Phase I Study of Combined Pegylated Liposomal Doxorubicin with Protracted Daily Topotecan for Ovarian Cancer

Deepu Mirchandani, Howard Hochster, Anne Hamilton, Leonard Liebes, Herman Yee, John P. Curtin, Sang Lee, Joan Sorich, Cornelia Dellenbaugh, and Franco M. Muggia

Abstract

Purpose: To determine the maximum tolerated dose and dose-limiting toxicity of Doxil with low-dose continuous infusion topotecan and subsequently with low-dose oral topotecan. Other specific aims were preliminary assessment of activity in advanced ovarian and tubal malignancies, pharmacokinetics of oral topotecan, and correlation of response with topoisomerase I and II expression in tumors.

Methods: Eligible patients had histopathologically documented advanced cancers beyond standard therapy, performance status ≤2, and adequate organ functions. Doxil (30-40 mg/m² i.v.) was given on day 1, with topotecan either oral topotecan 0.4 mg/m² bid for 14 days or continuous infusion topotecan (0.3-0.4 mg/m²/d) for 14 to 21 days, in 28-day cycles. Fifty-seven patients, 23 with epithelial ovarian or tubal cancers were enrolled. Plasma levels of lactone form of topotecan were determined on patients receiving oral topotecan.

Results: Grade 4 neutropenia and thrombocytopenia and grade 3 diarrhea were dose-limiting toxicities at the highest dose levels explored. Doxil (40 mg/m²/day 1) and continuous infusion topotecan at 0.4 mg/m²/days 1 to 14 could be safely given and is the recommended phase II dose. Oral topotecan was limited by low and erratic plasma topotecan levels and frequent gastrointestinal toxicity. Particularly long partial responses and stable disease were observed in patients with epithelial ovarian or tubal cancers. Clinical benefit (objective responses and stable diseases) correlated with elevated expression of both topoisomeraseas by immunohistochemistry in four of six epithelial ovarian or tubal cancer tumor samples.

Conclusion: Doxil with 14-day topotecan infusion is a well-tolerated regimen and suitable for study in platinum-resistant or refractory ovarian or tubal cancers. Frequent gastrointestinal toxicity and/or erratic absorption complicate treatment with a longer topotecan infusion or with oral topotecan, respectively, and these combinations are not recommended.

Doxil, a formulation of pegylated liposomal doxorubicin, has been shown to have antitumor activity against Kaposi sarcoma, breast, ovarian, and endometrial cancers (1-6). Pegylation of the liposomal bilayer reduces uptake of liposomes by the reticuloendothelial cells leading to long circulation times. This, in turn, achieves high liposomal drug accumulation in tumor sites, presumably because of highly permeable blood vessels coupled with their poor lymphatic drainage (7, 8). On the other hand, reduced entry into normal tissues results in attenuation of the myelosuppression, alopecia, emesis, and cardiotoxicity associated with free doxorubicin.

Because doxorubicin inhibits topoisomerase II catalytic activity (9) and exposure to topoisomerase I-targeting drugs such as the camptothecins may result in elevations of topoisomerase II protein and vice versa (10, 11), we became interested in exploring combinations of these two agents clinically. Topotecan with doxorubicin results in higher in vitro cytotoxicity, particularly following prolonged exposure to topotecan (12).

Topotecan, a semisynthetic water-soluble camptothecin analogue interacts with the DNA chain at the site of single-strand cleavage by topoisomerase I. This results in stabilization of topoisomerase I and DNA “cleavable complex” that is presumed not sensed by DNA repair mechanisms. In cells replicating their DNA, DNA strand breaks and irreversible DNA damage ensue when the replication fork in the alternate strand collides with the complex. Preclinical studies with topotecan have shown efficacy against several cell lines, including those with elevated topoisomerase I expression, and those that are anthracycline resistant (13-15). Topotecan has shown efficacy in platinum refractory ovarian cancer, when given i.v. bolus × 5 days, every 4 weeks, but myelosuppression is a prominent toxicity (16). Prolonged low-dose drug delivery may facilitate combination with other myelosuppressive drugs (17-19) and may be particularly suitable for oral administration. The
recommended phase II dose of single agent low-dose oral topotecan is 0.5 mg/m² twice daily for 21 days every 4 weeks, with dose-limiting toxicity being grade 4 diarrhea (20, 21). In a randomized study of oral (2.3 mg/m²/d in two divided doses) versus i.v. (1.5 mg/m²/d) topotecan over 5 days, activity in ovarian cancers (i.v., 22%; oral, 21%) was similar, with the oral group having lower grade 3 and 4 hematologic toxicity (i.v., 57%; oral, 20%) but at the cost of some increase in severe gastrointestinal toxicity (i.v., 1%; oral, 3%; ref. 22).

