Preclinical Trials with Combinations and Conjugates of T101 Monoclonal Antibody and Doxorubicin

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ABSTRACT

We investigated the potential for additive therapy for malignancy using an anti-human T-cell monoclonal antibody, T101, and the chemotherapy agent doxorubicin (DOX). We compared the efficacy of T101 alone, DOX alone, T101 and DOX covalently linked to dextran to form an immunocomplex, T101 plus DOX mixed together and injected, T101 and DOX injected separately, and nonspecific murine IgG2a plus DOX mixed together. Inhibition of [3H]thymidine was examined in vitro, and the clinical efficacy of each treatment was tested on human T-cell tumors growing in athymic mice. In vitro experiments confirmed retention of immunoreactivity and cytotoxicity by the immunocomplex, but it was not superior to DOX alone. In efficacy experiments, all therapeutic arms were superior to placebo treatment (P < 0.05). However, the best results in the animal tumor model were obtained with T101 mixed with DOX, perhaps because of formation of weak complexes via hydrophobic bonds. This mixture was superior to all other treatments, both by growth curve analysis (P < 0.05) and by analysis of complete regression of tumor (P < 0.01). T101 mixed with DOX was superior to a mixture of nonspecific mouse immunoglobulin and DOX and superior to a combination of T101 injected i.v. and DOX injected i.p. The antitumor effect of T101 mixed with DOX was blocked by premodulating the target antigen with T101. These data suggest that further exploration into monoclonal antibody-anthracycline complexes is warranted.

INTRODUCTION

The introduction of hybridoma technology and large-scale production of MoAbs1 have opened several avenues for cancer treatment (1, 2). MoAbs alone may be given with either direct cytotoxic or regulatory intent, or they may be given conjugated to cytotoxic substances such as chemotherapeutic agents, natural toxins, or radioisotopes. Reported trials of MoAbs alone have yielded mixed results. While transient antitumor effects have been observed in several studies, particularly in hematological malignancies (3–9), durable complete remissions have not been seen, with one notable exception involving idiotypoid therapy (10). Several radioimmunodetection studies with radiolabeled MoAbs have been reported, but therapeutic efforts have been limited (11–14). In vivo trials, with MoAbs conjugated to toxins such as ricin A-chain or to chemotherapy agents, have only recently begun.

We conducted a study of passive immunotherapy with murine MoAb T101 in patients with CLL and CTCL (15). We demonstrated T101 binding to target cells; transient antitumor effects were seen in several patients; side effects and toxicities were limited. On the other hand, neutralizing anti-mouse antibodies were produced in several CTCL patients (15, 16), and antigenic modulation occurred in every patient (15, 17). Modulation of the T65 antigen, with which T101 reacts, is associated with internalization of both the antigen and antibody (17, 18). Modulation limits T101 cytotoxic effects by precluding Fc receptor binding to effector cells and decreasing the number of antigenic sites available for antibody-specific binding (19). However, ICs of T101 may be effective inasmuch as internalization is desirable for a localized cytotoxic effect.

DOX is a commonly used chemotherapy agent (20). It is occasionally used as a second-line therapy as a single agent or in combination with other agents in CLL and CTCL. Colony assays showed CLL colony inhibition with continuous exposure to DOX as opposed to pulses (21). Recently, there has been increased clinical interest in the use of DOX by prolonged infusions of 24–96-h duration (22–25).

The above constitutes a rationale for investigation of T101-DOX IC. In this manuscript, we report results of preclinical testing of T101-DOX-IC and other combinations of T101 and DOX. Results with IC are compared to those obtained with DOX or T101 alone or in combination.

MATERIALS AND METHODS

Antibody/Drug Reagents. T101 is an IgG2A murine MoAb that immunoprecipitates an antigen (M, 65,000–67,000) expressed on malignant and normal T-lymphocytes, thymocytes, and CLL cells (26, 27). The clinical-grade T101 used in these studies was provided by Hybritech, Inc. (San Diego, CA) (4). Doses used in the in vitro experiments are indicated in the results section. In the animal experiments described below, 500-μg T101 was used per injection, whether free or conjugated.

The clinical pharmaceutical Adriamycin (Adria Laboratories, Inc., Columbus, OH) was reconstituted with saline as per package insert. A dose of 3.6 μg/injection was used in animal experiments, and doses used for in vitro experiments are noted in "Results."

