Original Article

Immunomodulatory therapy in refractory/recurrent ovarian cancer


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ABSTRACT

Objective: To investigate the efficacy and toxicity of immunomodulatory therapy (IMT) alone or as an add-on to palliative/salvage chemotherapy in patients with refractory/recurrent epithelial ovarian cancer (EOC).

Materials and methods: We retrospectively analyzed the efficacy and toxicity of IMT in 15 patients with refractory/recurrent EOC who had previously received multiple chemotherapy regimens.

Results: The median age of the patients was 56 years (range, 41–75 years). Three patients were platinum-sensitive, two were platinum-resistant, and the remaining 10 patients were refractory to platinum-based front-line chemotherapy. IMT consisted of picibanil (OK-432) on Day 1, interleukin-2 and/or interferon-α on Day 2 administered by subcutaneous injection (every week or 2-weekly). Five patients never received metronomic oral cyclophosphamide. After IMT, three patients achieved partial remission (PR, lasting for 11 months, 12 months, and 16 months), and six patients had stable disease (SD). The disease stabilizing rate (PR + SD) was 60% (3/3 in platinum-sensitive and 6/12 in platinum-resistant/refractory patients). The absolute lymphocyte count (ALC) at 1 month after IMT was significantly higher in the PR + SD group (median 1242.0/μL) than in the progression group (median 325.0/μL) (p = 0.012). No Grade 3 toxicities were observed. The median post-IMT survival time was 12 months (range, 2–39 months).

Conclusion: IMT alone or add-on to palliative/salvage chemotherapy for refractory/recurrent EOC achieves a substantial disease stabilizing rate without severe toxicity, which might be a potential option in selected patients. The ALC 1 month after IMT could be an early indicator to disease stabilization.

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Introduction

Epithelial ovarian cancer (EOC) is the eighth most common malignancy and the seventh leading cause of cancer-related mortality in women worldwide [1]. In 2009, EOC accounts for 1113 new cases, 438 deaths, and ranks the second lethal gynecologic malignancy in Taiwan [2]. Although EOC is generally diagnosed at an advanced stage, primary cytoreductive surgery followed by platinum-based chemotherapy can ensure the achievement of a complete remission (CR) in 70–80% of patients. Unfortunately, 60–70% of advanced EOC patients will have disease relapse after primary treatment [3–5]. Furthermore, disease progression during first-line treatment is associated with a dismal prognosis.

In general, the response rates of platinum-sensitive patients to second-line chemotherapy are usually > 30%. Conversely, platinum-resistant patients show response rates as low as 10–20% [3,5,6]. In addition, current salvage strategies for patients with refractory/recurrent EOC are not effective and rarely curative. Therefore, novel therapy treatment strategies have been eagerly awaited.

Previous studies demonstrated the clinical usefulness of immunomodulatory therapy (IMT) in several solid malignancies, including lung cancer, hepatocellular carcinoma, prostate cancer, melanoma, and renal cell carcinoma [7]. Because EOC have immunogenic properties [8], the potential usefulness of immunological approaches for the treatment of advanced EOC is attracting...
increasing interest. In addition, the presence of tumor-infiltrating lymphocytes has been shown to predict survival in EOC patients, suggesting the prognostic relevance of immune surveillance mechanisms in the course of this malignancy [9]. Notably, the increase of regulatory T (Treg)-cells in patients with EOC has been associated with a reduced survival [10]. The efficacy and low toxicity of IMT has been observed [8,11,12]. These results prompt us to apply similar approaches in advanced EOC with refractory/recurrent disease.

Penicillin-killed Streptococcus pyogenes [OK-432; Toll-like receptor (TLR) 4 agonist] is administered subcutaneously to activate skin Langerhans cells to enhance antigen presenting and upregulation of CD25 expression. OK-432 can trigger dendritic cell (DC) activation via the TLR 4–MD2 signal pathway, inducing DC maturation and linking innate cells and adaptive immune cells in host immunosurveillance [13]. The clinical usefulness of OK-432 for the treatment of lymphangioma, gastric cancer, and lung cancer has been extensively investigated in Japan [13]. Interleukin (IL)-2 can restore the ability of the immune system to produce CD4⁺ T cells and increase IL-2-induced CD4⁺ CD25⁺ (CD4⁺ FOXP3⁺) T cells, which can in turn suppress Treg cells [14,15]. IL-2 has been approved in the United States for treating metastatic renal cell carcinoma and metastatic melanoma [16]. Interferons (IFNs) may exhibit important antitumoral and antiangiogenic effects and induce apoptosis in malignant cells [7]. Type I IFNs (α/β) systemically activate natural killer (NK) cells and support the differentiation, maturation, and migration of DCs [17]. A single administration of cyclophosphamide was shown to deplete CD4⁺ CD25⁺ T cells in tumor-bearing animals, delay tumor growth, and cure rats bearing established tumors when followed by an immunotherapy that has no curative effect when administered alone [18].