Either sequential or simultaneous administration of dual topoisomerase targeting drugs (irinotecan as the topoisomerase I and doxorubicin or etoposide as the topoisomerase II–targeting drug) have shown activity in clinical studies and suggestion of pharmacodynamic modulation by one drug on the target of the other (11, 23, 24). The rationale for combining Doxil and topotecan is further supported by the prolonged half-life of pegylated liposomal doxorubicin, as well as by their nonoverlapping toxicities. Moreover, each has single agent activity in epithelial ovarian cancer (4–6, 26–29). In fact, several groups (30–34) have reported on small studies of Doxil or doxorubicin i.v. with 3 to 5 days of i.v. topotecan, given concomitantly or sequentially. Dose-limiting toxicities have been neutropenia and thrombocytopenia. Responses have been noted in breast (30), lung primarily small cell lung carcinoma (31), and ovarian cancers (32–34).

In our study, Doxil was given on day 1, with topotecan from days 1 to 15, based on earlier studies by our group with the New York Gynecologic Oncology Group and Eastern Cooperative Oncology Group (ECOG) showing the feasibility of combining protracted low-dose topotecan with other drugs (35). We report the results of this phase I study and preliminary observations on the potential efficacy of this combination.

**Patients and Methods**

**Study objectives.** Primary objectives were to evaluate the dose-limiting toxicities and establish maximum tolerated dose and recommended phase II dose of Doxil with prolonged low-dose topotecan, whether by the i.v. or oral routes. Exploratory secondary objectives were to detect pharmacokinetic interactions between Doxil and oral topotecan as well as determine topotecan blood levels during oral topotecan, to identify objective tumor responses in specific tumor types and to correlate any responses with expression of topoisomerase I and II in the initial tumor biopsies.

**Patient selection.** Eligibility criteria were adults ≥18 years, with histopathologically proven advanced malignancy beyond first-line therapy, a performance status ≤2 (ECOG-Zubrod scale), life expectancy of at least 3 months, no chemotherapy or radiation therapy within past 3 weeks, no known bone marrow involvement, and no active infection. Adequate and stable organ function required for eligibility was defined by cardiac ejection fraction >50% by nuclear multigated (MILGA) scan; serum creatinine ≤1.5 mg/d; absolute neutrophil count ≥1,500 mm³; platelets ≥100,000/mm³; total bilirubin <2.0 mg/d; aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase <3× upper limit of normal; and no clinically significant coagulopathy. Women of childbearing potential were to be tested to exclude pregnancy and advised on effective modes of contraception. Because dual topoisomerase targeting could potentially overcome resistance to single topoisomerase-targeting drugs (10–12), prior treatment with either anthracycline or topotecan or both was allowed. All patients on continuous infusion topotecan had a semipermanent central venous access placed. Patients with ovarian or tubal cancers were classified as potentially platinum sensitive or resistant based on the initial definitions of Markman and Hoskins (36). The study was approved by the Institutional Review Boards of the New York University Medical Center. Written informed consent that fulfilled all institutional, state and federal regulations was obtained from all patients before beginning protocol therapy.

**Drug administration and dose escalation.** Escalation from the initial dose levels was planned as shown in Table 1. Cycles were repeated every 28 days. The dose escalation strategy commenced at low doses of topotecan and a relatively low dose of Doxil. Subsequently, topotecan was escalated to one dose level below the previously determined maximum tolerated dose for this drug when given alone by continuous infusion for 21 days (17). Next, Doxil was escalated to achieve a tolerable single agent dose intensity of 12.5 mg/m²/wk (37). All toxicities were graded at the ECOG toxicity criteria. Three patients were entered at the dose level observed and analyzed for a minimum of 3 weeks for dose-limiting toxicities before escalating to the next level. Dose-limiting toxicity was defined as grade 4 mucositis, any other grade 3 or 4 nonhematologic toxicity, or grade 4 myelosuppression, occurring within the first cycle of chemotherapy. If no dose-limiting toxicity was observed, the dose was to be escalated to the next level. If at least one of three patients had dose-limiting toxicity, two other patients were entered at the same dose level. If none of the other three patients had dose-limiting toxicity, the next higher dose level was explored. If more than one of six patients had dose-limiting toxicity, the maximum tolerated dose having been reached, further dose escalation ceased. Three additional patients were to be entered at doses one level below the maximum tolerated dose, and if not more than one of six patients experienced dose-limiting toxicity at that dose, this dose level defined the recommended phase II dose.

