Original article

Annals of Oncology 13: 392–398, 2002
DOI: 10.1093/annonc/mdf030

Preclinical and phase I study of oxaliplatin and topotecan in combination in human cancer

G. Tortora[1]*, F. Ciardiello[1], V. Damiano[1], M. De Laurentiis[1], E. Matano[1], S. Pepe[1], M. Pensabene[1], G. Catalano[2], S. De Placido[1] & A. R. Bianco[1]

[1]*Dipartimento di Endocrinologia e Oncologia Molecolare e Clinica, Università di Napoli Federico II, Napoli, Italy;* [2]*Dipartimento di Medicina Sperimentale F. Magrassi, Seconda Universitá di Napoli, Napoli, Italy*

Received 18 May 2001; revised 23 August 2001; accepted 6 September 2001

Background: DNA damage caused by platinum agents is frequently followed by induction of topoisomerase I, providing a rationale for use of platinum-based compounds with topoisomerase I inhibitors.

Materials and methods: We studied the effect of a sequential schedule of oxaliplatin on day 1 and topotecan on days 2–5, in human colon and ovarian cancer cells *in vitro*, in nude mice bearing human cancer xenografts and finally in cancer patients in a phase I trial.

Results: We demonstrated a supra-additive effect of this combination on inhibition of colony formation and induction of apoptosis *in vitro*. We then demonstrated that the two agents in combination markedly inhibit tumor growth in nude mice. We translated these results into a clinical setting, conducting a phase I study in cancer patients with oxaliplatin 85 mg/m^2 on day 1 and topotecan at doses escalating from 0.5 to 1.5 mg/m^2 on days 2–5. Sixty cycles of treatment were administered to 18 patients affected prevalently by ovarian and colorectal cancer. Combination with topotecan 1.5 mg/m^2 caused a dose-limiting toxicity. Therefore the maximum tolerated dose of topotecan was 1.25 mg/m^2, at which six patients experienced a mild hematological and gastrointestinal toxicity. We also obtained evidence of clinical activity, particularly in ovarian cancer.

Conclusions: Our results provide a solid biological and clinical rationale for a phase II trial at the recommended doses of oxaliplatin 85 mg/m^2 and topotecan 1.25 mg/m^2, possibly in ovarian cancer patients.

Key words: ovarian cancer, oxaliplatin, phase I, topotecan

Introduction

Oxaliplatin is a 1,2-diamine-cicloesane derivative of cisplatin exhibiting several peculiar properties as compared to the parental compound. It is able to cause bulky DNA intrastrand adducts and conformational distorsions, which prevent the binding of the mismatch repair protein complex and lead to apoptosis [1]. Moreover, oxaliplatin is partly non-cross-resistant to cisplatin and carboplatin, causing a moderate hematological toxicity and a reversible peripheral neurotoxicity. It is also active against different types of cancer, including advanced ovarian and colon cancer [2].

Topotecan is a camptothecin derivative selective for topoisomerase I [3], currently used in a variety of tumors at a standard dose of 1.5 mg/m^2 for 5 days, including ovarian cancer patients who had received failed treatment with platinum derivatives and paclitaxel [4].

It has been shown that DNA damage produced by platinum agents is frequently followed by induction of topoisomerase I-dependent cleavage activity. *In vitro* studies have demonstrated that oxaliplatin induces topoisomerase I-mediated single strand breaks and that subsequent addition of topotecan increases DNA damage and cell death [5]. These data represent the biochemical rationale for the reported cooperative effect of platinum agents in combination with topoisomerase I inhibitors [6, 7]. A phase I clinical trial conducted on this basis (oxaliplatin 85 mg/m^2 given on day 1 and topotecan 0.5–1 mg/m^2 given for 5 days starting on day 1) has shown a severe and partly unexpected myelotoxicity resulting in the recommendation of careful selection of patients, based on previous treatment, for future studies [8]. However, the interesting biological rationale and the responses observed have led to the conclusion that further exploration of this combination of agents is worthwhile.