T101-DOX-dextran IC was produced using a technique described previously (28). Briefly, dextran was oxidized with periodate to form a thiohemdic acid. This preparation was then reduced with sodium cyanoborohydride. The IC was purified using Sephadex G100 gel filtration. Based on UV-visible absorbents at 495 and 280 nm, approximately 3.5–4 molecules of the drug bound per molecule of antibody. The molecular weight of the T101-DOX-dextran IC was estimated at 6.5 × 105 by high-performance liquid chromatography using TSK 400 and TSK 250 columns in series in 10 mM sodium sulfate, 20 mM sodium phosphate, and 10% dimethyl sulfoxide, pH 6.8. The molecular weight was estimated as 1.5 × 106 by sucrose gradient centrifugation of 125I-labeled conjugate. High-performance liquid chromatographic analysis and extraction with dichloromethane yielded no free DOX from the IC. Immunoreactivity was measured by association of trace iodinated IC with a solid-phase antigen. Conjugate immunoreactivity was consistently within 20% of free T101.

Cell Lines. For in vitro binding and cytotoxicity studies, Molt-4 (29), 8402 (30), and 8392 (30) cell lines were maintained as continuous cell lines.
suspensions in RPMI 1640 media with 10% fetal calf serum (Irvine Scientific, Irvine, CA). Molt-4 and 8402 are human T-cell leukemia lines with which T101 reacts, and 8392 is the B-cell autologous lymphoblastoid counterpart to 8402. Cells in log phase with viabilities exceeding 90% by trypan blue exclusion were used.

Immunofluorescence Assays. Indirect immunofluorescence assays were used to demonstrate binding of T101 and various T101-DOX IC to cells. Aliquots of 10^6 cells were incubated with the test reagent for 30 min at 4°C, washed twice, then incubated for another 30 min with fluorescein-conjugated, affinity-purified goat anti-mouse F(ab')2 (Boehringer-Mannheim, Indianapolis, IN). Direct DOX binding was determined using DOX red autofluorescence. Fluorescence analysis was performed on an Ortho Cytofluorograf-50H with a 2100-H computer connection (Ortho, Westwood, MA). Nonspecific mouse IgG2A RPC5 (Litton Bionetics, Fredrick, MD) was a negative control for indirect assays.

Cell Growth Inhibition Assays. The incorporation of [3H]thymidine was used to measure cell growth inhibition. Triplicate cultures were grown in 96-well, flat-bottomed microtiter plates (Costar, Cambridge, MA). Aliquots of 2 x 10^5 cells were mixed with various concentrations of MoAb/drug reagents in 200 µl of RPMI 1640 medium, with 2.5% human serum. Agents and cells were incubated at 24, 48, 72, or 96 h and [3H]thymidine (0.5 µCi) was added to each well 18 h before cells were harvested on the glass filter papers (Titertek; Flow Laboratories, McLean, VA). Counts per minute were measured in a liquid scintillation counter (Beckman Instruments, Irvine, CA), and results reported as percentage of control cpm.

Animal Tumor Studies. In vivo animal tumor studies were carried out in athymic mice bearing palpable, measurable, s.c. tumors of the Molt-4 cell line. The reproducibility of tumor establishment, stability of the T65 antigen, and T101-mediated T65 modulation in this system have been reported in detail elsewhere (31). Briefly, 5- to 6-week-old female BALB/c nu/nu mice were irradiated with 200 CgY once a week for 3 weeks and then given s.c. injections of 10^5 cultured Molt-4 cells and 10^5 irradiated HT-1080 fibrosarcoma cells. Animals were examined 10-14 days later for the presence of palpable, measurable tumors and then distributed among various treatment arms so that sums of cross-sectional diameters of measured tumors were approximately equal in all groups.

In preliminary experiments we confirmed T101-DOX-dextran IC binding to Molt-4 tumor in athymic mice using methodology described previously (31). In two separate experiments, tumor-bearing animals were sacrificed at 2, 4, or 24 h after i.p. injection of 500 µg T101, 3.6 µg DOX, or 500 µg T101-DOX-dextran IC containing 3.6 µg DOX. This was the maximal T101-DOX-dextran IC dose that could be given safely because of the volume required for solubility. Tumors were removed and teased into a cell suspension that was then incubated with either a control murine antibody or T101 in vitro. Thus, we measured direct T101 binding in vivo as well as residual T101 binding sites.

