Combined Immunotherapy with Low-dose IL-2 Plus IFN-α for Metastatic Renal Cell Carcinoma: Survival Benefit for Selected Patients with Lung Metastasis and Serum Sodium Level

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Objective: To clarify the survival benefit of immunotherapy for renal cell carcinoma patients with lung metastasis using low-dose interleukin-2 plus interferon-α, we examined survival outcomes and factors associated with prognosis.

Methods: This was a multicenter prospective study. Nephrectomized renal cell carcinoma patients with lung metastasis were treated with interleukin-2 (0.7 × 10^6 unit, 5 days a week) and interferon-α (6 × 10^6 IU, 3 days a week) for the first 8 weeks, and then with both interleukin-2 and interferon-α, 2 or 3 days a week for 16 additional weeks.

Results: Median follow-up period for 42 patients was 28.3 months (range: 4.2–43.8). Two-year overall survival rate was 82% and the probability of 3 year survival rate was 71%. Median progression-free survival was 10.4 months. While no difference was found in survival among patients assessed as complete response, partial response and no change, survival of patients assessed as NC or better was significantly better than those assessed as progressive disease ($P < 0.0001$). Furthermore, multivariate analyses identified pre-treatment serum sodium ($P = 0.004$) as an independent prognostic factor. The sodium level was also statistically associated with tumor response ($P = 0.035$). Patients with normal sodium level survived significantly longer ($P = 0.0005$) than those with low sodium level showing median survival of 12.2 months.

Conclusions: Combination immunotherapy with low-dose interleukin-2 plus interferon-α showed survival benefit for patients with lung metastasis whose tumor responded as no change or better. This combination immunotherapy could be beneficial for patients selected by metastatic organ and their pre-treatment serum sodium level.

Key words: renal cell carcinoma – interleukin-2 – interferon-α – lung metastasis – sodium

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INTRODUCTION

The prognosis for patients with advanced renal cell carcinoma (RCC) is poor. It is reported that the median survival for patients with advanced RCC is 10 months and 5-year survival rate is <15% (1). RCC is highly resistant to conventional cytotoxic chemotherapy, while RCC evokes an immune response, which occasionally results in spontaneous remission (2,3). Such observations provide the rationale for developing immunotherapeutic approaches to treatment and have led us to a clinical investigation of immunostimulatory cytokines, such as interleukin-2 (IL-2) and interferon-α (IFN-α). Positive response rates of 10–20% are reported with these cytokines and some patients achieve a complete and long-lasting remission (4–6).

Among the effective immunotherapy options, administering a high-dose bolus i.v. IL-2, IFN-α and low/intermediate dose of IL-2 plus IFN-α have shown some evidence of anti-tumor activity, but no impact on overall survival (7). A number of uncontrolled studies, however, have shown that low doses of IL-2 plus IFN-α are associated with less toxicity and capable of inducing partial and complete remission with a comparable effect on median survival (8–10). Naito et al. (11) have recently reported a large retrospective study of 1463 Japanese patients that cytokine-based therapy, including IL-2 and IFN-α, improved the prognosis of advanced RCC patients.

Many studies have suggested that the great benefits of the cytokines can be achieved when applied to appropriately selected patients (12,13). Improvements in patient selection will be necessary to ensure that patients who might attain durable remission with IL-2 will not miss this opportunity. The important issue is how these individuals can be selected more accurately. A prognostic model by the Memorial Sloan Kettering Cancer Center (MSKCC) (14) is the most extensively used guide for optimal treatment. In terms of histological characteristics, it has been reported that patients with RCC of clear cell histology respond well to cytokine therapy (15). Although many efforts have been undertaken to clarify clinical or molecular factors associated with response to cytokines, the potential remains largely untapped.

Recently, novel molecular-targeted agents have been developed for the treatment of metastatic RCC (16). These include tyrosine kinase inhibitors, such as sorafenib and sunitinib as well as mammalian target of rapamycin inhibitors. 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 remissions of metastatic RCC (17,18).

