Clinical relevance of human cancer xenografts as a tool for preclinical assessment: example of in-vivo evaluation of topotecan-based chemotherapy in a panel of human small-cell lung cancer xenografts

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Prediction of human tumor response based on preclinical data could reduce the failure rates of subsequent new anticancer drugs clinical development. Human small-cell lung carcinomas (SCLC) are characterized by high initial sensitivity to chemotherapy but a low median survival time because of drug resistance. The aim of this study was to evaluate the therapeutic relevance of a panel of human SCLC xenografts established in our laboratory using one compromising drug in SCLC, topotecan (TPT). Six SCLC xenografts derived from six patients were used: three were sensitive to a combination of etoposide (VP16), cisplatin (CDDP), and ifosfamide (IFO), and three were resistant, as published earlier. Growth inhibition was greater than 84% for five xenografts at doses of 1–2 mg/kg/day. TPT was combined with IFO, etoposide (VP16), and CDDP. IFO improved the efficacy of TPT in three of the five xenografts and complete responses were obtained even with the less TPT-sensitive xenograft. VP16 increased the efficacy of two of four xenografts and complete responses were obtained. The combination of TPT and CDDP did not improve TPT responses for any of the xenografts tested. Semiquantitative reverse transcriptase-PCR of genes involved in drug response, such as topoisomerase I, topoisomerase IIα, multidrug resistance 1 (MDR1), multidrug resistance-associated protein (MRP), lung resistance-related protein (LRP), and glutathione *S*-transferase π (GSTπ), did not explain the variability in drug sensitivity between SCLC xenografts. In conclusion, these preclinical data mirror those from published clinical studies suggesting that our panel of SCLC xenografts represents a useful tool for preclinical assessment of new treatments. *Anti-Cancer Drugs* 21:25–32 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Human small-cell lung carcinoma (SCLC), accounting for approximately 15–20% of all lung cancers, is an aggressive tumor with a high propensity for early regional and distant metastases. Chemotherapy is the primary treatment option for patients with SCLC, leading to a 5-year survival of approximately 20% in limited disease (LD), and less than 5% in extensive disease (ED). Although the initial tumor response rate to chemotherapy is very high (up to 96% for LD and up to 65% for ED), SCLC relapses after approximately 4 months in ED and 12 months in LD [1,2]. Although combinations of cyclophosphamide, adriamycin, and vincristine (CAV) or cyclophosphamide, adriamycin, and etoposide (CAE) have been widely used, cisplatin or carboplatin in combination with etoposide is now considered to be the standard first-line treatment of SCLC [3].

Topotecan (Hycamtin, TPT) is currently approved for the treatment of patients with SCLC who have failed or relapsed after first-line chemotherapy and who are not candidates for reinduction treatment. TPT, a water-soluble analog of camptothecin, belongs to the family of cytotoxic agents that inhibit topoisomerase I (Topo I). The main role of Topo I is relaxation of DNA, essential for transcription and replication processes. Topo I inhibitors reversibly stabilize the Topo I enzymatic complex and cleave DNA, leading to the formation of cleavage complexes that interfere with replication forks. The ensuing arrest of replication forks is accompanied by generation of permanent double-strand breaks that are thought to be responsible for the antiproliferative properties of TPT [4–7]. The cytotoxicity of anti-Topo I is therefore S-phase-specific, supporting prolonged therapeutic exposure. Moreover, TPT has been shown to be a

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potent inhibitor of HIF-1α and HIF-2α subunits leading to decreased vascular endothelial growth factor expression and antiangiogenic activity [8–11].