Dose modifications on continuous infusion topotecan were as follows: for patients with grade 3 hematologic toxicity, or grade 2 nonhematologic toxicity, the next cycle could be delayed for a maximum of 2 weeks, to allow recovery to grade 1 or better. A 25% dose reduction of topotecan was prescribed for patients being treated at dose level 1. At all other levels, dose reduction to the prior dose level was prescribed when similar episodes of such toxicity occurred.

After determining the maximum tolerated dose for 21-day continuous infusion topotecan (dose level 3) the protocol was amended to administer topotecan 0.4 mg/m² on days 1 to 15. Once the tolerance for 14-day continuous infusion topotecan was established, the protocol was again amended to substitute continuous infusion topotecan by oral topotecan at an analogous schedule of 0.4 mg/m² bid for days 1 to 15. In this part of the study (Table 1), patients with more than two prior therapies or pelvic radiation were assigned to Doxil 30 mg/m²/day 1 (stratum A), and those with one or less prior chemotherapy were administered.

**Table 1. Planned dose escalation of Doxil with Topotecan T-CIV or T-PO**

<table>
<thead>
<tr>
<th>Topotecan</th>
<th>Doxil 1-h infusion d1 (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose level 1 (T-CIV)</td>
<td>0.3 mg/m²/d1-14</td>
</tr>
<tr>
<td>Dose level 2 (T-CIV)</td>
<td>0.3 mg/m²/d1-21</td>
</tr>
<tr>
<td>Dose level 3 (T-CIV)</td>
<td>0.4 mg/m²/d1-21</td>
</tr>
<tr>
<td>Dose level 4 (T-CIV)</td>
<td>0.4 mg/m²/d1-21</td>
</tr>
<tr>
<td>Dose level 4a (T-CIV)</td>
<td>0.4 mg/m²/d1-14</td>
</tr>
<tr>
<td>Dose level 5 (T-CIV)</td>
<td>0.5 mg/m²/d1-21</td>
</tr>
<tr>
<td>Dose level 6 (T-CIV)</td>
<td>0.5 mg/m²/d1-21</td>
</tr>
<tr>
<td>Stratum A (T-PO)</td>
<td>0.4 mg/m² bid d1-14</td>
</tr>
<tr>
<td>Stratum B (T-PO)</td>
<td>0.4 mg/m² bid d1-14</td>
</tr>
</tbody>
</table>

Abbreviations: T-CIV, continuous infusion topotecan; T-PO, oral topotecan.
assigned to Doxil 40 mg/m²/day 1 (stratum B). Provisions for dose escalation were included on oral topotecan: for patients not having significant hematologic toxicity in the first two cycles, dose would be increased to 0.5 mg/m² bid in the third cycle. The oral dose was based on the <50% bioavailability, and the schedule on the known relationship of diarrhea with increasing duration of exposure (20, 21). Pharmacokinetic studies as previously described (see below) were included in this portion of the study to compare steady-state blood levels of continuous infusion topotecan from our previous study (17), with those being achieved after oral topotecan. To explore for any interaction between Doxil and topotecan possibly affecting pharmacology, patients were randomized to receive one cycle of oral topotecan alone in cycle one or cycle two. A total of 10 patients were to be entered on each stratum.

Supportive measures include premedication with granisetron before onset of chemotherapy and in subsequent days during topotecan infusion as needed; dexamethasone with ranitidine was given before Doxil. Granulocyte colony-stimulating factor (filgrastim) was not given prophylactically and provided at the discretion of the physician following neutropenic fever.

**Clinical evaluation.** History, physical examination, performance status assessment, and routine laboratory tests (complete blood count and differential, liver and renal chemistries, and metabolic tests) were done on each patient at most 2 weeks before treatment and weekly during the first two cycles of treatment, these were repeated prior to each cycle thereafter. Any laboratory values that were abnormal before start of treatment were repeated within 48 hours of treatment. Tumor measurements, by exam, chest X-rays or computerized tomography scans, and tumor marker levels, were done at most 2 weeks before and every 8 weeks on treatment. Response criteria were those previously published by ECOG (38). Patients remained on study if they showed a complete response, partial response, or had stable disease. Ovarian cancer patients with nonmeasurable disease were also assessed by Rustin's criteria using CA125 as previously described (6). Patients were removed from the study for progressive disease or at their request.

