Antitumor Efficacy of Interleukin-2 Alone and in Combination with Adriamycin and Dacarbazine in Murine Solid Tumor Systems

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ABSTRACT

Recombinant interleukin-2 (IL-2)/chemotherapy combinations have recently entered clinical trial. The rationale for sequencing has primarily been empiric or based on in vitro data. To establish in vivo models for chemotherapy trials, we investigated IL-2 alone and in combination with dacarbazine (DTIC) and Adriamycin. IL-2 (as a single agent given i.v. at 1-3 x 10^6 Cetus units once daily for 5 days, repeated 7-10 days later), was highly active against an immunogenic line of colon adenocarcinoma no. 11/A [tumor growth inhibition (T/C) = 0% with cures]. It was modestly active against colon adenocarcinoma no. 38 (T/C = 39%), mammary adenocarcinoma no. 16/C (T/C = 18%), and B16 melanoma (T/C = 21%). IL-2 was inactive against colon adenocarcinoma no. 7/A (T/C = 83%). Combination trials were done using DTIC and IL-2 against colon no. 7/A and upstaged colon no. 11/A. The combination of Adriamycin and IL-2 was tested against mammary adenocarcinoma no. 16/C. In the DTIC/IL-2 combination trials, the combination was superior over either agent used alone. In the IL-2/Adriamycin trials, the combination was no better than Adriamycin alone at optimum dosages.

INTRODUCTION

With the wider availability of IL-2 made possible by recombinant DNA technology, reports of clinical trials utilizing this lymphokine are increasing (1-3). IL-2 used as a single agent has shown activity both preclinically and clinically against a wide spectrum of tumors (4-9). Several preclinical trials have combined IL-2 with cytotoxics and demonstrated synergy (8-14).

The mechanism of the reported synergy between IL-2 and chemotherapeutic agents is speculative. Synergy may be due to: (a) expansion and increased activity of immune effector cells; (b) IL-2 stimulated production of cellular products that can alter the susceptibility of tumor cells to cytotoxic agents; or (c) alteration of vascular permeability increasing drug delivery to tumor sites (15).

We have evaluated the antitumor activity of IL-2 used alone and sequentially with cytotoxics to discern if combination IL-2/chemotherapy was more efficacious than either agent alone. These preclinical trials were precipitated by clinical protocols at our institution utilizing DTIC and IL-2 in combination for the treatment of malignant melanoma. Using a sequence schedule similar to that utilized in the clinical protocol, we investigated both DTIC and Adriamycin in combination with IL-2 in our murine solid tumor models. Except for one model (a subline of colon no. 11/A), these models historically are nonimmunogenic. Colon no. 11/A met the following criteria for an immunogenic tumor: (a) poor take rate upon rechallenge (only 75% of mice grew tumors upon rechallenge); (b) very delayed doubling time after tumor rechallenge (doubling time approached twice the normal); and (c) initial growth with subsequent regression of established tumor. Treatment was evaluated by measurement of tumors at the primary implantation site in early- and advanced-stage disease.

Mice

Inbreds (C57BL/6, C3H/He, and BALB/c), hybrids [B6D2F1, (C57GL/6 females x DBA/2 males), and C57F1, (BALB/c females x DBA/2 males)] mice were bred in-house from strains obtained from the Frederick Cancer Research Facility, Frederick, MD.

Tumors

The following transplantable solid tumors of mice were used for in vivo testing: colon adenocarcinomas no. 07/A (17), no. 11/A (17), and no. 38 (17-20), mammary adenocarcinoma no. 16/C (21) and B16 melanoma (21). All tumors are in the DTP frozen tumor repository, maintained by the Biological Testing Branch, Frederick, MD. Each has a detailed description, code identification number, and list of references at the National Tumor Repository.

Tumors were maintained in the mouse strain of origin and were transplanted in the appropriate F1 hybrid (or the strain or origin) for therapy trials. All mice weighed over 17 g at the start of therapy; the range of individual body weights in each experiment was within 2 g. The mice were supplied food and water ad libitum.

Antitumor Agent

Highly purified recombinant human IL-2 from Escherichia coli was supplied by Cetus Corporation, Emeryville, CA; 1.2 ml of distilled water was added to each vial of 1.2 mg (18 x 10^6 IU/mg; 3 x 10^7 CU/mg); 5% dextrose water was the diluent utilized for additional dilution. The drug was utilized within 72 h of preparation. The product was stored at 42°F. IL-2 was administered i.v. in all trials. DTIC was purchased from Miles Pharmaceuticals. The drug was diluted with distilled water. Additive agents were accounted for in the weight of the product. DTIC was administered p.o. and utilized within 6 h of preparation. The drug was stored at 42°F. Adriamycin was purchased from Adria Pharmaceuticals. The drug was diluted with distilled water. Adriamycin was administered i.v. and utilized within 1 h of preparation. In all combination trials, chemotherapy invariably preceded IL-2.