The clinical profile of TPT in SCLC was established in several phase II studies [12–15], and was confirmed in randomized phase III trials [16–18]. Owing to the poor outcome of the disease and chemotherapy-induced toxicity, particularly with cisplatin-based regimens [19], the management of patients with initial or relapsed SCLC therefore remains challenging. To select the most active molecules, preclinical investigation of new antitumor compounds is an important step in the process of drug development before their clinical use. The choice of preclinical tumor models used to evaluate new compounds is therefore a crucial step to obtain comparable results between preclinical assessments and human clinical trials. The aims of this study were therefore to evaluate the therapeutic relevance of a panel of human SCLC xenografts and determine the tumor molecular profile according to genes involved in the response to TPT-based chemotherapy. This study reports the efficacy of TPT administered alone or in combination with chemotherapeutic drugs, such as etoposide, ifosfamide, and cisplatin, and discusses the relevance of these preclinical results in relation to published clinical data.

Materials and methods

Small-cell lung carcinoma xenografts and in-vivo tumor growth

Tumor specimens were obtained from patients during surgical resection with their consent. Tumor samples were established as xenografts by subcutaneous implantation of a tumor fragment into the scapular area of nude mice and sequentially transplanted. For experimental therapeutic trials, 6- to 10-week-old female mice received a subcutaneous graft of tumor fragments with a volume of approximately 15 mm^3. Tumors appeared at the graft site 2–5 weeks later. Mice bearing tumors with a volume of 60–400 mm^3 were individually identified and randomly assigned to the control or treatment group (four to eight animals per group, as detailed in the tables and legends of figures) and treatment started on day 1. Animals with tumor volumes exceeding this range were excluded. Mice were weighed weekly. Tumor-bearing mice were killed when their tumor volume reached 2500 mm^3. Tumor volumes were calculated by measuring two perpendicular diameters using a caliper. Each tumor volume (V) was calculated according to the following formula: $V = (a \times b^2)/2$, where a and b are the largest and smallest perpendicular tumor diameters. Relative tumor volumes (RTVs) were calculated by the formula: $RTV = (V_x/V_1)$, where V_x is the tumor volume on day x and V_1 is the tumor volume at initiation of therapy (day 1). Growth curves were obtained by plotting median RTV values on the y-axis against time (expressed as days after initiation of therapy). Antitumor activity was evaluated according to three criteria: (i) tumor growth inhibition (TGI) calculated as 100 – [(RTVt/RTVc) × 100], where RTVt was calculated for individual tumors and RTVc was the mean RTV in the control group at a given time, (ii) growth delay index (GDI) calculated as the median growth delay in the treated group divided by the median growth delay in the control group, with each individual growth delay calculated as the time, in days, required for the individual tumor to reach a five-fold increase in volume, as published earlier [20], and (iii) complete regression (CR) rate defined as the absence of any palpable nodule at the graft site; the duration of CR was also recorded. The statistical significance of observed differences between individual RTVs for treated mice and control groups was calculated by a paired Student's t-test.

For in-vivo experiments, Swiss nu/nu female mice were bred in the animal facilities of Institut Curie, Paris, France. Animals were maintained under specific pathogen-free conditions. Animal care and housing complied with institutional guidelines of the French Ethical Committee (Ministère de l'Agriculture et de la Forêt, Direction de la Santé et de la Protection Animale, Paris, France), under the supervision of authorized investigators.

Drug formulation and administration

TPT (a gift from GlaxoSmithKline, Nanterre, France) was diluted in 0.9% sodium chloride solution and administered orally or intraperitoneally (i.p.) in a 0.2 ml volume to tumor-bearing mice on days 1–5. Different daily doses were tested from 0.5 to 2.5 mg/kg per day. The doses of etoposide, ifosfamide, and cisplatin have been reported previously [21]. Etoposide (VP16, Vepeside, Sandoz, France) was diluted in 0.9% sodium chloride solution and administered by i.p. injection in a volume of 0.2 ml to tumor-bearing mice on days 1–3 at a dosage of 12 mg/kg/day. Ifosfamide (Holoxan, Asta Medica, Bordeaux, France) was diluted in 0.9% sodium chloride solution and administered by i.p. injection in a volume of 0.2 ml to tumor-bearing mice on days 1–3 at a dosage of 90 mg/kg/day. Cisplatin (CDDP, Cisplatyl, Roger Bellon, France) was reconstituted in water and diluted in 0.9% sodium chloride solution and administered by i.p. injection in a volume of 0.2 ml volume to tumor-bearing mice on day 1 at a dosage of 6 mg/kg/day. All drugs were extemporaneously prepared. In tests of drug combinations, each drug was injected separately to the animals. Mice in the control groups received 0.2 ml of the drug-formulating vehicle with the same dose schedule as treated animals.