There were 6 therapeutic arms in the initial animal trials, and 2 schedules of administration utilizing a single i.p. injection or 5 daily i.p. injections. Each individual experiment involved 3-5 animals per arm. The 6 treatment groups included: (a) a placebo arm consisting of either PBS or 500 µg of nonspecific mouse protein RPC5; (b) 500 µg T101; (c) 3.6 µg DOX; (d) injections of T101-DOX-dextran IC containing 500 µg T101 and 5.4 µg DOX; (e) separate i.p. injections of T101 and DOX; and (f) 500 µg T101 and 3.6 µg DOX mixed in the same syringe. In subsequent experiments, T101 plus DOX was compared to T101 plus a nonspecific mouse IgG2A (UPC10) mixture, in addition to arms with PBS, DOX alone, and T101 alone. In another set of experiments, animals were pretreated with 100 µg T101 (to induce modulation) or PBS and then given injections of PBS, additional T101, DOX, or T101 mixed with DOX. In still another set of experiments, controls were compared with T101, DOX, T101 i.v. and DOX i.p., and T101 plus DOX mixed and injected i.p.

All animals had palpable, measurable s.c. tumors of about 4 x 5 mm or 20 mm^2 at the time therapy was started. Cross-sectional diameters were measured using Mitutoya calipers (Fisher Scientific, Irvine, CA). Measurements were obtained on the day of treatment and at 3-4 day intervals thereafter. The mean of 3 separate determinations of cross-sectional diameter products was recorded as tumor size. Animals were observed daily for general well-being and survival. All surviving animals were sacrificed 21 days after the start of therapy. All animals tolerated the injections without any apparent acute or delayed toxicities.

Statistical Analysis. For purposes of analysis, a tumor that totally disappeared following therapy was considered as a CR, and a decrease in size to <50% of the product of pretreatment cross-sectional diameters was defined as a PR if it was sustained for at least 1 week. For tumor-response curve analysis, the cross-sectional diameters for all tumors of all animals in each subgroup were averaged and the mean and the median plotted versus the number of days after start of therapy. In addition, a tumor index was calculated for each animal at each measurement by dividing the cross-sectional products at each time point by the pretreatment cross-sectional diameter product. Inasmuch as we were primarily interested in detecting response differences among the treatment groups and because of difficulties maintaining large groups of animals for prolonged periods of time, survival data were not generated in this study, and the design did not include curative intent.

Both mean and median tumor sizes for each group were plotted versus time as tumor growth curves. Only the mean tumor size plots are presented; the median tumor size plots were quantitatively quite similar. Because tumor growth curves for individual animals within particular treatment groups could be markedly heterogeneous, a non-parametric statistical procedure was utilized to compare tumor growth curves (32, 33). Examination and comparison of the individual growth curves for each group of animals was undertaken with this statistical technique. Data from 3 or 4 experiments, each with the same treatment arms, were combined for purposes of analysis. In addition, multiple comparisons among individual treatment groups were performed with the Bonferroni technique (34). Differences among tumor response rates were assessed with standard x² statistics (35), and differences in response rates between specific arms were determined using Fisher's exact test.

RESULTS

Binding to Cell Lines in Vitro. Table 1 shows the data for binding of the T101-DOX-dextran and DOX alone to B- and T-cell lines. The IC bound only to T-cells, suggesting that binding via antibody was much greater than any nonspecific binding taking place via any receptors for DOX. Binding to both B- and T-cell lines was apparent when sufficient quantities of DOX were added to solution, but the sensitivity of the assay was insufficient to detect DOX in the small quantities attached to 10 or 20 µg of T101 in the T101-DOX-dextran IC. Binding of DOX to B- and T-cells was demonstrated, but only at concentrations >100-fold over the amount of DOX in saturating quantities of T101-DOX-dextran IC.

Binding to Tumor in Vivo. Fig. 1 shows a series of cytographs histograms confirming the ability of the T101-DOX-dextran IC to bind to the Molt-4 tumor growing in the athymic mice. Both binding and modulation of the T65 antigen were

| Table 1 Indirect immunofluorescence with T101-DOX or DOX alone as determined by proportion of cells positive for fluorescence |
|-----------------|----------------|----------------|
| Concentration (µg/ml) | T101-DOX 8402 ([%]) | T101-DOX 8392 ([%]) | DOX alone 8402 ([%]) | DOX alone 8392 ([%]) |
| 200 | 99 | 97 | |
| 20 | 93 | 52 | 81 |
| 10 | 38 | 75 | |
| 5 | 72 | ND | 30 | 39 |
| 2 | 35 | ND | 8 | 11 |
| 1 | 13 | ND | 6 | 8 |
| 0.15%* | 6 | 6 | |
| 0.075* | 6 | 6 | |
| BKGD | 5 | 12 | |

* ND, not determined; BKGD, background.
* Amount of DOX contained in 20 µg T101-DOX-dextran.
* Amount of DOX contained in 10 µg T101-DOX-dextran.