With the aim of reducing toxicity whilst preserving potential antitumor activity, we have studied the effect of oxaliplatin

Correspondence to: Dr G. Tortora, Cattedra di Oncologia Medica, Dipartimento di Endocrinologia e Oncologia Molecolare e Clinica, Università di Napoli Federico II, Via S. Pansini 5, 80131 Napoli, Italy.
Tel: +39-081-7462061; Fax: +39-081-7462066;
E-mail: gtortora@unina.it

and topotecan in combination on a sequential schedule from cell culture to clinical settings. In the present study we first evaluated the activity of the combination on cancer cell proliferation and apoptosis *in vitro*. Then we confirmed these data *in vivo*, in nude mice bearing human tumor xenografts. Finally, we designed and conducted a phase I study in patients refractory to standard treatments.

Materials and methods

Cell cultures

GEO colon and OVCAR-3 ovarian human cancer cell lines were grown in McCoy medium or in a 1:1 mixture of DMEM and Ham's-F12 medium, respectively. All media were purchased from Flow Laboratories (Irvine, UK) and were supplemented with 10% heat inactivated fetal bovine serum, 20 mM Hepes pH 7.4, 5 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (Flow Laboratories). Cells were maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. For cell growth experiments in soft agar 10^4 cells/well were seeded in 24 multiwell cluster dishes as described previously [9] and treated with different concentrations of drugs as follows: oxaliplatin 0.1, 0.5 or 1 µg/ml on the day of seeding (day 0) and/or topotecan 1, 5 or 10 nM on days 1–4. Twelve days after the last treatment cells were stained with nitroblue tetrazolium (Sigma Chemicals, Milan, Italy) and colonies larger than 0.05 mm were counted [9].

Flowcytometric analysis of cell cycle

Cells were harvested, fixed in 70% ethanol, stained with a propidium iodide staining solution and the DNA content analyzed in duplicate by a FACScan flow-cytometer (Becton and Dickinson, Mountain View, CA, USA) as described previously [9]. Cell cycle data analysis was performed by a CELL-FIT program (Becton and Dickinson).

Apoptosis

The induction of apoptosis was determined by the Cell Death Detection ELISA Plus Kit (Roche Molecular Biochemicals, Mannheim, Germany) which detects cytosolic histone-associated DNA fragments. OVCAR-3 and GEO cells (5×10^4 cells/dish) were seeded into 35 mm dishes and treated with different concentrations of oxaliplatin (0.1 µg/ml on the day of seeding, day 0) and/or topotecan (1, 5 or 10 nM on days 1–4). On day 5, cells were washed once with PBS and lysed for 30 min then supernatant was recovered and assayed for DNA fragments as recommended by the manufacturer [10]. Each treatment was performed in quadruplicate. The total number of cells was measured with a hemocytometer in additional plates receiving an identical treatment. The optical density at 405 nm was normalized for cell number and the optical density ratio (treated:untreated cells) was considered an apoptotic index and expressed in arbitrary units [10].

In vivo studies in nude mice

Five- to six-week-old female Balb/c athymic (nu+/nu+) mice were purchased from Charles River Laboratories (Milan, Italy). Mice were maintained in accordance to the institutional guidelines of the University of Naples Animal Care and Use Committee. They were acclimatized to the University of Naples Medical School Animal Facility for 1 week prior to s.c. injection with 10^7 GEO cells resuspended in 200 µl of Matrigel (Collaborative Biomedical Products, Bedford, MA, USA) as described previously [11]. After 7 days, when well-established tumors of ~0.2 cm^3 were detected, 10 mice per group were i.p. treated with the following doses and schedules: oxaliplatin alone (either 10 mg/kg on day 1 every week for 4 weeks or 15 mg/kg on day 1 every 2 weeks); topotecan alone (2 mg/kg on day 1 every week for 4 weeks, or 2 mg/kg on days 1 and 2 every 2 weeks, or 0.5 mg/kg on days 1–4 every 2 weeks); oxaliplatin (10 mg/kg on day 1) in combination with topotecan (0.5 mg/kg on days 2–5) repeated every 2 weeks for a total of three cycles of treatment. Tumor size was measured twice weekly using the formula: $(\pi/6) \times$ larger diameter $\times$ (smaller diameter)^2.

Phase I study

Patient selection. Patients enrolled had a histologically confirmed solid tumor refractory to conventional therapy, no treatment of the malignancy for 6 weeks before study entry, a minimum life expectancy of 3 months and were at least 18 years old. Minimum eligibility requirements included: Eastern Cooperative Oncology Group performance status ≤2; neutrophil count ≥1500/µl; platelet count ≥100 000/µl; hemoglobin ≥9.5 g/dl; serum creatinine ≤2.0 mg/dl; total bilirubin <1.5 mg/dl; and aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities ≤2.5 × the upper limit of normal. Informed, signed consent was obtained as a condition of patient registration.

