Drugs of the Future 2004, 29(1): 9-22

CCC: 0377-8282/2004

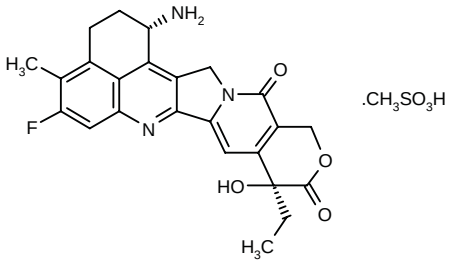
Exatecan Mesilate

Prop INNM; USAN

Anticancer Agent
DNA Topoisomerase I Inhibitor

DX-8951f

(1*S*,9*S*)-1-Amino-9-ethyl-5-fluoro-9-hydroxy-4-methyl-2,3,9,10,13,15-hexahydro-1*H*,12*H*-benzo[*de*]pyrano[3',4':6,7]-indolizino[1,2-*b*]quinoline-10,13-dione mesylate



$C_{24}H_{22}FN_3O_4.CH_4O_3S$

Mol wt: 531.5584

CAS: 169869-90-3

CAS: 171335-80-1 (as free base)

CAS: 197720-53-9 (as hydrate)

CAS: 144008-87-7 (as monohydrochloride)

EN: 197987

Abstract

Exatecan mesilate is a second-generation topoisomerase inhibitor that prevents rapidly dividing cells from replicating by interrupting DNA transcription, ultimately leading to cell death. Preclinical studies showed exatecan to have broad-spectrum antitumor efficacy. Its major adverse events were hematological and gastrointestinal. Phase I studies were subsequently conducted in patients with various drug-refractory malignancies. Exatecan administration was associated with significant myelotoxicity, but a relative paucity of other side effects. Neutropenia and thrombocytopenia were described as dose-limiting toxicities in these studies. A 30-min i.v. infusion over 5 days every 3 weeks was selected as the most appropriate schedule for phase II trials. Phase II analyses failed to demonstrate the same level of anticancer efficacy as initially suggested from preclinical analyses. However, exatecan did show some promise in the treatment of refractory ovarian and pancreatic cancer, maintaining the potential for clinical utility in certain situations. The potential for use in combination therapy also remains a valid option.

Synthesis

Friedel-Crafts acylation of 2-fluorotoluene (I) with succinic anhydride (II) in the presence of $AlCl_3$ affords 4-(4-fluoro-3-methylphenyl)-4-oxobutyric acid (III), which is reduced by hydrogenation over Pd/C to furnish the arylbutyric acid (IV). Esterification of compound (IV) by means of $SOCl_2$ in MeOH provides the methyl ester (V), which is submitted to aromatic ring nitration with KNO_3 and H_2SO_4 to yield 4-(4-fluoro-5-methyl-2-nitrophenyl)-butyric acid methyl ester (VI). After basic hydrolysis of the methyl ester of compound (VI), the resulting arylbutyric acid (VII) is subjected to intramolecular cyclization in hot polyphosphoric acid, producing the tetralone (VIII). Reduction of the keto group of tetralone (VIII) with $NaBH_4$, followed by dehydration of the resultant alcohol (IX) under acidic conditions, results in the dihydronaphthalene (X). Hydrogenation of the olefinic double bond and simultaneous reduction of the nitro group of compound (X) in the presence of PtO_2 gives the amino tetralin (XI), which is further protected as the acetamide (XII) employing Ac_2O and Et_3N. Regioselective benzylic oxidation of compound (XII) with $KMnO_4$ in acetone provides tetralone (XIII), which by functionalization of the α-position using butyl nitrite and *t*-BuOK affords the keto oxime (XIV). This oxime (XIV) is then reduced with zinc in the presence of Ac_2O to yield the diacetamide (XV), which is hydrolyzed under acidic conditions to give diamine (XVI). Finally, selective acylation of compound (XVI) at the aliphatic amino group with ethyl trifluoroacetate produces the trifluoroacetamide (XVII) (1, 2). Scheme 1.

Hydrolysis of the known pyranoindolizine acetal (XVIII) with 90% trifluoroacetic acid gives the trione (XIX), a known intermediate in the campthothecins synthesis, which is condensed with the trifluoroacetamide (XVII) in refluxing toluene to yield the hexacyclic compound (XX) as a diastereomeric mixture. Finally, this compound is submitted to acidic hydrolysis of the trifluoroacetamido

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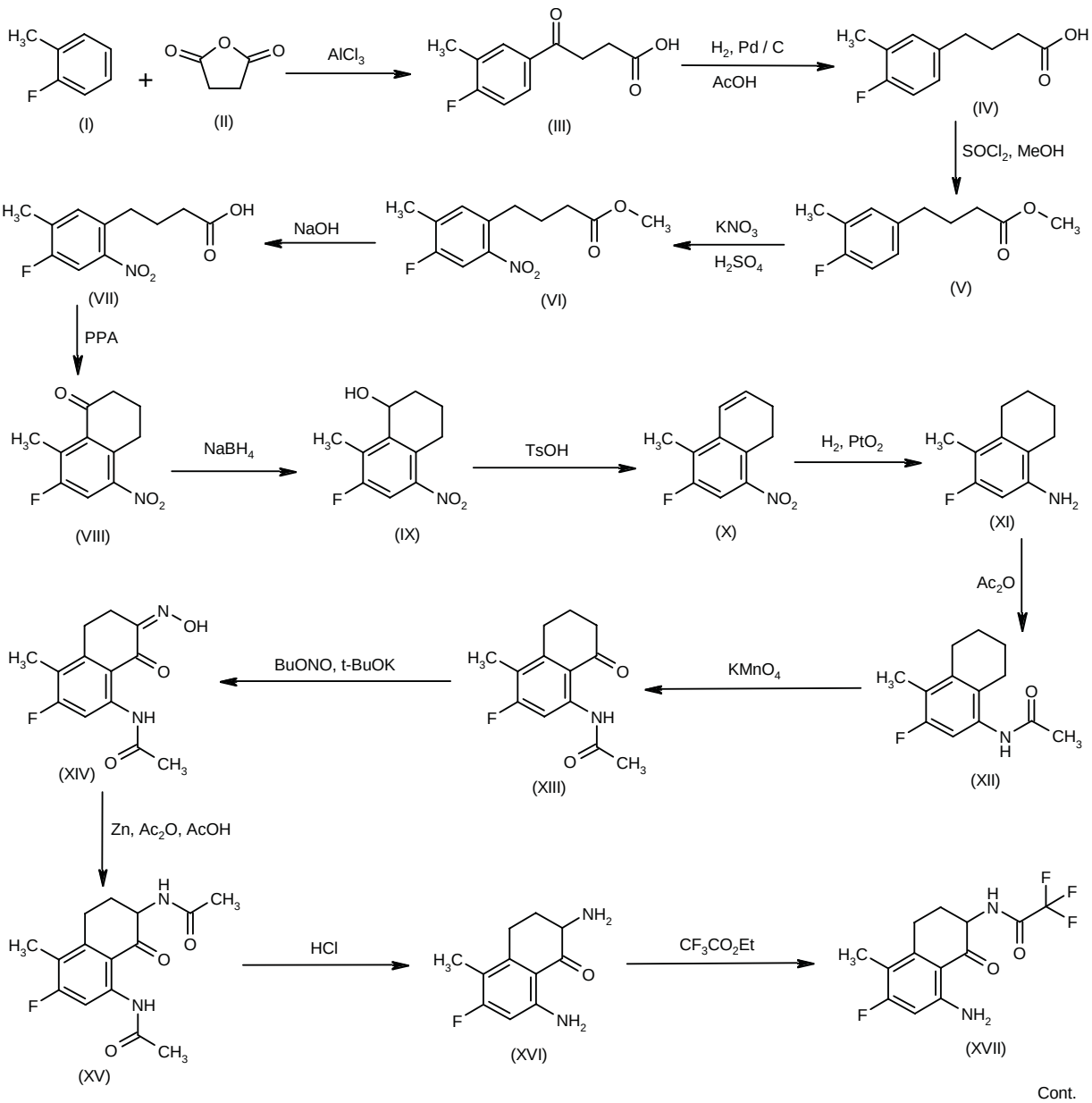
Scheme 1: Synthesis of Exatecan Mesilate