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PRECLINICAL TRIALS WITH T101 AND DOX

Fig. 1. Histograms showing binding of T101 and T101-DOX-dextran to Molt-4 tumor cells growing in athymic mice. Control background (white area), reactivity with secondary antibody (shaded area). Two h following an i.p. injection of 500 μg T101, there was substantial T101 binding and modulation of the T65 target antigen as evidenced by the decreased fluorescence of the secondary antibody. By 4 h, the tumor cells had been saturated in vivo, and, at 24 h, saturation persisted with even more modulation. In contrast, even after 24 h, there was only partial in vivo binding and limited antigen modulation in the presence of T101-DOX-dextran IC.

Fig. 2. Inhibition of [3H]thymidine incorporation. Specificity of the T101-DOX-dextran IC was not demonstrated in vitro. Fig. 2 shows the uptake of [3H]thymidine as a percentage of control for the various cell lines in the presence of T101, DOX, T101-DOX-dextran, or T101 plus DOX for the optimal 72-h incubation period. There was no increase in cytotoxicity with a 96-h infusion. T101 appeared to have no effect on [3H]thymidine uptake while DOX alone, the IC, and T101 plus DOX produced similar inhibition patterns with an IC₅₀ (the concentration inhibiting uptake of [3H]thymidine to 50% of control) of 5 × 10⁻⁸ M for a 72-h incubation.

In Vivo Tumor Growth Curves. Fig. 3 depicts tumor growth curves from various animal experiments with T101 and DOX. It should be emphasized again that these tumors were established and measurable at the time treatment was started. Values shown are the means of measurements from cohorts of 11-15 animals from 4 different experiments, all of which included a placebo arm and arms with T101 alone, DOX alone, T101-DOX-dextran, T101 plus DOX mixed together and injected, or T101 plus DOX injected separately. Because these are mean values, shrinkage of several tumors may have resulted in only a modest or no decrease in the mean of cross-sectional diameter products or growth index because of a few rapidly growing tumors reaching very large sizes.

The best arm in each individual experiment, and for group data, was T101 plus DOX mixed together prior to injection.
PRECLINICAL TRIALS WITH T101 AND DOX

Table 2 T101 therapy in nude mouse Molt-4 tumor model: summary of therapeutic efficacy following a single injection or 5 consecutive daily injections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Single injections</th>
<th>Multiple injections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mice With CR</td>
<td>mice With PR</td>
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<tr>
<td>Control</td>
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<td>0</td>
</tr>
<tr>
<td>T101</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>T101-DOX-dextran</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>DOX</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>T101 + DOX</td>
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<td>0</td>
</tr>
<tr>
<td>T101-DOX-complex</td>
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<td>4</td>
</tr>
</tbody>
</table>

* T101 + DOX injected separately i.p.
*b T101 + DOX drawn into same syringe, then injected.

Table 3 Summary of T101-DOX and UPC10-DOX trials in Molt-4 tumor model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mice</th>
<th>CR</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPC10</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T101</td>
<td>12</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>DOX</td>
<td>12</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>UPC10 + DOX</td>
<td>12</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>T101 + DOX*</td>
<td>12</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

* Both premixed in syringe, then injected i.p.

In Vivo Tumor Regressions. Table 2 summarizes the various therapies in terms of complete or partial tumor regression. There were significant differences among the various treatments (P < 0.001). The best results were obtained with the T101 plus DOX mixture with a 27% CR rate following a single injection, and 79% CR following 5 daily injections. When the results of both treatment schedules were combined, there was a 52% CR rate for the complex versus 7-20% for any other DOX-containing regimen. For single injections, there were 8 of 15 responses (CR + PR) for T101 plus DOX mixed together compared to the second best arm, T101-DOX IC (3 of 15) responses (P = 0.13). For multiple injections, 11 of 14 CR for T101 plus DOX mixed was better than 3 of 11 CR for T101 plus DOX injected separately (P < 0.05), and 5 of 14 for T101-DOX IC (P < 0.06).

