Successful Outcomes Using Combination Therapy of Interleukin-2 and Interferon-α for Renal Cell Carcinoma Patients with Lung Metastasis

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Objective: In our previous study, a combination therapy of interleukin-2 and interferon-α was found to be more effective than monotherapy, especially for lung metastasis. In order to determine the genetic markers of those who positively responded, a multi-institutional open study was conducted on the patients with lung metastasis. In this paper, the clinical response to our combination therapy is reported.

Methods: Untreated patients with lung metastasis were enrolled in this study. Patients received interleukin-2 (0.7 × 10^6 U/day) and interferon-α (6 × 10^6 IU/day): interleukin-2, 5 days a week and interferon-α, 3 days a week for the first 8 weeks, and then both interleukin-2 and interferon-α, 2 or 3 days a week for 16 additional weeks.

Results: Forty-two patients were able to be evaluated for response. The overall positive response rate was 35.7% (15 of 42) including 2 patients with complete response. Progression-free patients were observed more frequently in patients with lung metastasis only (80.6%) than those with lung plus other organ metastasis (54.5%). Tumor shrinkage was observed in 81.0% (34 of 42) of patients. Progression-free survival rate at 200 days was 63.6%. Toxicities observed were primarily flu-like symptoms due to the cytokines and were typical of those observed with each single agent.

Conclusions: Combination therapy of interleukin-2 and interferon-α was confirmed to be effective for renal cell carcinoma patients with lung metastasis. Identification of genetic markers is now ongoing with the tissue samples from this trial.

Key words: renal cell carcinoma — interleukin-2 — interferon-α — cytokine — combination therapy — lung metastasis

INTRODUCTION

Renal cell carcinoma (RCC) is highly resistant to conventional cytotoxic chemotherapy and the prognosis for patients with advanced RCC is poor. Spontaneous remissions have been reported with RCC, probably as a result of immune responses (1). Such observations provide the rationale for
developing immunotherapeutic approaches to treatment, with
the aim of stimulating or augmenting these apparently effec-
tive responses. Cytokine therapy is the only immunothera-
peutic approach that has so far been integrated into routine
clinical practice for RCC, although other strategies have
been investigated. The most widely used and extensively
studied cytokines for treatment of RCC are interleukin-2
(IL-2) and interferon-α (IFN-α). Positive response rates of
10–20% are reported with these therapies and some patients
achieve a complete and long-lasting remission (2–4). In
Japan, IL-2 at doses of 0.7–2.1 × 10^6 U/day, much lower
than those in the USA and Europe, has been used and has
produced similar response rate, with substantially less tox-

cicity (5,6). Treatment with the low-dose IL-2 has also been
reported to produce complete response (CR) in some patients
(7,8).

Recently, novel molecular-targeted agents have been
developed for the treatment of metastatic RCC. These
include tyrosine kinase inhibitors such as sorafenib and sunitinib, as well as the inhibitors of the mammalian target of
rapamycin. These agents have been designed to target tumor-
related angiogenesis and signal transduction. Although we
now have an increasing number of effective new agents for
patients, extensive experience has shown that they rarely
induce durable regressions of metastatic RCC (9). In order
to improve treatment strategies for metastatic RCC, a rational
refinement of the therapy is required through combination
regimens and patients selection.

In our previous report (10), we showed that the combined
use of IL-2 and IFN-α was successful in treating metastatic
RCC patients, especially for those with lung metastasis. In
addition, the combination therapy was tolerable, and no
additional adverse event was observed in this study to those
observed with monotherapy using either IL-2 or IFN-α. In
order to improve the patients’ response to this combination
therapy, we hypothesized that molecular markers in RCC
would provide us with better information regarding diagno-
sis, treatment and follow-up. In order to determine which
molecular markers are effective in predicting better patient
response, a new prospective trial was initiated to explore the
connection between molecular markers and the combination
therapy of IL-2 and IFN-α for patients with lung metastasis.
In this paper, we report on the efficacy of this combination
therapy.

PATIENTS AND METHODS

PATIENTS

We conducted the study at 35 medical centers in Japan. Patients who fulfilled the following criteria were selected as
subjects: histologically diagnosed as RCC; measurable lung
metastasis; patients feasible for collecting blood and speci-
mens from primary tumors or metastatic lesions for marker
analysis; no previous systemic treatment; complete surgical
excision of primary RCC; Eastern Cooperative Oncology
Group performance status (ECOG PS) score ≤1; age 20
years or over and < 80 years; life expectancy ≥3 months;
and patients who provided written informed consent. The
study was approved by the institutional review board at each
center.

