth vaccination. The metastatic tumors show significant remission after the fifth vaccination compared with before vaccination (70% reduction).

tetramer-positive CTL among CD8-positive T cells before and after the fourth vaccination. The frequency of tetramer-positive CTL was increased from 0.77% to 2.04% and from 0.05% to 0.20%, in cases 9 and 10 respectively.

### Discussion

Many tumor-associated antigens have been identified and clinical trials utilizing them have been conducted.<sup>(3-6)</sup> However, most such clinical trials were aimed at the treatment of advanced melanoma and there are few reports on the treatment of patients with solid cancers. Although the immunogenicity of these non-melanoma-associated antigens is relatively weak, a specific number of tumor antigens were determined. The HLA-A24-restricted CTL epitope survivin-2B80-88 derived from survivin-2B has high potency for CTL induction in various cancer patients, including those with breast cancer, colorectal cancer, gastric cancer and oral cancer.<sup>(10,18-20)</sup> Based on the findings of these studies *in vitro*, a phase I clinical study of survivin-2B peptide vaccine therapy began in September 2003 for patients with advanced or recurrent oral cancer, following those for colorectal cancer and breast cancer. In many clinical trials, patients received the peptide in combination with certain adjuvants such as incomplete Freund's adjuvant (IFA) and cytokines for the purpose of enhancing the immune responses against cancer. In the present study, patients received the survivin-2B peptide dissolved in physiological saline without any adjuvant in order to strictly evaluate the clinical effect of the peptide alone.

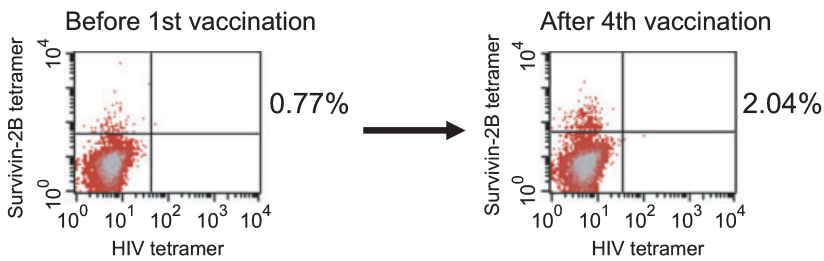
A dose-escalation trial was chosen to estimate the safe and optimal doses. Dosage groups of 0.1 and 1.0 mg were set up, consisting of six and four patients, respectively. None of the patients had any sign of toxicity. Therefore, the survivin-2B



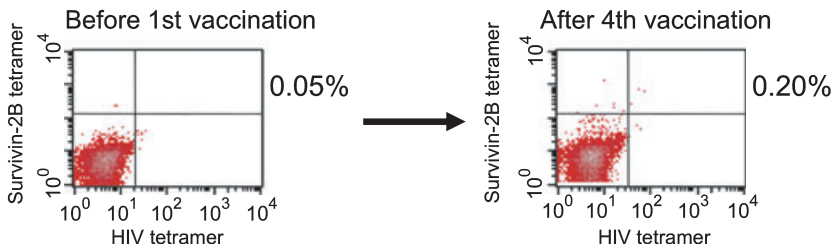
**Fig. 2.** Changes in the serum squamous cell carcinoma (SCC) antigen level during the vaccination in case 10. The dotted line indicates the cut-off point for the SCC antigen level. The arrows indicate the times of vaccination. The SCC antigen level significantly decreased to 0.7 ng/mL after the sixth vaccination. The cut-off value was 1.5 ng/mL.

peptide vaccine was safe and could be repeatedly injected into patients without serious side-effects. In terms of the clinical responses, the levels of tumor markers were temporarily decreased in comparison with the pretreatment status in two patients in the 1.0 mg dosage group. No patients in the 0.1 mg dosage group experienced a decrease in tumor markers. A PR was observed in one patient who was administered 1.0 mg of peptide. Therefore, the 1.0 mg dosage group appeared to have a better clinical outcome than the 0.1 mg dosage group. Based on these results, the recommended survivin-2B vaccine dose was 1.0 mg. Furthermore, we set up two distinct injection routes, s.c. into the ipsilateral neck or intratumorally. Intratumoral injection was concretely done by

