Yondelis® (trabectedin, ET-743): the development of an anticancer agent of marine origin

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Yondelis® (trabectedin, ET-743) is a novel antitumor agent derived from a marine source, the Caribbean tunicate *Ecteinascidia turbinata*. Preclinical studies demonstrated activity at low concentrations against a variety of tumors. The mechanism by which ET-743 exerts its antitumor activity has not been completely elucidated yet. Binding to the minor groove of DNA which causes a bend towards the major groove has been demonstrated. Furthermore, ET-743 interferes with DNA binding proteins and transcription factors. Clinical studies have been initiated as phase I dose-finding studies at four different treatment regimens. Dose-limiting toxicities were hematological, including neutropenia and thrombocytopenia. Furthermore, significant liver toxicity was observed, especially as a rise in transaminase levels. Antitumor activity in phase I and phase II trials was studied in multiple tumor types, including soft tissue sarcomas, melanomas and breast cancer. ET-743 is currently being extensively investigated in advanced soft tissue sarcomas. The present review describes the development of ET-743, highlighting chemical properties, mode of action, metabolism and preclinical and clinical studies. *Anti-Cancer Drugs* 14:487–502 © 2003 Lippincott Williams & Wilkins.

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Introduction

In the continuous search for effective anticancer therapy, nature provides an attractive source of new therapeutic candidate compounds [1,2]. At present, over 60% of the currently approved drugs for the treatment of cancer are derived from natural sources, including plant-derived agents, such as the taxanes paclitaxel and docetaxel, and microbe-derived agents, such as bleomycin and doxorubicin [3].

Currently, there is a growing interest for potential cytotoxic agents originating from organisms in the sea [2,4,5]. Due to the advances in diving techniques and deep-sea sample collection, the marine ecosystem became an accessible source for new chemical classes. Furthermore, new technologies in aquacultures provided a means for the production of these potential drugs on a larger scale, which facilitated the development of these agents [6,7]. The first living organisms in the sea appeared about 700 million years ago and, ever since, evolution has provided these organisms with defense mechanisms to survive in the hostile environment [8]. It was suggested that their survival is partly due to the excretion of highly toxic products [8]. Extracts of several of these marine organisms are currently in clinical development for the treatment of cancer, such as dolastatins, didemnin B, aplidin and kahalalide-F [9–12].

Ecteinascidia turbinata is a translucent tunicate that grows preferentially on mangrove roots in the Caribbean Sea. The potent cytotoxicity of its extracts was first discovered in the late 1960s; however, the purification of active compounds was not established until 1986 [13,14]. Yondelis® (trabectedin, ET-743) was one of the isolated compounds and based on its promising cytotoxic activity as well as its abundance in the tunicate, it was selected for further development as an anticancer agent [15]. Recently, the features of ET-743 have been briefly reviewed in relation to its potential use in the treatment of soft tissue sarcomas [16].

The present review describes the development of ET-743, highlighting its chemical properties, mode of action, metabolism, and preclinical and clinical studies.

Chemistry

Rinehart *et al.* were the first to isolate the active components from the extract of the *E. turbinata* [17]. Separation and identification of the different compounds was established using chromatography and fast-atom bombardment mass spectrometry (FABMS) [17]. More than 10 potentially active compounds were isolated and were assigned as ecteinascidins (ETs), followed by a number indicating the highest ion mass of originally observed for the compounds [17–20]. The initially

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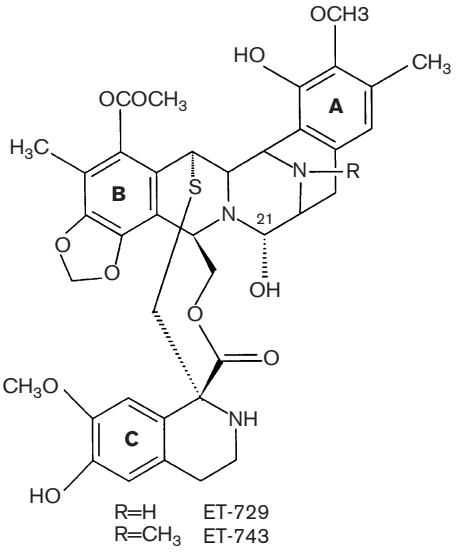
assessed molecular weight of ET-743 was found to be actually 761, due to the rapid loss of water.

ET-729 (*N*-desmethyl ET-743) displayed high *in vitro* and *in vivo* antitumor activity in various murine and human tumor assays, but limited availability of the drug in tunicate lead to the subsequent evaluation of alternative analogs [21]. Further *in vitro* and *in vivo* studies comparing ET-729 with ET-743 indicated that these compounds have similar potency. However, as ET-743 was more abundant in the tunicate it was selected for further development [15]. The molecular structures of ET-743 and ET-729 are shown in Figure 1.

Fig. 1



Structural formula of ET-743 and ET-729.