Determination of gene expression

The tumor expression of various genes involved in drug resistance was evaluated by reverse transcriptase (RT)-PCR methods. These genes included multidrug resistance 1 (MDR1), multidrug resistance-associated protein (MRP), lung resistance-related protein (LRP), glutathione

S-transferase π (GSTπ), Topo I, and Topo IIα. Isolation of total RNA, RT-PCR conditions, and primer sequences, except for Topo I, have been described earlier [21]. The primer sequences for Topo I were as follows: S: 5′-AAA AGT CCA AGC ATA GCA ACA G-3′; AS: 5′-AGG AAC AAA ATA GCC ATC ATC T-3′; amplicon length: 345 bp, PCR cycle: 25. RT-PCR conditions were the same as those for the above genes.

Results

Characteristics of small-cell lung carcinoma xenografts

Six human SCLC xenografts were included in the study: three were derived from primary tumor sites and three were derived from metastases. Xenografts were histologically defined either as oat cells or variant cell types, as shown in Table 1. Three SCLC xenografts were obtained from patients before treatment and three were derived from patients who were treated earlier with etoposide and cisplatin combination plus radiotherapy. The patient's survival time ranged between 8 weeks and 30 months. The *p53* gene was inactivated in all cases by deletions or mutations. Finally, the in-vivo tumor growth of transplanted xenografts differed with a doubling time ranging between 3 and 6 days (data not shown).

Antitumor efficacy of single-agent topotecan chemotherapy

The efficacy of i.p. administration of TPT was tested as single-agent therapy on the various SCLC xenografts at dosages of 0.5, 1, 1.5, and 2 mg/kg/day for 5 days. As shown in Table 2, tumor GDI and optimal TGI were dose-dependent. Three xenografts (SCLC-61, SCLC-101, and SCLC-96) showed GDI greater than 2 and TGI $\geq$ 90% for all four dosages tested, two xenografts (SCLC-108 and SCLC-74) showed significant GDI (>2) and TGI (>80%) at higher dosages of TPT, and one xenograft (SCLC-6) showed a maximum GDI of 2 and a maximum TGI of 65% at the highest dose of TPT. Inversely, and despite high GDI and TGI, CR rates ranged between 0 and 33% in all xenografts tested with a CR duration ranging between 2 and 16 days. Oral and i.p. administration of four doses, 0.5, 1, 1.5, and 2 mg/kg, of TPT (Fig. 1) were compared in the highly sensitive SCLC-61 xenograft and no difference was observed between the two routes of administration. Finally, TPT was well tolerated, as only one of the seven mice showed a weight loss greater than 10% at a dosage of 2 mg/kg/day.

Table 1 Clinical and biological features of the six human SCLC xenografts

Tumor xenograft	Histology	Human origin	Patient treatment before graft	Patient survival[a]	p53 status (altered codon)
SCLC-61	Oat cell	Primary	No	L	Mutated (175)
SCLC-6	Variant	Lymph node	No	L	Mutated (283)
SCLC-74	Variant	Lymph node	Yes	S	Deletion (175)
SCLC-101	Oat cell	Primary	Yes	I	Mutated (204)
SCLC-96	Variant	Primary	No	S	Mutated (163)
SCLC-108	Variant	Skin metastasis	Yes	S	Mutated (159)

SCLC, small-cell lung carcinoma.
[a]I, intermediate, 4–12 months; L, long, up to 30 months; S, short, <4 months.

