Biochemical Pharmacology, Vol. 54, pp. 755–759, 1997.

ELSEVIER

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00116-0

COMMENTARY

DNA Topoisomerase II Rescue by Catalytic Inhibitors

A NEW STRATEGY TO IMPROVE THE ANTITUMOR SELECTIVITY OF ETOPOSIDE

Peter Buhl Jensen and Maxwell Sehested†‡*

*Laboratory of Experimental Medical Oncology, Finsen Center, and
†Department of Pathology, Laboratory Center Rigshospitalet, DK-2100 Copenhagen, Denmark

ABSTRACT. The nuclear enzyme DNA topoisomerase II (topo II) is the target of important antitumor agents such as etoposide. Recent work has classified topo II targeting drugs into either topo II poisons that act by stabilizing enzyme–DNA cleavable complexes leading to DNA breaks, or topo II catalytic inhibitors that act at stages in the catalytic cycle of the enzyme where both DNA strands are intact and, therefore, do not cause DNA breaks. Accordingly, catalytic inhibitors are known to abrogate DNA damage and cytotoxicity caused by topo II poisons. In this commentary, we have focused on the possibilities of enabling high-dose therapy with the topo II poison etoposide by protection of normal tissue with catalytic inhibitors, analogous to folinic acid rescue in high-dose methotrexate treatment. Thus, we have demonstrated recently that (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane (ICRF-187) enabled a 3- to 4-fold dose escalation of etoposide in mice. Two high-dose etoposide models are described, namely use of the weak base chloroquine in tumors with acidic extracellular pH and targeting of CNS tumors with protection of normal tissue by the bisdioxopiperazine ICRF-187. In conclusion, high supralethal doses of topo II poisons in combination with catalytic inhibitor protection form a new strategy to improve the antitumor selectivity of etoposide and other topo II poisons. Such an approach may be used to overcome problems with drug resistance and drug penetration. BIOCHEM PHARMACOL **54**;7:755–759, 1997.

KEY WORDS. topoisomerase II; etoposide; bisdioxopiperazine; high dose treatment; tumor selectivity; preclinical models; resistance

The nuclear enzyme DNA topo II§ is the target of important antitumor agents [1]. Topo II targeted drugs such as etoposide (VP-16) and doxorubicin (Adriamycin®) are used in diseases such as leukemia, malignant lymphoma, breast and testicular cancer, and small cell lung cancer (SCLC). Thus, etoposide as a single drug yields remissions in 70% of SCLC patients, and half of these patients obtain complete remissions [2]. This essential enzyme, which exists in both a 170 kDa α and a 180 kDa β isoform, is responsible for a cleavage/rejoining reaction of double-stranded DNA allowing the separation of intertwined DNA strands [3]. During its catalytic cycle, the enzyme binds covalently to DNA, creating a transient double-strand DNA break. Through this DNA break the enzyme allows the passage of another DNA double-strand helix. Then DNA is rejoined, and the enzyme dissociates from the DNA. Topo II targeting anticancer agents can be classified conveniently into poisons and catalytic inhibitors (Table 1). Both drug classes inhibit the enzyme, but only topo II poisons result in the accumulation of topo II linked DNA breaks. This accumulation of DNA breaks is due to an inhibition of the rejoining step of the enzyme. The breaks are called cleavable complexes, as their detection relies on SDS fixation and proteinase treatment to remove the covalently linked topo II from the DNA break. Thus, a topo II poison acts at a stage of the catalytic cycle of the enzyme where the gate DNA strand is open or cleaved (Fig. 1), whereas a catalytic inhibitor interacts with the enzyme at a stage where there are no DNA breaks, i.e. the gate DNA strand is intact. This distinction may have some practical implications; thus, a topo II poison converts the essential enzyme to a poisonous molecule, whereas the catalytic inhibitor appears to exhibit toxicity by blocking the activity of the enzyme. Accordingly, a reduction in the nuclear topo II content or activity as in the at-MDR (altered topoisomerase II multidrug resistance) phenotype results in resistance to topo II poisons [4, 5] and a lack of cross-resistance or even increased sensitivity to catalytic inhibitors, as demonstrated with aclarubicin [6], fostriecin [7], and the ICRF-187 analogs -159 and -193 [8]. A logical consequence of the distinction is that a catalytic inhibitor should be able to inhibit a topo II poison by interfering with the catalytic cycle in such a way as to reduce the amount of cleavable complex formation, in other words, to decrease the available target of the poison. This has been shown to be the case for aclarubicin [9, 10], merbarone [11], fostriecin [12], novobiocin [13], suramin [14], chloroquine [15], distamycin [16], and the bisdioxopiperazines [17–19]. Only small differences in the molecular structure of a drug can determine whether it is a