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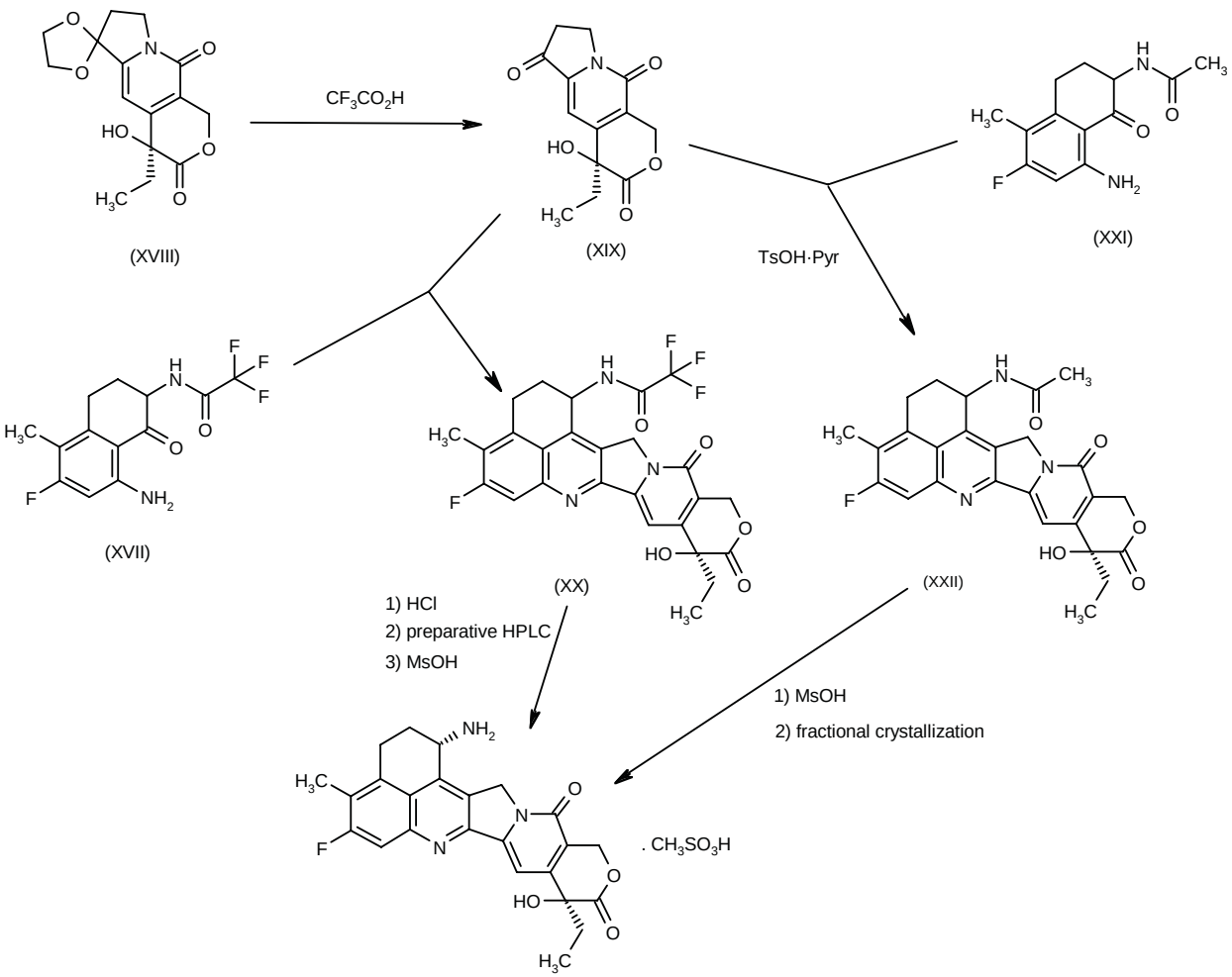
group, followed by isolation of the desired isomer by preparative HPLC (1, 2). Alternatively, condensation of trione (XIX) with the acetamido tetralone (XXI) provides the hexacyclic acetamide (XXII), which is hydrolyzed with methanesulfonic acid, and then the diastereomeric mixture of mesylate salts finally separated by fractional crystallization (3). Scheme 2.

Introduction

Topoisomerase I is a nuclear enzyme that plays a critical role in DNA replication, gene transcription and cell division. It causes DNA to unravel in preparation for transcription, through the relaxation of the torsionally strained duplex molecule. Topoisomerase I forms a covalent bond with double-stranded DNA, bringing about a nick in a single strand that ultimately results in a break. This intermediate is called the cleavable complex. The gap in the broken strand is subsequently resealed, and topoisomerase I dissociates from the complex (4).

Topoisomerase I inhibitors prevent the resealing of the DNA molecule by binding to the cleavable complex. This stabilization of the complex effectively prevents transcription from occurring, leading to the accumulation of DNA molecules with single-strand breaks. Double-strand

Scheme 2: Synthesis of Exatecan Mesilate



breaks are subsequently caused by collision of the single-strand molecules with the DNA-replication fork – a process that ultimately leads to cell death (4).