In Vivo Studies

Biological and Chemotherapy

The methods of protocol design, tumor transplantation, drug treatment, endpoint determination, definition of terms, toxicity evaluation, data analysis, quantification of tumor cell kill, and the biological significance of the drug treatment results with transplantable tumors have been presented (18-22). The following is a brief summary of those methods as they apply to the work described.
Because of limited supplies of IL-2 and to help ensure a more uniform tumor burden per mouse (thus reducing the requirement for greater numbers of mice per group), bilateral tumor implants were used. The animals necessary to begin an experiment were pooled, given s.c. bilateral implants on day 0 with 30- to 60-mg tumor fragments using a 12-gauge trocar, and again pooled before randomization to the various treatment and control groups. Chemotherapy was either started within 3 days after tumor implantation while the number of cells per mouse was relatively small (1 × 10^5 to 1 × 10^6 cells), or allowed to grow to palpation (2 to 4 × 10^6 cells) in a more advanced stage trial.

Tumors were measured with a caliper 1 to 3 times weekly (as needed) until tumors exceeded 1600 mg or cure was guaranteed. Tumor weights were estimated from 2-dimensional measurements:

\[ \text{Tumor weight (mg)} = a \times b^2/2 \]

where \( a \) and \( b \) are tumor length and width (mm), respectively.

**End Points for Assessing Antitumor Activity**

The following quantitative endpoints were used to assess antitumor activity.

**Tumor Growth Delay.** T-C value, where T is the median time (in days) required for the treatment group tumors to reach a predetermined size, and C is the median time (in days) for the control group tumors to reach the same size, was determined. Tumor-free survivors were excluded from these calculations (cures were tabulated separately).

**Calculation of Tumor Cell Kill.** For s.c. growing tumors, the log_{10} cell kill was calculated from the following:

\[ \log_{10} \text{cell kill (total)} = \frac{T-C}{(3.32)(T_D)} \]

where T-C is the tumor growth delay (in days) as described above, and \( T_D \) is the tumor volume doubling time (in days), the latter estimated from the best straight line from a log-linear growth plot of the control-group tumors in exponential growth (range, 500–1500 mg). The conversion of the T-C values to log_{10} cell kill is possible because the \( T_D \) of tumors regrowing after treatment approximated the \( T_D \) values of the tumors in untreated control mice.

**Determination of Activity by Tumor Growth Inhibition (T/C Value).** This is the most universally used method for the determination of antitumor activity and is the method used by the DTP and National Cancer Institute for early-stage disease.

Measurement takes place simultaneously in both the treatment and control groups. When the control group tumors reached approximately 750–1500 mg in size (median of group), the median tumor weight of each group was determined (including zeros). The T/C value as a percentage is an indication of antitumor effectiveness. A T/C <42% is considered significant antitumor activity by the DTP. A T/C value <10% is considered high antitumor activity and is the level used by the National Cancer Institute to justify further development if other requirements are met (termed DN-2 level activity).

A weight loss nadir of 20% per mouse or greater (mean of group) or 20% or more drug-deaths is considered an excessively toxic dosage. Animal body weights included the weights of tumors.

**RESULTS**

IL-2 was tested against several syngeneic transplantable murine solid tumors to determine its single-agent efficacy. Synergy of IL-2 with 2 cytotoxic agents (adriamycin and DTIC) was also determined. The cytotoxic agents in each trial historically had significant T/C values against the tumor utilized.

**Efficacy of Single-Agent IL-2 against Transplantable Solid Tumors:** Mammary No. 16/C (i.v. Efficacy Trial). Early-stage mammary no. 16/C was treated (day 1), \( T_D = 1.2 \) days with i.v. administered IL-2 using a once daily for 6 days schedule. Response of this model to i.v. IL-2 yielded a T/C of 18% (day 10; Table 1) at a MTD of 1.8 × 10^6 CU (30 mg/kg). At the highest dose, a LD_{50} occurred. At the next lower dose (TD = 1.2 × 10^6 CU), there were no toxicities and a T/C of 20% was obtained (day 10). The log_{10} cell kill was 0.8 at these 2 dosages.