‡ Corresponding author: Maxwell Sehested, M.D., Ph.D., Department of Pathology, Rigshospitalet 5444, DK-2100 Copenhagen, Denmark. Tel. (+45) 3545-5432; FAX (+45) 3545-5414; E-mail: maxwell@rh.dk

§ *Abbreviations:* topo II, topoisomerase II; MTX, methotrexate; and ICRF-187, (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane.

TABLE 1. Topoisomerase II targeted drugs divided into enzyme poisons and catalytic inhibitors and subdivided into DNA intercalators and non-intercalators

Topoisomerase II poisons	Topoisomerase II catalytic inhibitors
Intercalators	
Daunorubicin	Aclarubicin
Doxorubicin	Chloroquine
msacrine	
Ellipticine	
Mitoxantrone	
Non-intercalators	
Etoposide	Bisdioxopiperazines
Teniposide	(ICRF-159, -187, -193)
CP-115,953	Merbarone
Clerocidin	Fostriecin
	Suramin
	Novobiocin

topo II poison or a topo II catalytic inhibitor. Thus, we have demonstrated that the presence of a $COOCH_3$ moiety at the C-10 position in the anthracycline class of drug molecule is, in itself, enough to alter its status from a topo II poison to a catalytic inhibitor [6]. While topo II poisons are restricted to a relatively short part of the catalytic cycle of the enzyme, the catalytic inhibitors have several possible steps of interaction. These steps have been characterized in some detail for the intercalators and the bisdioxopiperazines. Thus, intercalating agents such as aclarubicin, ethidium bromide, and chloroquine act early in the catalytic cycle by hindering the access of the enzyme to its DNA substrate [15, 20, 21] (Fig. 1). Presumably, DNA minor groove binders such as distamycin act in the same way [16], while the bisdioxopiperazines appear to lock the enzyme in its closed-clamp post-religation step [22–24] (Fig. 1). One noticeable difference between topo II poisons and catalytic inhibitors is that while the former have supplied some of the most clinically potent and widely used anticancer agents, the only catalytic inhibitor that has been successful in clinical oncological practice has been aclarubicin. Thus, in a randomized phase III trial, aclarubicin + ara-C was found to be superior to daunorubicin + ara-C as first-line treatment of acute myeloid leukemia [25]. Curiously, this did not stimulate an interest in aclarubicin, especially as there is a lack of cross-resistance in the at-MDR phenotype between the topo II poison daunorubicin and the topo II catalytic inhibitor aclarubicin [6]. Such data suggest that their sequential administration would be beneficial. Unfortunately, this has not been tested, and today aclarubicin appears to approach pharmacoptosis—the end stage of a drug's life.

