A Phase I Trial of CD3/CD28-activated T Cells (Xcellerated T Cells) and Interleukin-2 in Patients with Metastatic Renal Cell Carcinoma

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ABSTRACT

Purpose: Xcellerated T Cells (Xcyte Therapies, Seattle, WA) are autologous T cells that have been activated and expanded ex vivo using antibodies to CD3 and CD28 coimmobilized on magnetic beads. This study assessed the safety, immunostimulatory effects, and antitumor activity of Xcellerated T Cells and interleukin-2 (IL-2) in patients with metastatic renal cell carcinoma (RCC).

Experimental Design: Twenty-six patients with measurable metastatic RCC after prior nephrectomy underwent leukapheresis. Peripheral blood lymphocytes were stimulated ex vivo for 8 days. Two cycles of therapy separated by 28 days were planned, with each cycle consisting of i.v. Xcellerated T Cells on day 1 and s.c. IL-2 at 10 × 10^6 units on days 1–10.

Results: Forty-nine cycles of therapy were administered to 25 patients. A mean (±SD) of 21.8 × 10^9 (±5.4 × 10^9) Xcellerated T Cells were administered each cycle. The infused cells were 94% ± 3% CD3^+, 64 ± 12% CD4^+, and 25 ± 12% CD8^+ (mean ± SD). Adverse events (most commonly, fever and an influenza-like syndrome) were mild to moderate. Two patients developed significantly elevated human antimouse antibody titers (HAMA). No complete or partial clinical responses were observed. However, two patients experienced significant tumor regression in bone metastases. Median survival was 21 months. The number of cells infused correlated with the peak absolute lymphocyte count achieved, and there was a trend to increased post-infusion survival in patients achieving higher peak absolute lymphocyte counts.

Conclusions: Adoptive immunotherapy with Xcellerated T Cells and IL-2 can be carried out safely on an outpatient basis in patients with advanced RCC. Further investigation of this therapy is warranted.

INTRODUCTION

The annual incidence of RCC in the United States is ~32,000 (1), accounting for roughly 3% of all adult cancers (2). Although the five-year survival rate has improved during the past 20 years, nearly 12,000 patients die each year as a result of this malignancy (3). This is in large part because 30% of RCC patients have metastatic disease at the time of diagnosis. Furthermore, as many as 40% of RCC patients eventually develop distant metastases, primarily to the lung, bone, liver, and brain (4).

Conventional chemotherapy and hormonal therapies are usually ineffective against metastatic RCC. However, this tumor is known for its responsiveness to immunotherapeutic agents such as IL-2. High-dose regimens of IL-2 can induce objective responses, including some durable CRs in patients with metastatic RCC (5–7). Regimens using low to moderate doses of IL-2 can be administered safely on an outpatient basis and have also been reported to be immunostimulatory and to induce objective responses (8, 9). The exact mechanism by which IL-2 exerts its antitumor effect is not known, but it is thought to stimulate the activation and proliferation of T cells capable of recognizing tumor antigens (6). Maximal rebound lymphocytosis in patients treated with high-dose IL-2 correlates with the achievement of a CR (6), suggesting the possibility that augmentation of lymphocyte numbers could improve therapeutic outcomes in cancer patients.

The Xcellerate Process (Xcyte Therapies, Inc., Seattle, WA) is designed to produce large quantities of activated T cells (Xcellerated T Cells) for the treatment of patients with cancer or immunodeficiency. In this procedure, human PBMCs are activated ex vivo using murine antihuman CD3 and murine antihuman CD28 monoclonal antibodies covalently attached to superparamagnetic microbeads (10–13). Gene-marking studies have documented the long-term survival of T cells expanded ex vivo with beads coated with anti-CD3 and anti-CD28 antibodies (14, 15). In a clinical trial of HIV-infected individuals, infusion of CD4^+ T cells, activated and expanded with a process similar to Xcellerate, was well tolerated (16). The therapy led to dose-
dependent increases in CD4⁺ T cell counts, demonstrating that the infused cells were capable of significant expansion in vivo. Furthermore, recent studies have documented that T cells, activated and expanded by CD3 and CD28 co-stimulation, maintain a broad T-cell repertoire (17, 18), which is important for mounting an effective immune response to infection, cancer, and vaccination (19).

