Phase I Trial of Interleukin 2 in Combination with the Soluble Tumor Necrosis Factor Receptor p75 IgG Chimera

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ABSTRACT
Our purpose was to determine the effective biological dose and/or maximum tolerated dose of recombinant human tumor necrosis factor receptor: IgG chimera (rhuTNFR:Fc; Immunex, Seattle, WA) in combination with interleukin 2 (IL-2) with regard to reduction in IL-2 toxicity and modulation of biological effects of high-dose IL-2 administration. Twenty-four patients with metastatic cancer were treated with escalating doses of rhuTNFR:Fc at 1, 5, 10, and 20 mg/m² i.v. on days 1 and 15 (dose levels 1–5) or 10, 20, and 30 mg/m² days 1 and 15 plus 50% dose on days 3, 5, 17, and 19 (dose levels 6–8) prior to IL-2 at doses of 300,000 IU/kg (dose level 1) and 600,000 IU/kg (dose levels 2–8) i.v. every 8 h on days 1–5 and 15–19. The median number of IL-2 doses was 24, and central nervous system, skin, and cardiac arrhythmias were the major dose-limiting toxicities. TNF bioactivity was inhibited, and the polymorphonuclear leukocyte chemotactic defect normally seen with IL-2 was not observed. Increases in C-reactive protein, IL-6, IL-8, and IL-1 receptor antagonist levels were partially suppressed relative to historical controls, whereas peripheral blood mononuclear cell phenotypes, urinary nitrate, endothelial adhesion molecule expression in skin biopsies, and cellular infiltrates in tumor biopsies were consistent with findings in patients treated with IL-2 alone. Four patients developed thyroid dysfunction. There were five responses: two complete responses (both melanoma) and three partial responses (response rate, 21%). rhuTNFR:Fc may modulate the toxicity and some of the biological effects of IL-2 while preserving antitumor activity. Dose level 6 (10 mg/m² on days 1 and 15, and 5 mg/m² on days 3, 5, 17, and 19) has been chosen for a randomized, double-blind, placebo-controlled trial of IL-2 with and without rhuTNFR:Fc.

INTRODUCTION

IL-2 is a potent immunomodulator with well-documented activity in renal cell carcinoma and melanoma, producing response rates of 15–25% with complete and durable responses in up to one-third of responders (1–6). Preclinical data indicate that response is dose dependent (7), and clinical trials seem to indicate that the best responses in humans are achieved with the highest doses (8). The high-dose regimen consisting of 600,000 IU/kg i.v. every 8 h on days 1–5 and 15–19 has been approved by the Food and Drug Administration as the treatment of choice in selected patients with metastatic renal cell carcinoma.

The utility of IL-2 has been limited by toxicity, much of which is indistinguishable from bacterial sepsis. This includes hypotension requiring pressor support, arrhythmias, myocardial infarction, pulmonary edema, fever, eosinophilia, anemia, thrombocytopenia, transient increase in bilirubin, oliguria, erythroderma, neurotoxicity, increased capillary permeability (9), catheter-related sepsis (10, 11), and, rarely, death (11). IL-2 also causes alterations in laboratory parameters similar to those seen in sepsis, including increases in hepatic acute phase proteins (12, 13), endothelial expression of leukocyte adhesion molecules such as ICAM-1 and E-selectin (14) and induction of IL-1, IL-15, TNF (15, 16), and NO (1, 19, 20) synthesis. There is considerable evidence that TNF plays a role in the morbidity of IL-2 therapy (21). Dexamethasone administered to patients receiving IL-2 blocks plasma TNF induction and prevents hypotension and fever, allowing for administration of three to four times the standard MTD (22).

The role of TNF in the antitumor activity of IL-2 is unknown. In one preclinical study, Fraker et al. (23) found that the combination of IL-2 and an anti-TNF antibody not only reduced toxicity, allowing more IL-2 doses to be administered, but also increased efficacy, eradicating tumors resistant to IL-2 alone. However, in a later study, the same investigators found that anti-TNF antibodies may have resulted in decreased survival in animals treated with IL-2 (24). The significance of these observations for IL-2 treatment of human malignancies is uncertain.