Study design. The study protocol was approved by the institutional Scientific Review Committee. Patients underwent a physical examination and performance status determination. Electrocardiogram and chest X-ray, complete blood count with platelet and differential counts, coagulation tests, serum chemistry profile and determination of serum tumor markers were performed within 14 days before initiating therapy and, at indicated times, during the entire period of study.

We combined oxaliplatin (Eloxatine) 85 mg/m^2 administered in 2 h i.v. on day 1 with topotecan (Hycamtin) at doses ranging from 0.5 mg/m^2 up to 1.5 mg/m^2 in five different dose levels administered in 30 min i.v. on days 2–5. Each cycle of treatment was repeated every 3 weeks. Patients were scheduled to receive at least three courses of therapy at the same dose level. Cohorts of three patients were scheduled for entry at each dose level. Escalation of the dose to the next higher level proceeded after all three patients received the first cycle of therapy and had been observed for at least 21 days without evidence of a dose-limiting toxicity (DLT), as defined below. Drug-related toxicities were evaluated during each cycle of therapy and graded according to the NCI Common Toxicity Criteria version 1 [12]. A DLT was defined as any of the following events: grade 4 neutropenia, grade 4 thrombocytopenia, any drug-related non-hematological toxicity greater than or equal to grade 3 (except alopecia). At the occurrence of a DLT in three patients from any cohort, an additional three patients were enrolled at the preceding dose level which was considered the maximum tolerated dose (MTD). Patients experiencing toxicities that were not dose-limiting could be retreated at the same dose level upon full recovery. Treatment was discontinued upon occurrence of a DLT or tumor progression.

Evaluation of response. A baseline assessment of all measurable disease sites using appropriate radiological techniques was performed within 21 days before the first cycle of therapy. Tumor burden was calculated as the sum of the products of the longest perpendicular diameters of all measurable lesions and response was evaluated by common criteria [13]. Duration of response was measured from the date that the response was first recorded to the date of documented disease progression.

Results

Effects on cancer cell growth and apoptosis *in vitro*

We measured the dose–response effect of oxaliplatin and topotecan on the soft agar growth of GEO colon and OVCAR-3 ovarian cancer cells, obtaining an IC_{50} of ~0.5 μg/ml for oxaliplatin and 15 nM for topotecan (data not shown). The two agents in sequential combination (oxaliplatin on day 0 and topotecan on days 1–4) caused a marked supra-additive inhibitory effect in both GEO and OVCAR-3, this is more evident with lower doses of each agent (Figure 1A and B). The first of each pair of bars shows, as stacked bars, the individual effects of each drug when used alone. Thus, the total height of these stacked bars also represents the expected total inhibition if drugs have an additive effect. The second bar of each pair shows the effect obtained when the drugs were used in combination. Therefore, the comparison between the height of the first bar and that of the second bar of each pair shows whether a supra-additive effect is obtained and the magnitude of this effect.

Cell cycle analysis demonstrated that oxaliplatin (0.5 μg/ml) alone markedly increased the percentage of cells in G_2-M phase, while topotecan (at the low dose of 5 nM) increased the S phase almost 2-fold. The two agents in combination caused a 3-fold and 6-fold increase in the percentage of cells in S phase and G_2-M phase, respectively (data not shown).

We studied the effect of oxaliplatin and topotecan, alone and in combination, on the induction of apoptosis in OVCAR-3 and GEO cells. As shown in Figure 1C, oxaliplatin or topotecan alone at the low doses of 0.1 μg/ml and 1 nM, respectively, did not induce apoptosis in ovarian OVCAR-3 cells, as compared with untreated cells. A 2-fold and a 2.3-fold induction were observed with topotecan alone at the higher doses of 5 and 10 nM, respectively. When oxaliplatin was used in combination with topotecan we observed at least an additive effect with topotecan 1 and 5 nM and a supra-additive effect with topotecan 10 nM (Figure 1C). The same doses of the two agents also caused at least an additive effect in GEO cells (data not shown).