We therefore hypothesized that cytokine-based therapy in combination with oral metronomic chemotherapy (as a form of IMT) for refractory/recurrent EOC may improve survival. The purpose of this study is to review the efficacy and toxicity obtained in patients treated with IMT alone or add-on to palliative/salvage chemotherapy after failure of front-line treatment in our hospital.

**Patients and methods**

**Patients**

This study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital, Taoyuan, Taiwan (100-3902A3). We retrospectively reviewed the medical records of 15 patients with refractory/recurrent advanced EOC who were treated with cytokine-based IMT between December 2004 and January 2013. Inclusion criteria were as follows: (1) age ≤ 75 years at the time of diagnosis of ovarian cancer; (2) pathologically-confirmed EOC; (3) initial treatment with surgical cytoreduction and platinum-based chemotherapy; (4) evidence of disease progression during first-line chemotherapy (refractory), or evidence of disease recurrence within 6 months after an initial response to first-line chemotherapy (platinum-resistant), or disease recurrence > 6 months after an initial complete response to first-line chemotherapy (platinum-sensitive) but failed salvage systemic chemotherapy; and (5) use of IMT either alone or as an add-on to chemotherapy.

**Immunomodulatory therapy**

IMT was performed with the following drugs: OK-432 [5 Klinische Einheit (KE, a unit of OK-432 dose)/vial, Picibanil; Chugai Pharmaceutical Co., Ltd. Tokyo, Japan], IL-2 (18 MIU/vial, Alde-sleukin; Chiron B.V., Amsterdam, The Netherlands), peginterferon (PEG-IFN)-α-2a (3 MIU/0.5 mL/syringe, Roferon-A; F. Hoffmann-La Roche Ltd., Basel, Switzerland), or IFN-β-1b [0.3 mg (9.6 MIU)/vial, Betaferon; Novartis Vaccines and Diagnostics, Inc., Emeryville, California, USA]. All of the patients received IMT either with or without metronomic chemotherapy at the physician’s discretion. IMT was administered in the hospital by subcutaneous injections. The use of OK-432 and IL-2 has been introduced since December 2004, whereas the administration of IFNs started in September 2006. Patients started IMT with a single agent, and other drugs were subsequently added in patients who did not show severe side effects. After 2011, the IMT regimens evolved into one vial of OK-432 on Day 1, one vial of IL-2, and/or one vial of IFN-α-2a on Day 2 (or Day 2 and Day 3) weekly or 2-weekly. Cytokine agents were discontinued in patients who showed severe chemotherapy-related leukopenia, infections, clinical signs of target organ failure due to disease progression, or who refused to continue IMT. Salvage/palliative chemotherapy was administered after IMT if there was no neutropenia or thrombocytopenia against its use. Metronomic chemotherapy consisted of oral cyclophosphamide 50 mg once per day after salvage/palliative chemotherapy until the next course IMT.

Taiwan Food and Drug Administration approved the use of OK-432 for the treatment of gastrointestinal cancer, head and neck cancer, thyroid cancer, and lung cancer; IL-2 for metastatic renal cell carcinoma and metastatic melanoma; PEG-IFN-α-2a for Kapoisi’s sarcoma, renal cell carcinoma, hairy cell leukemia, chronic hepatitis B and C, chronic myeloid leukemia, cutaneous T cell lymphoma, and non-Hodgkin’s lymphoma [19]. All of the patients were informed about the off-label use of IMT for the treatment of EOC.