Table 2 Response of SCLC xenografts to various doses of single-agent TPT

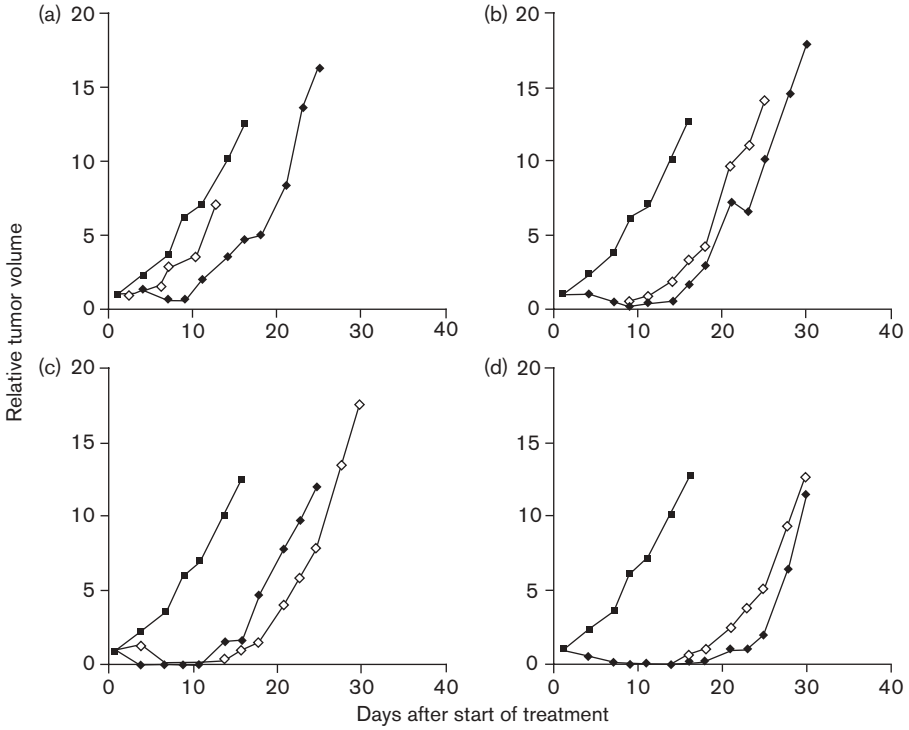
Tumor xenograft	TPT (mg/kg/day)[a]	Growth delay index (days)[b]	Growth inhibition (%)[c]	No. of complete regressions/ group	Duration of complete regressions (days)[c]
SCLC-61	0.5	2	93	0/3	–
	1	2.3	92	0/5	–
	1.5	2.6	98	1/6	7
	2	2.3	98	1/5	2
SCLC-6	0.5	1.1	45	0/5	–
	1	1.2	52	0/6	–
	1.5	1	58	0/5	–
	2	2	65	0/5	–
SCLC-74	0.5	1.6	57	0/6	–
	1	2	89	0/8	–
	1.5	1.9	84	0/5	–
	2	3	90	0/7	–
SCLC-101	1	2.3	90	1/5	2
	1.5	3.1	94	0/5	–
SCLC-96	0.5	2.7	95	1/5	9
	1	3.3	98	1/4	10
	2	4.1	98	1/4	11
SCLC-108	0.5	1.8	80	0/6	–
	1	2.8	94	0/6	–
	1.5	3.4	97	1/6	9
	2	3.9	99	2/6	16

SCLC, small-cell lung carcinoma; TPT, topotecan.
[a]TPT was given intraperitoneally at doses of 0.5–2 mg/kg/day, days 1–5.
[b]Growth delay index was calculated as the ratio between the median growth delay in treated mice and in control mice, as described in the Materials and methods.
[c]Expressed as the median value.

