Phase II Trial of Sequential Radiation and Interleukin 2 in the Treatment of Patients with Metastatic Renal Cell Carcinoma

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ABSTRACT
We have previously demonstrated that local tumor irradiation effectively enhanced the therapeutic effect of interleukin 2 (IL-2) therapy in an experimental murine renal adenocarcinoma model. Based on these preclinical studies, we have designed and initiated a Phase II trial of irradiation combined with IL-2 for the treatment of metastatic renal cell carcinoma. Patients received 800 cGy to the primary or metastatic lesion on days 1 and 15 followed by IL-2 (600,000 IU/kg i.v.) every 8 h on days 4–8 and 18–22. Sixteen patients were entered; all completed treatment and are therefore evaluable for toxicity and response. Two partial remissions were seen for a response rate of 12.5% (95% confidence interval, 0–28.7). There was no increase in toxicity over that which is anticipated from IL-2 alone. The antitumor activity seen in this trial is consistent with what would be expected from high-dose IL-2 alone.

INTRODUCTION
The treatment of metastatic RCC2 remains an area of intense clinical research. Considered resistant to standard forms of treatment such as radiation therapy and chemotherapy, most endeavors have centered around immunological approaches to this disease. High-dose IL-2 remains the only therapy for metastatic RCC that is approved by the United States Food and Drug Administration. Although effective in 15–20% of treated patients with evidence of durable remissions, high-dose IL-2 treatment remains restricted to a select group of patients due to its toxicity (1). Numerous investigators have evaluated approaches to either decrease the toxicity or increase the efficacy of IL-2 in the treatment of RCC (reviewed in Ref. 2).

Radiation treatment before immunotherapy has been reported to increase the efficacy of immunotherapy in preclinical murine tumor models. Using the murine Renca metastatic renal adenocarcinoma syngeneic to BALB/c mice, we have shown that local tumor irradiation before IL-2 therapy augments the antitumor activity of IL-2 (3, 4). In a spontaneous metastatic model induced by intrarenal injection of Renca cells, irradiation of the primary kidney tumor followed by systemic IL-2 therapy induced a significant reduction in the size of the primary tumor and eliminated spontaneous pulmonary metastasis (3). In a pulmonary metastasis model induced by i.v. injection of Renca cells, we demonstrated that local irradiation of left lung metastases followed by IL-2 therapy inhibited metastases in both lungs (4). In both models, the combination of radiation and IL-2 therapy induced a greater antitumor response than either modality alone that was observed in irradiated sites as well as in nonirradiated tumor sites. These data suggest that irradiation can augment the systemic effects of IL-2 therapy. Histological evaluation of Renca-bearing lung tumors revealed that either tumor irradiation or IL-2 therapy induced vascular damage, resulting in multifocal hemorrhages and mononuclear cell mobilization in the lung tissue, an effect that was amplified with the combined therapy (5).

Radiation caused an influx of macrophages (an important antigen-presenting cell) into tumor sites, and IL-2 caused T-cell infiltration. The combined therapy resulted in massive mobilization of macrophages, T cells, and natural killer cells in irradiated and nonirradiated tumor nodules, suggesting a role for these immune cells in the antitumor effect mediated by the combination therapy (5). We and others have also shown that tumor irradiation can increase the efficacy of IL-2 in other tumor models, provided that the tumor is responsive to immunotherapy (6, 7). We reported that the best therapeutic efficacy of the combined protocol was observed when a single radiation fraction of 800 cGy was followed by systemic IL-2 administration (4). We have translated our preclinical studies into a clinical trial for the treatment of metastatic RCC in which either the primary renal tumor or metastatic site was irradiated before IL-2 therapy. We report the results of this Phase II clinical trial of sequential irradiation and high-dose IL-2 therapy.

PATIENTS AND METHODS
Patients. Patients with metastatic RCC who were eligible for high-dose IL-2 and had either the renal primary lesion in place or a metastatic lesion of ≥2 × 2 cm were eligible for this trial. To be eligible for high-dose IL-2, patients had to have a performance status (Eastern Cooperative Oncology Group) of at least 2, serum creatinine of ≤2.0 mg/100 ml, total bilirubin of ≤1.5 mg/100 ml, platelet count of ≥100,000/mm3, WBC count...
of \( \geq 3000/\), and an anticipated life expectancy of \( >3 \) months. Patients also had to have a normal cardiac stress test and a FEV1 \( \geq 2 \) liters/min or \( \geq 80\% \) predicted. All patients were required to have at least one bidimensionally measurable lesion (not being irradiated). Patients with an active autoimmune disease requiring corticosteroids or with brain metastasis were excluded. All patients gave written informed consent approved by the institutional review board.