In the setting of cancer, the administration of large numbers of activated and expanded autologous T cells has the potential to increase the pool of T cells capable of reacting against tumor, as well as to lower the activation threshold for these cells when they encounter tumor targets. Release of secondary cytokines such as IFN-γ by the T cells could further enhance antitumor effects.

We conducted a Phase I trial of Xcellerated T Cells given in conjunction with s.c. IL-2 in patients with metastatic RCC to assess the toxicity, immunostimulatory effects, and clinical antitumor effects of this therapy. Previous studies in patients with RCC have shown that T cells, expanded and activated with the Xcellerate Process, can be produced at high yields, similar to those obtained from healthy donors (20). In this clinical trial, patients were scheduled to receive two doses of Xcellerated T Cells separated by 28 days. Each dose of Xcellerated T Cells was followed by a 10-day course of s.c. IL-2. The results of this trial, which provide both safety information and preliminary response data, are reported here.

PATIENTS AND METHODS

Patients. The Institutional Review Boards of the two participating institutions (University of Washington Medical Center and UCLA Medical Center) approved the study and informed consent. All of the patients provided written informed consent before undergoing any study-related procedures.

Eligible patients had histologically confirmed RCC on the basis of nephrectomy performed at least 30 days before registration, as well as radiographic confirmation of evaluable and/or measurable metastatic disease obtained less than 60 days before registration. Patients with a history of brain or central nervous system metastases were not eligible to participate in this study. All of the patients were over age 18 and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and a life expectancy of at least 3 months. Negative serological tests for human immunodeficiency virus type 1 and 2 and hepatitis B and C viral markers were required, as were adequate hematological, hepatic and renal function (WBC ≥4,000/mm³; neutrophils ≥1,500/mm³; lymphocytes ≥1,000/mm³; hemoglobin ≥10.0 g/dl; platelets ≥100,000/mm³; bilirubin and alanine aminotransferase ≤1.5 times the upper limit of normal; creatinine <2.0 mg/dl). Serious infection within 3 weeks before study entry, symptomatic hypercalcemia, or serum calcium ≥11 mg/dl rendered a patient ineligible, as did clotting factor abnormalities (prothrombin time or partial thromboplastin time >1.5 times the control). Prior chemotheraphy, immunotherapy, or cytokines (granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, erythropoietin), or other investigational therapy were not permitted; prior local radiation, hormonal, and/or steroid therapy had to have been completed at least 4 weeks before enrollment on study. Pregnant or lactating women were excluded from this study, as were patients with a history of autoimmune disease, allergies to mice or murine mouse proteins, detectable HAMA titers, or cancer other than RCC within 5 years before study entry.

Because of the possibility that the clinical status of these patients could deteriorate rapidly, two pretreatment eligibility evaluations were performed. The first occurred before leukapheresis; the second, during PBMC processing, was conducted no more than 10 days before the commencement of protocol therapy. Disease progression at the second evaluation did not preclude continuation in the study, although patients must have continued to meet eligibility criteria.

HAMA levels were measured 3 weeks after the first Xcellerated T Cell infusion, immediately before the second Xcellerated T Cell infusion, and at 3 and 7 months after initiation of protocol therapy. TSH levels were measured at the same time points to monitor for evidence of autoimmune reactivity.

Leukapheresis and Manufacture of Xcellerated T Cells. The treatment schema is summarized in Fig. 1. Leukapheresis to collect the PBMCs was performed on a continuous flow cell separator machine using a two-arm venous-access technique or, in one patient, a double-lumen central-venous catheter. Acid citrate dextrose was used as the anticoagulant. The end point of each leukapheresis collection was the processing of 10–12 liters of whole blood or 4 h of “run time” on the apheresis machine. After the Xcyte Therapies Cell Processing Facility (Seattle, WA) confirmed that the leukapheresis product met quality control specifications, T cells were activated and expanded from a fraction of the PBMCs using the Xcellerate Process. Other PBMC fractions were cryopreserved for later activation and expansion for the second cycle of treatment.