The question naturally arises whether the knowledge gained on the different drug interactions of topo II catalytic inhibitors and poisons could be put to clinical use. This situation is similar to that of the mid-1970s when folinic acid rescue was introduced for use in combination with high dose MTX. Thus, folinic acid is an antidote that is able to abolish completely the effects of MTX. This external salvage pathway has enabled manipulation of MTX pharmacology. High dose MTX with folinic acid rescue has been studied in a number of settings such as overcoming problems of drug resistance and poor drug penetration. Thus, MTX penetrates poorly into the cerebrospinal fluid. However, after high dose MTX, active dose levels can be achieved. These regimens, which employ otherwise lethal infusions of MTX, have now found application in the treatment of lymphomas, osteogenic sarcoma,

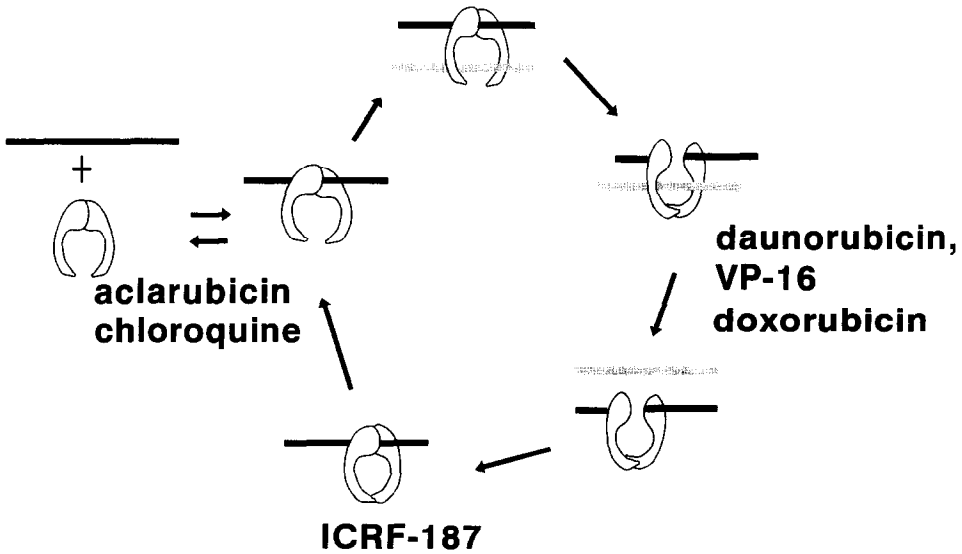

FIG. 1. The topoisomerase II catalytic cycle with the proposed interaction sites of the drugs mentioned. The enzyme is shown as a homodimer, the passing DNA strand is grey, and the cleaved DNA strand is black. VP-16 = etoposide.