Camptothecin, a natural alkaloid isolated from the Chinese plant Camptotheca acuminata in 1966, was the first topoisomerase I inhibitor to be developed. Camptothecin administration, however, was associated with relatively poor cytotoxicity, as well as a poor safety profile (2-5). Topotecan (Hycamptin®) and irinotecan (Camptosar®) were subsequently developed as camptothecin analogues, and are currently commercially available for the treatment of various carcinomas. Topotecan is particularly effective in patients with refractory/relapsed metastatic breast or small cell lung cancer, while irinotecan is effective in patients with refractory metastatic colorectal and lung carcinomas. Irinotecan is associated with gastrointestinal side effects, clinically severe diarrhea being particularly troublesome. Irinotecan is a prodrug that is activated by an enzyme that exists in distinct polymorphic forms. The differing actions of these polymorphs lead to increased interpatient variability, and are the cause of the severe and often unpredictable side effects associated with irinotecan administration. Exatecan, a second-generation topisomerase I inhibitor and a synthetic analogue of camptothecin, was subsequently designed to enhance the antitumor efficacy of these first-generation derivatives (4).

Pharmacological Actions

Exatecan exhibits favorable chemotherapeutic properties, indicating a therapeutic advantage over the topoisomerase I inhibitors topotecan and irinotecan. Exatecan differs from its predecessors in that it is water-soluble, displays reduced interindividual variability and

exhibits low cross-resistance. Furthermore, exatecan is active itself and does not require enzymatic (esterase-dependent) activation.

In vitro and *in vivo* experiments using human colon and ovarian cancer cells/tumors indicated particular promise for exatecan in ovarian cancer. The compound was more active than SN-38, the active metabolite of irinotecan, against most cell lines and its activity was not affected by the expression of various multidrug resistance (MDR)-associated proteins; rather, resistance to exatecan was related to the induction of breast cancer resistance protein (BCRP). Studies in nude mice demonstrated no activity against colon cancer xenografts, whereas high activity (51-94% growth inhibition) was seen against ovarian cancer xenografts. The compound was equally effective following i.p. and i.v. administration and was more active when administered weekly rather than daily (6).

Results from comparisons of different camptothecins showed exatecan to have potent antitumor activity against colon, breast, gastric and esophageal cancer cell lines. Exatecan had superior antitumor efficacy compared to topotecan and irinotecan against all human malignancies tested, with mean IC_{50} values of 30.8, 48.2, 43.6 and 70.6 ng/ml, respectively, against esophageal, gastric, colorectal and breast cancer lines (10).

In vitro and *in vivo* analyses using human tumors have shown exatecan to inhibit topoisomerase with a potency 20, 10 and 3 times greater than that exhibited by camptothecin, topotecan and SN-38, respectively. Exatecan showed greater antitumor activity against tumor cell lines, particularly breast, lung, gastric and colorectal carcinoma cells. It was also more effective than the other camptothecins against human gastric adenocarcinoma xenografts (11).

Significant antitumor activity was reported in mice bearing solid tumors treated with exatecan, and it had a wider effective dosse range compared to irinotecan and topotecan. Moreover, it was effective against tumor xenografts not responding well to irinotecan (12). Exatecan exhibited potent antitumor efficacy in a severe combined immunodeficient (SCID) mouse model of acute myelogenous leukemia (AML). An increase in dose was associated with prolonged survival on a 1-day schedule, which was generally well tolerated. A 3-day schedule significantly improved survival and was well tolerated. A low-dose 5-day dosing schedule was also well tolerated and significantly improved survival, but higher doses were toxic. Overall, it was concluded that both efficacy and toxicity of exatecan are schedule-dependent (13, 14).

In head-to-head comparisons, using 1-h exposure, exatecan was more effective than topotecan against adult tumor specimens, including head and neck, renal, hepatic, lung, ovarian, prostatic, breast, mesothelioma and colorectal tumors. Equivalent efficacy was observed, however, for equimolar concentrations of the drugs with continuous exposure. The maximum tolerated dose (MTD) of exatecan was 3 times that of topotecan, allowing for higher doses to be administrated. Exatecan also exhibited good activity against pediatric tumor specimens (15).

Pharmacokinetics and Metabolism

Exatecan is metabolized by the hepatic cytochrome P-450 enzymes CYP3A4 and CYP1A2. The major urinary metabolites in rats and humans are the 4-hydroxymethyl and the 4-hydroxylated forms (UM-1 and UM-2, respectively). UM-1 is the major metabolite in liver microsomes, being metabolized by the CYP3A4 isozyme (16, 17).

The preclinical and clinical toxicokinetics of exatecan were compared using data from dogs (the most sensitive species in terms of toxicity) and 6 phase I clinical trials in patients administered a starting dose equivalent to one-third of the toxic dose low (TDL) in dogs, given as a single dose every 3 weeks, daily x 5 every 3 weeks and weekly x 3 every 4 weeks, by 30-min or 24-h i.v. infusion. Clinical toxicity was similar to in dogs, neutropenia being dose-limiting. Pharmacokinetic analysis in humans revealed much slower clearance than in dogs and an elimination half-life of 8.9 h. In contrast to in dogs, the toxicokinetics in humans appeared to be independent of schedule (18).

Exatecan displayed linear pharmacokinetics following both single and multiple doses in phase I and II studies in cancer patients. Data from a number of phase I studies have been compiled in Table I (19-34).

Clinical Studies

A large number of phase I studies have been conducted in order to determine the antitumor efficacy and tolerability of exatecan in different cancers. The aim of phase I analyses was also to determine dose-limiting toxicity (DLT), MTD and the most appropriate dosing schedule for phase II studies.Exatecan was administered via 24-h continuous infusion every 3 weeks to patients with advanced solid tumors in a dose-escalating phase I study. Twenty-two patients with previously treated colon (n=21) and lung (n=1) carcinoma were administered exatecan 0.15 mg/m^2 (equivalent to one-third of the toxic dose in dogs). The dose could be escalated up to 3.6 mg/m^2 depending on treatment response and adverse events profiling. Patients were administered 1-8 (median of 2) treatment cycles throughout the course of the study (21-24). The MTD was found to be 2.4 mg/m^2 in this group of patients with solid tumors. The DLT of exatecan was granulocytopenia and granulocytopenia/thrombocytopenia in minimally pretreated and heavily pretreated patients, respectively. The authors recommended that the 2.4 mg/m^2 dose be used in the former, although a lower starting dose (1.2-2.4 mg/m^2) should be employed in patients with more extensive prior chemotherapy. Preliminary antitumor efficacy analysis showed that 82% patients experienced progressive disease, with no reports of either complete or partial responses in this setting. Stable disease was achieved by 18% of patients (21). The results of this study and most of those that follow are summarized in Table II.

Table I: Pharmacokinetic parameters of exatecan after intravenous infusion to cancer patients (from Prous Science Integrity®).