For the manufacture of the Xcellerated T Cells, the following procedure was performed. First, the PBMC leukapheresis product was washed and depleted of monocytes. Monocyte depletion was achieved by incubating the cells with Dynabeads M-450 Epoxy T (Dynal A.S.A., Oslo, Norway), allowing the phagocytic cells to adhere to and/or ingest the microbeads. The microbeads and any attached phagocytic cells were then removed by processing over a MaxSep Magnetic Separator (Nexell Therapeutics, Irvine, CA). Next, the T cells were activated and culture-expanded ex vivo in a closed system in an adaptation of a process described previously (10). Briefly, the T cells were costimulated with Xcyte-Dynabeads (Xcyte Therapies, Seattle, WA), which are antihuman CD3 (Ref. 21; Xcyte Therapies) and antihuman CD28 (Ref. 22; Xcyte Therapies) monoclonal antibodies coimmobilized to super-paramagnetic Dynasphere MS-4,5-REK particles (Dynal A.S.A.). Approximately 5 × 10⁸ CD3⁺ T cells were cultured in 3-liter cell culture bags (Baxter, Deerfield, IL) containing 100,000 IU of recombinant IL-2 (Chiron, Emeryville, CA) at a 3:1 bead:T-cell ratio for 8 to 9 days at 37°C in 5% CO₂ incubators. Cells were split 1:4 every 2–3 days. Cytokines, including IFN-γ, IL-2, IL-4, and IL-10, were measured in the culture supernatant by Luminex 100 capture sandwich immunoassay (Luminex Corp., Austin, TX) before splitting or harvesting of the cells. Cells were monitored for expression of markers of T-cell activation by flow cytometry, using monoclonal antibodies to CD25 and CD154 (Becton Dickinson, San Jose, CA). Residual beads in the final product were determined by centrifugation of a sample and counting under a microscope.
Once the cells were activated and the cellular expansion was complete, the beads and covalently attached antibodies were magnetically removed from the culture using a MaxSep Magnetic Separator. The Xcellerated T Cells were harvested and then were formulated in PlasmaLyte (Baxter, Round Lake, IL) containing 4% human serum albumin.

**Xcellerated T Cell Infusion and IL-2 Treatment.** After quality control verification and quality assurance release, the Xcellerated T Cells were packaged in EnduroTherm containers (ISC, Phoenix, AZ) and were shipped fresh to the outpatient infusion center at the University of Washington or UCLA for infusion into the patient within 48 h of formulation. Cycle 1 of treatment with Xcellerated T Cells and IL-2 commenced 10 days after leukapheresis and within 24 h of receipt of the Xcellerated T Cell product from the cell-processing facility. All of the patients were premedicated with acetaminophen 650 mg p.o. After 30 min they received a s.c. injection of IL-2, 10^6 IU, followed 30 to 60 min later with an i.v. infusion of Xcellerated T Cells in a volume of 400 ml at a rate of 15 cc/min over 30 min. All of the patients were monitored clinically for 4 h after the initiation of Xcellerated T Cell infusion and then were discharged home.

Subsequent doses of IL-2 were administered s.c. at 10 × 10^6 IU daily for 9 consecutive days after the initial IL-2 injection and Xcellerated T Cell infusion. An 18-day rest period followed, during which no treatment was given. Patients who experienced no significant toxicity, then received cycle 2 of Xcellerated T Cells and IL-2 therapy. Cells cryopreserved after leukapheresis were used to generate the second Xcellerated T Cell product. Planned dosages and scheduling were identical to those in cycle 1.

The dose of IL-2 was reduced by 50% for patients experiencing grade 3 toxicity. For patients with grade 4 toxicity, IL-2 was discontinued and restarted at 50% of the dose when the grade 4 toxicity resolved. After receiving the final IL-2 injection, no further treatment on this protocol was given. Patients were monitored for adverse effects for 7 months after treatment or until cancer progression. Patients were followed for cancer progression and survival for up to 31 months after Xcellerated T Cell therapy.

**Statistical Considerations.** The primary variable in this Phase I safety study was the incidence of dose-limiting (grade 3 and 4) toxicity of Xcellerated T Cell and IL-2 therapy. The analyses were primarily descriptive. The sample size of 25 patients was determined from the computation of a CI of a specified size from the exact binomial distribution. CI calculations were performed with StatXact 4.0.1. Pearson correlation coefficients were calculated to determine whether the total number of nucleated cells infused was associated with the peak absolute lymphocyte counts achieved in the patients. A Cox model analysis for censored survival data were performed to determine whether the peak lymphocyte count achieved after each Xcellerated T Cell infusion correlated with subsequent survival. For both the Pearson coefficient and Cox model calculations, the natural log of the peak absolute lymphocyte count was used because of significant skewing of peak lymphocyte data values. Overall and progression-free survival estimates were performed using the method of Kaplan and Meier (23). Data were most recently analyzed in December 2002, 29 months after enrollment of the first patient on study.