Colon Adenocarcinoma No. 07/A (i.v. Efficacy Trial). Colon no. 07/A was treated with single-agent IL-2 in both early and upstaged disease (i.e., a point at which the tumor in all treated mice is palpable but is <200 mg consistently). In the early staged groups, i.v. IL-2 was administered on days 3–7 and days 13–17 at 1 × 10^6 CU to 3 × 10^6 CU/injection for a TD of 1 × 10^6 to 3 × 10^6 CU. At all dose levels, IL-2 was ineffective against colon no. 07/A early staged disease with a minimum T/C at the highest dose of 83% (Table 2).

Colon no. 07/A was then upstaged. In the upstaged disease, all tumors were palpable but the median tumor size was not >200 mg. Mice were treated on days 7–11 and days 17–21 with the same dosages of i.v. IL-2. At the MTD (TD 3 × 10^6 CU), a T/C of 77% was obtained (Table 2). Thus, colon no. 07/A, upstaged, was equally unresponsive to i.v. IL-2 as colon no. 07/A early staged disease.

Colon Adenocarcinoma No. 38 (i.v. Efficacy Trial). IL-2 was administered to colon no. 38-implanted mice with early staged disease (day 3 after tumor implant) on a once daily for 5 days schedule at dosages ranging from 1 × 10^5 CU/injection to 3 × 10^5 CU/injection (1.26 mg/kg/injection to 4.2 mg/kg/injection; TD = 5.0 × 10^5 CU/injection to 1.5 × 10^6 CU/injection). The MTD was 1.5 × 10^6 CU, which yielded a T/C of 39% (marginal activity) with a log cell kill value of 0.43 (Table 3). There were no drug deaths at this dose.

B_{16} Melanoma (i.v. Efficacy Trial). Early-staged B_{16} melanoma was treated with single-agent IL-2 on days 1–6 after tumor implantation (Table 4). Dosages of i.v. IL-2 of 1 × 10^5 CU/injection to 3 × 10^5 CU/injection were administered. TD of IL-2 given ranged from 6 × 10^5 CU to 1.8 × 10^6 CU. At the MTD (1.8 × 10^6 CU) a T/C of 21% occurred, however, the log cell kill was 0.2 (Table 4). At the MTD, a LD_{50} occurred.

**Efficacy Studies with an Immunogenic Tumor**

Colon No. 11/A (i.v. Efficacy Trial). Treatment of an immunogenic subline of colon no. 11/A with single-agent IL-2 was begun on day 17, at a time when the tumor was upstaged. Dosages of single-agent IL-2 were 3 × 10^5 CU/injection (3.98 mg/kg/injection) and 1 × 10^6 CU/injection (1.2 mg/kg/injection) for a TD of 3 × 10^6 and 1 × 10^6 CU (Table 5). The mice were treated on days 17–21 and 31–35. At the highest dose, 1 of 6 mice (LD_{50}) died from drug toxicity. This death occurred on day 36 of the trial. No other drug-related deaths were noted. A T/C of 0% was obtained with both doses of IL-2. At day 180, there were 3 of 5 tumor-free survivors in the top dose and 1 of 7 tumor-free survivors at the 1 × 10^6 CU TD. Thus, this immunogenic subline of colon no. 11/A was very responsive to single-agent IL-2. Initial rechallenge of these mice yielded a 75% take rate (normal take rate for this subline of colon no. 11/A is 98–100%).

**Combination Chemotherapy/IL-2 Studies**

Colon Adenocarcinoma No. 07/A (DTIC/IL-2 Synergy Trial). DTIC and IL-2 were used in combination in the treatment of colon no. 07/A. In the single-agent arms, colon no. 07/A was totally unresponsive to IL-2 but was markedly responsive to DTIC (Table 6). A T-C and log cell kill calculation were used for synergy determination.

With single-agent DTIC (66 mg/kg every 2 h for 3 days, administered on days 3 and 15), a T-C of 20 days was obtained. When this same dose of DTIC was used in combination with the highest dose of IL-2, a LD_{50} occurred. At the next lower dose (TD = 1.2 × 10^6 CU), there were no toxicities and a T/C of 20% was obtained (day 10). The log_{10} cell kill was 0.8 at these 2 dosages.
Table 1: Treatment of early-stage mammary adenocarcinoma no. 16/C with i.r. administered IL-2