Antitumor efficacy of topotecan-based chemotherapy

To define the most effective drug combinations, TPT was combined with etoposide, ifosfamide, or cisplatin in the five human SCLC models SCLC-61/6/74/101/108. As in our earlier experiments, TPT was administered by i.p. injection at four different dosages, 0.5, 1, 1.5, or 2 mg/kg/day. Mice bearing each SCLC xenograft were treated by etoposide, ifosfamide, or cisplatin alone, and by the triple combination of these drugs (VIP), considered as standard treatment. As shown in Table 3, all models, apart from SCLC-74 and SCLC-108, were very sensitive to the VIP regimen with an optimal TGI ranging between 98 and 100%, an optimal GDI ranging between 3.1 and 10, but a CR rate ranging between 0 and 100%. For all SCLC xenografts expressing primary resistance to etoposide (SCLC-6 and SCLC-74), ifosfamide (SCLC-74), or cisplatin (SCLC-74) administered alone, concomitant administration of TPT induced a significant TGI and GDI (Fig. 2). However, a significant increase of the CR rate was only observed with the ifosfamide and TPT combination. Finally, combinations of

Fig. 1



Effect of single-agent topotecan (TPT) administered orally or intraperitoneally to mice bearing small-cell lung carcinomas (SCLC)-61 xenografts. TPT was administered orally (◇) or intraperitoneally (◆) at dosage of 1 (a), 1.5 (b), 2 (c), or 2.5 (d) mg/kg/day on days 1–5. Control group (■) received 0.2 ml of the drug-formulating vehicle with the same schedule as the treated animals.

Table 3 Response of SCLC xenografts to TPT, VP16, IFO, and CDDP as single agents and in combination with TPT

		VIP[a]	Etoposide				Ifosfamide				Cisplatin	
Topotecan[b] (mg/kg/day, days 1–5)		0	0	0.5	1	1.5	0	0.5[e]	1	1.5	0	0.5
SCLC-61	TGI[c]	100	100	nd	100	100	100	99	nd	nd	75	nd
	GDI[d]	10	2.9	nd	>19	6	3.3	3.5	nd	nd	2	nd
	CR[f]	3/3	3/5	nd	7/8	8/8	7/7	2/4	nd	nd	0/5	nd
SCLC-6	TGI	98	48	nd	63	nd	79	87	90	100	76	nd
	GDI	4.7	1.6	nd	2.4	nd	2	5	5	6	1.3	nd
	CR	0/8	0/7	nd	1/6	nd	0/7	2/7	0/8	4/6	2/5	nd
SCLC-74	TGI	70	49	nd	nd	88	29	81	91	nd	0	87
	GDI	1.8	1	nd	nd	2	0.9	2	2.3	nd	0.8	2
	CR	0/6	0/8	nd	nd	0/5	0/7	0/7	0/6	nd	0/8	0/5
SCLC-101	TGI	99	50	nd	nd	nd	80	nd	100	100	65	nd
	GDI	3.1	1.1	nd	nd	nd	2.2	nd	4.9	5.5	1.5	nd
	CR	3/8	0/6	nd	nd	nd	0/5	nd	7/11	7/11	0/5	nd
SCLC-108	TGI	52	nd	75	nd	nd	nd	77	nd	nd	nd	77
	GDI	1.5	nd	1.6	nd	nd	nd	1.9	nd	nd	nd	1.9
	CR	0/6	nd	0/7	nd	nd	nd	0/7	nd	nd	nd	0/8

CR, complete regression; GDI, growth delay index; nd, not done; SCLC, small-cell lung carcinoma; TGI, tumor growth inhibition; TPT, topotecan; VIP, etoposide/ifosfamide/cisplatin combination.

[a]VP16 (etoposide) was administered at a dosage of 12 mg/kg/day, days 1–3; IFO (ifosfamide) was administered at 90 mg/kg/day, days 1–3; CDDP (cisplatin) was administered at a dosage of 6 mg/kg/day, day 1.

[b]TPT was given at dose from 0.5 to 1.5 mg/kg/day, days 1–5.

[c]Optimal TGI calculated from the curves of median tumor growth at the optimal antitumor effect and expressed as the median value; in parentheses, number of complete regressions per group.