**RESULTS**

**Patients.** A total of 26 patients were registered on the study and underwent leukapheresis. Patient characteristics are listed in Table 1.

**T Cell Activation and Expansion in Vitro.** The Xcelerate Process was not initiated for one patient because the two leukapheresis products collected failed to meet sterility (Gram's stain negative) or cell-characteristic acceptance (CD4:CD8 ≥ 1) criteria. This patient was removed from the protocol. Production of Xcellerated T Cells for cycle 2 was unsuccessful for one patient because the final product did not meet CD3 purity release criteria (≥85%). This patient remained on the protocol. Production of Xcellerated T Cells for cycle 2 was unsuccessful for one patient because the final product did not meet CD3 purity release criteria (≥85%). This patient received neither the second cycle of Xcellerated T Cells nor additional IL-2. Thus 49 Xcellerated T Cell products were successfully produced from 25 patients. Table 2 summarizes the final Xcellerated T Cell profiles for products that were infused into patients. A mean
The Xcellerate Process expands T cells. The total number of cells in vitro was assessed at the initiation of the Xcellerate Process (day 0), and at days 3, 5, and 8. Shown are results for 49 Xcellerate Processes from 25 patients.

As a measure of their activity, the ability of Xcellerated T Cells to become restimulated with plate-bound anti-CD3 and anti-CD28 antibodies was assessed. Restimulated cells demonstrated strong induction of CD154 expression in both the CD4 and CD8 T-cell populations (Fig. 4).

Adverse Events. Twenty-five patients received the first cycle of treatment, and 24 of these patients also received the second cycle. Table 3 summarizes all of the adverse events with three or more occurrences and/or of Grade 3 severity, which were designated to be definitely, probably, or possibly related to protocol treatment. The majority of these events were mild-to-moderate in nature. Of the 25 patients who received Xcellerated T Cells and IL-2, 6 (24%) of 25 (95% CI, 9-45%) of the patients experienced at least one Grade 3 adverse event, judged to be at least possibly related to Xcellerated T Cell and IL-2 treatment. Further details of the Grade 3 adverse event are included in Table 3. No patient among the 25 experienced any Grade 4 adverse events; the estimated rate is 0% with a 95% CI of 0-14%.

Leukapheresis was well tolerated. Only two patients reported minor (Grade 1) adverse events (tingling of face and fingers, swelling and aching in hands) in connection with the procedure. These episodes ceased after treatment with calcium carbonate or calcium gluconate.

IL-2 was discontinued because of toxicity in three patients. One patient received 7 of the 10 planned doses of IL-2 in cycle 1, another patient received 7 of the 10 planned doses of IL-2 in cycle 2, and a third patient received 7 and 8 doses of IL-2 in his first and second cycles of therapy, respectively. The reasons for early discontinuation of IL-2 included cellulitis, pain, shortness of breath,

### Table 1 Baseline patient characteristics (n = 26)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Summary</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>Median (Range) 58 (45–78)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 19 (73%) Female 7 (27%)</td>
</tr>
<tr>
<td>Race</td>
<td>Caucasian 25 (96%) African American 0 (0%) Asian 0 (0%) Hispanic 0 (0%) Racially mixed 0 (0%) Other 1 (4%)</td>
</tr>
<tr>
<td>Performance status</td>
<td>0 (0%) 1 (62%) 2 (12%) 3 (12%)</td>
</tr>
<tr>
<td>Time from initial histological diagnosis to registration (mo)</td>
<td>0–6 13 (50%) 7–12 3 (12%) 13–18 0 (0%) 19–24 3 (12%) 25–30 4 (15%) &gt;30 3 (12%)</td>
</tr>
<tr>
<td>Metastatic sites</td>
<td>Bone 4 (15%) Brain/Central nervous system 0 (0%) Liver 2 (8%) Lung 17 (65%) Lymph nodes 3 (12%) Other 9 (35%)</td>
</tr>
<tr>
<td>Disease evaluability</td>
<td>Measurable disease 16 (62%) Evaluable disease only 10 (38%)</td>
</tr>
</tbody>
</table>