<table>
<thead>
<tr>
<th>CU/injection</th>
<th>Schedule</th>
<th>Total dose (CU)</th>
<th>Mean body weight change</th>
<th>Drug deaths</th>
<th>Median tumor burden day 10</th>
<th>T/C (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>d 1-6</td>
<td>1.8 x 10⁶</td>
<td>+0.5</td>
<td>d 6</td>
<td>4988</td>
<td>2180–9082</td>
<td>18</td>
</tr>
<tr>
<td>300,000</td>
<td>d 1-6</td>
<td>1.2 x 10⁶</td>
<td>+0.8</td>
<td>d 6</td>
<td>985</td>
<td>383–2921</td>
<td>20</td>
</tr>
<tr>
<td>100,000</td>
<td>d 1-6</td>
<td>6 x 10⁵</td>
<td>+0.6</td>
<td>d 6</td>
<td>1184</td>
<td>221–1484</td>
<td>23</td>
</tr>
</tbody>
</table>

* Weight change between days 1 and 6.

Table 2: Treatment of early- and advanced-stage colon adenocarcinoma no. 7 with i.V. administered IL-2

<table>
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<th>CU/injection</th>
<th>Schedule</th>
<th>Total dose (CU)</th>
<th>Mean body weight change</th>
<th>Drug deaths</th>
<th>Median tumor burden on day 10</th>
<th>T/C (%)</th>
<th>Comments</th>
</tr>
</thead>
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<tr>
<td>Early stage</td>
<td>Control</td>
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<td>+2.4</td>
<td>d 8</td>
<td>1082</td>
<td>540–1637</td>
<td></td>
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<tr>
<td>300,000</td>
<td>d 3-7</td>
<td>3.3 x 10⁶</td>
<td>+1.4</td>
<td></td>
<td>901</td>
<td>196–1001</td>
<td>83</td>
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<tr>
<td>200,000</td>
<td>d 3-7</td>
<td>2.2 x 10⁶</td>
<td>+1.4</td>
<td></td>
<td>1951</td>
<td>888–2081</td>
<td>&gt;100</td>
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<tr>
<td>100,000</td>
<td>d 3-7</td>
<td>1.1 x 10⁶</td>
<td>+1.2</td>
<td></td>
<td>1058</td>
<td>351–2448</td>
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* IL-2: (Cetus) Log LP-377. Drug preparation: white crystalline powder 3.6 x 10⁶ CU (1.2 mg)/vial; prepared with 1.2 ml of distilled H₂O, then diluted with 5% dextrose water to appropriate concentration, stable solution. CDF; males; average weight = 25.8 g; born 2/7/88; trocarred 5/13/88; colon adenocarcinoma no. 7, passage no. 36. * Weight change between days 3 and 24.

Table 3: Treatment of early-stage colon adenocarcinoma no. 38 with i.v. administered IL-2

<table>
<thead>
<tr>
<th>CU/injection</th>
<th>Schedule</th>
<th>Total dose (CU)</th>
<th>Mean body weight change</th>
<th>Drug deaths</th>
<th>Median tumor burden on day 10</th>
<th>T/C (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>d 1-6</td>
<td>1.8 x 10⁶</td>
<td>+0.3</td>
<td>d 8</td>
<td>1342</td>
<td>126–2628</td>
<td></td>
</tr>
<tr>
<td>300,000</td>
<td>d 3-7</td>
<td>1.5 x 10⁶</td>
<td>−0.5</td>
<td></td>
<td>525</td>
<td>32–992</td>
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<td>573–1965</td>
<td>91</td>
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<td>100,000</td>
<td>d 3-7</td>
<td>5 x 10⁵</td>
<td>−0.2</td>
<td></td>
<td>636</td>
<td>172–1175</td>
<td>47</td>
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* IL-2: (Cetus) Log LP-377. Drug preparation: white crystalline powder 3.6 x 10⁶ CU (1.2 mg)/vial; prepared with 1.2 ml of distilled H₂O, then diluted with 5% dextrose water to appropriate concentration; stable solution. Average weight; C57 = 23.7 g; colon adenocarcinoma no. 38, passage no. 77. * Weight change between days 3 and 8.