Dose	$t_{1/2}$ (h)	Cl (l/h/m^2)	V_{ss} (l/m^2)	MRT (h)	C_{max} (ng/l)	$AUC_{0\rightarrow\infty}$ (ng·h/l)
30-min i.v. infusion o.d. x 5 d 1x/3 wks						
0.1	8.4	1.4	11.7	9.7	29	106
0.2	8.1	1.4	12.5	8.9	40	118
0.3	10.0	1.5	13.1	11.6	52	317
0.4	8.2	1.7	14.6	9.6	55	247
0.5	8.4	1.7	13.5	9.2	77	308
0.6	9.5	1.6	14.6	10.8	78	490
30-min i.v. infusion 1 x/wk						
1.0	8.1	1.7	14.4	10.7	120	775
1.8	8.0	1.1	10.6	9.8	284	1530
2.3	7.7	1.9	14.4	8.1	280	1580
3.0	10.2	1.9	20.0		320	1840
30-min i.v. infusion 1 x/3 wks						
4.0	7.3	1.7	13.2	7.6	680	2580
5.3	7.8	2.1	17.7	9.2	608	3420
7.1	8.4	2.1	20.0	10.2	1150	4820
24-h i.v. continuous infusion						
0.3-1.2	9.5	1.6	15.3	16.8		

Abbreviations: $t_{1/2}$, elimination half-life; Cl, systemic plasma clearance; V_{ss}, volume of distribution at steady state; MRT, mean residence time; C_{max}, peak plasma concentration; $AUC_{0\rightarrow\infty}$, area under the concentration-time curve.

The 24-h continuous dosing regimen was tested in another phase I study in 27 patients with colorectal (n=15), esophageal (n=5), pancreatic (n=4), hepatic (n=2) and gastric (n=1) cancer. Exatecan 0.05-1.2 mg/m^2/day was administered via weekly i.v. infusion for 3 of every 4 weeks. The MTD was 0.8 and 0.53 mg/m^2 in minimally and heavily pretreated subjects, respectively. The DLT was found to be neutropenia and thrombocytopenia in this analysis. Diarrhea, constipation, nausea, vomiting, asthenia and neurosensory problems were among the nonhematological malignancies observed in the study. Five patients with colorectal, pancreatic or hepatocellular cancer experienced stable disease as their best response (19, 25).

Exatecan was delivered via protracted 21-day continuous infusion in patients with advanced solid malignancies. Thirty-one patients were included in the study and had been diagnosed with colorectal, lung, breast, renal and other miscellaneous cancers, with a performance status of 0-2. The study was performed in 2 distinct phases. In the first phase, investigators followed the continual reassessment method to prolong the dosing regimen of exatecan 0.15 mg/m^2/day. Drug administration was progressively increased from 5 to 21 days at a constant dose. In the second phase, the exatecan dose was increased in order to determine the MTD following a 21-day continuous infusion. Investigators reported negligible toxicity at infusion durations under 21 days. The MTD was determined as 0.15 mg/m^2/day by 21-day continuous infusion. Seven patients required dose reduction due to adverse events. One patient was a significant outlier, experiencing an abnormally high toxicity profile consisting of grade 4 neutropenia and thrombocytopenia. The patient had been taking Essiac tea for a long period of time, and continued to do so throughout the course of the study. It was suggested that the combination of Essiac tea with exatecan may have caused these serious adverse events. The use of these agents in combination is therefore contraindicated. Two patients achieved a partial response (PR) and objective evidence of antineoplastic activity (*i.e.*, a reduction in measurable disease) was obtained in 3 other patients (26, 35, 36).

Weekly 30-min infusions of exatecan 1-3.13 mg/m^2 were administered every 3 of 4 weeks in patients with advanced solid malignancies. Thirty-five patients were included in this analysis. Primary tumors were located at colorectal, lung, skin, ovarian and other sites. Patients were administered 1-10 (median of 2) cycles of exatecan therapy. The MTD on this dosing schedule was 2.75 and 2.1 mg/m^2 for minimally and heavily pretreated patients, respectively. The DLTs were neutropenia and neutropenia/thrombocytopenia in these respective patient groups. Hematological side effects, including anemia, leukopenia, neutropenia and thrombocytopenia, were experienced by the majority of patients. Myelosuppression was found to be reversible in these patients, however. Nonhematological toxicity (*i.e.*, nausea, vomiting, diarrhea, fever, alopecia, fatigue, headache and anorexia) did not appear to be dose-dependent. Twenty-four patients withdrew from the study due to the occurrence of adverse events. Thirty-one patients were evaluable for response, of whom 2 had a PR, 12 stable disease and 17 progressive disease (27, 28, 37).

In another study, weekly 30-min infusions of exatecan 3, 5 and 6.65 mg/m^2 were delivered every 3 weeks to 15 patients with advanced solid tumors. Chemo- and radio-

Table I: Clinical studies of exatecan (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Cancer	Open	Exatecan, 0.15 mg/m^2 iv over 24 h 1x/3 wk (n=3) Exatecan, 0.3 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 0.6 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 1.2 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 2.4 mg/m^2 iv over 24 h 1x/3 wk (n=9) Exatecan, 3.0 mg/m^2 iv over 24 h 1x/3 wk (n=5) Exatecan, 3.6 mg/m^2 iv over 24 h 1x/3 wk (n=2)	22	Exatecan was mostly associated with hematological toxicities and incuded stable disease in 4 of 22 patients with refractory solid tumors. The maximum tolerated dose for exatecan once every 3 weeks was 2.4 mg/m^2 in both minimally pretreated and heavily pretreated patients	21, 24
Cancer	Open	Exatecan, 0.15 mg/m^2 iv over 24 h 1x/3 wk (n=3) Exatecan, 0.3 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 0.6 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 1.2 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 2.4 mg/m^2 iv over 24 h 1x/3 wk (n=2)	8	Exatecan at doses up to 2.4 mg/m^2 once every 3 weeks was well tolerated and was not associated with any dose-limiting toxicities in patients with solid tumors	22
Cancer	Open	Exatecan, 0.15 mg/m^2 iv over 24 h 1x/3 wk (n=3) Exatecan, 0.3 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 0.6 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 1.2 mg/m^2 iv over 24 h 1x/3 wk (n=1) Exatecan, 2.4 mg/m^2 iv over 24 h 1x/3 wk (n=3) Exatecan, 3.6 mg/m^2 iv over 24 h 1x/3 wk (n=1)	10	Exatecan was associated with dose-limiting toxicities when administered to cancer patients at a dose of 3.6 mg/m^2 iv over 24 h once every 3 weeks	23
Cancer	Open	Exatecan, 0.05 mg/m^2 iv over 24 h 1x/w 3x/4 wk (n=1) Exatecan, 0.1 mg/m^2 iv over 24 h 1x/wk 3x/4 wk (n=1) Exatecan, 0.15 mg/m^2 iv over 24 h 1x/wk 3x/4 wk (n=1) Exatecan, 0.23 mg/m^2 iv over 24 h 1x/wk 3x/4 wk (n=1) Exatecan, 0.35 mg/m^2 iv over 24 h 1x/wk 3x/4 wk (n=1) Exatecan, 0.53 mg/m^2 iv over 24 h 1x/wk 3x/4 wk (n=5) Exatecan, 0.8 mg/m^2 iv over 24 h 1x/wk 3x/4 wk (n=8) Exatecan, 1 mg/m^2 iv over 24 h 1x/wk 3x/4 wk (n=4) Exatecan, 1.2 mg/m^2 iv over 24 h 1x/wk 3x/4 wk (n=5)	27	Exatecan was well tolerated at doses up to 0.8 mg/m^2 in minimally pretreated cancer patients and up to 0.53 mg/m^2 in heavily pretreated cancer patients	25
Cancer	Open	Exatecan, 0.075 mg/m^2 iv cont od 5x/21 d (n=1) Exatecan, 0.075 mg/m^2 iv cont od 10x/21 d (n=3) Exatecan, 0.12 mg/m^2 iv cont od 21x/21 d (n=1) Exatecan, 0.15 mg/m^2 iv cont od 5x/21 d (n=4) Exatecan, 0.15 mg/m^2 iv cont od 10x/21 d (n=1) Exatecan, 0.15 mg/m^2 iv cont od 15x/21 d (n=1) Exatecan, 0.15 mg/m^2 iv cont od 21x/21 d (n=20) Exatecan, 0.23 mg/m^2 iv cont od 21x/21 d (n=6) Exatecan, 0.30 mg/m^2 iv cont od 21x/21 d (n=3)	31	Exatecan showed evidence of antitumor activity when administered as a protracted continuous intravenous infusion to patients with advanced solid tumors. Doses higher than 0.15 mg/m^2 of exatecan were associated with unacceptable hematological toxicity in both minimally pretreated and heavily pretreated patients	26, 35
Cancer	Open	Exatecan, 1 mg/m^2 iv over 30 min 1x/wk 3x/4 wk (n=3) Exatecan, 1.33 mg/m^2 iv over 30 min 1x/wk 3x/4 wk (n=3) Exatecan, 1.77 mg/m^2 iv over 30 min 1x/wk 3x/4 wk (n=3) Exatecan, 2.10 mg/m^2 iv over 30 min 1x/wk 3x/4 wk (n=6) Exatecan, 2.35 mg/m^2 iv over 30 min 1x/wk 3x/4 wk (n=10) Exatecan, 2.75 mg/m^2 iv over 30 min 1x/wk 3x/4 wk (n=7) Exatecan, 3.13 mg/m^2 iv over 30 min 1x/wk 3x/4 wk (n=3)	35	Exatecan induced partial response and stable disease in 6% and 39% of patients with advanced refractory solid tumors, respectively. The dose-limiting toxicities were neutropenia for minimally pretreated patients and neutropenia and thrombocytopenia for heavily pretreated patients. The toxicity profile suggested a dose of 2.75 mg/m^2/week for minimally pretreated patients and 2.10 mg/m^2/week for heavily pretreated patients	27