**Pharmacokinetics.** Plasma topotecan levels on oral topotecan were measured on days 1 and 8, initially on cycles 1 and 2 (10 patients) but subsequently only on the cycle without coadministration of Doxil. Specimens on day 1 were obtained at baseline, and 1 and 4 hours after drug administration, and on day 8 before (trough sample) and within 1 hour of administration. Rigorous timing of the second post treatment sample was not prescribed because our objective was to generally monitor variability of levels across three studies of oral topotecan ongoing in our institution, rather than establishing bioavailability. Plasma specimens were processed and topotecan levels measured according to methods previously described (17). Briefly, plasma specimens were processed by a solid-phase extraction step followed by high-performance liquid chromatography reverse-phase chromatographic analysis as detailed below. Three to 5 mL of plasma from the clinical samples were centrifuged at 1,000 x g for 10 minutes followed by addition of buffers and the internal standard camptothecin (kindly given by the late Dr. Monroe Wall, Research Triangle Institute, Research Triangle Park, NC) dissolved in 0.1 mol/L NaH₂PO₄ (pH 3.5; concentration, 1 mg/mL) and was added in sufficient quantity to yield a final concentration of 10 ng/mL of the undiluted plasma along with an equal volume of [0.1 mol/L (NH₄)HPO₄ (pH 6.0). 1 mmol/L DOSS] before the application to the SPE extraction columns (Bond-elute, Varian, Torrance CA). Topotecan and the camptothecin internal standard were then eluted from the analytic column within 5 minutes. A standard set of six plasma samples containing topotecan ranging from concentrations of 0.1 to 10 ng/mL were processed and analyzed with each clinical specimen to provide a standard curve. For the analysis of the total topotecan content, typically, 0.2 mL of the extract was mixed with 0.1 mL of 0.05 mol/L H₂PO₄ allowed to sit at room temperature for 1 hour and then analyzed for total drug with the injection of 50 to 100 μL. The plasma standards were treated identically as for the clinical samples. The high-performance liquid chromatography system used a 15 cm x 4.6 mm 3-μm phenoscale C18 column (Phenomenex, Torrance, CA) conditioned with 67% methanol containing 0.01 mol/L DOSS, 1 mmol/L NH₄PO₄ (pH 6.0), and 0.3% triethylamine at a flow rate of 1 mL/min. Fluorescence detection was with an Applied Biosystems 970 fluorometer (Perkin-Elmer, Norwalk, CT) set at 380 nm excitation with a 470-nm cutoff filter, and data collected at 0.01 μA full scale. The chromatographic system consisted of A knauer (Sonnten, Inc., Upper Saddle River, NJ) model 42 pump and a Waters (Waters Associates, Milford, MA) model 712 WISP autosampler, controlled by an Axxiom model 747 PC controller/data system (Axxiom Chromatography, Inc., Moorpark, CA). Each sample was run in duplicate and the results were expressed as an average of the closed (lactone) values. In general, the samples were processed within an hour after collection and the percentage of closed determinations ranged from 60% to 90% of the total topotecan content in the clinical sample.

**Immunohistochemistry.** Responders were identified and their baseline tumors selected for study. We paired tumor from these patients with those of nonresponders evaluable for response receiving the same treatment dose level. Tissue sections 5-μm thickness were placed on charged glass slides from formalin-fixed paraffin-embedded tissues and deparaffinized by baking overnight at 60°C followed by three washes of xylene and three washes through graded alcohol (100%, 70%) to distilled water. The rehydrated slides were stained with antibodies to topoisomerases I and II (Novocastra Laboratories, United Kingdom; clones 1D6 and 3F6, respectively, both at dilutions 1:50) via an automated immunostainser, NexES (Ventana Medical Systems, Tucson, AZ) following appropriate antigen epitope retrieval by heating the slides to boiling in a 0.01 mol/L citrate buffer solution at pH 6 for 20 minutes. Positive (tonsilar lymphocytes) and tumor (a topoisomerase I– and II– negative and a positive topoisomerase I and II colon cancer) controls were used. Negative control studies were done in the absence of primary antibody. Primary antibody incubation was done overnight at room temperature followed by standard detection using a streptavidin-biotin peroxidase complex with 3,3-diaminobenzidine as the chromogen. Immunostains were graded as follows: negative or 0, when none of the cells stained for the topoisomerase enzyme; 1+, when 1% to 25% of the cells stained positively; 2+, when 26% to 50% of the cells stained positively; 3+, when 51% to 75% of the cells stained positively; and 4+, when >75% of the cells stained positive for the topoisomerase enzyme. Only nuclear localization was considered positive.

**Results**

**Patient characteristics.** Fifty-seven patients were enrolled on this protocol: 34 on continuous infusion topotecan and 23 on oral topotecan; one responder with papillary serous ovarian cancer on continuous infusion topotecan was moved at her request to oral topotecan, when this section of the study opened. She received two full cycles before returning to continuous infusion topotecan administration, again at her request.