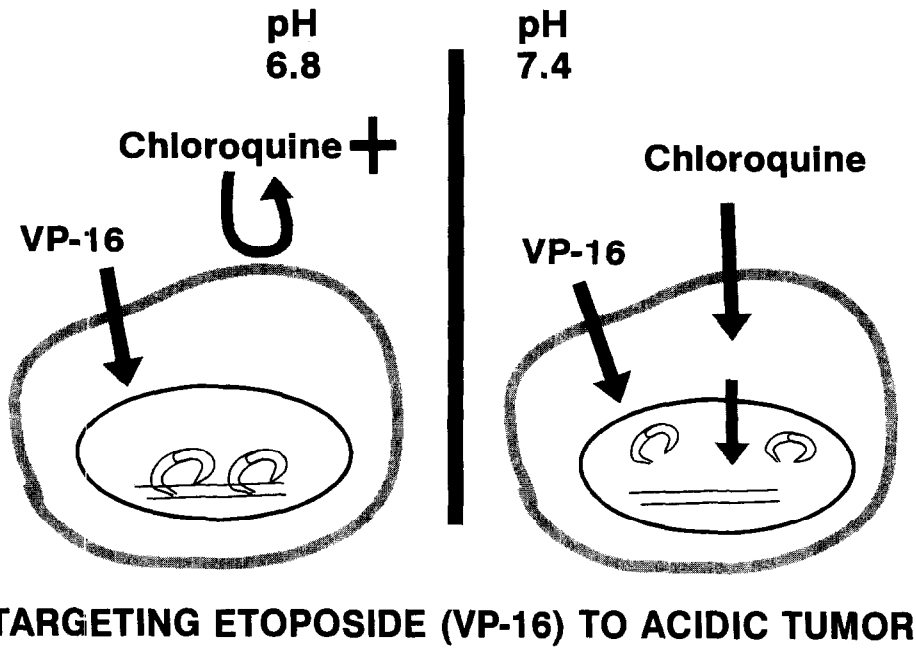

FIG. 2. Model depicting a pH-mediated regulation of topoisomerase II. An acidic extracellular pH is a common feature in many solid tumors. The neutral topoisomerase II poison etoposide (VP-16) traverses the plasma membrane at both neutral and acidic pH. However, the catalytic inhibitor chloroquine as a weak base binds a proton and is positively charged at low extracellular pH, and thus unable to cross the plasma membrane, while it is uncharged and freely permeable at neutral pH. In this way, chloroquine protects cells at neutral pH such as in normal tissue, but not in acidic cancerous tissue (see Ref. 15 for further details).

and acute leukemia [26]. Interestingly, similar options now appear with topo II rescue. The possibility of using high dose topo II poisons in combination with catalytic inhibitor protection is an intriguing idea to overcome problems with resistance and to exploit differential penetration. We therefore found it very encouraging when we realized that the bisdioxopirazine ICRF-187 within a wide range of non-toxic doses was able to protect against otherwise lethal