Continued

Table I Cont.: Clinical studies of exatecan (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Cancer	Open	Exatecan, 1 mg/m^2 iv over 30 min od 3x/28 d [on days 1, 8 and 15] Exatecan, 2.35 mg/m^2 iv over 30 min od 3x/28 d [on days 1, 8 and 15] Exatecan, 3.13 mg/m^2 iv over 30 min od 3x/28 d [on days 1, 8 and 15]	31	Exatecan showed antitumor activity in patients with solid tumors, although dose-limiting toxicities were found with doses of 2.35 and 3.13 mg/m^2	28
Cancer		Exatecan, 4 mg/m^2 iv over 30 min 1x/3 wk [to a maximum of 3 infusions] (n=3) Exatecan, 5.33 mg/m^2 iv over 30 min 1x/3 wk [to a maximum of 3 infusions] (n=4) Exatecan, 7.1 mg/m^2 iv over 30 min 1x/3 wk [to a maximum of 3 infusions] (n=1)	8	Doses equal to or lower than 7.1 mg/m^2 of exatecan once every 3 weeks were well tolerated but induced no antitumor response in heavily pretreated patients with cancer	31
Cancer		Exatecan, 0.1 mg/m^2 iv over 30 min od 5x/3 wk (n=3) Exatecan, 0.2 mg/m^2 iv over 30 min od 5x/3 wk (n=1) Exatecan, 0.4 mg/m^2 iv over 30 min od 5x/3 wk (n=10)	14	Exatecan at doses equal to or lower than 0.2 mg/m^2/day was well tolerated in heavily pretreated and minimally pretreated cancer patients. The 0.4 mg/m^2/day dose was associated with dose-limiting toxicities	32
Cancer	Open	Exatecan, 0.1 mg/m^2 iv over 30 min od 5x/3 wk (n=3) Exatecan, 0.2 mg/m^2 iv over 30 min od 5x/3 wk (n=1) Exatecan, 0.4 mg/m^2 iv over 30 min od 5x/3 wk (n=5)	9	A preliminary analysis suggested an acceptable toxicity profile for exatecan for 5 days every 3 weeks in patients with solid tumors	33
Cancer	Open	Exatecan, 0.1 mg/m^2 iv over 30 min od 5x/3 wk (n=3) Exatecan, 0.2 mg/m^2 iv over 30 min od 5x/3 wk (n=4) Exatecan, 0.3 mg/m^2 iv over 30 min od 5x/3 wk (n=7) Exatecan, 0.4 mg/m^2 iv over 30 min od 5x/3 wk (n=10) Exatecan, 0.5 mg/m^2 iv over 30 min od 5x/3 wk (n=6) Exatecan, 0.6 mg/m^2 iv over 30 min od 5x/3 wk (n=6)	36	Exatecan given for 5 days once every 3 weeks showed an acceptable toxicity profile and induced objective antitumor response or stable disease for at least 4 treatment courses in 36% of patients with advanced solid tumors. The recommended doses were 0.5 mg/m^2/day for minimally pretreated patients and 0.3 mg/m^2/day for heavily pretreated patients	34
Cancer	Open	Exatecan, 0.15 mg/m^2/day iv cont x 5-21 d Exatecan, 0.3 [changed to 0.15] mg/m^2/day iv cont x 5-21 d	23	Preliminary results suggested that a protracted continuous infusion regimen with exatecan showed antitumor activity and was well tolerated in cancer patients	36
Cancer		Exatecan, 1 mg/m^2 iv over 30 min 1x/wk 3x/4 wk Exatecan, 1.33 mg/m^2 iv over 30 min 1x/wk 3x/4 wk Exatecan, 1.77 mg/m^2 iv over 30 min 1x/wk 3x/4 wk	8	Exatecan was well tolerated at doses up to 1.77 mg/m^2 once weekly but induced no antitumor response in cancer patients	37
Cancer	Open	Exatecan, 3 mg/m^2 iv over 30 min 1x/3 wk (n=3) Exatecan, 5 mg/m^2 iv over 30 min 1x/3 wk (n=6) Exatecan, 6.65 mg/m^2 iv over 30 min 1x/3 wk (n=6)	15	Exatecan showed an acceptable toxicity profile in patients with advanced refractory cancer. The recommended dose for future phase II clinical trials was 5 mg/m^2 iv over 30 min once weekly for 3 weeks	38, 39
Cancer	Open	Exatecan, 4 mg/m^2 iv over 30 min 1x/3 wk (n=3) Exatecan, 5.33 mg/m^2 iv over 30 min 1x/3 wk (n=6) Exatecan, 7.1 mg/m^2 iv over 30 min 1x/3 wk (n=3)	12	Exatecan once every 3 weeks induced stable disease in 50% of patients with advanced refractory solid tumors. The recommended dose for patients previously treated with chemotherapy was 5.33 mg/m^2	39, 40