Our previous pilot study has shown that combination treatment with low-dose IL-2 (0.7 × 10^6 unit/person) plus IFN-α is effective for metastatic RCC patients, especially those with metastasis limited to lung (19). In addition, the combination therapy was tolerated well and no additional adverse event was observed in comparison with the monotherapy using either low-dose IL-2 or IFN-α. Thus, in order to confirm the efficacy of the treatment and to explore genetic markers that may be useful in patient selection, we have tried a new prospective and multicenter trial of the combination therapy on patients who had radical nephrectomy, lung metastasis and no previous systemic therapy. The efficacy for tumor responses has already been described in our recent report (20); briefly, the efficacy for patients with metastasis limited to lung has been reproduced with similar response rate of 35.5% and the disease control rate of 80.6%. A separate paper reports that expression levels of HLA-DQA1 and HLA-DQB1 are candidate markers for predicting the tumor response to this combination therapy using oligoDNA microarray analysis after enrichment of the cancer cells with laser microbeam microdissection technology (21).

In this paper, we report survival outcomes of this study and examined factors associated with the prognosis of patients receiving the combination therapy with low-dose IL-2 plus IFN-α. We show that the combination therapy produced superior survival outcomes with a 2-year overall survival rate of 82%. Furthermore, better survival was shown to be significantly associated with tumor responses including NC (no change) and with normal baseline serum sodium level, indicating that the combination immunotherapy will be beneficial to patients selected by their pre-treatment serum sodium in addition to their metastatic organ limited mainly to lung.

PATIENTS AND METHODS

PATIENTS AND TREATMENT

Study design and patient inclusion criteria have been previously described (20). Briefly, this was a prospective, multicenter and open-label trial for Japanese patients with metastatic RCC, who had received radical nephrectomy, measurable lung metastasis, the possibility of providing blood and specimens from primary tumors to determine genetic markers, and who had received no previous systemic treatment. Patients were enrolled from September 2006 to April 2008. The study was approved by the institutional review board at each center.

Administration of IL-2 (Imunace, Shionogi, Osaka, Japan) and IFN-α (Sumiferon, Dainippon Sumitomo, Osaka, Japan) was commenced simultaneously and continued for 8 weeks at following doses: IL-2 administrated by intravenous infusion at 0.7 × 10^6 unit/person per day, 5 days a week and IFN-α subcutaneously or intramuscularly at dose 6 × 10^6 IU, 3 days a week. From week 9 to week 24, IL-2 and IFN-α were administered 2 or 3 days a week to patients showing evidence of objective response or NC. When this 24-week treatment was completed, progressive disease was detected, or this regimen could not be continued because of severe side effects, subsequent therapy was determined on each case by each center. The patients who were assessed as PD could continue to receive treatment with IL-2 and/or IFN-α.
(continuous cytokine therapy) when centers determined it to be beneficial to them, because continuation of cytokine treatment despite progression of disease was reported to add a survival benefit to patients (11) and alternative agents (molecular target drugs) other than cytokines had not been approved in Japan by April 2008. Before their official approval, however, target drugs became available for clinical trials during the present study and were given to some patients who experienced relapse.

OUTCOME VARIABLES

The efficacy of tumor response has reported in our recent paper (20). Tumor response was assessed by up to 24 weeks plus an additional 4-week follow-up after commencement of the treatment according to the criteria of the Japanese Urological Association (JUA) (22) which is similar to the WHO criteria (23). We used JUA criteria instead of RECIST in order to compare the efficacy with our previous pilot study (19). Response evaluation was reviewed by external independent radiologists following investigators’ assessment and further confirmed by central assessment. Progression-free survival (PFS) was defined as the time from the date of registration to disease progression or death, whichever occurred first. Overall survival was defined as the time from registration until death from any cause. Baseline serum sodium was determined in each center and low sodium level was determined based on the criterion of each center.

STATISTICAL ANALYSIS

For time-to-event endpoints, medians and 95% confidence intervals (CI) were estimated using the Kaplan–Meier method and the differences were assessed using log rank test. Uni- and multivariate survival analyses were based on the Cox proportional hazards regression model. Univariate parameters with $P < 0.05$ were used in the multivariate analyses using the backward selection.