Molecular structure

The ETs belong to the class of the tetrahydroisoquinoline compounds, which also comprises antibiotic agents such as saframycins, safracins and naphthyridinomycins [19]. The ETs have the most complicated structure in this class, comprising three tetrahydroisoquinoline systems, designated units A, B and C. Units A and B are also present in saframycins, safracins, but only unit A is present in naphthyridinomycins [19]. ET-743 differs from most other ecteinascidins and related alkaloids by the structure of its C subunit, which is attached to the rigid bis(tetrahydroisoquinoline) A–B subunit via a flexible 10-membered lactonic ring.

The structures of most ET extracts were assigned using nuclear magnetic resonance (NMR) techniques and FABMS. The stereochemical assignment had to be established through X-ray analyses of the re-crystallized compound [18]. It appeared that two independent molecules are associated as a dimer through an interaction of the B rings of molecules. The molecule has a compact shape and is hydrophobic. The hydroxyl group at the C21 position (see Fig. 1) points away from the bottom side of ring A and this orientation was shown to be a prerequisite for successful binding to DNA without steric hindrance [18,19].

Supply and synthesis of ET-743

After the identification of ET-743 as a potential anticancer agent, a method had to be established to ensure its supply, as large-scale harvesting of the tunicate was neither practical nor ecologically acceptable. However, for the preclinical and clinical evaluation, and ultimately commercial development, a sufficient and guaranteed supply of the drug substance was a prerequisite. A method was developed which enabled growth of colonies of the tunicate in aquaria equipped with natural photoperiod, artificial seawater, filters, controlled temperature, aeration and salinity, and this provided a source of ET-743 raw drug substance [15]. The main source of natural supply has been the mariculture plants, where the organism is cultured in its natural milieu under controlled conditions.

A multistep chemical synthesis was also developed. The first total synthesis of ET-743 was accomplished by Corey *et al.* [22] and this process involved the enantio-selective formation of ET-743 using several building blocks and an intermediate pentacyclic structure. The process was optimized with the development of a new synthetic route for the intermediate structure, which improved the overall yield of this intermediate from 35 to 57%. Furthermore, this procedure appeared to be simpler and with an improved reproducibility, in addition, no difficulties were encountered in the product purification or scale-up [23]. Another option for the synthesis of ET-743 has been described by Cuevas *et al.*, starting from cyanosafracin B [24]. This cyano-derivative of the antibiotic safracin B is available through fermentation of the bacteria *Pseudomonas fluorescens*, and appeared to be robust and cheap starting material for the synthesis of multigram amounts of ecteinascidin compounds. Saito *et al.* developed the synthesis of the ABC ring structure in the ET-743 molecule, but the application of this structure in the total synthesis of ET-743 is still under investigation [25]. Recently, Zhou *et al.* described a faster route to synthesize the A and C unit of the molecule as part of the total synthesis of ET-743 [26].

The knowledge on the synthesis process of ET-743 provided access to other members of the ecteinascidin family and it enabled the search for more simple and stable structure analogs of ET-743 which could then be

evaluated for their cytotoxic potential. Phthalascidin (Pt 650) is one of these structurally related compounds, and appeared to have an antitumor profile and activity comparable to ET-743 [27]. The compound is synthesized through the pentacyclic intermediate structure and it was more stable in solutions than ET-743 [22,27]. In the Pt 650 molecule, the 10-membered lactone bearing the C unit is replaced by the phthalimide moiety. It has been shown that modifying the C unit in the ET-743 molecule changes the drug's ability to inhibit cell division and that substitution of the unit by a phthalimide moiety does not affect the antiproliferative activity. Replacement of the benzenoid A ring in the molecule by a quinoid A ring resulted in a 10-fold reduction of the cytotoxic potency. Furthermore, it appeared essential that the functional group at the C21 position is either a cyano or a hydroxyl group [27,28]. This requirement is consistent with the studies on the mechanism of action of ET-743, showing that under physiological conditions a reactive iminium is formed at the C21 position, which is responsible for the binding to DNA [29].

Pharmaceutical formulation

ET-743 has limited aqueous solubility. However, by adjustment of the pH to 4, adequate concentrations of ET-743 could be reached. Instability of ET-743 in aqueous solution necessitated lyophilization in order to increase the storage stability of the pharmaceutical product. ET-743 is currently formulated as a sterile lyophilized product containing 250 μg active substance per dosage unit, 250 mg mannitol as a bulking agent and 0.05 M phosphate buffer at pH 4 in order to solubilize ET-743. This formulation is unstable with long-term storage at refrigerated and room temperature, and should therefore be stored between −15 and −25°C, protected from light. Reconstitution is performed by adding 5 ml Water for Injection, with subsequent dilution in normal saline before i.v. infusion. The reconstituted solution is stable at ambient temperature for up to 24 h [15,30].

Mechanism of action

The exact way ET-743 exerts its antitumor activity has not been completely elucidated yet, although extensive research has been performed. Several mechanisms have been proposed and they are listed in Table 1. Overall, it seems that ET-743 has a mechanism of action different from other known anticancer agents. A discussion of the proposed mechanisms is given in the current section.