**Treatment.** Treatment consisted of a single fraction of radiation on days 1 and 15 followed by high-dose IL-2 administered on days 4–8 and 18–22. Radiation was administered at a dose of 800 cGy to a lesion \( \leq 5 \times 5 \) cm with an irradiated margin of 2 cm around the lesion. The same lesion was treated on days 1 and 15. IL-2 (Chiron, Emeryville, CA) was given at a dose of 600,000 IU/kg IVPB over 15 min every 8 h, beginning at 4 p.m. on days 4 and 18 for a planned 14 doses. Concomitant medications with IL-2 administration included acetaminophen (650 mg) every 4 h, indomethacin (25 mg) every 6 h, and ranitidine (150 mg) every 12 h. Meperidine was administered for rigors due to IL-2, and diphenhydramine was given for pruritus. Antiemetics and antiarrhythmic agents were left to the discretion of the treating physician. Doses of IL-2 were omitted rather than delayed for refractory hypotension (defined as not responsive to 5 \( \mu \)g/kg/min dopamine), anuria, or respiratory distress. IL-2 was discontinued for mental confusion, sustained tachydysrhythmia, and/or evidence of myocardial ischemia.

Patients were evaluated daily while on treatment by the treating physician. Daily electrolytes, serum creatinine, and serum ionized calcium were obtained together with a complete blood count and chemistry profile on days 4, 8, 18, and 22. Patients were evaluated for antitumor responses at 4 and 8 weeks after IL-2 administration. Standard response criteria were used. Patients were eligible for retreatment (without radiation) if they exhibited a sustained partial response to their initial treatment. Complete responders were followed without additional treatment.

**Histology and Immunohistochemistry.** Surgical specimens from a renal tumor were fixed in 4% paraformaldehyde and paraffin-embedded. Tissue sections were stained with H&E or used for immunohistochemical detection of macrophages and T cells. Mouse monoclonal antibodies directed against human CD3 for T cells and CD68 for human macrophages (DAKO Corp., Carpinteria, CA) were used followed by a peroxidase-based ABC method (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions that included diaminobenzidine as a chromogenic substrate (Sigma Chemical Co., St. Louis, MO). Before mounting, the immunostained sections were lightly counterstained with hematoxylin. Negative controls were included by incubating sections with appropriate IgG isotypes instead of each antibody as described previously (5).

**RESULTS**

Patient characteristics are presented in Table 1. Eight patients had a prior nephrectomy and therefore had irradiation to a metastatic site. The sites irradiated included the primary site, lung nodule, liver metastasis, bone metastasis, and lymph node. All 16 registered patients completed treatment and are evaluable for response and toxicity. The mean number of doses of IL-2 administered was 11 on days 4–8 and 8 on days 18–22. The mean number of IL-2 doses administered is not different from our previous experience7 or that reported in the literature (1). Doses of IL-2 were omitted for refractory hypotension or anuria. One patient had a spontaneous perforation of her sigmoid colon several days after completing therapy that required resection and a colostomy. The perforation occurred in a section of the sigmoid colon without other pathological abnormality. This patient received irradiation to her left kidney as part of the protocol, and review of her irradiation port films showed that the sigmoid colon was not in the field of irradiation. Another patient experienced an episode of atrial fibrillation with a rapid ventricular response after 13 doses of IL-2 during the first week of treatment that resolved. After the fourth dose of IL-2 during the second week of treatment, the patient had several episodes of nonsustained ventricular tachycardia, and IL-2 was discontinued. The patient had no evidence of myocardial ischemia and recovered from the tachydysrhythmia without sequelae. This patient received irradiation to a right lung lesion; therefore, the heart was not in the field of irradiation.

There were 2 partial responses in 16 patients, for a response rate of 12.5% (95% confidence interval, 0–28.7). One patient had irradiation to a renal primary and had total regression of multiple pulmonary nodules after IL-2 therapy as well as more than a 50% regression of the renal primary. This patient subsequently underwent radical nephrectomy 4 months after treatment and remains disease free at over 2.5 years. A renal tumor specimen from this patient was obtained after nephrectomy and processed for histological staining. Some sections were completely necrotic without significant numbers of surviving ma-

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3 B. G. Redman, unpublished observations.
lignant cells. Other tumor sections showed multifocal hemorrhages and inflammatory infiltrates among residual tumor tissue (Fig. 1, A and B). The tumor specimen consisted mostly of fibrous tissue, with few remaining malignant cells (Fig. 1, A and B). A massive infiltration of T cells (Fig. 1C) and macrophages (Fig. 1D) in the tumor was identified by immunohistochemistry.

The second patient had metastatic RCC to the retroperitoneal, mediastinal, and left supraclavicular lymph nodes 1 year after undergoing a left radical nephrectomy for RCC. The patient received irradiation to the left supraclavicular lymph nodes and, after IL-2 administration, had a greater than 80% reduction in all sites of disease. The patient subsequently received retreatment with high-dose IL-2 without irradiation and remains in partial remission at 9+ months.