RESULTS

PATIENT CHARACTERISTICS

From September 2006 to April 2008, a total of 44 Japanese patients were enrolled in this study and treated with low-dose IL-2 plus IFN-α therapy as a first-line setting. One patient was excluded due to violation of inclusion criteria and one discontinued treatment in the first week by withdrawal of consent. The baseline characteristics of 42 patients, which have been previously described in part (20), are shown in Table 1. All patients had undergone radical nephrectomy and had lung metastasis. Thirty-one patients (73.8%) had metastasis limited to lung. Others (11 patients) had multiple organ metastases, including lymph node, bone, liver, pancreas, adrenal gland and/or cardiac membranes in addition to lung. The number of measurable metastatic

| Table 1. Patient characteristics | $n$ | % |
|----------------------------------|----|---|
| Gender                           |    |   |
| Male                             | 32 | 76.2 |
| Female                           | 10 | 23.8 |
| Age                              |    |   |
| Less than 65                     | 28 | 66.7 |
| 65 or greater                    | 14 | 33.3 |
| ECOG PS                          |    |   |
| 0                                | 33 | 78.6 |
| 1                                | 9  | 21.4 |
| Nephrectomy                      |    |   |
| Yes                              | 42 | 100 |
| No                               | 0  | 0   |
| Pathological T stage             |    |   |
| pT1                              | 9  | 21.4 |
| pT2                              | 9  | 21.4 |
| pT3                              | 23 | 54.8 |
| pT4                              | 1  | 2.4 |
| Histology                        |    |   |
| Clear cell                       | 38 | 90.5 |
| Papillary                        | 1  | 2.4 |
| Mixed                            | 3  | 7.1 |
| Metastatic organ                 |    |   |
| Lung                             | 42 | 100 |
| Lymph node                       | 7  | 16.7 |
| Bone                             | 5  | 11.9 |
| Others                           | 7  | 16.7 |
| Number of metastatic organ       |    |   |
| Single (lung only)               | 31 | 73.8 |
| Multiple                         | 11 | 26.2 |
| Number of metastatic lesion      |    |   |
| 1                                | 3  | 7.1 |
| 2                                | 9  | 21.4 |
| 3–5                              | 16 | 38.1 |
| 6–10                             | 12 | 28.6 |
| 17–26                            | 2  | 4.8 |
| MSKCC risk group                 |    |   |
| Favorable                        | 1  | 2.4 |
| Intermediate                     | 29 | 69  |
| Poor                             | 12 | 28.6 |

ECOG, Eastern Cooperative Oncology Group; Others, included liver, pancreas and cardiac membrane; MSKCC, Memorial Sloan Kettering Cancer Center.

lesions in each patient varied from 1 to 26 with a median number of 4. Among patients with only lung metastasis, the number of lesions varied from 1 to 16 with a median
number of 3. Thirty-eight (90.5%) of 42 patients had pure clear cell carcinoma, 1 papillary and others (3 patients) had mixed cell type with clear cell carcinoma. Based on MSKCC prognostic criteria (14), patients were categorized mostly in the intermediate (69.0%) and poor (28.6%) risk groups with only one patient categorized as favorable group (2.4%). To utilize the primary tumor specimens for marker analysis, the present study had mainly enrolled patients (92.9%: 39/42) who had metastasis at nephrectomy, which is one of the risk factors in the MSKCC criteria.

OVERALL SURVIVAL AND PFS
Median follow-up period for 42 patients was 28.3 months (range: 4.2–43.8). The overall survival had not reached the median by June 2010. In the first 12 months and the next 12 months after the registration, 3 and 4 deaths had occurred, during these respective periods. The 1- and 2-year overall survival rates were 89.9% (95% CI: 75.4–96.1) and 82.0% (66–91%), respectively. Figure 1 shows the overall survival curve estimated by the Kaplan–Meier method. The probability of 3-year survival rate was estimated to be 70.9% (54–83%). The patients (n = 7) who died in 2 years had either multiple organ metastases (n = 4) or poor risks (n = 5) by MSKCC criteria (14), although 7 of 12 poor risk patients have survived for over 2 years (data not shown).