[d]Index of GDI calculated as the ratio between the median growth delay in treated mice and control mice.

[e]Ifosfamide was administered at a dose of 30 mg/kg/day on days 1–3.

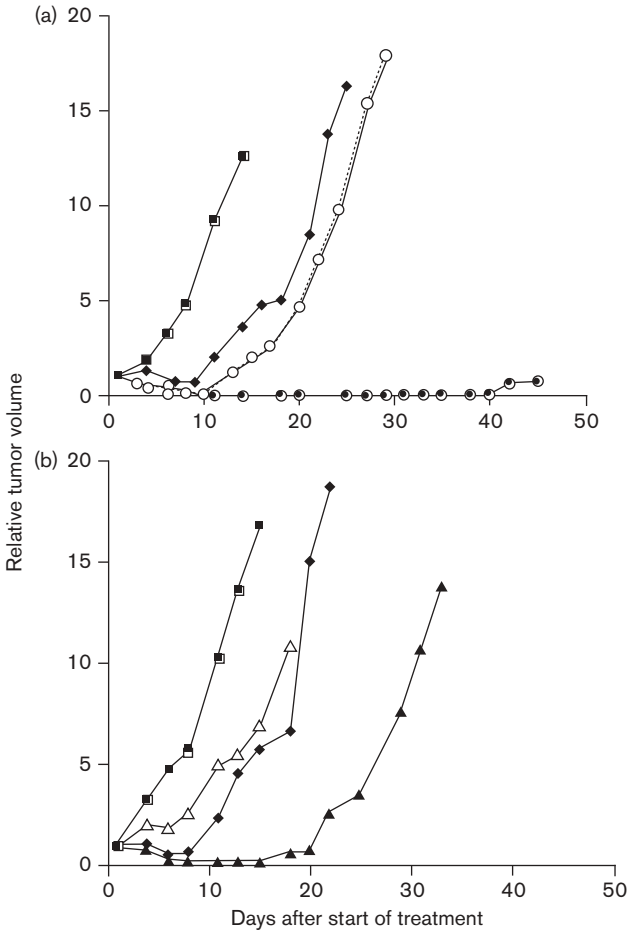
[f]Number of CR per total number of mice per group.

TPT with etoposide or ifosfamide seemed to be as effective as the VIP regimen, suggesting that cisplatin-related toxicities could be avoided without loss of therapeutic efficacy. The TPT and cisplatin combination was very toxic leading to the death of all treated mice.

Expression of different drug resistance genes

Semiquantitative RT-PCR was used to analyze the expression of various genes involved in drug resistance. Total RNA was isolated from the six human SCLC xenografts. The relative expression of the genes of interest was calculated within the linear amplification range and determined with respect to the internal standard β2-microglobulin (β2m) gene (Table 4). Expression of

Fig. 2



Effects of topotecan (TPT) single-agent therapy or in combination with etoposide (a) or ifosfamide (b) in mice bearing small-cell lung carcinomas (SCLC)-61 xenografts. (a) TPT alone was administered at a dosage of 1 mg/kg/day days 1–5 (♦), etoposide alone was administered at a dosage of 12 mg/kg/day days 1–3 (○), and TPT was administered in combination with etoposide (●). (b) TPT alone was administered at a dosage of 0.5 mg/kg/day, days 1–5 (♦), ifosfamide alone was administered at a dosage of 30 mg/kg/day days 1–3 (△), and TPT was administered in combination with ifosfamide (▲). Control group (■) received 0.2 ml of the drug-formulating vehicle with the same schedule as the treated animals.