Table 4: Treatment of early-stage B16 melanoma with i.v. administered IL-2

<table>
<thead>
<tr>
<th>CU/injection</th>
<th>Schedule</th>
<th>Total dose (CU)</th>
<th>Mean body weight change</th>
<th>Drug deaths</th>
<th>Median tumor burden on day 10</th>
<th>T/C (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>d 1-6</td>
<td>1.8 x 10⁶</td>
<td>+0.3</td>
<td>d 7</td>
<td>1342</td>
<td>1584–3940</td>
<td>6 Mice</td>
</tr>
<tr>
<td>300,000</td>
<td>d 3-7</td>
<td>1.2 x 10⁶</td>
<td>−0.7</td>
<td></td>
<td>1512</td>
<td>126–1756</td>
<td>48</td>
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<tr>
<td>100,000</td>
<td>d 3-7</td>
<td>6 x 10⁵</td>
<td>+0.9</td>
<td></td>
<td>1877</td>
<td>1252–2224</td>
<td>60</td>
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</tbody>
</table>

* IL-2: (Cetus) Lot LP-377. Drug preparation: white crystalline powder 3.6 x 10³ CU (1.2 mg)/vial. Prepared with 1.2 ml of distilled H₂O then diluted with 5% dextrose water to appropriate concentration; stable solution. C57 males used for B16; B16 melanoma, passage no. 66. * Weight change between days 1 and 7.

Table 5: Treatment of immunogenic colon adenocarcinoma no. 11/A with i.v. administered IL-2

<table>
<thead>
<tr>
<th>CU/injection</th>
<th>Schedule</th>
<th>Total dose (CU)</th>
<th>Mean body weight change</th>
<th>Drug deaths</th>
<th>Median tumor burden on day 48 (mg/mouse)</th>
<th>T/C (%)</th>
<th>Comments</th>
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</thead>
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<td>3 x 10⁶</td>
<td>+0.3</td>
<td>d 36</td>
<td>1912</td>
<td>1584–3940</td>
<td>6 Mice</td>
</tr>
<tr>
<td>300,000</td>
<td>d 17–21, 31–35</td>
<td>3 x 10⁶</td>
<td>+0.3</td>
<td>d 36</td>
<td>0</td>
<td>0</td>
<td>3/5</td>
</tr>
<tr>
<td>100,000</td>
<td>d 17–21, 31–35</td>
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<td>+0.3</td>
<td>d 36</td>
<td>0</td>
<td>405</td>
<td>21</td>
</tr>
</tbody>
</table>

* Drug preparation: white crystalline powder; 3.6 x 10³ CU/vial (1.2 mg/vial); mixed with 1.2 ml of distilled H₂O vial, diluted to appropriate concentration with dextrose water; stable, clear solution; colon adenocarcinoma 11/A, passage no. 43. IL-2 (Cetus) Lot LP-377. CDF; females; born 11/4/87; trocarred 3/4/88. Mean weight 25.1 g at start of drug treatment (day 12). Trial advanced stage only; tumors palpable, median size 80 mg (0–163 mg) in control (representative cage). * Only death of entire trial.
IL-2 (3.0 x 10^6 CU/injection, 6-10 days and 18-22), the T-C was increased to 31 days. With the combination, a log cell kill of 1.5 was obtained (compared with a 0.9 log cell kill for single-agent treatment). This combination demonstrated a modest increase in efficacy over single-agent therapy. There was no appreciable therapeutic improvement with the lower dosage of IL-2 (1 x 10^6 CU/injection, days 6-10 and 18-22) in combination with DTIC. In fact, this dosage of IL-2, used in combination with the higher dose of DTIC, yielded a response lower than DTIC alone. The first occurred on day 142 of the trial, and 6 of 6 mice was cured (through day 180). When combined with 3 x 10^6 TD IL-2, there were 3 of 6 cures. When combined with 3 x 10^6 TD IL-2, there were 5 of 6 cures.

There were 2 separate incidents of tumor rechallenge with colon no. 11/A. The first occurred on day 142 of the trial, and the second rechallenge occurred on day 203. Both rechallenges included bilateral tumor implants using a 12-gauge trocar. Thirty- to 60-mg tumor fragments were used in the first rechallenge; 45- to 80-mg tumor fragments were used in the second rechallenge. In the first rechallenge, there was only a 75% take rate. The first rechallenge occurred in a DTIC-only treatment and IL-2-only treatment arm. The second rechallenge included all tumor-free mice in all remaining arms (49 mice).

DISCUSSION

The use of biologic response modifiers in cancer therapy is a rapidly expanding practice and is presently being considered as the “fourth modality of cancer treatment” (23). Because other therapeutic modalities used in combination are often more beneficial than used alone, the use of biological agents that induce immunomodulation or other effects may also offer a meaningful therapeutic improvement in combination with various cytotoxic agents or other modalities. Combination chemotherapy/biological therapy can be advantageous since these 2 therapeutic modalities often have nonoverlapping toxicities. Combination chemotherapy may therefore permit superior antitumor activity in comparison with either agent used alone at equitoxic dosages. In preclinical therapy, this is known as the combination therapeutic index (24). Preclinically, the 10% lethal dose has traditionally been accepted by experimentalists as the MTD and provides a basis for comparison of the effects of one treatment with another. Traditionally, to claim therapeutic synergism of a 2-drug combination, the dose of each agent...

