Abstract. We report on Wilms tumor (WT1) peptide immunotherapy in a patient with intractable ovarian cancer patient over an extended period. Case Report: Immunotherapy using WT1 peptide has been undergoing clinical trials for gynecological cancer. We used WT1 peptide vaccination to treat a 53-year-old woman suffering from ovarian cancer with peritoneal dissemination. After 2 months, her pleural and cardiac effusion had disappeared, and the sum of the longest diameter of the target lesion (in the pelvic mass) was reduced. There was a weak positive correlation between CA125 and mononuclear phagocyte/lymphocyte ratio (Spearman’s $\rho=0.275$, $p=0.015$). Intradermally administered WT1 peptide vaccination in a case of intractable ovarian cancer stabilized the disease over the course of one year. However, the immunotherapeutic mechanism of WT1 peptide and immunological escape mechanism for carcinoma cells remain to be elucidated.

Ovarian cancer is one of the most common gynecological malignancies in Japan. Its frequency has dramatically increased in the last decade. Although there are well-established surgical and chemotherapeutic treatments, the need for molecular-target therapy has increased, especially for recurrent disease that has acquired radio- or chemoresistance.

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The Wilms’ tumor gene WT1 has been isolated and identified as a gene responsible for a childhood renal neoplasm, Wilms’ tumor (1-3). This gene encodes a zinc finger transcription factor and plays important roles in cell growth and differentiation (4, 5). Although WT1 was first categorized as a tumor-suppressing gene, it was recently demonstrated that the wild-type WT1 possessed an oncogenic rather than tumor-suppressing function in many kinds of malignancies (6). WT1 is highly expressed in hematological malignancies and solid tumors, including ovarian cancer (7, 8).

WT1 is now regarded as a molecular target for immunotherapy in various malignant tumor types. Clinical trials of WT1 peptide-based cancer immunotherapy are ongoing: WT1 peptide vaccination has been shown to be safe and clearly effective against several kinds of malignancies (9-13).

Ohno et al. reported that twelve patients with WT1/human leukocyte antigen (HLA)-A*2402-positive gynecological cancer were included in a phase II clinical trial of WT1 vaccine therapy. This study evaluated clinical response after a WT1 vaccine was administered 12 times over three months and found that WT1 vaccine therapy for patients with gynecological cancer was safe and produced clinical responses: stable disease (SD) in 3 patients and progressive disease (PD) in 9 patients (13).

In the following study, we report a case of intractable ovarian cancer in which an intradermally administered WT1 peptide vaccination stabilized the disease over the course of a year.

Clinical study. This case report concerns a patient from our WT1 peptide vaccination study. Entry criteria for the study were as follows: 16-79 years of age, immunohistochemical expression of WT1 in cancer cells of more than 3 months, performance status 0-1, no severe organ function impairment and the written informed consent of the patient. At least 4
weeks prior to immunotherapy, the patient had to be free from antitumor treatments such as surgery, chemotherapy and radiotherapy. The protocol was approved by the Institutional Review Board and the Ethical Committee at Kanazawa University.

**WT1 peptide treatment plan.** The WT1 peptide vaccine consists of an HLA-A*2402-restricted, modified 9-mer WT1 peptide (amino acids 235-243 CYTWNQMNL), in which Y is substituted for M at amino acid position 2 (the anchor position) of the natural WT1 peptide. The WT1 peptide [Good Manufacturing Practice (GMP) grade] was purchased from Multiple Peptide Systems (San Diego, CA, USA) as lyophilized peptide.

Patients received intradermal injections of 3.0 mg HLA-A*2402-restricted adjuvant (EPPIC S.A., Paris, France). Vaccinations were scheduled weekly for 12 consecutive weeks (13). Efficacy was based on computed tomography (CT) obtained at baseline and after 4, 8 and 12 weeks exposure to the vaccine.

<table>
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<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
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<td>-14.7</td>
<td>-17.2</td>
<td>-17.2</td>
<td>-16.9</td>
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**Case Report**

A 53-year-old woman was diagnosed as having serous ovarian adenocarcinoma in November 2007. After omentectomy of a pelvic mass with peritoneal dissemination, tri-weekly combination chemotherapy with paclitaxel and carboplatin produced SD and tumor shrinkage of 25%. This was followed by weekly administration of docetaxel, which also contributed to SD.