Table 4 Relative expression of chemoresistance-related genes of SCLC xenografts

Tumor xenograft	Topo I	Topo IIα	MDR1	MRP	LRP	GSTπ
SCLC-61	0.57	1.03	0.37	5.59	0.00	3.38
SCLC-6	1.24	1.22	0.08	2.38	0.15	0.88
SCLC-74	0.50	0.19	0.00	1.84	2.83	1.64
SCLC-101	0.27	0.08	0.00	1.57	0.36	1.24
SCLC-96	0.09	0.69	0.00	1.9	0.54	0.00
SCLC-108	0.28	0.31	0.19	1.05	0.53	0.65

GSTπ, glutathione S-transferase π; LRP, lung resistance-related protein; MDR1, multidrug resistance 1; MRP, multidrug resistance-associated protein; SCLC, small-cell lung carcinoma; Topo I, topoisomerase I; Topo IIα, topoisomerase IIα.

Topo I, the target of TPT, was detected in all xenografts tested, but at different levels ranging between 0.09 (SLCL-96) and 1.24 (SCLC-6). Expression of Topo IIα, the pharmacological target of etoposide, differed in all tumors with relative gene expression varying between 0.08 (SCLC-101) and 1.22 (SCLC-6); this level of expression did not explain the difference of in-vivo efficacy of etoposide single-agent therapy, as SCLC-61 and SCLC-6 expressed high levels, but their DGI were 100 and 48%, respectively. MDR1 was expressed in three xenografts (SCLC-61/SCLC-6/SCLC-108) and absent in the other three tumors. MRP was expressed in all tumors and the highest level was detected in SCLC-61. LRP was expressed in all SCLC xenografts except for SCLC-61. Finally, GSTπ was expressed at different levels in all SCLC xenografts except for SCLC-96. No significant correlation was established between the relative expression of the various genes studied and the in-vivo response to TPT single-agent therapy.

Discussion

Prediction of human tumor response from preclinical data could reduce failure rates of clinical development of new anticancer drugs. The crucial question is therefore to define whether established preclinical models, that is, xenografts, retain the characteristics of the human tumors from which they are derived. To address this issue, we have developed a panel of six SCLC xenografts derived directly from patient tumors, which has already been used for preclinical studies using various chemotherapy regimens, such as CCAV (cyclophosphamide, cisplatin, doxorubicin, and etoposide), and VIP (etoposide, ifosfamide, and cisplatin) [21–24]. The aims of this study were therefore to evaluate the therapeutic relevance of this panel of human SCLC xenografts, using TPT, and to analyze the tumor molecular profile according to genes putatively involved in the response to TPT-based chemotherapy.

In this study, three xenografts (SCLC-61, SCLC-101, and SCLC-96) showed TGI $\geq$ 90% at all four dosages of TPT tested alone, two xenografts (SCLC-108 and SCLC-74) showed significant TGI (> 80%) at higher dosages of TPT alone, and one xenograft (SCLC-6) showed a maximum TGI of 65% at the highest dose of

TPT alone. Clinical phase II studies have shown that TPT given for 5 consecutive days at a dose of 1.5 mg/m^2/day intravenously (i.v.) is active in patients with recurrent SCLC. Three phase II studies of single-agent i.v. TPT have shown responses rates of 14–38% among sensitive patients with response rates of 2–6% among refractory patients. Median survival time was 26–28 weeks for sensitive patients compared with 16–20 weeks for refractory patients [12–14]. Furthermore, a phase III randomized study in patients with relapsed sensitive SCLC showed that outcomes with TPT were at least as good as those with CAV regimen [17].

Oral and i.p. administration of four doses of TPT (0.5, 1, 1.5, and 2 mg/kg) were compared in the highly sensitive SCLC-61 xenograft and no difference was observed between the two routes of administration. An oral formulation of TPT showed similar efficacy to the i.v. formulation in patients with relapsed SCLC [15,25]. In a phase III study of patients with relapsed sensitive SCLC, response rates with oral and i.v. TPT were 18.3% and 21.9%, respectively [25].