In this study, we performed a Phase I dose escalation of rhuTNFR:Fc (Immunex Corp. Seattle, WA), a chimeric mole-

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3 The abbreviations used are: IL, interleukin; ICAM, intercellular adhesion molecule; TNF, tumor necrosis factor; NO, nitric oxide; NOS, nitric oxide synthase; EBD, effective biological dose; MEBD, minimum EBD; MTD, maximum tolerated dose; rhuTNFR:Fc, recombinant human TNF receptor: IgG chimera; NEMC, New England Medical Center; CRP, C-reactive protein; IL-1Ra, IL-1 receptor antagonist; PBMC, peripheral blood mononuclear cell; CR, complete response; PR, partial response; MR, minor response; DLT, dose-limiting toxicity.
cule composed of two molecules of the extracellular domain of the p75 TNF receptor linked by an IgG Fc (25), in combination with high-dose IL-2. rhuTNFR:Fc has extremely high binding affinity relative to cell surface-associated TNF receptors and a longer half-life than the monomeric soluble TNF receptor (26). Treatment with rhuTNFR:Fc substantially improves survival in mice given otherwise lethal injections of TNF or lipopolysaccharide (27), and rhuTNFR:Fc in doses known to neutralize TNF activity does not diminish the antitumor activity of IL-2 in mice.** A Phase I dose escalation safety trial of rhuTNFR:Fc in healthy adults demonstrated no serious adverse effects in doses from 1–60 mg/m².** The objectives of this study were to determine the EBD of rhuTNFR:Fc in combination with IL-2, to determine if rhuTNFR:Fc reduced IL-2 toxicity, allowing administration of more doses of IL-2, and to determine the effects of rhuTNFR:Fc on clinical and biological parameters altered by high dose IL-2.

MATERIALS AND METHODS

Eligibility

Eligible patients had to have advanced carcinoma which was either refractory to standard therapy or for which no standard therapy was available, or have renal cell carcinoma, for which high-dose IL-2 is standard therapy. Patients were required to have measurable or evaluable disease, an Eastern Cooperative Oncology Group Performance status of 0 or 1 to have measurable or evaluable disease, an Eastern Cooperative Oncology Group Performance status of 0 or 1, and age. All patients over the age of 35 were required to pass an exercise treadmill test. Patients with evidence of congestive heart failure, symptoms of coronary disease, serious arrhythmias, evidence of past myocardial infarction on EKG, contraindications to the use of pressor agents, seropositivity for HIV by ELISA, history of organ allografts, brain metastases, seizure disorders, any significant medical disease other than malignancy, or who were likely to require corticosteroids for intercurrent disease, were excluded. Prior therapy was restricted to no more than two prior chemotherapy regimens and no more than two prior biological therapy regimens and had to be completed at least 4 weeks prior to beginning treatment on this protocol. At least 6 months had to have elapsed since any prior IL-2 therapy. This protocol was approved by the Investigational Drug Branch, Cancer Therapy Evaluation Program and the Biological Response Modifier Program, Division of Cancer Treatment, National Cancer Institute; the Food and Drug Administration; Immunex Corp. (Seattle, WA); Chiron Corp. (Emeryville, CA); and the NEMC Human Investigational Review Committee and Clinical Study Unit Advisory Committee. All patients were required to provide voluntary, written informed consent prior to participation in this study.

Treatment

IL-2 was provided by Chiron Corp. (Emeryville, CA) until January 1994, when commercially available drug was used (Chiron). It was provided as a lyophilized powder of 1 mg/5ml vial and was reconstituted with 1.2 ml sterile water for injection, then diluted to 50 ml total volume with 5% dextrose, USP. rhuTNFR:Fc was supplied by Immunex (Seattle, WA) as a lyophilized powder containing 10 mg rhuTNFR:Fc, 40 mg mannitol, USP, 10 mg sucrose, NF, and 1.2 mg Tris (trinemethane), USP, per vial. It was reconstituted with 1.0 ml bacteriostatic water for injection USP containing 0.9% benzyl alcohol and then further diluted to a total volume of 100 ml in 0.9% sodium chloride for injection USP.