Table 6 Treatment of colon adenocarcinoma no. 7/A with combination dacarbazine and IL-2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>CU or mg/kg injection</th>
<th>Schedule</th>
<th>Total dose CU or mg/kg</th>
<th>Drug deaths</th>
<th>Median tumor burden on day 25</th>
<th>T/C</th>
<th>Tumor-free day 276</th>
<th>T-C (days)</th>
<th>Log_{10} cell kill (gross)</th>
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<tbody>
<tr>
<td>No treatment</td>
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</tr>
<tr>
<td>Single-agent therapy</td>
<td>DTIC</td>
<td>p.o.</td>
<td>102</td>
<td>Q2h x 3, d 3, 15</td>
<td>612</td>
<td>0/8</td>
<td>0</td>
<td>0</td>
<td>1/8</td>
<td>70</td>
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<td>DTIC</td>
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<td>1</td>
<td>0/8</td>
<td>20</td>
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<td>IL-2</td>
<td>i.v.</td>
<td>3 x 10^6</td>
<td>d 6–10; 18–22</td>
<td>3 x 10^6</td>
<td>1/6</td>
<td>2304</td>
<td>1312–3648 &gt;100</td>
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<td>d 6–10; 18–22</td>
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<td>p.o.</td>
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<td>Q2h x 3, d 3, 15</td>
<td>612</td>
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<td>0</td>
<td>0</td>
<td>0/6</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>i.v.</td>
<td>3 x 10^6</td>
<td>d 6–10; 18–22</td>
<td>3 x 10^6</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
<td>0/6</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>DTIC</td>
<td>p.o.</td>
<td>66</td>
<td>Q2h x 3, d 3, 15</td>
<td>396</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
<td>0/6</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>i.v.</td>
<td>1 x 10^6</td>
<td>d 6–10; 18–22</td>
<td>1 x 10^6</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
<td>0/6</td>
<td>28</td>
</tr>
</tbody>
</table>

* Q2h x 3, every 2 h for 3 days.
* Death day 24 (LD<sub>7</sub>).
* Too many cures to calculate T-C.
alone and in combination should include a range of closely spaced dosage levels, including frankly toxic levels. In this manner, the MTD of each single agent and the combination can be selected for comparison. This definition may not necessarily be the MTD. The more appropriate term, therefore, when discussing the 2-drug combination (IL-2/chemotherapeutic agent) should be therapeutic improvement of the combination as opposed to mere survival. By defining response in this manner, one does not imply synergy but rather alteration of biological activity.

In the adriamycin/IL-2 trial against mammary 16/C, a therapeutic improvement did not occur when the highest nontoxic dose of adriamycin was combined with IL-2. Interestingly, there was no clear enhancement of toxicity when the 2 agents were used in combination. At 9.2 mg/kg, adriamycin may have been too immunosuppressive, resulting in lymphocyte depletion and preventing therapeutic improvement of the combination adriamycin/IL-2 versus single-agent adriamycin. Therapeutic improvement was seen with the second and third dose levels of adriamycin in combination with 1.5 x 10^4 CU of IL-2 (but not at the fourth or lowest dose level). In addition to its cytotoxic properties, adriamycin has demonstrated immunopotentiating properties at moderate doses. This immunomodulatory effect of adriamycin may have contributed to the therapeutic improvement observed with the mid-dose adriamycin and mid-dose IL-2 combinations (14, 25, 26).

Adriamycin/IL-2 in combination has also been investigated in adoptive chemoimmunotherapy trials. Salup et al. (27), using broadly cytotoxic lymphocytes generated by in vitro culture in combination with adriamycin, were able to cure Stage II and III mouse renal cell cancer (RENA model) by bicompartimental (i.v./i.p.) therapy (27). Previous treatment of that model with either cytotoxic lymphocytes or adriamycin alone was much less effective (28).

DTIC was also investigated in combination with IL-2 against both colon no. 7/A and colon no. 11/A. The DTIC was administered 3 to 5 days prior to the IL-2. In the colon no. 7/A trial, there was a modest therapeutic improvement in 3 of 4 combination groups, compared with the matched single-agent treatments. Interestingly, IL-2 alone had no effect on this tumor model. In the colon no. 11/A trials it appeared that the combination had greater efficacy over single-agent IL-2 or DTIC at all matched groups (based on number of cures in each arm).