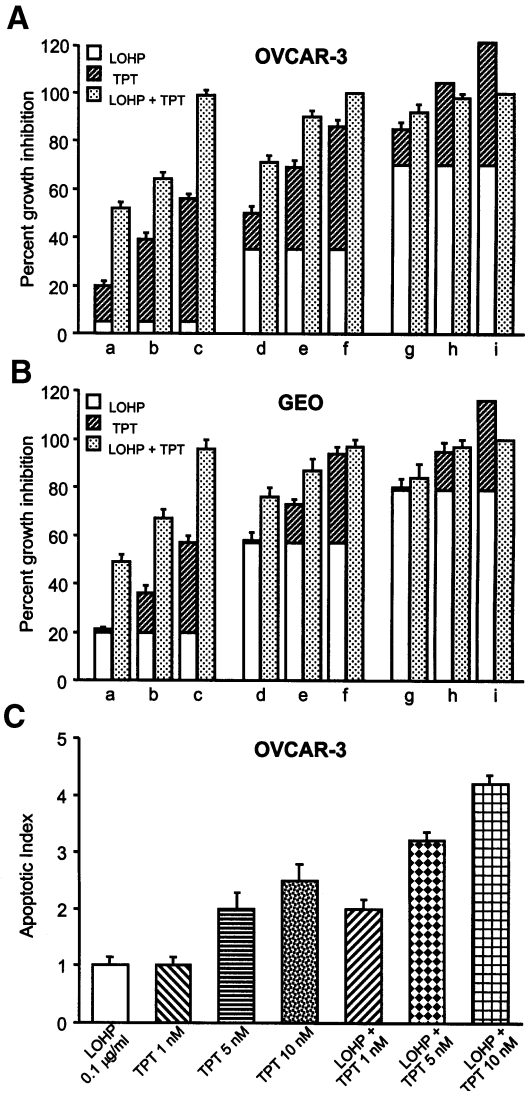

Figure 1. Effect of oxaliplatin (LOHP) and/or topotecan (TPT) on cancer cell growth (**A**), (**B**), and apoptosis (**C**). Effect of LOHP and TPT alone and in combination on the soft agar growth of OVCAR-3 (A) and GEO (B) cells. LOHP 0.1 μg/ml (a–c); 0.5 μg/ml (d–f); 1 μg/ml (g–i) was added on day 0; TPT 1 nM (a, d, g), 5 nM (b, e, h), 10 nM (c, f, i) was added on days 1–4. Cells were counted 12 days after seeding. Data are expressed as a percentage inhibition of colony formation compared to untreated control cells. The data represent means and standard errors of triplicate determination of at least two experiments. (C) Effect of LOHP and TPT, alone and in combination, on the induction of apoptosis in OVCAR-3 cells. Data are expressed in arbitrary units as relative increase compared with untreated cells, considered as 1. Data represent means and standard errors of quadruplicate determination of three experiments.

Effect *in vivo* in nude mice bearing human cancer xenografts

We translated the results obtained *in vitro* into a model of nude mice xenografted with GEO cells. We first studied the antitumor effect of different doses and schedules of oxaliplatin and topotecan, selecting moderately effective doses of each single agent. We observed similar effects for oxaliplatin either 10 mg/kg once a week for 4 weeks or 15 mg/kg once a week every 2 weeks (data not shown). We also demonstrated similar growth inhibitory effects with three different doses and schedules of topotecan, including the low dose 0.5 mg/kg for 4 days every 2 weeks (Figure 2A). Therefore, to be consistent with the *in vitro* model, we randomized mice to receive the combination regimen of oxaliplatin 15 mg/kg on day 1 and topotecan 0.5 mg/kg on days 2–5. Each cycle was repeated every 2 weeks for a total of three cycles of treatment (Figure 2B). We have shown that oxaliplatin alone was ineffective in inhibiting tumor growth since by day 35 all oxaliplatin-treated