All treatment was administered on an inpatient basis in the Biotherapy Unit located in the NEMC Clinical Study Unit, a setting capable of providing ICU level care. Patients with limited peripheral access had a central venous catheter placed under sterile conditions. IL-2 was administered as a 15-min i.v. infusion every 8 h on days 1–5 and 15–19, with a maximum of 14 doses during each week. rhuTNFR:Fc was administered as a single i.v. infusion over 30 min on day 1 prior to initiating IL-2 therapy (Table 1). After toxicity and pharmacokinetic analysis for dose level 5, the dosing schedule of rhuTNFR:Fc was changed so that for dose levels 6–8, rhuTNFR:Fc was administered on days 1, 3, and 5 and on days 15, 17, and 19. The following were administered to abrogate IL-2 toxicity: acetaminophen, indomethacin, ranitidine or cimetidine for prophylaxis of gastrointestinal bleeding, hydroxyurea hydrochloride or diphenyldramine for pruritis, and antiemetics and anti diarrheal agents as required. Oral ciprofloxacin was administered during treatment and through the week between admissions to prevent catheter-related sepsis. Corticosteroids were not permitted unless life-threatening symptoms developed. Patients were discharged when their side effects resolved sufficiently and were allowed to return home between the two courses of therapy. Patients were evaluated for response during weeks 7 and 11. Patients with minor, partial, or complete responses received a second cycle of therapy beginning at approximately week 12. Patients were eligible to receive a total of three cycles of treatment.

Study Design

Three patients were enrolled in each rhuTNFR:Fc cohort (Table 1), and a minimum of 2 patients in a cohort had to complete day 20 of their first cycle of therapy without DLT before any patients could be enrolled at the next dose level. There were no intrapatient dose escalations. The National Cancer Institute Common Toxicity Criteria were used to grade toxicity. DLT for the combination of IL-2 plus rhuTNFR:Fc was defined as intubation, myocardial infarction or ischemia (documented by EKG changes), life threatening sepsis, sustained ventricular arrhythmia or supraventricular arrhythmia causing hypotension and requiring cardiovascular, pericardial tamponade, bowel ischemia or perforation, renal dysfunction requiring dialysis, coma or other grade 4 neurotoxicity not reversible (to grade 2 or less) within 48 h of discontinuing treatment, or any other severe life-threatening toxicities (grade 4) not reversible (to grade 2 or less) within 72 h of stopping treatment. Because the toxicity of IL-2 is dose dependent and can be controlled by

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4 E. Trehu, unpublished data.
5 Consuelo Blosch, Immunex, personal communication.
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Table 1 Dose schedule

<table>
<thead>
<tr>
<th>Dose level</th>
<th>IL-2 (IU/kg) q8h × 5 days*</th>
<th>TNFR:Fc (mg/m²)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3 × 10⁵</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>6 × 10⁵</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>6 × 10⁵</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>6 × 10⁵</td>
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</tr>
<tr>
<td>5</td>
<td>6 × 10⁵</td>
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</tr>
<tr>
<td>6</td>
<td>6 × 10⁵</td>
<td>20(10/5/5)</td>
</tr>
<tr>
<td>7</td>
<td>6 × 10⁵</td>
<td>40 (20/10/10)</td>
</tr>
<tr>
<td>8</td>
<td>6 × 10⁵</td>
<td>60 (30/15/15)</td>
</tr>
</tbody>
</table>

* q8h, every 8 h; days 1–5 and 15–19; maximum of 14 doses per week.

Table 2 Patient characteristics

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Gender M/F</th>
<th>Performance status (ECOG)</th>
<th>Median age, years (range)</th>
<th>Prior therapy</th>
<th>Immunological (IL-2)</th>
<th>Chemotherapy</th>
<th>Radiation</th>
<th>Primary disease</th>
</tr>
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<tbody>
<tr>
<td>24</td>
<td>14/10</td>
<td>20/4</td>
<td>49 (23–68)</td>
<td>12 (5)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>16 Small cell cancer</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td>16 Cutaneous melanoma</td>
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<td></td>
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<td></td>
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<td>1 Ocular melanoma</td>
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<td></td>
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<td></td>
<td>1 Renal cell carcinoma</td>
</tr>
<tr>
<td>Sites of disease</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 Uterine cancer</td>
</tr>
<tr>
<td>One</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Ovarian cancer</td>
</tr>
<tr>
<td>Two</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Bladder cancer</td>
</tr>
<tr>
<td>Three</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Prostate cancer</td>
</tr>
</tbody>
</table>
| ≥Four           | 3          |                           |                           |               |                     |              |           | 1 | 3 | 1

* ECOG, Eastern Cooperative Oncology Group.