The median PFS was 10.4 months (5.6–14.8) (Fig. 1). While one of the two patients assessed as complete response (CR) has relapsed after a follow-up period of 13 months but surviving over 32.2 months, another patient remained with no evidence of disease for over 25 months by continued therapy with IL-2 plus IFN-α. One patient with papillary type RCC (type not classified) in the lung, who had responded to the combination therapy (assessed as PR), was progression free for 10 months and survived for over 29 months.

Survival was compared between patient groups with only lung metastasis (n = 31) and with extrapulmonary organs (n = 11). The difference was not statistically significant, but patients with only lung metastasis tended to survive longer than those with extrapulmonary metastasis (log-rank P = 0.0745, data not shown). The 2-year survival rates of patients with only lung metastasis and with extrapulmonary metastases were 89.7% (71.3–96.5) and 61.4% (26.6–83.5), respectively.

RELATIONSHIP BETWEEN TUMOR RESPONSE AND CAUSE-SPECIFIC SURVIVAL
In our subgroup analysis, a strong correlation was found between diagnosis of tumor response (20) (the response assessed by 24 weeks after the first dose) and cause-specific survival (Fig. 2). In the patient group achieving CR or PR (n = 15), only one death occurred in 24 months with a 2-year survival rate of 92.9% (59.1–99.0) and no death occurred in patients assessed as NC (n = 16) in 24 months. Thus, the 2-year survival rate was 96.6% (77.9–99.5) for patients achieving objective response or NC. A patient diagnosed as PR who had died after 12 months had baseline characteristics, including multiple organ metastases (lung plus mediastinal lymph node), 16 lung metastatic lesions and poor risk factors (<1 year from initial visit to metastasis, >10 mg/dl high corrected calcium and low hemoglobin) by MSKCC prognostic criteria.

In contrast, 6 deaths had occurred in patients diagnosed as PD (n = 11) in 24 months with a 2-year survival rate of 40.0% (12.3–67.0). The median survival time was 13.2 months (7.0–27.0) for the PD subpopulation. All of the 6 patients have been assessed as PD by 8 weeks from the first dose with a median of 4 weeks.

PROGNOSTIC AND PREDICTIVE FACTORS
To identify clinical factors predicting prognosis in patients who received the combined IL-2 plus IFN-α therapy,
univariate and multivariate analyses using the Cox proportional hazard regression model were performed on baseline parameters, including pathological, blood and urinary tests. Survival was significantly associated with corrected calcium, CRP, serum albumin, sodium and lymphocyte count on univariate analyses (Table 2). Multivariate analyses showed that baseline serum sodium (P = 0.004), lymphocyte count (P = 0.005) and corrected calcium (P = 0.010) were independent risk factors for shorter survival, although a small number of patients in the present study seemed to exclude some potential factors. Serum sodium level was also found to be associated with tumor response to this therapy (Table 3; responder (CR/PR) vs. non-responder: P = 0.035). Furthermore, more strong correlation (P = 0.020) was found between patients with clinical benefit (CR/PR/NC) and without benefit (PD). Using the Kaplan–Meier estimate and log-rank test, serum sodium levels were also shown to be statistically significant predictor of survival time (P = 0.0005, Fig. 3). The 2-year survival rates for patients with normal sodium level was 90.7% (73.9–96.9) and 42.9% (9.8–73.4), respectively. The median survival time of patients with low sodium level was 12.2 months.

In MSKCC risk factors (14), corrected calcium was shown to be the only factor associated with survival on multivariate analyses. Prognostic groups by MSKCC criteria were also found to have a correlation with survival. Because only one patient was categorized in a favorable group, survival for intermediate (n = 29) plus favorable group was compared with that of the poor group (n = 12), and the difference was statistically significant (P = 0.036, data not shown). The 2-year survival rates for the favorable/intermediate and poor groups were 92.9% (74.3–98.2) and 58.3% (27.0–80.1), respectively. The median survival time of patients with low sodium level was 12.2 months.