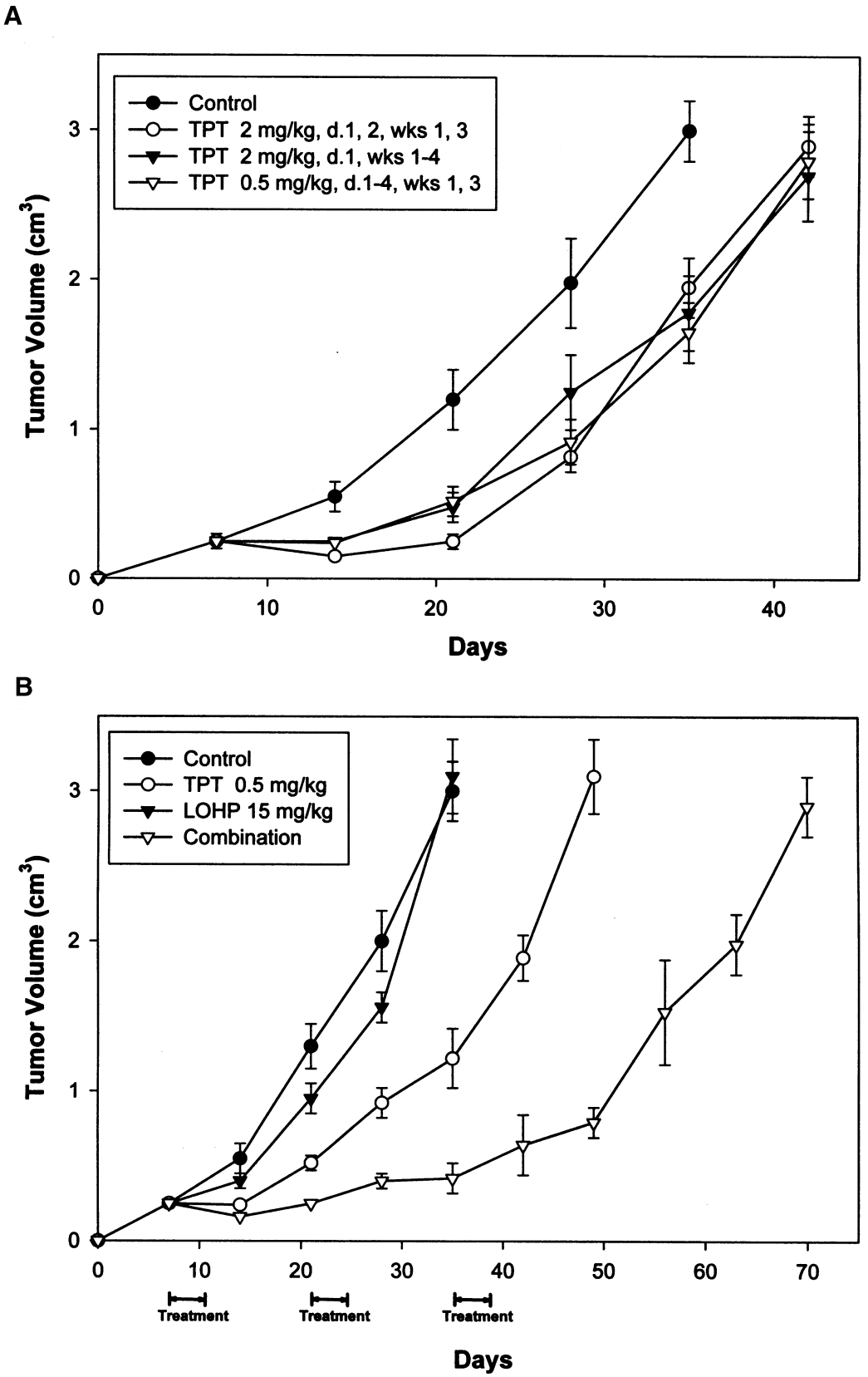

Figure 2. Effect of the treatment with topotecan (TPT) and/or oxaliplatin (LOHP) on the growth of GEO tumor xenografts. (**A**) Effect of different doses and schedules of administration of TPT. (**B**) Effect of LOHP and TPT alone and in combination. The schedule of administration of each single agent, alone or in combination, for the three cycles of treatment were as follows: LOHP 15 mg/kg on days 7, 21, 35; TPT 0.5 mg/kg on days 8–11, 22–25, 36–39 after tumor cell injection (day 0).