Continued

Table I Cont.: Clinical studies of exatecan (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Cancer	Open	Exatecan, 0.1 mg/m^2 iv over 30 min od 5x/3 wk (n=6) Exatecan, 0.17 mg/m^2 iv over 30 min od 5x/3 wk (n=3) Exatecan, 0.25 mg/m^2 iv over 30 min od 5x/3 wk (n=4) Exatecan, 0.35 mg/m^2 iv over 30 min od 5x/3 wk (n=3) Exatecan, 0.40 mg/m^2 iv over 30 min od 5x/3 wk (n=2) Exatecan, 0.45 mg/m^2 iv over 30 min od 5x/3 wk (n=6)	24	Exatecan was well tolerated at doses equal to or lower than 0.40 mg/m^2/daily for 5 days once every 3 weeks and showed evidence of antitumor activity in patients with solid tumors	41, 42
Leukemia	Open	Exatecan, 0.6 mg/m^2 iv over 30 min od 5x/3-4 wk (n=4) Exatecan, 0.9 mg/m^2 iv over 30 min od 5x/3-4 wk (n=9) Exatecan, 1.2 mg/m^2 iv over 30 min od 5x/3-4 wk (n=3) Exatecan, 1.35 mg/m^2 iv over 30 min od 5x/3-4 wk (n=2) Exatecan, 0.9 mg/m^2 iv over 30 min od 7x/3-4 wk (n=7)	25	Exatecan at daily doses of 0.9 mg/m^2 showed an acceptable toxicity profile and evidence of antitumor activity when administered to patients with leukemia	43
Leukemia	Open	Exatecan, 0.6 mg/m^2 iv over 30 min od 5x/3-4 wk (n=4) Exatecan, 0.9 mg/m^2 iv over 30 min od 5x/3-4 wk (n=6) Exatecan, 1.2 mg/m^2 iv over 30 min od 5x/3-4 wk (n=3) Exatecan, 1.35 mg/m^2 iv over 30 min od 5x/3-4 wk (n=2)	15	Exatecan showed antitumor activity in 73% of paients with leukemia. A dose of 0.9 mg/m^2/day for 5 days once every 3-4 weeks was recommended for future phase II clinical trials	44
Pancreatic cancer	Open Open	Exatecan, 1.5-3.5 mg/m^2 iv over 30 min od 2x/3 wk + Gemcitabine, 750 mg/m^2 iv over 30 min od 2x/3 wk Exatecan, 1.5-3.5 mg/m^2 iv over 30 min od 2x/3 wk + Gemcitabine, 850 mg/m^2 iv over 30 min od 2x/3 wk Exatecan, 1.5-3.5 mg/m^2 iv over 30 min od 2x/3 wk + Gemcitabine, 1000 mg/m^2 iv over 30 min od 2x/3 wk	70	The combination of exatecan and gemcitabine was well tolerated and showed antitumor activity in minimally and heavily pretreated patients with pancreatic cancer. The recommended doses for this regimen in future phase II-III clinical trials were 2.0 mg/m^2/day of exatecan and 1000 mg/m^2/day of gemcitabine	45
Cancer	Open	Exatecan, 1.5 mg/m^2 iv over 30 min od 2x/3 wk + Gemcitabine, 750 mg/m^2 iv over 30 min od 2x/3 wk (n=9) Exatecan, 2.0 mg/m^2 iv over 30 min od 2x/3 wk + Gemcitabine, 750 mg/m^2 iv over 30 min od 2x/3 wk (n=6) Exatecan, 2.5 mg/m^2 iv over 30 min od 2x/3 wk + Gemcitabine, 750 mg/m^2 iv over 30 min od 2x/3 wks (n=4) Exatecan, 3.0 mg/m^2 iv over 30 min od 2x/3 wk + Gemcitabine, 750 mg/m^2 iv over 30 min od 2x/3 wk (n=6) Exatecan, 3.5 mg/m^2 iv over 30 min od 2x/3 wk + Gemcitabine, 750 mg/m^2 iv over 30 min od 2x/3 wk (n=1)	26	Evidence of antitumor activity was found for exatecan combined with gemcitabine in minimally pretreated and heavily pretreated patients with advanced solid tumors	46
Cancer	Open	Exatecan, 0.25 mg/m^2 iv over 30 min od 5x/3 wk (n=3) Exatecan, 0.35 mg/m^2 iv over 30 min od 5x/3 wk (n=6) Exatecan, 0.45 mg/m^2 iv over 30 min od 5x/3 wk (n=13) Exatecan, 0.55 mg/m^2 iv over 30 min od 5x/3 wk (n=5)	27	Intravenous exatecan showed antitumor activity in pediatric patients with advanced solid tumors. The maximum tolerated dose was established at 0.45 mg/m^2/day for 5 days once every 3 weeks	47

Continued

Table I Cont.: Clinical studies of exatecan (from Prous Science Integrity®).

Indication	Design	Treatments	n	Conclusions	Ref.
Adeno-carcinoma	Open	Exatecan, 0.5 mg/m^2 iv over 30 min od 5x/3 wk	15	No significant antitumor activity was found with this dose level of exatecan in patients with advanced or metastatic adenocarcinoma of the colon or rectum	48
Non-small cell lung cancer	Open	Exatecan, 0.5 mg/m^2 iv over 30 min od 5x/3 wk	23	Exatecan was well tolerated and showed antitumor activity in patients with advanced non-small cell lung cancer	49
Breast cancer	Open	Exatecan, 0.2 mg/m^2 iv over 30 min od 5x/21 d (n=2) Exatecan, 0.3 mg/m^2 iv over 30 min od 5x/21 d (n=16) Exatecan, 0.4 mg/m^2 iv over 30 min od 5x/21 d (n=28) Exatecan, 0.5 mg/m^2 iv over 30 min od 5x/21 d (n=5)	39	Exatecan was associated with an acceptable toxicity profile and moderate antitumor activity in patients with metastatic breast cancer refractory to anthracyclines and taxanes	50, 51
Ovarian and peritoneal cancer	Open	Exatecan, 0.3 mg/m^2 iv over 30 min od 5x/3 wk	9	Exatecan was well tolerated and showed efficacy in inducing disease stabilization in patients with refractory advanced ovarian, tubal or peritoneal cancer	52
Ovarian cancer	Open Double-blind, Multicenter	Exatecan, 0.3 mg/m^2 iv over 30 min od 5x/3 wk (n=31) Exatecan, 2.1 mg/m^2 iv over 30 min 1x/wk 3x/4 wk (n=16)	47	Exatecan was well tolerated and active in the treatment of relapsed, refractory ovarian cancer. The strongest activity was found with the 5-daily regimen	53
Pancreatic cancer	Open Multicenter	Exatecan, 0.5 mg/m^2 iv over 30 min od 5x/3 wk	39	Exatecan showed significant antitumor activity in previously untreated patients with pancreatic cancer	54-56
Liver cancer	Open, Multicenter	Exatecan, 0.5 mg/m^2 iv over 30 min od 5x/3 wk	28	Exatecan monotherapy only induced minor responses in patients with confirmed hepatocellular carcinoma	57
Biliary and gallbladder cancer	Multicenter	Exatecan, 0.5 mg/m^2 iv over 30 min d 1-5 q 22 d	42	Exatecan had modest activity in advanced biliary tree cancers. Toxicity was predictable and manageable	58