**Clinical assessment**

The patients were classified as sensitive, resistant, or refractory to platinum-based therapy depending on the interval between the initial response and the first relapse [20]. Routine blood counts, liver function tests, renal function test, and serum CA-125 levels were serially assessed prior to IMT and at follow-up. Lymphocyte subsets were determined on a Beckman Coulter’s Epics XL-MCL™ Flow Cytometer and analyzed using the XL SYSTEM II™ software. We evaluated tumor response [CR, partial remission (PR), stable disease (SD), and progressive disease (PD)] according to medical records, imaging studies by Response Evaluation Criteria in Solid Tumors (RECIST) criteria [21], and the Rustin guidelines by measurement of serum CA-125 [22,23]. Data on treatment toxicity were extracted from clinical charts and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (CTCAE v 4.0).

**Statistical analysis**

In patients with refractory disease, overall survival (OS) was calculated from the date of diagnosis to the date of death. In patients with recurrent disease, survival after recurrence (SAR) was calculated from the date of first recurrence to the date of death. Post-IMT progression-free survival (PFS) and post-IMT survival (PIS) were determined from the date of IMT administration to the date of the subsequent recurrence or death. The disease stabilizing rate was defined by summing up the percentages of CR, PR, and SD lasting for at least 6 months. Descriptive statistics were calculated for all characteristics of the study patients. Survival curves (PFS, OS, SAR, and PIS) were generated using the Kaplan–Meier method. Differences in median values between groups were analyzed with the Mann–Whitney U test for comparison of the two independent groups and the Wilcoxon signed ranks test for comparison of the
two related groups. All calculations were performed using the SPSS statistical software (version 19.0; IBM, Somers, NY, USA). Two-tailed \( p < 0.05 \) were considered to be statistically significant.

**Results**

**Patients**

The clinical characteristics of the 15 patients are summarized in Table 1. All of the patients received platinum-based front-line chemotherapy. The median age was 56 years (range, 41–75 years). Three patients were platinum-sensitive to first-line chemotherapy, two were platinum-resistant, and the remaining 10 patients were refractory to platinum-based front-line chemotherapy.

**Treatment**

The treatment schemes and the clinical outcomes are shown in Table 2. Patients were treated with IMT as an add-on to salvage/ palliative chemotherapy, either with or without metronomic oral cyclophosphamide. However, some patients received IMT alone when the general conditions did not allow the use of chemotherapy (e.g., in the presence of thrombocytopenia).

The salvage/palliative chemotherapy used in this study was usually at low doses, including cisplatin 40–100 mg/m² 3-weekly, paclitaxel 50–80 mg/m² 2-weekly, oral etoposide alone 50 mg/d for 14 days 3-weekly, cyclophosphamide 600–750 mg/m² 3-weekly, oral melphalan alone 0.2 mg/kg for 5 days 4-weekly, topotecan 2.5–3.5 mg/m² weekly, pegylated liposomal doxorubicin (Lipodox) 12–40 mg/m², gemcitabine 650–800 mg/m² or 5-FU 600–1000 mg/m² monthly, as a single agent or doublets, occasionally with bevacizumab 5 mg/kg (Table 2).

**Outcomes**

At the date of the last follow-up (June 15, 2013), 14 patients died of their disease and only one patient (Case 3) was alive with their disease. After IMT, three patients (Cases 3, 13, 14) achieved PR (lasting for 11 months, ≥12 months, and 16 months, respectively), six patients had SD, whereas the remaining six patients showed PD (Table 2).

In the entire study cohort, the disease stabilizing rate (PR+SD) was 60% [100% (3/3) in the platinum-sensitive group, and 50% (6/12) in the platinum-resistant/refractory group, respectively]. The median PIS time was 12 months (range, 2–39 months). The 1-year PIS rate was 53.3% (Fig. 1). The mean SAR after the first recurrence was 38.8 ± 30.9 months for patients with recurrent disease (platinum-sensitive patients, 57.3 months; platinum-resistant patients, 11 months). The mean OS for patients in the refractory group was 18.2 ± 11.1 months, and six patients had a survival longer than 12 months.

**Immune cyt profiles**

The data of immune cyt profiles in patients with PR+SD and PD after IMT is compared in Table 3. White blood cells (WBCs) and absolute lymphocyte counts (ALCs) at baseline were within the normal range. Prior to IMT, there were no significant differences in ALC between the PR and PD groups. After the administration of IMT, the median values of WBC (47.14 ± 18.54) and ALC (4.15 ± 1.88) in the PR group were significantly higher than in the PD group. The median values of ALC in the PR+SD group could be maintained at 1 month and 3 months after IMT, whereas this difference was not statistically significant in the PD group.