Table 1. Patient demographics and disease characteristics

Patients	18
Age (years)	
Median	54
Range	30–71
Sex (m/f)	4/14
Primary site of tumors	
Ovarian cancer	10
Colorectal cancer	6
Testis	1
Lung	1
Prior therapies	
Chemotherapy (2 lines)	12
Chemotherapy (3 lines)	5
Chemotherapy (2 lines) + radiotherapy	1
Performance status	
0	5
1	10
2	3

mice, as well as mice in the control group, were dead. In the group treated with topotecan alone we observed a growth inhibitory effect accompanied by increased mice survival. A marked inhibitory effect was observed in mice treated with the two agents in combination. In fact, at 7 weeks following tumor injection, tumor volume was 90% smaller than that observed in mice treated with topotecan alone and tumors did not achieve a size incompatible with normal life until at least 5 weeks after treatment withdrawal (Figure 2B).

Phase I study

We designed a phase I trial based on the results obtained in the preclinical studies. We combined oxaliplatin 85 mg/m^2 on day 1 with escalating doses of topotecan on days 2–5. We studied five dose levels with topotecan escalating from 0.5 mg/m^2 (level I) to 1.5 mg/m^2 (level V) increasing the dose of topotecan by 0.25 mg/m^2 at each level. We enrolled 18 patients, prevalently affected by ovarian and colorectal cancer, who had failed standard treatments under at least two different treatment regimens (Table 1). As shown in Table 2 three patients were treated at each dose level, with three additional patients (giving a total of six) treated at the MTD, level IV. Starting from the second dose level several patients received multiple cycles of treatment that were repeated every 3 weeks. A total of 60 cycles of treatment were administered.

The most relevant toxic effects observed were neutropenia, thrombocytopenia and diarrhea (Table 3). Combination with topotecan at 1.5 mg/m^2 (dose level V) caused a DLT in each of the three patients studied who suffered febrile or grade 4 neutropenia, grade 2 diarrhea and mucositis at the first cycle of treatment (Table 3).

Table 2. Total number of patients and cycles administered

Level	Topotecan dose (mg/m^2)[a]	Patients	No. of cycles
I	0.5	3	3
II	0.75	3	12
III	1	3	12
IV	1.25	6	30
V	1.5	3	3

[a]All patients also received oxaliplatin 85 mg/m^2 in each cycle.

In the combination with topotecan, a dose of 1.25 mg/m^2 was considered the MTD. At this dose level three patients received four cycles of treatment and three received six cycles, experiencing a moderate toxicity. Neutropenia and thrombocytopenia appeared after the first cycle with an average onset time of 7 days, while diarrhea had a comparatively early onset occurring in the first week of treatment in most cases. Neutropenia and diarrhea were of short duration and only a few cases required medical support, such as G-CSF and loperamide, respectively. Two patients who had previously received severely myelotoxic regimens experienced grade 3 neutro-

Table 3. Toxicity according to WHO criteria

	Level II[a]	Level III[a]	Level IV[a]	Level V[a]
Neutropenia				
Grade 3	1 (18)	2 (12)	7 (30)	–
Grade 4	–	1 (12)	1 (30)	2 (3)
Febrile neutropenia	–	–	–	1 (3)
Thrombocytopenia				
Grade 3	1 (18)	–	3 (30)	2 (3)
Grade 4	–	–	–	1 (3)
Anemia				
Grade 3	–	–	2 (30)	–
Grade 4	–	–	–	–
Vomiting				
Grade 3	–	–	4 (30)	1 (3)
Grade 4	–	–	–	1 (3)
Diarrhea				
Grade 1	–	1 (12)	7 (30)	–
Grade 2	–	–	1 (30)	2 (3)
Mucositis				
Grade 1	–	–	3 (30)	–
Grade 2	–	–	–	2 (3)

[a]Numbers refer to patients experiencing toxicity. Figures in parenthesis indicate the total number of cycles administered at that dose level.

penia, thrombocytopenia and anemia, while in only one cycle out of 30 was grade 4 neutropenia experienced. Interestingly, no peripheral neurotoxicity was observed in spite of the fact that >50% of patients had previously received platinum agents. In general, side effects at this dose level were manageable. Patients receiving multiple cycles of treatment did not show signs of cumulative toxicity.

We obtained evidence of the clinical activity of this regimen, particularly in ovarian cancer, although all patients had already received treatment with paclitaxel and a platinum agent. In fact, we observed a complete response maintained for >9 months in two stage IV ovarian cancer patients and stabilization of disease in two patients with ovarian cancer and two patients with metastatic colorectal cancer. Moreover, most ovarian cancer patients also obtained a reduction of serum CA 125 levels at the lower dose levels.