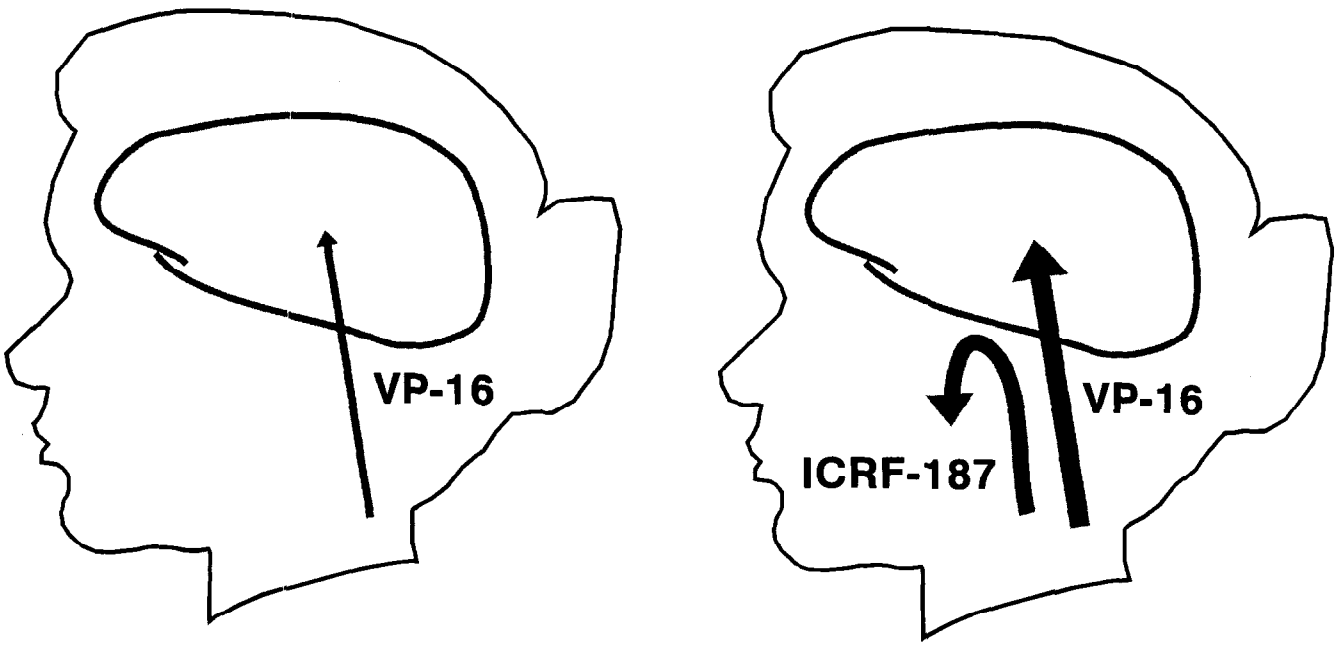

FIG. 3. Model showing high-dose therapy of CNS tumors by etoposide (VP-16) enabled by differences in permeability of the blood–brain barrier of the lipophilic topoisomerase II poison VP-16 and the hydrophilic catalytic inhibitor ICRF-187. Standard dose VP-16 therapy is displayed at the left (thin arrow), while at the right high-dose VP-16 (thick arrow) is made possible by protecting normal tissue with ICRF-187, which does not pass the barrier. This latter combination enables the use of supralethal etoposide doses. This means that higher etoposide concentrations are reached in primary and metastatic CNS tumors. The increased etoposide concentration in neural tissue is not considered a problem as it is low in target topoisomerase II content (see Ref. 27 for results).

TABLE 2. Increased survival of mice with CNS tumors treated with high-dose etoposide + ICRF-187 compared with an equitoxic dose of etoposide

		Increased life span (%)		
	N	Etoposide (33 mg/kg)	Etoposide + ICRF-187 (125 mg/kg + 120 mg/kg)	P
Group I	18	13	38	<0.01
Group II	18	21	38	<0.01
Group III	18	13	63	<0.01

Three groups of mice (each containing 18 animals) were inoculated intracerebrally on day 0 with 10^4 L1210 tumor cells and then were treated on day 2 with either a standard dose of etoposide or high-dose etoposide with ICRF-187 rescue. The increased life span was compared with that of the saline-treated controls. The Mann-Whitney test was used for statistical evaluation (from Ref. 27).

doses of etoposide in mice [27]. This was in contrast to the intercalator aclarubicin where the window of opportunity was much smaller [28].

The demands for a meaningful interaction are: (1) the obtained antagonism should selectively protect normal tissue and not tumor cells, and (2) the interaction should enable significant dose escalations *in vivo*. We have investigated two models for improving selectivity, one being antagonist trapping by low extracellular pH [15], while the other is utilization of differences in drug pharmacokinetics across the blood–brain barrier [27].

In the first model demonstrated in Fig. 2, chloroquine, a weak base intercalator, selectively protects cells at physiological pH against etoposide-induced cytotoxicity, etoposide being a neutral drug with an unaltered membrane transport at different pH levels. As solid tumors are known to have an acid extracellular pH, these would be targeted as chloroquine is ionized at acid pH and thus unable to cross the plasma membrane. Though effective *in vitro* [15], chloroquine appears too toxic for use *in vivo* in mice (unpublished); therefore, less toxic analogs are needed. However, these data convincingly demonstrate that pH-mediated regulation of etoposide-induced cytotoxicity is indeed feasible.

The second model, as shown in Fig. 3, regards the utilization of the blood–brain barrier and is based on the assumption that the bisdioxopiperazine ICRF-187, being highly water soluble, will not pass the blood–brain barrier to any significant degree, whereas the more lipophilic drug etoposide will. In contrast to chloroquine, ICRF-187 is much less toxic; *in vivo* studies have been carried out demonstrating that (a) non-toxic doses of ICRF-187 can protect healthy mice, enabling an increase of the LD_{10} by 360%, and (b) mice with L1210 inoculated into the CNS have a significantly better median survival when treated with a high-dose etoposide/ICRF-187 combination than when treated with an equitoxic etoposide dose ([27] and Table 2). These encouraging results have led us to instigate more detailed *in vivo* studies including different schedules, other topo II poisons, and other murine CNS tumor types with continued promising outcomes.

This work was supported by the Danish Cancer Society.