**Toxicity**

In this study, all of the patients were hospitalized and were given hydration therapy through an intravenous line both prior to and during IMT. After the administration of OK-432, 11 patients experienced Grade 2 fever (treated with acetaminophen) and one patient showed Grade 1 fever. All of the 14 patients treated with IL-2 had Grade 1/2 fever, but no cases of hypotension or arrhythmia were observed. Two patients experienced pain and erythematous changes (Grade 2) at the site of injection, whereas two other patients showed mild tenderness (Grade 1). No cases of renal or liver impairment were noted.

**Discussion**

The prognosis of patients with refractory/recurrent EOC treated by conventional salvage therapy is generally poor. Moreover, these patients can experience severe adverse effects that may significantly impair the quality of life.

The clinical trials of single-agent chemotherapy (Lipo-Dox [6,24–25], topotecan [6,27], gemcitabine [24], weekly paclitaxel [28,29], and oral etoposide [30,31]) for refractory/resistant-recurrent EOC have reported overall response rates of 6–35%, median PFS of 2–6 months, and median OS of 5–20 months.
Table 2
Detailed treatment and outcome information of 15 patients with recurrent/refractory ovarian cancer on front-line chemotherapy who received IMT.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (y)</th>
<th>FIGO stage</th>
<th>ECOG/Platinum-free interval</th>
<th>Prior treatment</th>
<th>IMT + metronomic C/T (No. of courses)</th>
<th>Salvage/palliative systemic C/T during IMT (No. of courses)</th>
<th>Sites of Dx during IMT</th>
<th>Response to IMT</th>
<th>Post-IMT PFS(^{(d)}) (mo)</th>
<th>OS/SARe (mo)</th>
<th>OS(^{(f)}) (mo)</th>
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<td>Sensitive to 1st line C/T</td>
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<tr>
<td>1</td>
<td>45</td>
<td>IIIC</td>
<td>3/15 months</td>
<td>CEKLP (4)</td>
<td>OK-432 (12) IL-2 (2) OK-432+IL-2 (2) oral Y (1)</td>
<td>Pelvic tumor, carcinomatosis</td>
<td>KPCL (13)</td>
<td>SD</td>
<td>15</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>IIIC</td>
<td>3/15 months</td>
<td>CEKP (2)</td>
<td>OK-432 (8) IFN-β (1) OK-432+IFN-α (8) OK-432+ IFN-β (1) IL-2+IFN-α (1) OK-432+IL-2+IFN-α (4) oral Y (2)</td>
<td>Brain, pelvic tumor, carcinomatosis</td>
<td>KPTFLYG (17)</td>
<td>SD</td>
<td>14</td>
<td>85</td>
<td>39</td>
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<td>3</td>
<td>48</td>
<td>IIIC</td>
<td>2/10 months</td>
<td>CKPLT (3)</td>
<td>OK-432 (3) OK-432+IL-2 (3) OK-432+IL-2+IFN-α (17) oral Y (18)</td>
<td>Distal colon mass, carcinomatosis</td>
<td>P (14)</td>
<td>PR</td>
<td>12()</td>
<td>37()</td>
<td>12()</td>
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<td>Resistant to 1st line C/T</td>
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<tr>
<td>4</td>
<td>54</td>
<td>IIIC</td>
<td>2/5 months</td>
<td>KP (1)</td>
<td>OK-432 (2) IL-2 (1) OK-432+IL-2 (1) OK-432+IFN-α (1) oral Y (2)</td>
<td>Liver, spine, carcinomatosis</td>
<td>MP (2)</td>
<td>SD</td>
<td>10</td>
<td>12</td>
<td>11</td>
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<tr>
<td>5</td>
<td>56</td>
<td>IIIC</td>
<td>3/5 months</td>
<td>CKPY (2)</td>
<td>Yes (subphrenic and subumbilical mass) OK-432 (4) OK-432+IL-2 (1) OK-432+IFN-α (1) oral Y (2)</td>
<td>Pelvic tumor, carcinomatosis</td>
<td>CP (3)</td>
<td>PD</td>
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<td>5</td>
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<td>Refractory to 1st line C/T</td>
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<tr>
<td>6</td>
<td>49</td>
<td>IIIC</td>
<td>4/NA</td>
<td>CKMPY (4)</td>
<td>N/A</td>
<td>Pancreas, carcinomatosis</td>
<td>MP (3)</td>
<td>PD</td>
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<td>8</td>
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<td>75</td>
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<td>CPY (2)</td>
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<td>Lung, MLN</td>
<td>CYP (4)</td>
<td>PD</td>
<td>N/A</td>
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<td>6</td>
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<td>47</td>
<td>IIIC</td>
<td>3/NA</td>
<td>CPY (2)</td>
<td>Yes (PALN, lung) OK-432 (3) IL-2 (1) OK-432 (3) IL-2 (1) OK-432+IL-2 (2) OK-432+IFN-α (2) oral Y (3) OK-432+IL-2 (3) oral Y (1) OK-432 (3) IL-2 (1) OK-432+IL-2 (3) oral Y (1)</td>
<td>Lung, liver, PALN</td>
<td>CDPY (3)</td>
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<td>CKP (2)</td>
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<td>CPEM (4)</td>
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<td>Pelvic tumor, carcinomatosis</td>
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<td>16</td>
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</table>