On continuous infusion topotecan, there were 16 men and 18 women (eight Hispanic, 17 Caucasian, three African American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asian or Pacific Islander), ranging in age from 37 to 80, with a median of 54 years. The majority had an American, and six Asia
sarcoma \((n = 1)\), and unknown primary \((n = 3)\); adenocarcinoma, squamous, and small cell carcinoma). On the two oral topotecan strata, all were women (four Hispanic, 17 Caucasian, one African American, and one Asian). Ages ranged from 43 to 68 years, with a median of 54 years. Tumor types included ovarian \((n = 14)\), tubal \((n = 3)\), gastric \((n = 1)\), non–small cell lung carcinoma \((n = 1)\), endometrial \((n = 1)\), nasopharyngeal \((n = 1)\), unknown primary adenocarcinoma \((n = 1)\), and mixed mullerian tumor \((n = 1)\).

Overall, 23 patients with ovarian or tubal cancers: seven were enrolled on continuous infusion topotecan and 17 on oral topotecan (one patient noted above was also enrolled in the continuous infusion topotecan part). Three patients had tubal adenocarcinomas including one with mixed clear cell and endometroid features. Twenty patients had ovarian cancers: six serous papillary, 12 otherwise unspecified ovarian adenocarcinomas, and two with mixed clear cell and endometroid features. All had received prior platinum and paclitaxel based regimens, and only four were in the potentially platinum-sensitive category (relapse, 6 m or longer after their last platinum regimen). Two of seven on i.v. topotecan had received both doxorubicin and topotecan earlier, whereas earlier exposure to either drug was noted in only 2 of 17 patients on oral topotecan, plus the one patient transiently shifting to oral topotecan from i.v. topotecan had received prior doxorubicin.

**Treatment given.** On continuous infusion topotecan, 34 patients were enrolled and received a total of 157 cycles (range, <1-19; median, 6); 10 received more than six cycles. Only 28 patients were evaluable for toxicity. Patients receiving either no i.v. topotecan or >1 week of infusion (five patients) and no Doxil (one patient) were deemed inevaluable. Other early features. All had received prior platinum and paclitaxel based regimens. Two of seven on i.v. topotecan had received both doxorubicin and topotecan earlier, whereas earlier exposure to either drug was noted in only 2 of 17 patients on oral topotecan, plus the one patient transiently shifting to oral topotecan from i.v. topotecan had received prior doxorubicin.

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The seven ovarian patients on continuous infusion topotecan received 63 cycles (range, <1-19; median, 9) with six patients receiving at least six cycles. On oral topotecan: 23 patients received a total of 105 cycles (range, <1-18; median, 3); five received more than six cycles. The 16 ovarian or tubal cancer patients on oral topotecan received 75 cycles (range, <1-18; median, 4); only three patients received at least six cycles.

**Treatment toxicities on continuous infusion topotecan.** Of the 28 patients evaluable for toxicity, no dose-limiting toxicities occurred at the first three dose levels. At dose level 4, the second patient enrolled had grade 4 thrombocytopenia and four more patients were entered (one had grade 4 neutropenia and one had both grade 4 neutropenia and grade 4 thrombocytopenia). Hence, three additional patients were enrolled at dose level 3. One of these, patients experienced grade 3 diarrhea at completion of cycle dosing. To ensure feasibility of every 4 week dosing, further dose modification at Doxil (40 mg/m²/day 1, dose level 4a) with continuous infusion topotecan at 0.4 mg/m²/day 1 to 14 was therefore introduced, and six patients were enrolled at this dose level. Only one patient had dose-limiting grade 4 neutropenia and grade 4 thrombocytopenia (Table 3); dose level 4a was defined as the recommended phase II dose.

Other grade 2 and grade 3 toxicities included anemia (one each), neutropenia (grade 2, \(n = 5\)); grade 3, \(n = 3\)), thrombocytopenia (grade 2, \(n = 2\); grade 3, \(n = 4\)), nausea (grade 2, \(n = 3\), vomiting (grade 2, \(n = 1\)), mucositis (grade 2, \(n = 6\)), and fatigue (grade 2, \(n = 4\)). We documented infusion-related events in six patients who developed port infections during cycle 2 requiring hospital admission for i.v. antibiotics. One of these patients went on to have *Clostridium difficile* colitis with grade 3 diarrhea, occurring during her hospitalization for *Staphylococcus aureus* sepsis. One patient had *Pneumocystis carinii* pneumonia after cycle 3 and improved following trimethoprim-sulfamethoxazole and steroids.