### Table 2 Cell product summary

<table>
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<tr>
<th>Xcellerated T Cell product (n = 49)</th>
<th>Mean</th>
<th>SD</th>
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<tbody>
<tr>
<td>Total nucleated cell count × 10⁹</td>
<td>21.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Total CD3⁺ count × 10⁹</td>
<td>20.5</td>
<td>5.3</td>
</tr>
<tr>
<td>%CD3⁺ (T lymphocytes)</td>
<td>93.8</td>
<td>3.3</td>
</tr>
<tr>
<td>%CD3⁺CD4⁺</td>
<td>63.9</td>
<td>11.8</td>
</tr>
<tr>
<td>%CD3⁺CD8⁻</td>
<td>25.0</td>
<td>11.7</td>
</tr>
<tr>
<td>CD4⁺/CD8 ratio</td>
<td>3.5</td>
<td>2.4</td>
</tr>
<tr>
<td>%CD19⁻CD20⁺ (B lymphocytes)</td>
<td>3.9</td>
<td>3.1</td>
</tr>
<tr>
<td>%CD14⁺ (monocytes)</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>%viability</td>
<td>95.0</td>
<td>3.1</td>
</tr>
</tbody>
</table>

(±SD) of 21.8 × 10⁹ (±5.4 × 10⁹) Xcellerated T Cells were administered each cycle. The cells were of high T cell (CD3⁺) purity (93.8% ± 3.3%) and viability (95.0% ± 3.1%). Both CD4 and CD8 T cell subsets expanded well; the mean CD4⁺/CD8 ratio increased from 2.6 ± 1.5 to 3.5 ± 2.4 following the Xcellerate Process. A five-log depletion of beads was achieved in the final products, with a mean of 3 × 10⁴ residual beads measured. For a 70-kg person, this represents 0.00006% of the dose of Dynabeads M-450 on a per-kg basis that have been safely infused in rats (24).
hyperkalemia, renal dysfunction, fever and chills, nausea, diarrhea, and induration and erythema at the injection sites.

Four patients received 50% dose reductions of IL-2. One patient received a dose reduction for the last four doses of cycle 2, and one for the fifth dose of cycle 1. A third patient received 50% of the planned IL-2 dose for the last six doses of cycle 2; this was the patient who had discontinued cycle 1 after seven IL-2 injections. A fourth patient received 50% of the planned IL-2 dose for the last four doses of cycle 1. IL-2 doses were reduced because of fever, chills, and gastrointestinal symptoms (cramping, diarrhea, constipation).

A marked elevation of HAMA titer occurred in two patients. In one patient, the HAMA titer increased from <20 ng/ml to 1,194 ng/ml at day 27 of cycle 2. The titer subsequently

**Fig. 3** The Xcellerate Process activates T cells. The activation of T cells was assessed by flow cytometry at days 0, 3, 5, and 8 of the Xcellerate Process for forward scatter [measure of cell size (A)], CD25 (IL-2 receptor) expression (B), and CD154 (CD40 ligand) expression (C). Secretion of cytokines in the culture supernatant was also assessed (D). Values plotted are mean ± 95% CI for 49 Xcellerate Processes (A–C) or for a subset of 10 (D).

**Fig. 4** Xcellerated T Cells express CD154 on restimulation. Xcellerated T Cells from day 8 were restimulated for 4 h with plate-bound anti-CD3 and anti-CD28 antibodies. CD154 expression was assessed by flow cytometry. Values plotted are mean ± 95% CI for 49 Xcellerate Processes.
returned to the normal range 10 months later. In a second patient, the HAMA titer increased from <20 ng/ml to 3,108 ng/ml at the 7-month follow-up visit. It had decreased to 1,400 ng/ml 8 months later. Six additional patients had slightly elevated HAMA titers (range, 28–158 ng/ml). No patients, including the two who had detectable HAMA levels at day 21 of cycle 1 (41 and 52 ng/ml), developed clinical signs or symptoms of significant allergic reactions during the second T cell infusion. Two patients had low TSH levels at the 8-week follow-up visit, and an additional three patients had elevated TSH levels (range, 2.7–17.0 mIU/liter) at the 3- or 7-month follow-up visits. No patients demonstrated clinical evidence of hyperthyroidism or hypothyroidism.