Discussion

A rational approach to cancer treatment is based on the combination of non-cross-resistant agents interfering with selected molecular targets, possibly functionally related to each other, in order to exploit a potential cooperative effect. The combination of platinum derivatives, which cause DNA damage and can induce topoisomerase I enzyme, with specific topoisomerase I-selective agents might have relevant therapeutic implications. Oxaliplatin exhibits peculiar biochemical properties, partial non-cross-resistance and a different toxicity profile compared with cisplatin and carboplatin, which make it a suitable candidate for combination with topoisomerase I drugs [1]. In this regard, preclinical studies have shown that oxaliplatin has an additive effect with SN-38, a metabolite of irinotecan [14], and an additive or synergistic effect with topotecan [5]. A phase I clinical study in which topotecan was given for 5 days, starting the same day as oxaliplatin, has demonstrated severe toxicity in a subgroup of patients [8]. Therefore, we decided to evaluate the effect of these two agents in combination using a sequential schedule, giving oxaliplatin on the first day and topotecan on the following 4 days. We conducted studies in a preclinical setting, *in vitro* and in animal models, and in a clinical context with a phase I trial in cancer patients refractory to standard treatments.

We demonstrated that oxaliplatin and topotecan used in sequential combination cause a supra-additive inhibition of growth in human GEO colon and OVCAR-3 ovarian cancer cells, expecially evident with lower doses of each agent. These effects were associated with enhancement of apoptosis and perturbation of cell cycle distribution with increased accumulation of cells in S and G_2-M phases.

We confirmed these findings *in vivo*, in nude mice bearing GEO tumor xenografts, using different doses and schedules of the two agents alone, then selecting the doses to be used in combination with the same sequential schedule used *in vitro*. Animals received three cycles of treatment. All mice untreated or treated with oxaliplatin alone were dead 5 weeks after tumor injection. Treatment with topotecan alone caused ~60% inhibition of growth at the end of treatment as compared with the previous groups. However, 2 weeks following treatment withdrawal all topotecan-treated mice were dead. Conversely, at the same time point, animals receiving oxaliplatin and topotecan in combination were all alive and had a tumor volume 90% smaller than mice treated with topotecan alone. In addition, once the tumors recovered their growth they did not cause the death of the mice before 5 weeks following treatment withdrawal. No signs of acute or delayed toxicity were observed in treated mice.

On this basis, we designed and conducted a phase I study. We used oxaliplatin on day 1 at 85 mg/m^2, a dose commonly used and well tolerated in combination regimens [2] and topotecan on days 2–5 at five different dose levels ranging from 0.5 to 1.5 mg/m^2. We administered 60 cycles of treatment to 18 patients. The treatment regimen was generally well tolerated and toxic effects were mainly hematological (neutropenia and thrombocytopenia) and gastrointestinal (mucositis and diarrhea). The DLT was attained in the combination with topotecan at 1.5 mg/m^2. Three patients affected by ovarian cancer and three by colon cancer received a total of 30 cycles of treatment at the MTD, with topotecan 1.25 mg/m^2. Although patients at this dose level had been pretreated with drugs responsible for hematological toxicity and diarrhea, they experienced only manageable side effects during the study, with moderate bone marrow and gastrointestinal toxicity, except for one case of grade 4 neutropenia. Several patients received multiple cycles of treatment, up to six, without signs of cumulative toxicity. Interestingly, we have not observed neurotoxicity, a peculiar toxicity attributed to oxaliplatin, even in patients who had previously received platinum derivatives and taxanes.

Both topotecan and oxaliplatin have been widely studied in combination regimens with different agents. In the majority of studies the relative recommended doses have been between 85 and 100 mg/m^2, on day 1, for oxaliplatin and up to 1 mg/m^2 for 5 days for topotecan [2, 3]. The schedule used in our study allows the administration of approximately two-thirds of the standard dose of topotecan as a single agent.

Topotecan is used as a single agent to treat various types of cancer, including ovarian cancer [3, 4]. At the standard dose of 1.5 mg/m^2 it induces an average response rate of 14% in ovarian cancer patients, varying between 12% and 20% in those refractory or sensitive to cisplatin, respectively [4, 15]. However, in other types of cancer, such as those of the gastrointestinal tract, the percentage of responses is relatively poor [16].