TREATMENTS

Administration of IL-2 (Imunace, Shionogi, Osaka, Japan)
and IFN-α (Sumiferon, Dainippon Sumitomo, Osaka, Japan)
was started simultaneously and continued for 8 weeks at fol-
lowing doses: IL-2 administrated by intravenous infusion at
0.7 × 10^6 U/person per day, 5 days a week and IFN-α subcu-

taneously or intramuscularly at dose 6 × 10^6 IU, 3 days a
week. From week 9 to week 24, IL-2 and IFN-α were admi-

nistered 2 or 3 days a week.

EFFECTIVENESS ANALYSIS

Tumor response was classified in accordance with Japanese
Urological Association (JUA) evaluation criteria (11). Radiologic tumor measurements were performed every 4
weeks for the first 8 weeks, then every 4–8 weeks during
treatment, and 4 weeks after the last dose. Tumor evaluation
was reviewed by external independent radiologists.

TOLERANCE EVALUATION

Patients given one or more doses of IL-2 or IFN-α were
evaluated at each visit for tolerance, which included the
assessment of adverse events according to National Cancer
Institute-Common Terminology Criteria for Adverse Events
(NCI-CTCAE), version 3.0.

RESULTS

PATIENTS CHARACTERISTICS

From September 2006 to April 2008, a total of 44 patients
were enrolled. One patient was excluded from full analysis
set due to violation of inclusion criteria. Table 1 shows the
baseline characteristics of the remaining 43 patients. The
most frequently occurring tumor subtypes were clear-cell
carcinoma (88.4%), spindle cell carcinoma (2.3%), papillary
RCC (2.3%) and mixed type (7.0%). All patients had lung
metastasis. The other metastatic sites were lymph nodes in
seven patients, bones in five patients and others in seven
patients. The majority (74.4%) of patients had lung meta-
stasis only, and the remaining patients had metastases in lung
plus other organs. According to Memorial Sloan-Kettering
Cancer Center (MSKCC) prognostic risk groups (12), only
one patient belonged to the favorable risk group, because
most of the patients (39 of 43) had started the treatment
within 1 year after nephrectomy to serve the tumor speci-
mens for marker analysis. Twenty-nine patients (67.4%)
belonged to the intermediate risk group and 13 patients (30.2%) to the poor risk group.

**EFFICACY**

Forty-two patients were able to be evaluated for response. In order to compare the efficacy with our previous study (10), we used the same (JUA) criteria for the evaluation of tumor response as that in the previous study. Table 2 summarizes response to the treatment. According to the independent central review, two patients (4.8%) achieved a CR, and 13 patients (31.0%) achieved a partial response (PR), for an overall response rate of 35.7% (95% CI, 21.6–52.0%). Among patients with metastasis to only lung, the response rate (35.5%: 95% CI, 19.2–54.6%) was not different from the overall response rate, but the proportion (80.6%) of \( \geq \)NC (CR + PR + MR + NC) was higher than that (54.5%) in patients with metastasis in other organs than just the lung. During the study, a total of 34 patients (81.0%) had some degree of tumor shrinkage from the baseline values (Fig. 1). We also applied RECIST criteria for the evaluation. The response rate was 37.5% (2 CR + 10 PR) in eligible patients \( (n = 32) \). Twelve (37.5%) and 8 (25%) patients were evaluated as stable and progressive disease, respectively.

Median time to progression had not been reached during the study, and the proportion of patients free from progression was 63.6% at 200 days from the first dose (Fig. 2). Figure 3 shows the changes in individual tumor burden during treatment. The median time to respond (the time needed until tumor size decreased to 50%) was 56 days (range, 28–166 days) in 15 patients with CR or PR, and the median duration of response had not been reached at the time of the analysis (range 27–146+ days). As shown in Fig. 3, a majority (12 of 15: 80.0%) of CR and PR patients remained on therapy at 22–28 weeks with responses lasting. Patients with good performance status (PS = 0) or with a normal value of C-reactive protein tended to respond well to this combination therapy, but the difference was not statistically significant (data not shown). Among the risk groups based on MSKCC criteria (12), the response rate in the intermediate risk group was 41.4% with 2 CR patients, and that in the poor risk group was 25.0%. One favorable risk group patient was NC.