**DISCUSSION**

Our previous pilot study has shown that combination therapy with low-dose IL-2 plus IFN-α is effective for metastatic

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### Table 2. Univariate and multivariate analyses of baseline parameters for overall survival of patients receiving IL-2 plus IFN-α

| Risk factors | Categories       | Univariate analyses |  |  |  |
|--------------|------------------|---------------------|---|---|---|
|              |                  | Hazard ratio | 95% CI | P   | Hazard ratio | 95% CI | P   |
| Sodium       | Low vs. N         | 6.48     | 1.94–21.6 | 0.002 | 16.1     | 2.45–105 | 0.004** |
| Lymphocyte   | Low vs. N         | 7.91     | 2.04–30.8 | 0.003 | 14.7     | 2.25–96.6 | 0.005** |
| Corrected Ca | >10 mg/dl         | 5.51     | 1.56–19.4 | 0.008 | 13.2     | 1.83–94.2 | 0.010** |
| Albumin      | >0.3 mg/dl        | 5.35     | 1.15–24.9 | 0.032 | 1.04     | 0.14–7.57 | 0.966  |

aN, normal.
**P < 0.05 on multivariate analysis.

### Table 3. Correlation between pre-treatment serum sodium and tumor response to IL-2 plus IFN-α

| Sodium level, n (%) | Normal | Low |
|---------------------|--------|-----|
| n                   | 34     | 8   |
| Tumor response      |        |     |
| CR/PR               | 15 (44.1) | 0 (0) |
| NC                  | 13 (38.2) | 3 (37.5) |
| PD                  | 6 (17.6) | 5 (62.5) |
| Clinical benefit    |        |     |
| CR/PR/NC            | 28 (82.4) | 3 (37.5) |

\*p-value: Fisher’s precision test.

The tumor response was assessed by up to 24 weeks plus additional 4-week follow-up after the first dose (20). Response evaluation was reviewed by external independent radiologists following investigators’ assessment, and further confirmed by central assessment. **p-value**

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![Figure 3. Survival and baseline serum sodium level of patients treated with IL-2 plus IFN-α. Survival was significantly different between patients with normal and low sodium levels (P = 0.0005). The median survival time of patients with low sodium level was 12.2 months, while the survival for patients with normal sodium level has not been reached to median during observation period (median: 28.3 months, range: 4.2–43.8).](https://academic.oup.com/jjco/article-abstract/41/8/1023/815076)
RCC patients, particularly those with metastasis limited to lung (19). The present trial has confirmed the efficacy of tumor response for patients with lung metastasis (20) and the present study further showed that this regimen provides a good survival benefit. The treatment was well tolerated and no additional adverse events occurred to those observed with monotherapy using either low-dose IL-2 or IFN-α (20). The overall survival did not reach the median in the median follow-up of 28.3 months (range: 4.2–43.8). The median PFS was 10.4 months with 1- and 2-year survival rate of 89.9 and 82.2%, and the probability of 3-year survival rate of 70.9%. While the data from the USA showed that the 1- and 3-year survival rates were 54 and 19%, respectively, in 463 metastatic RCC patients who received IFN-α (14), a large retrospective study on Japanese patients (11), 82% of whom had received cytokine therapy, including IFN-α and/or IL-2, showed 64.2 and 35.2% of 1- and 3-year survival rates, respectively. The 1- and 3-year survival rates of the present study are similar to or even better than those (86 and 46%, respectively) of favorable risk subpopulation in a randomized trial of IFN-α with/without IL-2 and fluorouracil (24).

It is noted that patients enrolled in this study were categorized mostly in intermediate (69.0%) and poor (28.6%) risk groups with only one patient categorized as favorable in the MSKCC prognostic model. To utilize the primary tumor specimens for gene marker analysis, the present study had mainly enrolled patients who had metastasis at nephrectomy, which is one of the risk factors in the MSKCC criteria. Despite the small proportion of favorable patients, on the whole, the survival outcomes were superior.

One reason for the better outcomes in the present study can be attributed to our patient selection by the criteria that included prior radical nephrectomy, ECOG performance status of 0–1 and limited metastasis mainly to lung. Upfront nephrectomy has been shown to enhance survival time for immunotherapy of metastatic RCC patients (25). In fact, nephrectomy improved the median survival period from 10.3 to 14.3 months in patients with only lung metastasis (26). In addition, racial differences may affect the survival of metastatic RCC patients as reported in one study (27).