Fig. 1 rhuTNFR:Fc pharmacokinetics. The serum levels of rhuTNFR:Fc, as determined by ELISA, are shown for representative patients at dose levels 5–8. The 1/2 of rhuTNFR:Fc was 72 h, and a change in dosing from day 1 alone at dose level 5 to days 1, 3, and 5 with a 50% reduction of the day-1 dose administered on days 3 and 5 at dose levels 6–8 resulted in steady-state levels above 4000 ng/ml. Patient 5.1: DLT. patient 6.1: Δ, patient 7.1: ×, patient 8.1.

Table 3 IL-2 doses and DLTs per rhuTNFR:Fc dose level

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Mean no. of IL-2 doses (SD)</th>
<th>DLTs</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>27 (±2.0)</td>
<td>Hypotension, neurotoxicity</td>
</tr>
<tr>
<td>2</td>
<td>25 (±2.5)</td>
<td>Hypotension, neurotoxicity</td>
</tr>
<tr>
<td>3</td>
<td>23 (±1.5)</td>
<td>Diarrhea, neurotoxicity</td>
</tr>
<tr>
<td>4</td>
<td>21 (±5.9)</td>
<td>Atrial fibrillation, hypotension</td>
</tr>
<tr>
<td>5</td>
<td>25 (±2.3)</td>
<td>Neurotoxicity</td>
</tr>
<tr>
<td>6</td>
<td>27 (±2.3)</td>
<td>Hypotension</td>
</tr>
<tr>
<td>7</td>
<td>23 (±5.6)</td>
<td>Atrial fibrillation, neurotoxicity</td>
</tr>
<tr>
<td>8</td>
<td>21 (±4.5)</td>
<td>Hypotension, neurotoxicity</td>
</tr>
</tbody>
</table>

withholding doses of IL-2, the inability to receive ≥18 doses of IL-2 in a treatment cycle because of toxicity also constituted a DLT. Dose modification for IL-2 was accomplished by holding doses rather than reducing the dosage. Doses were held for serum bicarbonate less than 16 mmol/liter, grade 2 or worse cardiac dysrhythmias, dysfunction or ischemia, grade 3 pulmonary toxicity (requiring >2 liters O₂ nasal cannula to maintain O₂ saturation >90%), grade 1 neuro-cortical toxicity, any grade 3 toxicity tolerated poorly by the patient, or hypotension unresponsive to vasopressors or requiring both dopamine at ≥5 μg/kg/min and Neosynephrine. Treatment was restarted when toxicity returned to grade 2 or less, with the exceptions of cardiac and neurological toxicity where full recovery (grade 0) was required before restarting.

The MTD for rhuTNFR:Fc in combination with IL-2 was defined as the dose level below that at which at least three of six patients developed a DLT. In the absence of dose limiting toxicities sufficient to define the MTD, the plan was to halt dose escalation at one dose level beyond the MEBD or at a total rhuTNFR:Fc dose of 60 mg/m², which was the highest dose of rhuTNFR:Fc tested in Phase I studies in healthy subjects. The tentative MEBD of rhuTNFR:Fc to be administered in conjunction with the standard high-dose IL-2 regimen was defined as the dose at which two of three patients failed to develop an increase in day 3 serum CRP levels to >25% above baseline. At this level, the true MEBD was defined by the addition of at least two of the following biological parameters: plasma TNF bioactivity or IL-6 or IL-8 immunoactivity at 2 h < two times baseline, neutrophil chemotaxis <25% falloff relative to baseline, absence of endothelial adhesion molecule expression (ICAM and E-selectin), and urinary nitrate excretion <25% increase above baseline.

Clinical Assessments

Tumor measurements were performed by measuring the sum of the products of the longest diameter and the perpendicular diameter in the widest portion of each site of disease. Response assessment was made using standard criteria. CR was defined as disappearance of all clinical and laboratory evidence of disease for a minimum of 4 weeks. PR was defined as ≥50% decrease in the sum of the products of the perpendicular diameters of all measurable lesions for a minimum of 4 weeks without simultaneous increase in the size of any lesion or appearance of any new lesions. MR was defined as a 25–50% decrease in the sum of the products
Hypotension
- Requiring Pressors (<48 h)
- Persisting >48 h
Syncope
Renal
- Creatinine 2.5-5.0 x normal
- >5 x normal
- Oliguria (<300 ml in 8 h)
- Anuria >24 h
Gastrointestinal
- Nausea/vomiting
- Diarrhea (>7 stools/day)
- Bilirubin >1.5-3 x normal
- >3 x normal
- Alkaline phosphatase (> 5 x normal)
- Transaminases 5.1-20 x normal
- 20 x normal
Central nervous system
- Disorientation/somnolence
- Motor weakness
Cardiac
- Ventricular ectopy
- Ischemia
- Atrial fibrillation
- Elevated CPK
Weight gain >10% from baseline
Hematological
- Thrombocytopenia (<50,000/µl)
- Neutropenia (<0.9/µl)
Infection
- Requiring treatment
- Bacteremia
- Fever >40°C (104°F)
Rigors
Dermatological
- Symptomatic rash
- Exfoliative dermatitis