**SAFETY**

During the observation period (until 4 weeks after the last dose), no patients had died. A total of 44 patients were eligible for safety analysis. Adverse events are summarized in Table 3. The most common events were flu-like symptoms including fever (100%) and fatigue (88.6%), followed by gastrointestinal symptoms such as anorexia (70.5%) and nausea (22.7%). Grade 4 fever was observed in one patient, but was resolved during treatment. Abnormal laboratory results were: leukocyte disorders such as eosinophilia (79.5%), neutropenia (68.2%) and lymphopenia (31.8%), followed by abnormal liver function findings such as elevated aspartate aminotransferase (AST, 47.7%), elevated alanine aminotransferase (ALT, 40.9%) and elevated \( \gamma \)-glutamic pyruvic transaminase (\( \gamma \)-GTP, 34.1%). Neutropenia in six patients and lymphopenia in one patient were Grade 4. Five of the six patients showing neutropenia were resolved during treatment, and the remaining one patient was resolved by suspending administration of IFN-\( \alpha \). Lymphopenia disappeared during continued administration.

**DISCUSSION**

We observed a confirmed response rate of 35.7% with combined IL-2 and IFN-\( \alpha \) therapy in a population of previously untreated RCC patients with lung metastasis. Two patients (4.8%) achieved CR lasting during observation. The response rate in this study was similar to that (33.3%, 12 of 36) in patient subgroup who had metastasis in the lung in a previous study (10). In the present study, the response rate (35.5%) in patients with metastasis limited to lung was not different from the overall response rate, but the proportion

### Table 1. Patient characteristics \( (n = 43) \)

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>33</td>
<td>76.7</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>23.3</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>28</td>
<td>65.1</td>
</tr>
<tr>
<td>( \geq 65 )</td>
<td>15</td>
<td>34.9</td>
</tr>
<tr>
<td><strong>ECOG performance status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>33</td>
<td>76.7</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>23.3</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>38</td>
<td>88.4</td>
</tr>
<tr>
<td>Spindle cell</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>Papillary</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>Mixed</td>
<td>3</td>
<td>7.0</td>
</tr>
<tr>
<td><strong>Number of metastatic organs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>32</td>
<td>74.4</td>
</tr>
<tr>
<td>Multiple</td>
<td>11</td>
<td>25.6</td>
</tr>
<tr>
<td><strong>Metastatic site</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>43</td>
<td>100</td>
</tr>
<tr>
<td>Lymph node</td>
<td>7</td>
<td>16.3</td>
</tr>
<tr>
<td>Bone</td>
<td>5</td>
<td>11.6</td>
</tr>
<tr>
<td>Others</td>
<td>7</td>
<td>16.3</td>
</tr>
</tbody>
</table>

ECOG, Eastern Cooperative Oncology Group.

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(80.6%) of ≥NC (CR + PR + MR + NC) was higher than that (54.5%) in patients with metastases in lung plus other organs. Occurrence of lung metastases is most prominent in advanced RCC patients, and it is reported that almost 40% of those have lung metastasis only and none in other organs in Japan (13).

The toxicity profile observed in the present study is consistent with that reported in our previous study (10). The most common events were flu-like symptoms due to cytokines. Grade 4 neutropenia was observed in six (13.6%) patients, although the events have been recovered during the treatments. Abnormal serum levels relating to liver dysfunction (ALT, AST and γ-GTP) were observed in nearly half of the patients, but the majority of the liver dysfunction was temporary and manageable. During the observation period (until 4 weeks after the last dose), no death was observed for any reason in this study. Therefore, the regimen used in this study is considered to be well tolerated in Japanese patients with metastatic RCC.

Previously, several trials have examined the efficacy of IL-2 combined with IFN-α. These trials used high or intermediate doses of IL-2, and the response rates were reported to be 10–18% (14,15). Therefore, the result of the present study was better than those in the reports, even with much lower dose of IL-2. Vaglio et al. (16) have examined low-dose IL-2 (1 × 10^6 IU/m^2, subcutaneous) and IFN-α (1.8 × 10^6 IU/m^2, intramuscular) and have reported an overall response rate of 10.9% with median survival of 65.1 months for low-risk patients. They also showed that the chronic administration of low-dose IL-2 and IFN-α increases circulating lymphocyte subset (e.g. CD3^+CD56^+ cells) without exhausting the immunological response over time (17,18). The patient selection criterion and/or the treatment regimen may affect the difference in the efficacies from the present study. In addition, racial difference may affect the responsiveness to the cytokine therapy because some racial differences were suggested for the survival of metastatic RCC patients (13,19). Very recently, Miyake et al. (20) reported the outcome of clinical treatment with sequential combination of low-dose IL-2 and IFN-α. Of 52 patients, 3 patients achieved CR and 9 patients achieved PR with overall response rate of 23.1%. In this report, the response rate for patients with metastasis limited to lung was 28.6% (10 of 35). Although the treatment regimen used by Miyake’s group was somewhat different from that in the present study, the combination therapy with low-dose IL-2 and IFN-α produces superior efficacy for RCC patients with lung metastasis. Most recently, the prognosis of 1463