References

1. Chen AY and Liu LF, DNA topoisomerases—Essential enzymes and lethal targets. *Annu Rev Pharmacol Toxicol* **34:** 191–218, 1994.
2. Hansen HH, Management of small-cell cancer of the lung. *Lancet* **339:** 846–849, 1992.
3. Wang JC, DNA topoisomerases. *Annu Rev Biochem* **65:** 635–692, 1996.
4. Pommier Y, Kerrigan D, Schwartz RE, Swack JA and McCurdy A, Altered DNA topoisomerase II activity in Chinese hamster cells resistant to topoisomerase II inhibitors. *Cancer Res* **46:** 3075–3081, 1986.
5. Danks MK, Schmidt CA, Cirtain MC, Suttle DP and Beck WT, Altered catalytic activity of and DNA cleavage by DNA topoisomerase II from human leukemic cells selected for resistance to VM-26. *Biochemistry* **27:** 8861–8869, 1988.
6. Jensen PB, Sørensen BS, Sehested M, Demant EJF, Kjeldsen E, Friche E and Hansen HH, Different modes of anthracycline interaction with topoisomerase II. Separate structures critical for DNA-cleavage, and for overcoming topoisomerase II-related drug resistance. *Biochem Pharmacol* **45:** 2025–2035, 1993.
7. de Jong S, Zijlstra JG, Mulder NH and de Vries EGE, Lack of cross-resistance to fostriecin in a human small-cell lung carcinoma cell line showing topoisomerase II-related drug resistance. *Cancer Chemother Pharmacol* **28:** 461–464, 1991.
8. Ishida R, Hamatake M, Wasserman RA, Nitiss JL, Wang JC and Andoh T, DNA topoisomerase II is the molecular target of bisdioxopiperazine derivatives ICRF-159 and ICRF-193 in *Saccharomyces cerevisiae*. *Cancer Res* **55:** 2299–2303, 1995.
9. Jensen PB, Sørensen BS, Demant EJF, Sehested M, Jensen PS, Vindeløv L and Hansen HH, Antagonistic effect of aclarubicin on the cytotoxicity of etoposide and 4′-(9-acridinylamino)methanesulfon-*m*-anisidide in human small cell lung cancer cell lines and on topoisomerase II-mediated DNA cleavage. *Cancer Res* **50:** 3311–3316, 1990.
10. Jensen PB, Jensen PS, Demant EJF, Friche E, Sørensen BS, Sehested M, Wassermann K, Vindeløv L, Westergaard O and Hansen HH, Antagonistic effect of aclarubicin on daunorubicin-induced cytotoxicity in human small cell lung cancer cells: Relationship to DNA integrity and topoisomerase II. *Cancer Res* **51:** 5093–5099, 1991.
11. Drake FH, Hofmann GA, Mong S, Bartus JL, Hertzberg RP, Johnson RK, Mattern MR and Mirabelli CK, *In vitro* and intracellular inhibition of topoisomerase II by the antitumor agent merbarone. *Cancer Res* **49:** 2578–2583, 1989.
12. Boritzki TJ, Wolfard TS, Besserer JA, Jackson RC and Fry DW, Inhibition of type II topoisomerase by fostriecin. *Biochem Pharmacol* **37:** 4063–4068, 1988.
13. Lee FYF, Flannery DJ and Siemann DW, Modulation of the cell cycle-dependent cytotoxicity of adriamycin and 4-hy-