**Treatment toxicities on oral topotecan.** Because patients were randomized to topotecan alone or topotecan with Doxil in cycles 1 and 2 for pharmacology studies, the dose-limiting toxicity shown in Table 2 reflect only the first cycle of combined topotecan and Doxil. On stratum A, 14 patients were entered but two did not complete cycle 1 because of progression. A fatal episode of grade 4 leukopenia, febrile neutropenia, grade 4 thrombocytopenia complicated by *Escherichia coli* sepsis occurred on day 4 of cycle 1. Four other patients did not complete cycle 1: a grade 3 neutropenia and sepsis complicated by renal insufficiency and grade 3 diarrhea, nausea and vomiting and

### Table 2. Dose-limiting toxicities at each dose level

<table>
<thead>
<tr>
<th>DL</th>
<th>Doxil</th>
<th>T-CIV</th>
<th>Patient no.</th>
<th>Evaluable patients</th>
<th>DLT</th>
<th>G4n</th>
<th>G4t</th>
<th>G3d</th>
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</thead>
<tbody>
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<td>30</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
<td>30</td>
<td>0.3 × 21</td>
<td>4</td>
<td>3</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.4 × 21</td>
<td>8</td>
<td>3 + 3 (a)</td>
<td>0+1 (a)</td>
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<td>0</td>
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</tr>
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<td>1 (x)</td>
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</tr>
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<td>4a</td>
<td>40</td>
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<td>6</td>
<td>1</td>
<td>1 (y)</td>
<td>1 (y)</td>
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</tr>
<tr>
<td>Stratum</td>
<td>Doxil</td>
<td>T-PO</td>
<td>Patient no.</td>
<td>Evaluable patients</td>
<td>DLT</td>
<td>G3n</td>
<td>G4t</td>
<td>G3d/N/V</td>
</tr>
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<td>A</td>
<td>30</td>
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<td>12</td>
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<td>1</td>
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<tr>
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<td>9</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
</tbody>
</table>

NOTE: At DL3, first three patients had no DLT (a). At DL4, 3 of 11 patients had DLT. Thus another three patients were entered at DL3 and one patient had DLT. One patient with both G4 n and t (x). One patient with both G4 n and t (y), one of whom also had fatal sepsis (z, see text). Modified dose-level 4, recommended phase II dose (4a).

Abbreviations: DL, dose-level; G, grade; n, neutropenia; t, thrombocytopenia; d, diarrhea; N/V, nausea/vomiting; T-CIV, continuous infusion topotecan; T-PO, oral topotecan.
three patients who had grade 3 \((n = 1)\) on grade 2 \((n = 2)\) gastrointestinal events. Another patient experienced grade 3 neutropenia and fever and grade 3 diarrhea, nausea, and vomiting terminating treatment after completing cycle 1 of oral topotecan alone.

On stratum B, nine patients were entered. Two went off study early because of toxicity: one had a grade 3 reaction to Doxil and one experienced grade 3 diarrhea. Other grade 2 and 3 toxicities for both strata included anemia (grade 2, \(n = 3\)), neutropenia (grade 2, \(n = 4\)), thrombocytopenia (grade 2, \(n = 1\)), nausea (grade 2, \(n = 1\)), vomiting (grade 2, \(n = 2\)), diarrhea (grade 2, \(n = 5\)), mucositis (grade 2, \(n = 1\)), and fatigue (grade 2, \(n = 1\)). Finally, one patient who developed small bowel obstruction during cycle 1 underwent surgical correction and then resumed treatment for another three cycles.

**Cardiac assessment in patients with cumulative doses of Doxil >500 mg/m\(^2\).** As in our prior experience with Doxil (39), we chose a cumulative dose of >500 mg/m\(^2\) as our cut off for analysis. Four patients had ovarian cancer and no preexisting heart disease; one had a history of hypertension on treatment. Their baseline MUGA scans left ventricular ejection fractions ranged from 66% to 72%. Only one patient (who had received 300 mg/m\(^2\) prior free doxorubicin) experienced a decrease in left ventricular ejection fractions (66-58%). She eventually progressed after 29 cycles, and an end-of-study left ventricular ejection fraction was 72%. Two other patients showed no change from baseline left ventricular ejection fraction after 13 and 63 cycles of maintenance Doxil, and one other patient receiving 13 cycles of maintenance Doxil did not have repeat MUGA scans but underwent an uneventful bowel resection for tumor related obstruction 1 year later.

**Antitumor effects.** Table 3 provides details on objective responders with ovarian cancer: two nonmeasurable patients [one relapsing with rapidly rising CA125 to over 300 within 5 months of her initial carboplatin and paclitaxel induction and the other with surgically documented unresectable liver and peritoneal disease (persisting after i.p. platinum attained long-lasting (29 to >64 m) complete responses by CA125-only criteria after two cycles followed eventually by Doxil maintenance]. A third patient had a partial response lasting 26 m and three patients had stable disease over 18 to 34 months; one of these had been treated with single agent Doxil followed by single agent bolus \(\times 5\) day cycles of topotecan with only transient improvement. Of 16 patients with ovarian or tubal cancer on oral topotecan, one had a partial response by computerized tomography scan and normalization of CA125 at 6 months, lasting for 18 months. Seven had stable disease for 7 to >34 months, whereas three progressed after two cycles.