therapy was stopped in all patients 4 weeks prior to study onset. Patients were treated with 1-5 (median of 2) cycles of exatecan therap. The MTD was 5 mg/m^2 on this schedule. The DLTs in this study were hepatotoxicity and neutropenia, both of which occurred following the 6.65 mg/m^2 dose. A 100% dose escalation was planned for this study, but side effects were experienced at the first dose level, and a more conservative 67% dose escalation was undertaken. The main side effects reported were diarrhea, nausea and vomiting. The recommended dose for weekly 30-min i.v. infusions was 5 mg/m^2 (29, 38, 39).

Twelve patients with refractory solid tumors were administered a 30-min infusion of exatecan 4-7.1 mg/m^2 every 3 weeks in a European phase I study. Patients had been diagnosed with cervical (n=3), gastric (n=2), colorectal (n=2), lung (n=1), pancreatic (n=1), leiomyosarcoma (n=1), renal (n=1) or unknown primary (n=1) carcinoma and received 1-10 (median of 2) courses of treatment. Neutropenia was once again the DLT in this phase I trial. The MTD was defined as 7.1 mg/m^2, with 2 of 3 patients experiencing neutropenia following this dose. Investigators reported the death of 1 patient due to the development of acute respiratory distress syndrome. The causal relationship with exatecan could not be ruled out due to the fact that pulmonary toxicity has been reported with other topoisomerase inhibitors. The authors recommended an exatecan dose of 5.5 mg/m^2 for phase II studies based on this weekly infusion protocol (30, 31, 40).

A large number of phase I studies assessing the appropriateness of a daily 30-min i.v. infusion of exatecan over 5 days every 3 weeks have been carried out in the past few years. Results from these daily infusion schedules showed the recommended dose to be much lower than previous regimens (32, 41, 42). Exatecan 0.1-0.6 mg/m^2 was administered via 30-min i.v. infusion for 5 days every 3 weeks to 36 patients with advanced solid malignancies. Dose escalations of 100% were employed in this study design, until toxicity prevented further escalation. Myelosuppression (particularly neutropenia) was the DLT associated with this dosing schedule. The most

commonly reported adverse event was noncumulative neutropenia. Severe neutropenia was observed following exatecan 0.5 and 0.3 mg/m^2/day in minimally and heavily pretreated patients, respectively. The authors noted, however, that these episodes were brief, and were not associated with fever or sepsis. Nausea, vomiting and diarrhea were among the nonhematological side effects observed. None of these gastrointestinal effects were deemed severe, however. The recommended doses for minimally and heavily pretreated patients were 0.5 and 0.3 mg/m^2/day, respectively. Objective responses including stable disease were experienced by 36% of patients in this trial (20, 33, 34).

The antileukemic efficacy of exatecan was tested in a phase I study in a group of patients with refractory or relapsed acute hematological malignancies. Twenty-five adult and pediatric patients with acute myeloid leukemia (AML; n=21), acute lymphocytic leukemia (ALL; n=2), myelodysplastic syndrome (MDS; n=1) and chronic myelogenous leukemia (CML; n=1) were administered a daily 30-min infusion of exatecan 0.6-3.0 mg/m^2/day for 5-days on an every-3-week cycle. The DLT in this population was stomatitis, with a dose of 0.9 mg/m^2/day being recommended for phase II trials. Two AML patients achieved hematological improvement. Investigators suggested that exatecan be evaluated in combination therapy in AML and in other hematological malignancies (43, 44).

Exatecan plus gemcitabine combination therapy was assessed in a phase I study in patients with advanced solid malignancies. Seventy patients were administered 30-min infusions of exatecan 1.5-3.5 mg/m^2 and gemcitabine 750-1000 mg/m^2 on days 1 and 8 of a 3-week cycle. The MTDs for exatecan and gemcitabine were found to be 2 and 1000 mg/m^2, respectively, in the heavily pretreated patients and the DLT was neutropenia in this cohort. The main toxicities were hematological, with additional reports of nausea and fatigue. Patients with pancreatic cancer were particularly sensitive to the combination. These results are currently being followed up in phase II and III trials in patients with advanced pancreatic cancer (45, 46).

Exatecan showed moderate anticancer efficacy when tested in a pediatric population. Twenty-seven patients (median age = 16 years) with advanced solid tumors were administered exatecan 0.25-0.55 mg/m^2 in this phase I analysis. Neutropenia and thrombocytopenia were the DLTs in this study. The MTD was 0.45 mg/m^2/day. Of 32 evaluable patients, 2 and 3 showed evidence of a PR and an MR, respectively (47).

A phase II study was conducted in patients with fluorouracil-resistant metastatic colorectal cancer. Fifteen patients with a performance status of less than or equal to 2 were included and were administered exatecan 0.5 mg/m^2 over 30 min for 5 days every 3 weeks. Response was assessed following 1-7 (median of 3) treatment cycles. Exatecan was not effective as an anticancer therapy in these patients. No complete or partial responses were obtained in this population and minor responses, stable disease and progressive disease were reported in 1, 6 and 8 patients, respectively. Exatecan was well tolerated, however. Granulocytopenia (n=10), leukopenia (n=6), anemia (n=3) and thrombocytopenia (n=2) were among the grade 2-4 toxicities reported in this group. Other toxicities included fatigue, nausea and vomiting, diarrhea, stomatitis and alopecia (48).

Twenty-three patients with previously untreated non-small cell lung carcinoma (NSCLC) were assessed in another phase II trial of exatecan. Patients were administered a 30-min infusion of exatecan 0.5 mg/m^2 for 5 days on an every-3-week cycle, for a maximum of 6 cycles. Three of 16 evaluable patients experienced a PR and 7 had stable disease. Toxicity included neutropenia, thrombocytopenia, anemia, emesis and fatigue (n=18 for all adverse events). Exatecan therefore showed some promise in the treatment of de novo patients with NSCLC (49).