Three other patients had a decrease in the size of irradiated lesions, but with progression in nonirradiated sites. The lesions that were irradiated included a renal primary, a mediastinal lymph node, and a lung nodule. None of the regressions in these three irradiated lesions met the criteria for a partial response.

DISCUSSION

We report our clinical experience with the combination of radiation therapy and high-dose IL-2 in the treatment of metastatic RCC. The rationale for this trial was based on our preclinical investigations of sequential irradiation and immunotherapy in the murine Renca model. There is one other report in the literature of a pilot study evaluating the combination of radiation therapy and high-dose IL-2 (with and without tumor-infiltrating lymphocytes) in the treatment of patients with cancer (8). The investigators of this trial concluded that the combination did not seem to have a synergistic antitumor effect. Their trial differed from ours in the dose of radiation used and in the time interval from irradiation to initiation of IL-2 administration. Radiation was administered as either two or four fractions of 500 cGy separated by 4–6 h and given over 1 or 2 days, 2–24 h before the first course of high-dose IL-2. High-dose IL-2 was administered similar to our trial. Irradiation was not repeated again before the second half of high-dose IL-2 administration. The majority of patients received 2000 cGy. In our trial, a single fraction of 800 cGy was administered 3 days before both courses of IL-2.

The activity seen in our trial of the combination of irradiation and IL-2 could be attributable to the activity of high-dose IL-2, because the response rate is consistent with that reported in the literature for IL-2 alone. We are in agreement with the previous report in that the toxicity of irradiation combined with IL-2 is no greater than that seen with high-dose IL-2 alone and did not compromise our ability to administer IL-2. One patient had a spontaneous bowel perforation, which occurred in a site outside of the radiation field, and bowel ischemia/perforation is a recognized complication of high-dose IL-2 (9).

It is unclear whether irradiation of part of the tumor contributed to the IL-2 therapeutic effect observed in the two responders who received the combined therapy. Histological evaluation of the residual renal tumor of one of the responders, obtained by nephrectomy after the combined therapy, shows...
evidence of vascular damage with multifocal hemorrhages and extensive T-cell and macrophage infiltration with areas of necrosis. These findings in the human tumor specimen treated with the combined therapy are similar to our observations in the murine Renca tumor-bearing lung metastases treated with radiation and IL-2 therapy and may be suggestive of effects induced by both irradiation and IL-2 therapy in this patient (5). The vascular damage causing inflammatory mononuclear infiltrates in the tumor nodules and their microenvironment may play a role in the antitumor responses observed, because we have demonstrated in mice that depletion of T cells and natural killer cells abrogates the antitumor effect mediated by the combined therapy (4).

Although the number of patients in our clinical trial was low, we did not observe a greater response rate with the combination therapy than with high-dose IL-2 alone, in contrast to our findings in the murine model. These discrepancies between mouse and human studies may be due to the poor responsiveness of human RCC to irradiation. In our murine studies, the Renca tumors showed a dose-dependent response to local irradiation alone and a significant inhibition of lung metastases after treatment with a single dose of 800 cGy. The selection of 800 cGy for our human protocol was based on these findings, but it may be that higher doses of irradiation (>800 cGy) can be more effective when combined with IL-2 in the treatment of metastatic RCC. However, 800 cGy as a single fraction locally administered was found to be the largest tolerable dose that could be given to patients with less than a 5% chance of morbidity (10). As in the previous clinical trial, higher total doses of irradiation administered in multiple fractions did not seem to be synergistic when combined with IL-2 (8).

Alternatively, irradiation of human RCC cells may cause alterations different from those observed in the Renca cells. In our murine model studies, we have shown that irradiation increases the expression of class I MHC antigen expression in Renca cells and apoptosis, which may result in availability of tumor antigens and could increase the tumor immunogenicity (4, 5). It is possible that the failure of the clinical trials to show a synergistic effect from irradiation and IL-2 is the inability of irradiation to increase the expression of class I MHC antigens on human RCC. Several investigators have documented the loss of HLA class I expression in advanced RCC (11, 12). Whether irradiation can induce the up-regulation or expression of HLA class I antigens in human RCC is an area that requires further investigation.

We have failed to reproduce the preclinical findings of a synergistic effect of irradiation and IL-2 in the treatment of patients with metastatic RCC. We have shown in murine models that only tumors responsive to IL-2 were more effectively killed by the combination of irradiation and IL-2 therapy (6). The responsiveness of patients to the combined therapy may occur only in patients responding to IL-2 therapy. Modifications of the combined protocol may be needed; for example, irradiation of more than one tumor site may be more effective, and the time between irradiation and initiation of IL-2 treatment could be shortened. Because of the intriguing results obtained in the preclinical murine models, the interaction of irradiation and immunotherapy warrants additional investigations.

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REFERENCES


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