Of the 27 patients with nongynecologic tumors on continuous infusion topotecan, four patients were ineligible for response, due to line sepsis in cycles 1 and 2 (one patient) and surgical events or deterioration precluding dosing of the drugs (three patients). A partial response lasting 4 months was seen in one nasopharyngeal patient, stable disease was documented in 10 patients whereas 12 progressed. On oral topotecan, only five nongynecologic patients were evaluable for response (one came off study with a grade 3 infusion reaction to Doxil in cycle 1): a gastric cancer patient achieved a complete response after four cycles that lasted eight additional months, whereas the other four patients progressed after two cycles.

**Topotecan plasma levels.** Peripheral blood samples in both cycles 1 and 2 (i.e., topotecan alone or Doxil and topotecan) were obtained in 10 patients on oral topotecan, but a very wide variability was noted (0.26-4.72 ng/mL) while receiving drug. Data from 18 patients who were studied on topotecan alone had a mean blood level of topotecan lactone levels of 1.72 \(\pm\) 1.05 ng/mL (SD), whereas nine patients who were randomized to topotecan + Doxil showed a mean level of 1.46 \(\pm\) 1.32 (SD).

---

**Table 3. Description of responders among epithelial ovarian or tubal cancer patients**

<table>
<thead>
<tr>
<th>Characteristic at enrollment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurable disease</td>
<td>No</td>
<td>No(^*)</td>
<td>Yes, 4 cm</td>
<td>Yes, 3 cm</td>
</tr>
<tr>
<td>CA125 at enrollment</td>
<td>300</td>
<td>68(^*)</td>
<td>1,900</td>
<td>43</td>
</tr>
<tr>
<td>Relapse within 6 m of platinum</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>No. prior chemotherapies</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Exposure to prior anthracycline or topotecan</td>
<td>No</td>
<td>Doxorubicin</td>
<td>No</td>
<td>Topotecan</td>
</tr>
<tr>
<td><strong>Response to therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment received</td>
<td>T-CIV</td>
<td>T-CIV</td>
<td>T-CIV</td>
<td>T-PO</td>
</tr>
<tr>
<td>Response on CT scan</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Time to response on CT scan (mo)</td>
<td>—</td>
<td>—</td>
<td>3.7</td>
<td>6</td>
</tr>
<tr>
<td>Response by CA125</td>
<td>CR</td>
<td>CR</td>
<td>CR</td>
<td>CR</td>
</tr>
<tr>
<td>Time to maximal CA125 response (mo)</td>
<td>1.25</td>
<td>2</td>
<td>10</td>
<td>5.5(^1)</td>
</tr>
<tr>
<td>Duration of response (mo)</td>
<td>&gt;64</td>
<td>29</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>No. cycles of combination therapy</td>
<td>6</td>
<td>18(^\dagger)</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>No. months of maintenance Doxil</td>
<td>52</td>
<td>11</td>
<td>15</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^*\)This patient had documented disease in liver and pelvis at second-look surgery 7 weeks earlier.

\(^\dagger\) CA125 initially increased from 43 to 232 on cycle-1 of oral topotecan alone, then normalized at 4.5 months from starting combination Doxil and oral topotecan.

\(^1\)Includes two cycles on oral topotecan.
This variability was also noted in our other ongoing studies with oral topotecan and in marked contrast with our prior phase II study using 0.4 mg/m²/d of 21 day infusional topotecan where the mean range for topotecan lactone was 3.6 ± 0.84 ng/mL on day 8 (n = 15, mean ± SD). The trough levels from eight patients were all below the lower limit of detection of the topotecan assay (0.1 ng/mL).

**Immunohistochemistry and correlation with responses.** Immunohistochemical evaluation of topoisomerases I and II was done on tumor samples from 12 patients. Six of these patients were ovarian or tubal cancers. Of these six, four with >3 and >4 dual topoisomerase expression (Fig. 1) showed clinical benefit (defined as objective response or stable disease as best response). On the other hand, six nonovarian cancer cases showed no obvious relationship between tissue topoisomerase expression and clinical benefit (Table 4).