Table 4: Significant toxicity (grade 3 or 4) by dose level

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotension</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Persisting &gt;48 h</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Syncope</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyspnea at rest</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Renal</td>
<td>2.5-5.0 x normal</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>&gt;5 x normal</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Oliguria (&lt;300 ml in 8 h)</td>
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<tr>
<td>Gastrointestinal</td>
<td>Nausea/vomiting</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Diarrhea (&gt;7 stools/day)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>&gt;1.5-3 x normal</td>
<td>1</td>
<td>0</td>
<td>3</td>
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<td>2</td>
</tr>
<tr>
<td>&gt;3 x normal</td>
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<td>3</td>
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<td>3</td>
<td>1</td>
<td>1</td>
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<td>Alkaline phosphatase (&gt; 5 x normal)</td>
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<td>0</td>
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</tr>
<tr>
<td>Transaminases</td>
<td>5.1-20 x normal</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<td>&gt;20 x normal</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Central nervous system</td>
<td>Disorientation/somnolence</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>Motor weakness</td>
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<td>Ventricular ectopy</td>
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<td>2</td>
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<tr>
<td>Weight gain &gt;10% from baseline</td>
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<td>Hematological</td>
<td>Thrombocytopenia (&lt;50,000/µl)</td>
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<td>Infection</td>
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Table 5: Response

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<th>Tumor</th>
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* Response durations: CR, 24+ and 9 months; PR, 4, 5, and 13 months. CR/PR = 21%.

Laboratory Studies
CRP, routine laboratory tests, and thyroid studies including TSH, T₄, and anti-microsomal and anti-thyroglobulin antibodies were measured in the NEMC clinical laboratories.

rhuTNFR:Fc Pharmacokinetics. Serum levels of rhuTNFR:Fc were measured using the Human Soluble TNF Receptor II assay (R & D Systems, Minneapolis, MN), with a standard curve generated from serial dilutions of rhuTNFR:Fc. The levels of naturally occurring circulating TNFR p75 induced diameter of all measurable lesions without the development of new metastatic lesions. Progressive disease was defined as a 25% or greater increase in the sum of the products of the perpendicular diameters of all measurable lesions or development of new metastatic lesions.
Fig. 2. CRP levels. There was no dose-dependent difference in CRP levels, although at all levels of rhuTNFR:Fc, there was a delay in CRP rise until day 3 of treatment.

by IL-2 were too low to contribute to rhuTNFR:Fc level calculated with the commercial kit and were, therefore, not examined (28).

Secondary Cytokine Levels. Plasma levels of TNF, IL-6, IL-8, and IL-1RA were measured by RIA as described previously (29–32). Radioactive cytokines for these RIAs were obtained from Dupont NEN (Cambridge, MA). TNF bioactivity was measured by the L929 fibroblast cytotoxicity assay (33), using recombinant human TNF-α (Genzyme, Cambridge, MA) as a standard.

PBMC Immunophenotyping. Whole blood was collected in EDTA-containing tubes and was incubated immediately at room temperature with FITC-conjugated primary antibodies to CD3, CD4, CD8, CD14, and CD19, and phycoerythrin-conjugated primary antibodies to CD16, CD25, CD56, and HLA-DR (Becton Dickinson, San Jose, CA). RBCs were then lysed with FACS Brand Lysing Solution (Becton Dickinson), the tubes were centrifuged, and the pellets were resuspended in 0.1% azide. After repeat centrifugation, the pellets were resuspended in 1% paraformaldehyde in PBS and assayed in a Becton Dickinson FACS scanner, gated for the mononuclear cell population.