**In Vivo Immune Effects.** Fig. 5 shows the mean absolute lymphocyte and eosinophil counts for patients. Both lymphocyte and eosinophil counts increased significantly after each Xcellerated T Cell infusion, but most prominently after the second infusion. At the 7-month follow-up visit, mean lymphocyte counts remained significantly elevated compared with the values obtained at eligibility.

The total nucleated cells infused in cycle 1 correlated with the peak absolute lymphocyte count during that cycle, i.e., between the first and second infusion ($r = 0.58$, one sided $P = 0.001$). Correlation of the total nucleated cells infused in cycle 2 with the peak absolute lymphocyte count after infusion 2 was not statistically significant ($r = 0.26$, one sided $P = 0.11$). However, the number of cells infused in cycle 1 as well as the total number of nucleated cells infused in both cycle 1 and cycle 2 both correlated with the peak absolute lymphocyte count achieved in cycle 2 ($r = 0.35$, one-sided $P = 0.05$, and $r = 0.38$, one-sided $P = 0.03$, respectively). The higher peak lymphocytosis seen after cycle 2 infusion compared with after cycle 1 infusion (see Fig. 5A) would be consistent with the cells infused in cycle 1 persisting and influencing the second peak during cycle 2. The peak absolute lymphocyte counts achieved in cycle 1 and cycle 2 were highly correlated ($r = 0.72$, one sided $P < 0.001$).

A trend to increased postinfusion survival was noted in patients achieving higher peak absolute lymphocyte counts after the therapy. For each 2.7-fold (value of the base of the natural log) increase in the peak lymphocyte count achieved in cycle 1 and cycle 2, the death hazard was reduced by 39% (one sided $P = 0.14$) and 62% (one sided $P = 0.06$), respectively. A correlation between the number of cells infused and survival was not found.

**Clinical Outcomes.** All of the patients were evaluated for objective clinical response to protocol therapy. Two patients experienced radiographic regression of bone metastasis after the second cycle of therapy (Figs. 6, A and B). However, both patients had progression at other sites of metastatic disease and, therefore, did not meet criteria for a partial response or a CR.

The median progression-free survival from study entry is estimated to be 4 months (95% CI, 3–7 months). The median survival from study entry is estimated to be 21 months (95% CI, 14–23 months). The median follow-up in surviving patients is 26 months. Progression-free survival and overall survival data are provided in Fig. 7, A and B.

**DISCUSSION**

This Phase I trial demonstrates that Xcellerated T Cells can be reproducibly manufactured and administered. Forty-nine of 50 Xcellerated T Cell products (98%) were successfully manufactured from the 25 patients with adequate leukapheresis products. The T cells, expanded 66-fold ($\pm 18$) on average, maintained a high CD4:CD8 T-cell ratio (3.5 ± 2.4), and were of high purity (CD3$^+$ = 93.8% ± 3.3%). Consistent with potent activation, the T cells expressed high levels of CD25 and CD154 and also produced high levels of type-1 cytokines detected in the culture supernatant during the Xcellerate Process. Type-1 cytokine responses are considered to be important in immune responses directed against cancer (25). Furthermore, the Xcellerated T Cells were capable of reactivation on restimulation *ex vivo*, which suggests that they may be able to maintain their potency *in vivo*.

This clinical trial also demonstrates that the combination of Xcellerated T Cells and IL-2 can be administered safely to patients with metastatic RCC in an outpatient setting. The majority of patients were able to complete the planned course of therapy. Most adverse events were low-to-moderate in severity. Because Xcellerated T Cells and IL-2 were co-administered, it is not possible to definitively separate toxicity related to the Xcellerated T Cells from IL-2-related toxicity. However, no patients required IL-2 dose modifications because of treatment-related toxicity sooner than the 5th day of the treatment cycles, which suggests that there were no immediate dose-limiting toxicities related to the infusion of Xcellerated T Cells. It remains possible that the Xcellerated T Cells may have increased the subsequent IL-2 toxicities. However, the nature and severity of the toxicities observed were comparable with a similar regimen of IL-2 alone (26, 27).