droperoxycyclophosphamide by novobiocin, an inhibitor of mammalian topoisomerase-II. *Cancer Res* **52:** 3515–3520, 1992.
14. Bojanowski K, Lelievre S, Markovits J, Couprie J, Jacquemin-Sablon A and Larsen AK, Suramin is an inhibitor of DNA topoisomerase-II *in vitro* and in Chinese hamster fibrosarcoma cells. *Proc Natl Acad Sci USA* **89:** 3025–3029, 1992.
15. Jensen PB, Sørensen BS, Sehested M, Grue P, Demant EJF and Hansen HH, Targeting the cytotoxicity of topoisomerase II directed epipodophyllotoxins to tumor cells in acidic environments. *Cancer Res* **54:** 2959–2963, 1994.
16. Woynarowski JM, Sigmund RD and Beerman TA, DNA minor groove binding agents interfere with topoisomerase II mediated lesions induced by epipodophyllotoxin derivative VM-26 and acridine derivative *m*-AMSA in nuclei from L1210 cells. *Biochemistry* **28:** 3850–3855, 1989.
17. Tanabe K, Ikegami Y, Ishida R and Andoh T, Inhibition of topoisomerase II by antitumor agents bis(2,6-dioxopiperazine) derivatives. *Cancer Res* **51:** 4903–4908, 1991.
18. Ishida R, Miki T, Narita T, Yui R, Sato M, Utsumi KR, Tanabe K and Andoh T, Inhibition of intracellular topoisomerase II by antitumor bis(2,6-dioxopiperazine) derivatives: Mode of cell growth inhibition distinct from that of cleavable complex-forming type inhibitors. *Cancer Res* **51:** 4909–4916, 1991.
19. Sehested M, Jensen PB, Sørensen BS, Holm B, Friche E and Demant EJF, Antagonistic effect of the cardioprotector (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane (ICRF-187) on DNA breaks and cytotoxicity induced by the topoisomerase II directed drugs daunorubicin and etoposide (VP-16). *Biochem Pharmacol* **46:** 389–393, 1993.
20. Sørensen BS, Sinding J, Andersen AH, Alsner J, Jensen PB and Westergaard O, Mode of action of topoisomerase II-targeting agents at a specific DNA sequence. Uncoupling the DNA binding, cleavage and religation events. *J Mol Biol* **228:** 778–786, 1992.
21. Rowe T, Kupfer G and Ross W, Inhibition of epipodophyllotoxin cytotoxicity by interference with topoisomerase-mediated DNA-cleavage. *Biochem Pharmacol* **34:** 2483–2487, 1985.
22. Roca J, Ishida R, Berger JM, Andoh T and Wang JC, Antitumor bisdioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp. *Proc Natl Acad Sci USA* **91:** 1781–1785, 1994.
23. Roca J and Wang JC, DNA transport by a type II DNA topoisomerase: Evidence in favor of a two-gate mechanism. *Cell* **77:** 609–616, 1994.
24. Sehested M and Jensen PB, Mapping of DNA topoisomerase II poisons (etoposide, clerocidin) and catalytic inhibitors (aclarubicin, ICRF-187) to four distinct steps in the topoisomerase II catalytic cycle. *Biochem Pharmacol* **51:** 879–886, 1996.
25. Hansen OP, Pedersen-Bjergaard J, Ellegaard J, Brincker H, Boesen AM, Christensen BE, Drivsholm A, Hippe E, Jans H, Jensen KB, Killmann SA, Jensen MK, Karle H, Laursen B, Nielsen JB, Nissen NI and Thorling K, Aclarubicin plus cytosine arabinoside versus daunorubicin plus cytosine arabinoside in previously untreated patients with acute myeloid leukemia—A Danish national phase-III trial. *Leukemia* **5:** 510–516, 1991.
26. Ackland SP and Schilsky RL, High-dose methotrexate: A critical reappraisal. *J Clin Oncol* **5:** 2017–2031, 1987.
27. Holm B, Jensen PB and Sehested M, ICRF-187 rescue in etoposide treatment *in vivo*. A model targeting high-dose topoisomerase II poisons to CNS tumors. *Cancer Chemother Pharmacol* **38:** 203–209, 1996.
28. Holm B, Jensen PB, Sehested M and Hansen HH, *In vivo* inhibition of etoposide-mediated apoptosis, toxicity, and antitumor effect by the topoisomerase II-uncoupling anthracycline aclarubicin. *Cancer Chemother Pharmacol* **34:** 503–508, 1994.