**Figure 1.** Survival of the 15 patients after immunomodulatory therapy (IMT).

Although the use of combination chemotherapy or novel targeted therapies may result in higher response rates (17–39%), the median PFS (3.0–5.4 months) and median OS (12.6–18.6 months) were not improved [29,32–34].

In the present study, the use of IMT alone or add-on to salvage/palliative chemotherapy resulted in a median PIS time of 12 months (range, 2–39 months). The mean SAR after the first recurrence was 57.3 months in the platinum-sensitive group and 11 months in the platinum-resistant group. In the refractory group, the mean OS was 18.2 ± 11.1 months, and six patients achieved a survival longer than 12 months. Our results are noteworthy because 13 of the 15 patients had an ECOG performance status ≥ 3, with a mean number of previous regimens of 2.6 (Tables 1 and 2). By contrast, patients enrolled in previous Phase II/III clinical trials had a better performance status (ECOG or WHO performance status of 0–2) and generally failed only one previous regimen [6,24–34].

OK-432 can directly inhibit RNA synthesis in tumor cells, interact between the immune system and cancer cells, and may act as an adjuvant agent by inducing several cytokines [e.g., IL-1, IL-2, IL-6, IL-8, IL-12, IL-18, granulocyte colony stimulating factor (G-CSF), granulocyte myelocyte (GM)-CSF, IFN–α, and IFN–γ] [13]. IL-2 triggers chemokine receptor CXCR4 chemokine (C-X-C motif) receptor 4 expression on Treg cells, enables Treg cells migration toward chemokine CXCL12 chemokine (C-X-C motif) ligand 12 in tumor microenvironment, and may enforce Treg cell tumor accumulation [15]. Our IMT formula was evolving from OK-432 to combination with IL-2 and/or IFN–α. Unfortunately, the IMT was not used under a prospective study, and the patients were treated in a palliative manner. Without a formal funding, we did not systematically check T-cell subpopulations initially; therefore, the correlation of response cannot be evaluated. In our series, we observed that the median of ALC (p = 0.012) at 1 month after IMT was significantly higher in the PR + SD group than the PD group. It might be explained that certain patients have a better response to IMT, which could expand and maintain cytotoxic lymphocytes.

The main adverse effects of IMT include fatigue, flu-like symptoms, fever, injection site reaction, and transient paradoxical
hematological effects (e.g., leukocytosis following OK-432 administration or leukopenia after IL-2 treatment). Because OK-432 contains penicillin, the risk of an allergic type hypersensitivity reaction cannot be excluded [13,35].

Several limitations with regards to this study merit consideration. First, most patients accepted this approach only when they showed a poor ECOG performance status. Second, the retrospective nature of our study and the small sample size may limit the ability to generalize our findings. Third, the characteristics of the patients were heterogeneous. Finally, the agents used for IMT and the timing of treatment were not uniform (Table 2).

In conclusion, despite the limitations of this study, we can still observe that IMT alone or add-on to salvage/palliative chemotherapy may achieve a good disease stabilizing rate for refractory/recurrent EOC without severe toxicity, and it may therefore be a potential option in selected patients. We also observed that the ALC 1 month after IMT could be an early indicator of disease stabilization. Future studies in larger sample sizes with exploratory immune subpopulation [e.g., an increase of CD4+ CD25+ (CD4+ FOXP3+) T cells and/or NK cells, CXCR4 expression of Treg] and cytokine profiles (such as ILs, G-CSF, GM-CSF, IFN-α, and IFN-γ) are needed to confirm our findings.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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