Several points need to be addressed in the colon no. 11/A trials. In contradistinction to the other models studied, single-agent IL-2 was curative in a dose-dependent manner. Second, DTIC was curative over a broad range of doses. Third, when cured animals were rechallenged, the doubling time was prolonged and there was only 75% regrowth. These observations imply marked immunogenicity of this subline of colon no. 11/A. Therefore the results of the trials with this model are likely to be relevant only to other immunogenic tumors.

In our trials, the chemotherapeutic agent was given prior to IL-2 administration. Mitchell (14) has recently discussed several studies addressing the issue of cytotoxic/biological combinations. His overall conclusion was that cytotoxic agents should be administered first for the following reasons: (a) this schedule would permit some recovery from the cytotoxic agent before...
attempting to stimulate the immune system; (b) some chemotherapeutic agents affect tumor cell membranes, making them more susceptible to immune lysis (i.e., adriamycin) (26); (c) other cytotoxic agents have demonstrated tumor cell membrane fluidity alteration through their effects on lipid metabolism (29, 30). Changes such as these can increase the sensitivity of the tumor cell to immune destruction, allowing the chemotherapeutic agent to play an immunopotentiating role (14, 26, 27). The primary mechanism of chemotherapy is cytoreduction. Chemotherapy administered before the biological agent would reduce the tumor burden with which the immune system has to contend. Immunotherapy preclinically has demonstrated greatest activity against small tumor burdens (10^3-10^4 tumor cells) (31). By administering chemotherapy first, cytoreduction would ensue, allowing for the most optimal biological treatment condition.

All of our combination trials utilized chemotherapy prior to biological therapy. Theoretically this may be the optimal schedule, however, definitive trials should be undertaken, altering sequencing to determine if indeed this is so. Several models were investigated in our trials with single-agent IL-2. Although a T-C of <42% was obtained in several models (mammary no. 16/C, colon no. 11/A, colon no. 38, B16 melanoma), in only one model (the presumed immunogenic subline of colon no. 11/A) was there also a biologically important log cell kill (i.e., >1.0) associated with single-agent IL-2 therapy. A consistent therapeutic improvement for the combination of IL-2 and a cytotoxic agent (compared with single-agent treatment) was obtained only with an immunogenic tumor (colon no. 11/A, Table 8). In that trial, all combination dosages were clearly superior to the matched single-agent treatments.

In the nonimmunogenic tumors, however, at individual dosage levels the combination of the cytotoxic and IL-2 was superior to single-agent therapy, (h) The addition of IL-2 never increased the host lethality of a given cytotoxic was rarely inferior to single-agent therapy. In conclusion, marked antitumor efficacy of single-agent IL-2 was demonstrated in the immunogenic subline of colon no. 11/A. Also, consistent therapeutic improvement with combination DTIC/IL-2 was only demonstrated in the immunogenic colon no. 11/A model. Combination adriamycin/IL-2 therapy demonstrated enhanced efficacy against the nonimmunogenic mammary no. 16/C tumor but only when moderate, and possibly immunopotentiating, doses of adriamycin were used. If indeed renal cell carcinoma and malignant melanoma can be classified as "immunogenic-type" tumors, then IL-2 in combination with a cytotoxic agent, such as DTIC (which historically