<p>| Table 2. Response to IL-2 + IFN-α combination therapy for RCC patients with lung metastasis |
|----------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th>n</th>
<th>CR</th>
<th>PR</th>
<th>MR</th>
<th>NC</th>
<th>PD</th>
<th>Response rate (%)</th>
<th>95% CI</th>
<th>≥NC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>42</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>12</td>
<td>11</td>
<td>35.7</td>
<td>21.6–52.0</td>
</tr>
<tr>
<td>Lung only</td>
<td>31</td>
<td>2</td>
<td>9</td>
<td>4</td>
<td>10</td>
<td>6</td>
<td>35.5</td>
<td>19.2–54.6</td>
</tr>
<tr>
<td>Lung + others</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>36.4</td>
<td>10.9–69.2</td>
</tr>
<tr>
<td>Lymph node</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>60.0</td>
<td>—</td>
</tr>
<tr>
<td>Bone</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>33.3</td>
<td>—</td>
</tr>
<tr>
<td>Others^a</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

Evaluation was confirmed and based on external independent assessment following investigators’ assessment. IL-2, interleukin-2; IFN-α, interferon-α; RCC, renal cell carcinoma; CR, complete response; PR, partial response; MR, minor response; NC, no change; PD, progressive disease; CI, confidence interval.

^aOthers included lymph node, bone, liver, pancreas, adrenal gland and cardiac membranes.
Japanese metastatic RCC patients has been reported (13). In the report, better survival was noted in the patient group treated with cytokine, IL-2 and/or IFN-α. Median survival time of patients treated with cytokine was 24.9 months, whereas that of patients not treated with cytokine was 11.3 months, although the comparison of the time is needed to attention because of the retrospective study.

In recent years, the molecular-targeted drugs were available for the treatment of metastatic RCC. It is reported that sorafenib and sunitinib are as effective for patients who are intolerant of cytokine treatment. Overall response rates and progression-free survival have been reported not to be significantly different between first- and second-line setting for both agents (21–25). From above, it is thought to be possible to improve the success rate in treating advanced RCC patients, especially those with lung metastases, if the combination treatment with IL-2 and IFN-α is chosen as the first-line treatment seeing it has a better outcome, even to the extent that CR can be expected. Furthermore, from our results of tracing changes in tumor burden (Fig. 3), the effectiveness of the treatment can be mostly observed from 8 to 12 weeks after the commencement of the treatment, which means in the case of a patient who is intolerant of this treatment, an alternative treatment with molecular targeting drugs or a combination treatment with molecular targeting drugs and cytokine can commence without delay.

In order to improve further the therapeutic management of metastatic RCC, the identification of molecular markers which predict the response to treatments is needed in addition to pathological selection of patients. The present trial involves the identification of gene markers for the responses to the combined immunotherapy as one of the primary endpoints. An analysis of gene expression profiling is now ongoing.

Table 3. Treatment-related adverse events occurring in at least 10% of patients

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Any grade, n (%)</th>
<th>Grade 3, n (%)</th>
<th>Grade 4, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>44 (100)</td>
<td>5 (11.4)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>39 (88.6)</td>
<td>1 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Anorexia</td>
<td>31 (70.5)</td>
<td>4 (9.1)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>10 (22.7)</td>
<td>1 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>24 (54.5)</td>
<td>3 (6.8)</td>
<td>0</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>35 (79.5)</td>
<td>1 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>30 (68.2)</td>
<td>14 (31.8)</td>
<td>6 (13.6)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>14 (31.8)</td>
<td>4 (9.1)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>Erythropenia</td>
<td>13 (29.5)</td>
<td>1 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin decreased</td>
<td>12 (27.3)</td>
<td>2 (4.5)</td>
<td>0</td>
</tr>
<tr>
<td>Hematocrit decreased</td>
<td>13 (29.5)</td>
<td>1 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>18 (40.9)</td>
<td>1 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Elevated AST</td>
<td>21 (47.7)</td>
<td>2 (4.5)</td>
<td>0</td>
</tr>
<tr>
<td>Elevated γ-GTP</td>
<td>15 (34.1)</td>
<td>1 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Albumin, serum-low</td>
<td>6 (13.6)</td>
<td>2 (4.5)</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss</td>
<td>21 (47.7)</td>
<td>1 (2.3)</td>
<td>0</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamic pyruvic transaminase.

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Conflict of interest statement

None declared.

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