Because the superior results obtained with T101 plus DOX mixed may have been due to a T101-DOX complex and altered DOX pharmacokinetics and improved bioavailability rather than antibody-directed targeting, we conducted an additional set of experiments. Table 3 and Fig. 4 show results of experiments comparing in vivo efficacy of a T101 plus DOX mixture versus a mixture of DOX and the nonspecific murine IgG2A (UPC10). The dose of DOX used in these experiments was somewhat higher than the previous experiments (6 μg/injection). There was a significant difference among treatments (P < 0.001). The best results were again obtained with T101 plus DOX mixed.
DOX, superior to the UPC10 plus DOX curve (P < 0.05). There were 0 of 12 CR for UPC10 plus DOX versus 7 of 12 CR for T101 plus DOX injected together (P < 0.01).

Inasmuch as a T101-DOX complex should produce an enhanced effect via T101 specific binding, additional experiments were conducted in the presence of decreased antigen expression. Table 4 shows the differences among various therapies following placebo or T101-induced modulation (P < 0.01). In this group of experiments, there were 7 of 18 responses for T101 plus DOX in the absence of premodulation versus only 2 of 18 in the presence of modulation (P = 0.12). Premodulation followed by DOX alone produced 4 of 18 responses compared to 2 of 18 for premodulation followed by T101 plus DOX. There was no significant difference for growth curves among the three arms containing DOX, although T101 plus DOX in the absence of modulation appeared slightly better (data not shown).

Table 5 shows results of experiments designed to further address the question of additive or synergistic effects of T101 plus DOX compared to results with a putative T101-DOX complex. T101 plus DOX mixed and injected i.p. produced responses in 10 out of 12 animals compared to 4 of 12 when T101 was injected i.v. and DOX i.p. (P < 0.06). Again, the best growth curve was for T101 plus DOX premixed and injected i.p. Combining data from all experiments in which there was both an arm with T101 plus DOX mixed, and an arm with T101 plus DOX injected separately, yields 37 of 59 responses for the complex versus 16 of 53 for the two injected separately (P < 0.003).

**DISCUSSION**

This study showed that: (a) A T101-DOX-dextran IC and combinations of T101 plus DOX produced tumor regressions in athymic mice bearing human T-cell malignancy. (b) T101 and DOX mixed together produced a greater antitumor effect than did T101-DOX-dextran IC in comparable amounts and had a greater antitumor effect than T101 alone, DOX alone, T101 plus DOX injected separately, or DOX and a nonspecific IgG2A mixed together. The superior effect of the drug-MoAb mixture was blocked by antigenic modulation consistent with a concept that a noncovalent T101-complex had been formed. (c) Difficulty in demonstrating cytotoxic specificity in vitro did no preclude in vivo specificity of such IC or complexes; in vitro, small volume and proximity factors may facilitate drug binding to cells by other than the antibody portion of an IC or complex.

It has been difficult to produce DOX-IC mixtures that have a high DOX/antibody ratio, are water soluble, and retain immunoreactivity and cytotoxicity. The dextran linkage circumvents these problems and has been previously used to form anthracycline IC (28). Unfortunately, such ICs are extremely large and, although they may appear effective in vitro, such large complexes are removed rapidly by the reticuloendothelial system in vivo in animals (J. M. Frincke and R. M. Bartholomew, unpublished observation). The in vivo stability of the T101-DOX-dextran linkage is also unproven. Noncovalent, direct linkage of immunoglobulin and certain drugs via hydrophobic bonds would yield an attractive immunopharmaceutical because the size would not be substantially greater than the normal immunoglobulin molecule. This is the same advantage that covalent-linked ICs have. However, a noncovalent binding might be even more advantageous inasmuch as the toxic agent might be more readily dissociated at the cell surface or intracellularly. One explanation for our results is that T101 and DOX may have formed such a complex. Lack of enhanced superiority of a nonspecific IgG plus T101 mixture mitigated against altered bioavailability of DOX as an explanation and suggested that antigen specificity was important in providing the improved antitumor effect. The fact that antigenic modulation blocked the superior antitumor effect of premixed T101 plus DOX strongly supports a putative T101-DOX complex. Other investigators have also suggested that such noncovalent IC might retain cytotoxic activity and, perhaps, be superior to IC with tight covalent binding (36), but none have conducted experiments to show specificity of a putative complex or to show that effects can be blocked by competitive inhibition of the target antigen.