In this study, TPT was well tolerated. The predominant toxicity in TPT-treated patients was hematologic, principally noncumulative, reversible neutropenia. Nonhematologic toxicities were generally grade 1 or 2, and the subjective tolerability of TPT was good. TPT has an incomplete, overlapping toxicity profile with other agents used in the treatment of SCLC. Reversible, nonoverlapping, nonhematologic toxicities and in-vitro antitumor synergy with platinum agents, taxanes, and Topo II inhibitors may make TPT an ideal candidate for use in combination with other chemotherapy agents [26–28]. Therefore, to improve its efficacy, TPT has been combined with other chemotherapies, including etoposide, ifosfamide, and cisplatin [29–32]. In the experiments reported here, the efficacy of TPT was improved by combination with ifosfamide (SCLC-6, SCLC-61, and SCLC-101) or etoposide (SCLC-6 and SCLC-61). Moreover, in VIP-refractory xenografts (SCLC-74 and SCLC-108), etoposide improved the efficacy of TPT. Administration of TPT before etoposide was found to be more effective, possibly by increasing Topo IIα levels [29,33]. This would lead to increased sensitivity of tumors to subsequent treatment with etoposide, as also shown by Saraiya and colleagues [34]. Combinations of TPT and etoposide or ifosfamide also seemed to be as effective as the VIP regimen, suggesting that cisplatin-related toxicities could be avoided without decreasing therapeutic efficacy.

The cisplatin and TPT combination would be a useful option in the treatment of SCLC because of the nonoverlapping toxicities of these agents and the potential for TPT to prevent repair of platinum DNA adducts. *In vitro*, CDDP is known to induce interstrand and intrastrand DNA adducts, such as a poisoning of the Topo I. Combined with TPT, this mechanism could be exacerbated [35]. Moreover, Van Waardenburg *et al.* [35] have shown that the in-vivo persistence of cisplatin–DNA adducts correlated with increased covalent Topo I–DNA complexes that might increase the sensitivity to TPT. Data from phase II studies of TPT with cisplatin as first-line therapy for ED-SCLC indicate efficacy in this setting, with response rates of 60–63% and median survival of 8.0–9.6 months [36,37]. However, in this study, the CDDP with TPT combination did not improve the efficacy of TPT. Furthermore, the TPT and cisplatin combination was very toxic with the death of all treated mice. Earlier studies have shown that hematologic toxicity with TPT/CDDP is sequence-dependent, and that toxicity is reduced when cisplatin is given on day 5 of TPT treatment instead of day 1, as in this study [38–40]. Owing to its toxicity, the TPT and cisplatin combination required low dosages of TPT, inducing similar efficacy of both TPT–CDDP and etoposide–TPT in SCLC-74 and SCLC-108 xenografts, similar to the results observed in clinical trials (63% for TPT/CDDP vs. 61 or 69% for TPT/VP16) [36,41,42]. However, cisplatin slightly increased the response to TPT–CDDP in SCLC-74 but not in SCLC-108, as compared with TPT alone. This observation could be explained by the higher content of GSTπ and related enzymes in these tumors [43,44]. In a randomized phase III trial, the combination of oral TPT and cisplatin was compared with the standard etoposide–cisplatin regimen in previously untreated patients with ED-SCLC. Oral TPT with cisplatin provided similar efficacy and tolerability to the standard regimen (etoposide with cisplatin) in untreated ED-SCLC and may provide greater patient convenience compared with i.v. etoposide and cisplatin [41].

Study of the expression of various genes involved in the response to camptothecin analogs showed that Topo I expression was the most important. Earlier reports have also suggested that the sensitivity of cells to TPT might be because of decreased accumulation of the drug in cells, but independently of the P-glycoprotein-mediated MDR and MRP. Breast cancer resistance protein (BCRP) has also been shown to play a role in intracellular drug accumulation [45–48]. However, no significant correlation between relative expression of the various genes studied and the in-vivo response to TPT administered alone or in combination was observed in our xenografts.

In conclusion, this panel of SCLC established xenografts seems to be very representative of human SCLC disease in terms of histology and drug response, confirming their value for preclinical assessment of new drugs or new drug combinations.

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