Patients with metastatic breast cancer were enrolled in an open-label phase II study. Thirty-nine patients were administered doses of 0.2-0.5 mg/m^2/day, depending on prior exposure to chemotherapeutic agents. Exatecan was delivered via 30-min i.v. infusion for 5 days every 3 weeks. Visceral metastases were present in 94% of patients. All patients in this analysis were resistant to taxane and anthracycline treatment. Hormone receptor status was negative in 49% of patients. Treatment response was assessed following 1-16 (median of 4) courses of exatecan therapy. Antitumor efficacy was only modest in this study, with 3 patients achieving a PR on 0.4 mg/m^2/day. Twenty further patients achieved either an MR (4 patients) or stable disease. Fourteen patients had progressive disease as their best response. Median survival was 14 months following exatecan therapy, with a median time to disease progression of 3 months. The DLT was neutropenia, with 46% and 33% of patients, respectivley, experiencing grade 3 and grade 4 neutropenia, and 5% of these patients developed neutropenic fever. Five patients receiving exatecan 0.5 mg/m^2/day underwent a dose reduction to 0.4 mg/m^2/day due to persistent neutropenia. Other commonly reported adverse events included fatigue, headache, nausea, myalgia, constipation, vomiting and paresthesias. Exatecan mesilate therefore had modest efficacy and an acceptable tolerability profile in these patients (50, 51).

The efficacy and tolerability of exatecan were tested in 9 patients with advanced ovarian, tubal or peritoneal cancer prefractory to platinum, taxane and topotecan chemotherapy. Refractory disease was characterized by treatment failure, disease progression in spite of chemotherapy or relapse within 6 months of treatment. Patients were required to have a tumor size equal to or more than 2 cm, with adequate hepatic, renal and bone marrow. Three of 5 evaluable patients had stable disease, with no evidence of partial or complete response being identified. Grade 3-4 toxicities included neutropenia (n=1) and thrombocytopenia (n=1), and 8 patients had grade 2 anemia. Nausea/vomiting and fatigue were among the nonhematological adverse events reported. Exatecan was therefore suggested to have potential as

second-line therapy in patients refractory to platinum, taxane and topotecan treatments (52).

Exatecan mesilate exhibited antitumor activity in a phase II trial in a group of patients with advanced refractory ovarian cancer. Forty-seven patients were randomized to receive exatecan 0.3 mg/m^2/day for 5 days every 3 weeks, or 2.1 mg/m^2/day once a week for 3 weeks every 4 weeks. The drug was administered via 30-min i.v. infusion. All patients had previously relapsed on or were refractory to prior therapy with platinum and taxane. Patient response was measured after 1-6 (median of 2) cycles of study medication. Of 23 evaluable patients, 2 achieved a PR (both of whom were on the daily x 5 dosing schedule). Five patients achieved stable disease and 16 experienced disease progression. The study authors concluded that the 5-day dosing regimen was superior to the weekly schedule in these patients, and recommended this for further investigations. The most significant toxicity was myelosuppression. Other commonly reported adverse events included neutropenia (n=7), nausea (n=3), alopecia (n=2), anemia (n=2), thrombocytopenia (n=1) and constipation (n=1). The investigators commented that exatecan was generally well tolerated by patients in this study (53).

The efficacy and tolerability of exatecan mesilate were also tested in 39 patients with advanced pancreatic cancer. Patients were administered a 30-min i.v. infusion of exatecan at a dose of 0.5 mg/m^2/day for 5 days every 21 days in this multicenter phase II study. Patients were allowed 1 adjuvant chemotherapeutic (n=20) or chemoradiation (n=8) regimen throughout the course of the study. Patient reponse was assessed after 1-20 (median of 2) cycles of study medication. Partial response, minor response and stable disease were achieved by 5%, 3% and 39% of patients, respectively. Median survival was 5.5 months, with a 1-year survival rate of 27% in these patients. These efficacy results are superior to those reported following gemcitabine therapy in the same treatment population. Grade 2-4 neutropenia, thrombocytopenia, fatigue and nausea/vomiting were experienced by 56%, 11%, 8% and 8% of patients, respectively. Nine patients were hospitalized due to adverse events attributable to exatecan administration. These promising efficacy and tolerability results indicate the potential use of exatecan as first-line therapy in the treatment of pancreatic cancer (54-56).

Patients with hepatocellular cancer only exhibited a minor response to exatecan mesilate therapy in a multicenter phase II trial in 28 patients who received a 30-min infusion of 0.5 mg/m^2/day for 5 days every 3 weeks. Extrahepatic metastases were detected in 57% of patients at the time of study onset. Patient response was evaluated following 1-10 (median of 3) courses of drug therapy. There were no complete or partial responses noted in any of the patients treated. Three minor responses were achieved, however, with 2 patients exhibiting a 25% decrease in alpha-fetoprotein and 1 patient exhibiting a tumor regression of 50%. Median survival was 40 weeks, with a 6-month survival rate of 70% in these patients. Neutropenia, anemia and thrombocytopenia were the main toxicities observed in these patients. Evidence of minor responses suggests the possibility of exatecan being used in comination with other chemotherapeutic agents, and indicates that its use should not be entirely ruled out in patients with hepatocellular carcinoma (57).

Exatecan mesilate also showed only modest activity in the setting of hepatobiliary cancer. Forty-two patients were assessed in this multicenter phase II study. Patients with advanced cholangiocarcinoma (n=25) or gallbladder cancer (n=17) were administered a 30-min infusion of exatecan 0.5 mg/m^2/day for 5 days every 22 days. Patients were minimally pretreated, having only undergone less than or equal to 1 prior chemotherapeutic regimen, and 60% of patients were previously untreated. Median ECOG performance status was 0-2. A PR was achieved by 5% of patients, 7% had an MR and 33% experienced stable disease, whereas 44% of patients had disease progression as their best response. Median survival was 7.8 months, with a median survival of 10.3 months in patients who had not undergone prior chemotherapy. Patients had a 1-year survival rate of 27%, with a median time to progression of 1.9 months. Exatecan mesilate showed some anticancer activity in these patients, although the effect was only moderate, and it was not recommended for first-line therapy in this case. Commonly reported adverse events included neutropenia (n=21), thrombocytopenia (n=10), nausea/vomiting (n=4) and fatigue (n=3), with 10% of patients withdrawing from the study and 38% undergoing a dose reduction because they were unable to tolerate the study drug. No treatment-related deaths were reported throughout the course of the study. The investigators concluded that the toxicity profile of exatecan was predictable and manageable, and did not rule out the possibility of using this agent in combination therapy in this setting (58).

Conclusions

Exatecan mesilate failed to exhibit the level of antitumor efficacy initially extrapolated from preclinical trials. Phase I and II studies showed mild to moderate anticancer activity at best. Patients undergoing exatecan treatment experienced myelosuppressive and gastrointestinal side effects. Myelosuppression was found to be dose-dependent and dose-limiting in most cases. Patients previously treated with chemotherapeutic agents showed increased sensitivity to myelosuppression compared with minimally pretreated patients. Stratification of chemotherapeutic history is therefore an important consideration in the design of future trials with this agent. Exatecan showed some promise when combined with other chemotherapeutic agents, and its clinical utility will ultimately need to be determined in randomized, controlled phase III trials.

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