Analysis of growth curves from all experiments suggested that there is an additive or synergistic effect of T101 plus DOX, but the best results were consistently obtained with the putative complex formed by premixing T101 plus DOX. Lending credence to this concept is the role of albumin in plasma as a carrier protein because of its ability to reversibly bind hydrophobic molecules and thus enhance their solubility. Indeed, this rationale is probably applicable to all immunoglobulins and any chemotherapeutic agents that are substantially hydrophobic.

Therapy-induced tumor regressions in animal models are crucial for establishing a rationale for clinical studies with IC. There are innumerable possible T101-IC and some basis for selection of one IC over another is an important issue. The athymic mouse tumor model provides such a screening system for antitumor efficacy as well as toxicity. In the past many investigators have relied on injections of immunotherapeutic agents at the time of tumor implantation and claimed benefit when subsequent tumor growth was retarded. Such models are not comparable to tumor-bearing models, which have been utilized for years in the screening of chemotherapeutic agents. With MoAb-IC, it is imperative to test the immunopharmaceutical in a tumor-bearing animal and to compare the effect with that produced by chemotherapy alone, antibody alone, and the two given separately.

The necessity for these controls was apparent in our experiments; T101 alone significantly retarded tumor growth compared to controls and produced regressions in some animals. This may have been due to mouse effector cell-mediated cellular cytotoxicity, inasmuch as murine IgG2A are known to be most effective in such systems (37, 38). DOX was given at a dose of about 1/200 to 1/20 of the lethal dose for 50% of mice. As a single injection this dose produced limited antitumor effects, and more tumor regressions were seen with 5 daily injections, or 5 times the dose of a single injection. T101 plus DOX, whether premixed, injected separately i.p., or injected separately i.p. and i.v., appeared to have some additive or synergistic effect.

Our initial experiments primarily examined the T101-DOX-dextran IC, but included a T101 plus DOX control arm. The two were drawn up into the same syringe in our first experiment, but injected separately in a second experiment. Because of the striking differences, we conducted additional experiments using both methods as separate arms. The final analysis of all of the

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**Table 5 Summary of T101 plus DOX trials in Molt-4 tumor model using i.v. T101 and i.p. DOX**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mice</th>
<th>CR</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS control</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T101 i.v.</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>DOX i.p.</td>
<td>12</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>T101 i.v. + DOX i.p.</td>
<td>12</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>T101 + DOX complex i.p.*</td>
<td>12</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

* Both premixed in syringe prior to injection.
experiments established that the T101 plus DOX mixture produced the best antitumor effect with no apparent toxicity and that this preparation was clearly superior to the T101-DOX-dextran IC, to T101 alone, to DOX alone, and to the potentially synergistic or additive combination of T101 plus DOX injected separately. We believe that simply mixing T101 and DOX resulted in a noncovalent T101-DOX IC that might be clinically useful. Experiments with an additional control, namely a mixture of T101 plus a nonspecific murine IgG2A, demonstrated that the improved antitumor effect was not simply due to altered pharmacokinetics and increased availability of DOX. Blocking of the effect by premodulation of the T65 antigen provided further support for an antibody-specific cytotoxic effect. Subsequent in vitro experiments have also provided evidence for a T101-DOX complex.

Our series of in vitro experiments suggested that T101-DOX-dextran IC could bind to appropriate targets and induce antigen modulation, but that the cytotoxicity of the preparation was not specific. We presumed that this was due to binding/uptake of free DOX or T101-DOX via DOX receptors or other mechanisms. The small volumes in which these experiments were performed are much different from the volume of distribution and binding that takes place in vivo. There were no carrier proteins to take the drug away, no reticuloendothelial system to remove large complexes, and a different environment for metabolism of drug and antibody. The important lesson from this is that, if specific binding and retention of cytotoxicity are confirmed, an IC should not be discarded simply because specific cytotoxicity cannot be demonstrated in vitro.

ACKNOWLEDGMENTS

The authors thank Tammy McCallister and Michele Miller for their assistance in the laboratory and June Kathleen Meyers for her excellent secretarial assistance.

REFERENCES


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Preclinical Trials with Combinations and Conjugates of T101 Monoclonal Antibody and Doxorubicin


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