### Case no. 9



### Case no. 10



**Fig. 3.** Tetramer staining before the first vaccination and after the fourth vaccination in cases 9 and 10. Flow cytometric analysis was performed using a FACSCaliber and CellQuest software (Becton Dickinson Biosciences). The frequency of the cytotoxic T lymphocyte (CTL) precursors was calculated as the number of tetramer-positive cells divided by the number of CD8-positive cells. The peptide-specific CTL frequency is indicated as the percentage of tetramer-positive CTL among CD8-positive T cells before the first vaccination and after the fourth vaccination. In cases 9 and 10, the frequency of tetramer-positive CTL was increased from 0.77% to 2.04% and from 0.05% to 0.20%, respectively. HIV, human immunodeficiency virus.

submucosal or subcutaneous vaccination into the peripheral parts of tumors, avoiding necrotic areas and vessels, for introral tumors and neck tumors, respectively. However, no significantly different findings as a whole were noted for the clinical and immunological responses.

In the present study, one patient (case 10) achieved a clinical PR. This demonstrated that the survivin-2B vaccination could yield an excellent response in oral cancer. The patient had received tegafur/uracil (UFT) as oral adjuvant chemotherapy and limited systemic chemotherapy for a few months prior to the vaccine treatment. She was judged to have PS1 in the ECOG classification. It is possible that peptide-based immunotherapy might be more effective in patients with reduced immune suppression as a result of recent intensive chemotherapy, as suggested by the previous clinical study of survivin-2B vaccination for colon cancer, although the study consisted of only a limited number of patients.<sup>(25)</sup> The results of the present trial were mostly compatible with the colon cancer studies in terms of the chemotherapeutic background. Furthermore, by immunohistochemistry, we preliminarily examined the infiltration of local immune cells in metastatic progressive tumor samples from her neck obtained before the first vaccination. Infiltration of CD8 T-cells into the peripheral parts of the tumor was markedly observed. On the other hand, a large number of tumor cells with strong survivin and HLA class I expression were observed. It was presumed that these findings indicated good conditions for immune responses in the tumor microenvironment. However, we failed to obtain a specimen during or after vaccination to evaluate the frequency of these cells (data not shown). Further studies to elucidate the immunoregulatory mechanisms of the immune escape by analyzing the infiltrating immune cells in local tumor sites will be necessary.

Although analysis of peripheral blood lymphocytes using HLA-A24/peptide tetramers actually revealed a slight increase in the peptide-specific CTL frequency in six patients, the immune responses had no relevance to the clinical responses in this study. It seems reasonable to conclude that the number of CTL induced by the vaccine was insufficient to induce tumor regression in patients with advanced or recurrent oral cancer, as vaccine-specific CTL might not be recruited into the tumor site, and the cytotoxic function of CTL might be suppressed in the tumor site by certain mechanisms such as regulatory T cells and immunosuppressive cytokines in the tumor microenvironment.

Overall, the survivin-2B peptide vaccination was well tolerated, but it is suggested that this vaccination protocol might provide only marginal immunological and clinical responses in most advanced or recurrent oral cancer patients. It is possible that advanced protocols such as a more intense immunization schedule and delivery in combination with a specific adjuvant and/or an immune-stimulatory cytokine might improve the efficacy of the survivin-2B peptide vaccine against oral cancer. Indeed, vaccination of the survivin-2B peptide mixed with IFA increased the frequency of peptide-specific CTL more than vaccination with the peptide alone in a phase I clinical trial for patients with advanced or recurrent breast cancer.<sup>(26)</sup> Based on the results of the present study and the other trials, a second clinical study of survivin-2B peptide vaccine has recently been started in combination with IFA and interferon-alpha.

### Disclosure Statement

The authors have no conflict of interest.

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