Urinary Nitrate. Beginning 3 days prior to admission for therapy with IL-2 plus rhuTNFR:Fc and continuing through the first week of treatment, patients were placed on a low nitrate diet. Twenty-four-h urine samples were obtained prior to treatment, days 2–3 of treatment, and days 6–7. A 10-mll fraction was obtained for nitrate measurement, and the remainder was analyzed for creatinine in the NEMC clinical laboratory. Samples were incubated with nitrate-reducing Escherichia coli in an ammonium formate buffer as described by Bartholomew (34). The suspension was added to the Greiss reagent (1% sulfanilamide, 0.5% naphthylethlyenediamine dihydrochloride, and 2.5% phosphoric acid; Ref. 35), and the absorbance at 550 nm was measured in a microplate reader. The concentration of nitrite was determined by least squares linear regression analysis of a standard curve generated with sodium nitrate standards. The amount of urinary nitrite was expressed as the ratio of nitrite to creatinine to allow for the decrease in creatinine clearance that occurs during IL-2 therapy. E. coli were obtained from the NEMC Clinical Microbiology Laboratory.

Neutrophil Chemotaxis. Whole blood was obtained from patients at dose level 8 on day 5 of therapy, and the chemotaxis of neutrophils in response to formylmethionyl-leucylphenylalanine and zymosan was measured as described previously (36).

Immunohistochemistry. Skin biopsies were obtained from patients at dose level 8 prior to starting therapy and on day 5. After local anesthesia with 1% Xylocaine, a 3-mm punch biopsy of forearm skin was obtained. Specimens were placed in OCT medium (Tissue Tek, Elkhart, IN) and snap frozen in acetone and dry ice. Four-μm cryostat sections were prepared on gelatin-coated slides and analyzed by immunohistochemistry using avidin-biotin (ABC) immunoperoxidase techniques (37, 38). Tissue sections were fixed in acetone, then sequentially incubated at room temperature with primary murine monoclonal antibodies against ICAM and E-Selectin (Boehringer-Ingelheim, Litchfield, CT), biotinylated second-step antibody, and an avidin-biotin-peroxidase complex (Vectastain ABC Kit; Vector Labs, Burlingame, CA). Slides were rinsed in PBS, stained with diaminobenzidine, and counterstained with 2% aqueous methyl green. Tumor biopsies were obtained from selected patients with s.c. or nodal metastases amenable to biopsy and were snap frozen and stained as above using primary antibodies to CD4, CD8, CD14, CD56, and HLA-DR (Biosource International, Camarillo, CA).

Statistics. To determine the effect of rhuTNFR:Fc on various parameters of IL-2 toxicity, standard inferential statistical analyses of the data were performed. However, given the exploratory nature of this study and the small sample sizes, the focus of statistical exploration was the collection of data and generation of hypotheses.
Fig. 3 Secondary cytokines. The levels of IL-6, IL-8, and IL-1RA are shown for dose levels 2–8. There was a dose-dependent suppression of IL-6 induction later in the week of treatment and suppression at all dose levels on day 1. There was suppression of IL-8 induction at all dose levels, particularly at day 1, time = 4 h, when IL-8 usually peaks in response to IL-2 therapy. The increase in IL-1RA typically seen with IL-2 treatment was delayed.
RESULTS

Patients

Twenty-four patients were treated at eight different dose levels. Characteristics of the patients are listed in Table 2. Most of the patients had renal cell carcinoma. The patient population differed from those of most high-dose IL-2 trials in that one-half had received prior immunotherapy, including IL-2. A majority of the patients had multiple sites of tumor involvement.

rhuTNFR:Fc Pharmacokinetics

Pharmacokinetic data for rhuTNFR:Fc from representative patients treated at dose levels 5–8 are shown in Fig. 1. At dose levels 5 and below, rhuTNFR:Fc t1/2 was 72 h, similar to that in patients receiving rhuTNFR:Fc alone, resulting in a decline in serum levels by day 4. At dose levels 6–8, the administration of a reduced dose of rhuTNFR:Fc on days 3 and 5 resulted in serum levels consistently above 4000 ng/ml with a dose-dependent increase in peak levels to 10,000 ng/ml at dose level 8.