### Table 8 Treatment of immunogenic colon adenocarcinoma no. 11 with combination i.v. administered IL-2/p.o. administered DTIC*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mg/kg/injection or CU/injection</th>
<th>Schedule</th>
<th>Total dosage (mg/kg or CU)</th>
<th>Body weight change at nadir g/mouse</th>
<th>Day of study</th>
<th>Drug deaths</th>
<th>Days death occurred</th>
<th>Median tumor burden on day 48 (mg/ mouse)</th>
<th>T/C (%)</th>
<th>No. of mice tumor-free day 180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTIC, 306 mg</td>
<td>102 mg/kg, q2h × 3</td>
<td>d 12, 27</td>
<td>612 mg/kg</td>
<td>-0.5</td>
<td>33</td>
<td>0</td>
<td>36</td>
<td>1912</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>DTIC, 201 mg</td>
<td>67 mg/kg, q2h × 3</td>
<td>d 12, 27</td>
<td>402 mg/kg</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>36</td>
<td>85</td>
<td>4</td>
<td>1/6</td>
</tr>
<tr>
<td>DTIC, 126 mg</td>
<td>42 mg/kg, q2h × 3</td>
<td>d 12, 27</td>
<td>252 mg/kg</td>
<td>-2.7</td>
<td>33, 36</td>
<td>32</td>
<td>2, 36</td>
<td>48</td>
<td>3</td>
<td>1/6</td>
</tr>
<tr>
<td>DTIC, 84 mg</td>
<td>28 mg/kg, q2h × 3</td>
<td>d 12, 27</td>
<td>168 mg/kg</td>
<td>-2.3</td>
<td>33</td>
<td>0</td>
<td>36</td>
<td>240</td>
<td>13</td>
<td>2/6</td>
</tr>
<tr>
<td>IL-2</td>
<td>300,000 U</td>
<td>d 17–21, 31–35</td>
<td>3 × 10^6 CU</td>
<td>-0.8</td>
<td>36, 36</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0/3/5</td>
</tr>
<tr>
<td>IL-2</td>
<td>100,000 U</td>
<td>d 17–21, 31–35</td>
<td>1 × 10^6 CU</td>
<td>-0.4</td>
<td>36</td>
<td>0</td>
<td>32</td>
<td></td>
<td>2</td>
<td>3/6</td>
</tr>
<tr>
<td>DTIC</td>
<td>67 mg/kg</td>
<td>d 17–21, 31–35</td>
<td>402 mg/kg, 1 × 10^6 CU</td>
<td>-0.5</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0/5/6</td>
</tr>
<tr>
<td>IL-2</td>
<td>100,000 U</td>
<td>d 17–21, 31–35</td>
<td>1 × 10^6 CU</td>
<td>-0.6</td>
<td>36</td>
<td>0</td>
<td>48</td>
<td></td>
<td>3</td>
<td>3/6</td>
</tr>
<tr>
<td>DTIC</td>
<td>42 mg/kg</td>
<td>d 17–21, 31–35</td>
<td>252 mg/kg, 1 × 10^6 CU</td>
<td>-0.3</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0/4/6</td>
</tr>
<tr>
<td>IL-2</td>
<td>100,000 U</td>
<td>d 17–21, 31–35</td>
<td>1 × 10^6 CU</td>
<td>+0.5</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0/5/6</td>
</tr>
<tr>
<td>DTIC</td>
<td>28 mg/kg</td>
<td>d 17–21, 31–35</td>
<td>108 mg/kg, 1 × 10^6 CU</td>
<td>+0.5</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0/5/6</td>
</tr>
<tr>
<td>IL-2</td>
<td>100,000 U</td>
<td>d 17–21, 31–35</td>
<td>1 × 10^6 CU</td>
<td>-1.4</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0/5/6</td>
</tr>
<tr>
<td>DTIC</td>
<td>102 mg/kg</td>
<td>d 17–21, 31–35</td>
<td>612 mg/kg, 3 × 10^6 CU</td>
<td>+0.6</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0/5/6</td>
</tr>
<tr>
<td>IL-2</td>
<td>300,000 U</td>
<td>d 17–21, 31–35</td>
<td>3 × 10^6 CU</td>
<td>-2.1</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0/5/6</td>
</tr>
<tr>
<td>DTIC</td>
<td>28 mg/kg</td>
<td>d 17–21, 31–35</td>
<td>168 mg/kg, 3 × 10^6 CU</td>
<td>-0.5</td>
<td>36</td>
<td>0</td>
<td>405</td>
<td></td>
<td>21</td>
<td>1/7</td>
</tr>
<tr>
<td>IL-2</td>
<td>100,000 U</td>
<td>d 17–21, 31–35</td>
<td>3 × 10^6 CU</td>
<td>-1.3</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0/5/6</td>
</tr>
</tbody>
</table>

Drug preparation: white crystalline powder. 3.6 × 10^6 CU/vial (1.2 mg/vial). Mixed with 1.2 ml of distilled H2O vial; diluted to appropriate concentration with dextrose water; stable, clear solution; colon adenocarcinoma no. 11/A, passage no. 43; IL-2 (Cetus) Lot LP-377. CDF, females: born 11/4/87; inoculated 3/4/88. Mean weight: 25.1 mg at start of drug treatment (day I 2). Trial advanced stage only; tumors palpable, median size 89 mg (0–163 mg) in control (representative cage). DTIC was administered before the biological agent would play an immunopotentiating role (14, 26, 27).

* Only death of entire trial.
has demonstrated some efficacy against malignant melanoma),
may be appropriate therapy.

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Patricia Mucci LoRusso, Sharon Lea Aukerman, Lisa Polin, et al.


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