**Table 4. Correlation of clinical benefit to tissue topoisomerase I and II immunostaining**

<table>
<thead>
<tr>
<th>Ovarian Cases</th>
<th>CB versus PD</th>
<th>Non-ovarian Cases</th>
<th>CB versus PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+, 4+</td>
<td></td>
<td>3+, 4+</td>
<td></td>
</tr>
<tr>
<td>Topo I and II</td>
<td>4 versus 0</td>
<td>Topo I and II</td>
<td>3 versus 2</td>
</tr>
<tr>
<td>Topo II</td>
<td>1 versus 0</td>
<td>Topo II</td>
<td>1 versus 1</td>
</tr>
<tr>
<td>Topo I</td>
<td>1 versus 1</td>
<td>Topo I</td>
<td>0</td>
</tr>
<tr>
<td>Both &lt;3+</td>
<td></td>
<td>Both &lt;3+</td>
<td>2 versus 1</td>
</tr>
</tbody>
</table>

NOTE: See text and Fig. 1 for definition of 0 to 4+ immunostaining. Abbreviations: CB, clinical benefit (responses and stable disease); PD, progressive disease; Topo, topoisomerase.

**Discussion**

This study sought to determine the appropriate dose of Doxil to be combined with topotecan by prolonged low-dose administration. The extended circulation time of the pegylated liposomal doxorubicin as well the protracted low-dose topotecan administration, allow the testing of the concept of prolonged dual topoisomerase inhibition as a therapeutic strategy. With the development of oral topotecan, the study was amended to explore its suitability in this combination and provide an opportunity to eliminate the discomfort, infection, and thrombosis associated with semipermanent venous access devices.

**Fig. 1.** A, ovarian cancer showing 90% nuclear staining for topoisomerase I (graded as 4+). B, same tumor sample showing 70% nuclear staining for topoisomerase II (graded as 3+). Patient experienced a partial response on protocol treatment. C, ovarian cancer showing 20% nuclear staining for topoisomerase I (graded 1+). Not shown is 50% staining for topoisomerase II. The patient experienced progressive disease on protocol treatment. D, ovarian cancer showing 20% nuclear staining for topoisomerase II (graded 1+). Not shown is 70% staining for topoisomerase I (grade as 3+). This patient also experienced progressive disease on protocol treatment.
However, whereas the continuous infusion topotecan in combination with Doxil was feasible over several dose ranges, the oral topotecan was associated with one fatal episode of sepsis and severe gastrointestinal intolerance to frequently debilitating nausea, vomiting, and diarrhea, toxicities that were more apparent in pretreated patients (stratum A). Topotecan levels on oral topotecan were erratic and with a lower mean that was ~50% lower, contrasting with our extensive experience with i.v. topotecan (18). The discovery that topotecan is a substrate of the breast cancer resistance protein (40, 41), an ATP-binding cassette xenobiotic exporter expressed in the biliary canalicular membrane, and to a lesser extent in the colonic mucosa (and in the renal epithelium; ref. 42) provides an explanation for this pharmacologic and toxicologic behavior. With poor patient tolerance and compliance and the substantial gastrointestinal toxicity encountered, we conclude that the combination with oral topotecan is not yet suitable for phase I/II trials. Development of breast cancer resistance protein inhibitors specific for the colonic mucosa and for the biliary canaliculi that modulate enterohepatic circulation could render the substitution of oral topotecan for i.v. topotecan more appealing (43, 44).

Fifty-seven patients were entered on this trial, with dose escalations as shown in Table 1. We were specifically interested in the responsiveness by patients with ovarian and tubal cancers to this combination, because both drugs show antitumor activity in second-line therapy for both platinum-sensitive and platinum-resistant patients. In fact, our observations including 23 patients with ovarian or tubal cancer are relevant to future phase II studies: two complete responses and two partial responses (17%) responded (three of seven in i.v. topotecan), and these were of very long duration, when complemented by maintenance with Doxil alone (45). Clinical benefit was found in all four epithelial ovarian or tubal cancer patients with elevated tissue levels of both topoisomerase enzymes. The ability to predict with immunohistochemistry whether epithelial ovarian or tubal cancer patients may respond to the combination requires further study.

In conclusion, we report on the safety profile of Doxil combined with a proracted low-dose infusion of topotecan on a 14-day schedule. The recommended phase II dose is Doxil 40 mg/m²/day 1 with topotecan by continuous infusion 0.4 mg/m²/days 1 to 14 every 28 days. Myelosuppression is the predominant toxicity observed on this schedule, as well as following topotecan by continuous infusion 0.4 mg/m²/days 1 to 21 with a lower dose of Doxil. However, this last schedule is more cumbersome to patients and gastrointestinal toxicity is more prominent. Presently, oral topotecan does not seem suitable for delivery in combination with Doxil but remains a viable option should breast cancer resistance protein inhibitors (42, 43) become available for future clinical trials.

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Phase I Study of Combined Pegylated Liposomal Doxorubicin with Protracted Daily Topotecan for Ovarian Cancer

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