Although two patients developed significantly elevated

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### Table 3 Most common adverse events

<table>
<thead>
<tr>
<th>Event</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthenia</td>
<td>13</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Fever</td>
<td>12</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Chills</td>
<td>14</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Injection site reaction</td>
<td>13</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>11</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Anorexia</td>
<td>11</td>
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</tr>
<tr>
<td>Rash</td>
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<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
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<td>2</td>
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<td>Insomnia</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Constipation</td>
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</table>

$^a$ Adverse events with three or more occurrences and/or of Grade 3 severity, which were designated to be definitely, probably, or possibly related to protocol treatment are listed.
HAMA titers, no symptoms indicating an allergic-type reaction were observed. Five patients (25%) had abnormal TSH levels during the treatment course, but none developed clinical evidence of hyperthyroidism or hypothyroidism. Thyroid dysfunction is well documented in patients receiving IL-2 therapy, occurring in >40% of patients in two large series (28, 29). Thus Xcellerated T Cells do not appear to increase the incidence of thyroid dysfunction beyond what would be anticipated from IL-2 therapy alone.

Xcellerated T Cells + IL-2 therapy led to a marked peripheral lymphocytosis that was greater after the second cycle of therapy. The lymphocyte count subsequently decreased to the normal range, but the mean lymphocyte count at the 7-month follow-up visit still remained significantly elevated compared with the count at the eligibility visit. The peripheral eosinophil counts increased after each cycle of therapy, similar to the lymphocyte counts. Both lymphocytosis and eosinophilia have been reported to occur during therapy with IL-2 (30–33). The degree of lymphocytosis varies with the dose and route of IL-2, so the contribution of Xcellerated T Cells to the lymphocytosis is difficult to determine based on previous reports. However, the lymphocytosis observed is significantly higher than with a comparable dose of s.c. administered IL-2 alone (31). The observation that the number of cells infused correlated with the patients’ peak absolute lymphocyte counts suggests that the infused T cells contributed to the lymphocytosis. The effect of Xcelle rated T Cells on peripheral lymphocyte counts will be better defined in an ongoing trial of this therapy in prostate cancer patients in which no IL-2 is being administered.

Although no patients achieved a complete or partial response, the responses observed in the bone metastases of two patients are noteworthy. Both patients also received pamidronate as palliative therapy for their bone metastasis. However, such striking regressions of bulky radiographically apparent bone metastases are uncommon with pamidronate alone; although bisphosphonates have been demonstrated to delay the progression of bone metastases in cancer patients, there is little evidence to suggest that bisphosphonates can promote the healing of such lesions (34, 35). Resolution of bone metastases with
IL-2 alone is also uncommon; bone lesions have been demonstrated to be an adverse prognostic variable in patients with metastatic RCC undergoing treatment with IL-2 (36). The healing of bone lesions in two of the four patients with bone involvement is, therefore, an unusual finding. Although the small number of tumor regressions observed in this study does not permit firm conclusions, one may hypothesize that Xcellerated T Cells + IL-2 may exert antitumor activity preferentially in bone. This could possibly result from preferential homing of the T cells to bone. In addition, activated T cells have been demonstrated to secrete factors, such as IFN-γ and receptor activator of nuclear factor-κB ligand, that have important effects on the process of bone remodeling (37). To further explore the possible role of activated T cells in bone healing, studies of Xcellerated T Cells in bone-tropic cancers including prostate cancer and multiple myeloma are in progress.

The median survival of 21 months observed in this trial compares favorably with the historical median survival of 1 year (38–41), as well as to the 11.1–17-month median survival found in the nephrectomized arms of recent randomized trials (42, 43). However, this was a small trial, and the favorable results could have been influenced by patient selection. The association between the peak of the absolute lymphocyte count achieved after the second infusion of T cells and subsequent survival, which approached statistical significance, is consistent with the Xcellerated T Cells + IL-2 therapy having an antitumor treatment effect. However, alternative explanations, such as the degree of lymphocytosis achieved serving as a marker for patients with a better prognosis or better underlying immune function, remain possible. These survival results as well as the bone effects observed in this trial warrant further investigation of activated T-cell therapy in RCC and other cancers.

ACKNOWLEDGMENTS

We thank P. Y. Liu for statistical consultation, and Susan Schulman for assistance in the preparation of the manuscript.

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A Phase I Trial of CD3/CD28-activated T Cells (Xcellerated T Cells) and Interleukin-2 in Patients with Metastatic Renal Cell Carcinoma

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