The baseline serum sodium was found to have a significant positive correlation with tumor response and survival. Most recently, Jeppesen et al. (28) have reported that the level of baseline serum sodium is one of the prognostic and predictive factors in metastatic RCC patients who have been treated with IL-2-based therapy with/without IFN-α. In their work, low serum sodium has been shown to be a prognostic factor for short survival and a predictive factor for a lack of response to the immunotherapy. In the present study, the responders were found only in patients with normal sodium levels. The survival was significantly longer in patients with normal sodium than those with low sodium ($P = 0.0005$). Thus, our observations in the present study were consistent both with prognostic and predictive values of the serum sodium. These results imply that the tumor response and survival can be further improved by patient selection with baseline serum sodium levels in addition to the pathological criteria, including limited metastasis to lung.

Furthermore, the present study showed that tumor responses were closely associated with survival. The survival of patients assessed as NC was not different from those as CR or PR, while survival for patients assessed as PD was significantly shorter than those assessed as the objective response or NC ($P < 0.0001$). Since similar observations have been shown in our previous pilot study of IL-2 plus IFN-α combination therapy (19), our two independent prospective trials demonstrated that patients showing objective responses or NC can anticipate a survival benefit from this combination therapy. This finding is in agreement with previous reports on IL-2-based immunotherapy (29,30). In the present study, patients who died within 2 years had been diagnosed as PD by 8 weeks from the first dose. Thus, it might be possible to consider that the patients who are assessed as not PD in the first 2 months could continue the combination therapy and could benefit from the treatment.

It is of interest to mention that IFN-α has recently been shown to play a role in the dynamic balance between activated regulatory and effector T cells (31,32). Pace et al. (31) have reported that IFN-α inhibits IL-2-induced regulatory T cell (Treg) proliferation and function through antigen-presenting cell activation. IL-2 plays important roles in tumor immunity by enhancing dendritic cell function, and T cell and NK cell effector activities, while IL-2 also delivers essential signals for the activation of Treg, which suppresses the functions of effector T cells in their homeostasis (33). Therefore, the combination of IL-2 with IFN-α may enhance antitumor activity through suppression of Treg with the aid of IFN-α as suggested by Tatsugami et al. (34).

Administration of targeted agents has become a routine practice for treatment of patients with metastatic RCC. However, none of the novel targeted agents seem to be curative. Furthermore, both randomized and expanded-access trials on sunitinib and sorafenib have shown that PFS and overall survival of both agents have been reported not to be significantly different between treatment-naive and cytokine-refractory patients (17,18,35–38), indicating that the agents are as effective for patients who are refractory to cytokines. From above, it is thought to be possible to improve the survival benefit for metastatic RCC patients, if the combination therapy with IL-2 plus IFN-α is chosen as the first-line treatment, seeing it has better outcomes, even to the extent that complete remission can be expected. In the case of a patient who is refractory to this treatment, an alternative treatment with targeted agents can commence without delay and provide additional benefits.

A more accurate patient selection would ensure that the benefits they receive from the treatment are maximized. Our separate paper reports that expression levels of HLA-DQA1 and HLA-DQB1, the genes known to form heterodimers in antigen presentation process, are candidate markers for predicting the tumor response to the combination therapy with
IL-2 plus IFN-α (21). Exclusion of patients with tumors lacking either expression of these two genes is likely to improve the response rate to IL-2 plus IFN-α from 36 to 67%, indicating that a pretreatment genetic test would provide useful information in narrowing down the patients in order to improve the efficacy of this treatment and reduce unnecessary medical costs. Thus, by extending the patient selection criteria to metastatic organs, baseline sodium levels and a genetic test, the efficacy of the treatment can improve further.

Although the present study is a non-randomized prospective study, including a relatively small number of patients with a short follow-up period, the results showed that the combination therapy with low-dose IL-2 plus IFN-α provides survival benefits for selected patients who had limited metastases mainly to lung. Furthermore, the present study suggests that if patients are selected by their baseline serum sodium levels, combined immunotherapy would be a great benefit for them.

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Conflict of interest statement

None declared.

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