The patient participated in our WT1 vaccine trial beginning in October 2008. She received HLA-A*2402, and met the inclusion criteria for the phase II clinical study. She was administered WT1 at weekly intervals.

**Decrease in tumor size and normalization of tumor marker (CA125).** According to the internationally approved Response Evaluation Criteria in Solid Tumors (RECIST) guidelines, sum of the longest diameter (SLD) of the target (pelvic) lesion was reduced: the length was 96.5 mm before administration, 97.8 mm (+1.3%) after 1 month, 84.2 mm (−12.8%) after 2 months,
and 82.7 mm after 3 months (−14.7%); after 3 months’ administration, SLD continued to decrease (Figure 1). In addition, pleural and cardiac effusions disappeared within two months of beginning administration, and remained absent (Figure 2). Dosage of vaccine was increased from 41 units/ml to 160 units/ml after 1 month of administration, and the level of CA125 normalized after 3 months. According to the patient’s wishes, we continued to administer the vaccine, with concomitant normalization/suppression of CA125 (Figure 3a).

No adverse effects of vaccination were observed other than a local inflammatory response with erythema at the injection sites.

Mononuclear phagocyte/lymphocyte ratio (Mo/Ly ratio) and CA125. To evaluate the immunological response to WT1 peptide vaccination, we analyzed the correlation between CA125 and the Mo/Ly ratio (Figure 3b). A weak positive correlation was observed (Spearman’s \( r = 0.275, p = 0.015 \)).

Although WT1 peptide vaccination was continued for one year, CA125 began to gradually increase approximately 9 months following initial administration (Figure 3a). The patient subsequently dropped out of the clinical trial due to receiving another newly approved chemotherapy regimen for ovarian cancer.

**Discussion**

Ovarian cancer is a common malignant gynecological cancer of perimenopausal women. Patients with metastatic disease have a poor prognosis, with 5-year progression-free survival usually less than 30% in Japan.

Our patient had primary disease in the ovary, with metastases in the uterus, peritoneum, pelvic lymph nodes and omental. She also developed a pelvic mass with ascites and pleural effusion during chemotherapy, indicating poor response to chemotherapy. Because of her poor prognosis, she was selected for WT1 peptide immunotherapy. Immediately following inception of peptide immunotherapy, the size of the pelvic mass increased, but within two months, a decrease in tumor size and normalization of the level of tumor marker
CA125 were observed. Despite the initial resistance to chemotherapy, stabilization of her disease for nearly a year suggests the efficacy of WT1 peptide vaccination.

Alterations of peripheral monocytes and lymphocytes might be good parameters for evaluating immunologic status and predicting recurrence in patients with gastric cancer (14). In our case, there was a weak positive correlation between CA125 and the Mo/Ly ratio (Spearman’s $\rho=0.275$, $p=0.015$). We believe in this case that the Mo/Ly ratio indicated immunologic status and predicted recurrence.

Belli et al. reported that in a study of 28 patients with resected metastatic melanoma, two showed complete response and three manifested long-term disease stabilization with HSPPC-96 (autologous tumor-derived heat-shock protein GP96-peptide complex) vaccine (15). Bolonaki et al. also reported that disease stabilization occurred in 8 out of 22 patients with advanced non-small cell lung cancer vaccinated with an optimized cryptic human telomerase reverse transcriptase peptide (16). Ohta et al. reported a case of WT1 peptide immunotherapy for metastatic childhood rhabdomyosarcoma, with patient remission continuing more than 22 months (17). This latter case is significant, as there are few reports of long-term WT1 peptide vaccination for cancer – apart from our own, where the vaccine stabilized intractable ovarian cancer over a year.

Unfortunately, the pathogenesis of ovarian cancer relapse is unknown. In addition, the immunotherapeutic mechanism of WT1 peptide and immunological escape mechanism for carcinoma cells remain to be elucidated.

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References