Toxicity

The median number of IL-2 doses administered to the 21 patients receiving full-dose IL-2 (600,000 IU/kg i.v. every 8 h) was 24 (Table 3). Grade 3 and 4 toxicities for all 24 patients are listed in Table 4. The most common DLT was neurotoxicity, which occurred in 16 patients, 2 at each dose level. Hypotension requiring pressors occurred in at least two of three patients at each dose level from 1–5 but in only one of three patients at each dose level from 6–8. Atrial fibrillation occurred in three patients, and an elevated creatine phosphokinase, indicating subclinical myocarditis, was present in five patients, predominantly at dose levels 4 and 7. Capillary leak, as represented by weight gain and edema, appeared to be reduced at the highest dose levels, but two patients at dose level 8 had dyspnea. Renal and gastrointestinal toxicities were similar at all dose levels. At 60 mg/m², the highest dose of rhuTNFR:Fc tested both in healthy subjects and in the IL-2-treated patients in this study, no toxicity was observed that could be attributed to the addition of the TNFR:Fc; therefore, the MTD of TNFR:Fc in combination with IL-2 cannot be defined by this study.

Response

There were two CRs of 24+ and 9 months duration, both in patients with melanoma (Table 5). There were three PRs, two in renal cell carcinoma of 4 and 13 months duration, and one in melanoma lasting 5 months. In addition, 5 of the 15 renal cell carcinoma patients had MRs and 2 had stable disease. The three partial responders had metastatic disease limited to the lungs, and one of the two complete responders had only s.c. disease. All responders received 23 or more doses of IL-2.

Thyroid Dysfunction

Thyroid dysfunction occurred in four patients at dose levels 2, 4, 5, and 6 and was clearly autoimmune in three (dose levels 2, 4, and 6), with elevated anti-microsomal antibody titers at 4 weeks after treatment. Three patients developed an elevated TSH and low T4 at 8 weeks, which was preceded in two patients by a transient increase in T4 at four weeks; the fourth patient’s TSH rose by 4 weeks after treatment. The patient at dose level 2 had a PR, and patients at dose levels 4 and 5 had MRs.

Laboratory Studies

CRP. The levels of CRP are shown in Fig. 2. CRP was predicted to be a sensitive indicator of TNF inhibition and was intended as the major criteria for definition of the EBD. CRP elevation was observed by day 3 in all patients, regardless of the rhuTNFR:Fc dose.

Secondary Cytokines. Fig. 3 shows the levels of IL-6, IL-8, and IL-1RA at dose levels 2–8. IL-6 and IL-8 induction were suppressed in a dose-dependent fashion compared to prior findings in patients treated with IL-2 alone (39, 40), particularly noticeable at dose levels 6–8 on day 1. IL-1RA induction appeared to be delayed compared to that seen in IL-2-treated patients (28). TNF immunoreactivity was significantly increased in a dose-dependent fashion as expected, since the assay does...
not distinguish between bound and free TNF. Despite these extreme elevations in TNF levels by RIA, functional assays revealed almost complete inhibition of TNF bioactivity (Fig. 4).

PBMC Immunophenotyping. The pattern of PBMC subsets pretreatment and on day 2 of therapy in the three patients at dose level 8 is shown in Fig. 5. Lymphocytes (CD3, CD4, CD8, and CD19) and natural killer cells (CD16 and CD56) disappeared from the circulation immediately.

Urinary Nitrate. Urinary nitrate excretion at dose levels 7 and 8 was elevated in three patients by day 3, and by day 7 in the other three patients, consistent with findings in IL-2-treated patients (19).

Neutrophil Chemotaxis. The neutrophil chemotactic defect previously reported in patients receiving high-dose IL-2 (36) was not observed in any of the three patients at dose level 8 (Fig. 6).

Endothelial Adhesion Molecule Expression. Expression of both ICAM and E-selectin on dermal capillaries and basilar keratinocytes was markedly increased on day 5 skin biopsies compared to pretreatment in all three patients at dose level 8 (Fig. 7).

Tumor Immunohistochemistry. Tumor biopsies were obtained before and after treatment on one patient at dose level 2 and two patients at dose level 5. The pretreatment biopsy from patient 2.3, who had a MR with subsequent progression, stained positively for CD4, CD8, CD14, CD56, and HLA-DR before treatment, but the posttreatment biopsy was positive only for CD4 and CD14. The biopsy from patient 5.3, who had progressive disease, was minimally positive for HLA-DR pretreatment and minimally positive for CD14 and HLA-DR after treatment. The biopsy from patient 5.2, who had a CR, showed minimal staining for HLA-DR alone before treatment but was positive for CD4 and CD8 and markedly positive for CD14 and HLA-DR after treatment.