We obtained evidence of clinical activity of this combination regimen, particularly in ovarian cancer patients. In fact, we observed complete responses, maintained for >9 months, in two heavily pretreated stage IV ovarian cancer patients. Like all the other ovarian cancer patients enrolled, both had previously received paclitaxel and a platinum agent, also one

of them had an early relapse after two different treatment regimens. Two other ovarian patients obtained a stabilization of the disease, while the majority of them experienced a reduction of serum CA 125 levels, even at the lower dose levels. We have also reported a stabilization of the disease in two patients with metastatic colorectal cancer.

For these reasons our results are encouraging and support further exploration of such a combination regimen in a phase II study at the recommended doses of oxaliplatin 85 mg/m^2 on day 1 and topotecan 1.25 mg/m^2 on days 2–5, in early relapsed or refractory ovarian cancer patients.

Acknowledgements

This study was partly supported by grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC).

References

1. Raymond E, Chaney SG, Taamma A, Cvitkovic E. Oxaliplatin: a review of preclinical and clinical studies. Ann Oncol 1998; 9: 1053–1071.
2. Misset JL, Bleiberg H, Sutherland W et al. Oxaliplatin clinical activity: a review. Crit Rev Oncol Hematol 2000; 35: 75–93.
3. Burris HA 3rd, Fields SM. Topoisomerase I inhibitors. An overview of the camptothecins analogs. Hematol Oncol Clin North Am 1994; 8: 333–355.
4. Bookman MA, Malmstrom H, Bolis G et al. Topotecan for the treatment of advanced epithelial ovarian cancer: an open-label phase II study in patients treated after prior chemotherapy that contained cisplatin or carboplatin and paclitaxel. J Clin Oncol 1998; 16: 3345–3352.
5. Goldwasser F, Bozec L, Zeghari-Squalli N, Misset JL. Cellular pharmacology of the combination of oxaliplatin with topotecan in the IGROV-1 human ovarian cancer cell line. Anticancer Drugs 1999; 10: 195–201.
6. Ma J, Maliepaard M, Nooter K et al. Synergistic cytotoxicity of cisplatin and topotecan or SN-38 in a panel of eight solid-tumor cell lines *in vitro*. Cancer Chemother Pharmacol 1998; 41: 307–316.
7. Romanelli S, Perego P, Pratesi G et al. *In vitro* and *in vivo* interaction between cisplatin and topotecan in ovarian carcinoma systems. Cancer Chemother Pharmacol 1998; 41: 385–390.
8. Misset JL, Goldwasser F, Riofrio M et al. Topotecan-oxaliplatin every three weeks: a phase I and pharmacological study. Proc Am Soc Clin Oncol 1999; 18: 845 (Abstr).
9. Ciardiello F, Pepe S, Bianco C et al. Down-regulation of RIα subunit of the cAMP-dependent protein kinase induces growth inhibition of human mammary epithelial cells transformed by c-Ha-ras and c-erbB-2 proto-oncogenes. Int J Cancer 1993; 53: 438–443.
10. Tortora G, Caputo R, Damiano V et al. A novel MDM2 anti-sense oligonucleotide has anti-tumor activity and potentiates cytotoxic drugs acting by different mechanisms in human colon cancer. Int J Cancer 2000; 88: 804–809.
11. Ciardiello F, Damiano V, Bianco R et al. Anti-tumor activity of combined blockade of epidermal growth factor receptor and protein kinase A. J Natl Cancer Inst 1996; 88: 1770–1776.
12. National Cancer Institute. Guidelines for reporting of adverse drug reactions. Bethesda, MD: Division of Cancer Treatment, National Cancer Institute, 1988.
13. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981; 47: 207–214.
14. Zeghari-Squalli N, Raymond E, Cvitkovic E, Goldwasser F. Cellular pharmacology of the combination of the DNA topoisomerase I inhibitor SN-38 and the diaminocyclohexane platinum derivative oxaliplatin. Clin Cancer Res 1999; 5: 1189–1196.
15. Coleman RL, Miller DS. Topotecan in the treatment of gynecologic cancer. Semin Oncol 1997; 24 (6 Suppl 20): 55–63.
16. Creemers GJ. Topotecan in advanced colorectal cancer. Semin Oncol 1997; 24 (Suppl 20): 42–48.