DISCUSSION

This study demonstrates that circulating TNF bioactivity can be neutralized by rhuTNFR:Fc in patients receiving high-dose IL-2 therapy without eliminating the antitumor effect of IL-2. rhuTNFR:Fc may also decrease the toxicity of IL-2, allowing more doses of IL-2 to be administered. It also provides evidence that some of the biological phenomena observed during IL-2 therapy, including induction of IL-6, IL-8, IL-1RA, and defective neutrophil chemotaxis, may be mediated through TNF. In addition, it shows that some other effects of IL-2, such as induction of autoimmune thyroiditis, lymphocyte and natural killer cell trafficking as reflected by their disappearance from the peripheral blood, NO production, and endothelial adhesion molecule expression may not be dependent on circulating TNF. Despite the fact that the MEBD of TNFR:Fc in combination with IL-2 was not reached as defined at the outset of the study, we feel that the clinical and immunological effects observed in this study deserve further evaluation.
It inhibits the adherence of neutrophils to and also reversed the neutrophil chemotactic defect seen with TNF as an early intermediary in the induction of these cytokines. Inhibition of TNF by rhuTNFR:Fc in this study is responsible for reversal of the neutrophil chemotactic defect. Alternatively, this may represent a direct effect of suppression of circulating TNF bioactivity.

Although administration of rhuTNFR:Fc appeared to reduce the degree of hypotension in these IL-2-treated patients relative to historical controls, it did not eliminate hypotension and did not affect urinary nitrate and nitrite excretion. The hypotension induced by bacterial endotoxin, TNF, and IL-2 is believed to be due to endogenous NO production by the endothelium (44–50), resulting in vasodilatation. NO is derived from the oxidative deamination of arginine by the enzyme NOS. Once formed, NO is an unstable metabolite rapidly converted to nitrite (NO₂⁻) and nitrate (NO₃⁻). Kilbourn et al. (45) have shown that TNF-induced hypotension can be reversed by the NOS inhibitor NMA (45). Hibbs et al. (19) have demonstrated a marked increase in the urinary excretion of arginine-derived nitrate and nitrite in patients undergoing intensive IL-2 treatment. Our results suggest that factors other than circulating TNF are responsible for NO production in response to IL-2 and that the hypotension caused by IL-2 may be multifactorial.

Endothelial adhesion molecule expression after treatment with IL-2 and rhuTNFR:Fc in this study was similar to that seen with IL-2 alone. Egress of cytolytic cells from the microvasculature into tumor tissue is critical to their tumoricidal activity, and adhesion to activated endothelium is a necessary first step. Expression of ICAM-1 and E-selectin in IL-2-treated patients may play a role in the trafficking of tumoricidal leukocytes (14). In vitro, cultured endothelial cells increase their expression of ICAM-1 and express E-selectin in response to IL-1 and TNF, but not IL-2 (14), implicating an intermediary cytokine in vivo. The increase in E-selectin and ICAM-1 on the dermal capillaries of patients in this study receiving IL-2 and rhuTNFR:Fc, together with similar findings in patients treated with IL-2 and dexamethasone (22), suggests that high levels of circulating TNF are not required for IL-2 related endothelial adhesion molecule expression.

Tumor biopsies were analyzed on three patients in this study, and there was an impressive increase in T-cell and macrophage infiltration and tumor cell HLA-DR expression in the patient who achieved a CR at dose level 5, which was not seen...
in the two nonresponders. In patients treated with IL-2, Rubin et al. (51) have reported positive correlations between response and posttreatment tumor HLA-DR expression and infiltration by T-cells and macrophages. Since IL-2 does not directly induce HLA-DR, a secondary cytokine such as TNF has been implicated as the intermediary (51, 52). Others have suggested that macrophages play an important role in the tumoricidal activity of IL-2 through an IFN-γ-mediated pathway (53). The finding of HLA-DR induction, infiltration by T cells and macrophages, and CR in a patient treated with rhuTNFR:Fc suggests that TNF may not be an absolute requirement for the local antitumor immunological effects of IL-2.

The results of this Phase I study suggest that some of the toxic and biological effects of IL-2 may be reversed by inhibition of circulating TNF activity with rhuTNFR:Fc, without eliminating the antitumor activity. These findings will be reassessed in a randomized, double-blind, placebo-controlled trial of IL-2 plus or minus rhuTNFR:Fc. The MEBD as defined at the outset of this study was never reached, but toxicity and immunological effects were similar at the top three dose levels; therefore, dose level 6 of rhuTNFR:Fc, consisting of 10 mg/m² on day 1 followed by 5 mg/m² days 3 and 5, was chosen for the next study.

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