Binding to the minor groove of DNA

Interactions of ET-743 with DNA were first proposed on the basis of X-ray crystallography [18] and molecular modeling [19]. Pommier *et al.* subsequently demonstrated that ET-743 specifically binds to DNA at the N_2 position of guanine sites, located in the minor groove of DNA [14]. They found that ET-743 non-covalently binds to duplex DNA and that the binding is fully reversible upon DNA denaturation.

A chemical mechanism that gives rise to the alkylation has been described by Moore *et al.* [29]. They proposed that intra-molecular acid-catalyzed activation of the carbinolamine moiety occurs, which subsequently results in dehydration and the formation of an iminium intermediate. It was demonstrated that this iminium intermediate is responsible for the alkylation of the 2-amino group of guanine. A requirement for the reactivity of the carbinolamine group is an internal catalytic proton adjacent to the leaving hydroxyl group [29]. Recently published simulation studies have indicated that binding of multiple ET-743 molecules to adjacent binding sites is structurally and energetically possible and could be preferable over binding to individual sites [31].

Initially, it was suggested that rings B and C in the ET-743 molecular structure (Fig. 1) were responsible for recognition and binding to DNA and that ring A was almost perpendicular to these two rings across the minor groove [19]. However, later an NMR-based model of ET-743 with duplex DNA indicated that the A and B subunits bind to DNA while the C subunit is projected out of the minor groove and makes limited contacts with the DNA [32]. Seaman *et al.* described that parallel hydrogen bonding networks are essential for the stabilization of the A and B unit binding complex with the base pairs of DNA [33]. This network was also suggested to

Table 1 Proposed mechanisms of action for ET-743

Mechanism		References
Binding to the minor groove of DNA	binding at specific sites, induces bending of DNA toward the major groove	14,18,19,29,31–38
Interaction with transcription factors and DNA binding proteins	suggested interactions with NF-Y, *MDR1* expression, P-gp, SXR	39–49
Disorganization of the microtubule network	disorganization of the network is induced but not through direct action on tubulins	51
Topoisomerase I	probably not essential for activity, occurs at concentrations much higher than the cytotoxic activity of ET-743	27,53–55
Perturbation of the cell cycle	G_1 phase cells are most sensitive to in cell cycle delay, resulting in a blockade in the G_2/M phase; p53 independent	55,56
Interference DNA repair pathways	NER-deficient cells are less sensitive to ET-743, at clinically relevant concentrations	55,57–59

direct the course of recognition between DNA and ET-743 by a direct read-out mechanism [33]. A molecular modeling study recently provided further evidence for this proposal [34]. In addition to the hydrogen bonding networks, it was suggested that the pre-organization of DNA by proteins also enhances the binding of ET-743 to DNA. For example, the binding to DNA of SP1, a zinc finger containing transcription factor, widens the minor groove and causes a DNA structure that is complementary to the shape of the ET-743 molecule [33–35].

The binding of ET-743 was shown to cover approximately 3–5 bases [14] and specific sequences have been identified which are favored for the association, i.e. 5′-PyGG (where Py is pyrimidine and is either T or C) and 5′-PuGC (where Pu is purine and is either A or G) [14,33]. The preferred sequences follow from the combination of the geometry of minor groove and the optimal donor/acceptor positions in the A and B subunit, for maximizing the number of DNA–drug hydrogen bonds [33]. Zewail-Foote *et al.* [36] investigated the differences in binding of ET-743 to favored (5′-AGC) and non-favored (5′-AGT) binding sites on DNA. They demonstrated that the alkylation reaction of ET-743 is reversible under non-denaturing conditions [36]. This contrasts with the previous findings of Pommier *et al.* who showed that DNA denaturation was required for the reversibility of this binding [14]. The reaction of ET-743 with both binding sites (favored and non-favored) occurred at the same rate. However, the reversibility of ET-743 from the non-favored binding site appeared to occur at an enhanced rate. Therefore, it was suggested that the rate of covalent reversibility and not the covalent reaction rate governs the observed sequence specificity of ET-743 [36,37].

A result of the bonding between ET-743 and DNA is the induction of a curvature in the DNA helix and the direction of this bending appears to be towards the major groove, as demonstrated by gel electrophoresis experiments [38]. Results of later molecular dynamics simulations were in agreement with this finding [34]. This property differentiates ET-743 from other minor groove binders, which bend DNA towards the opposite direction [38].

Interaction with transcription factors and DNA binding proteins

Bonfanti *et al.*. have investigated whether the antitumor activity of ET-743 could involve an interaction of ET-743 with DNA binding proteins and several transcription factors [39]. This may result in an impairment of gene regulation, as was described for other minor groove binders. An inhibition of DNA binding was observed for several transcription factors and activators; however, this effect was established at much higher concentrations (10–300 μM) than necessary for the cytotoxic effect [39]. Studies by Minuzzo [40] and by Jin [41] indicated that ET-743 affects the transcriptional activation by the transcription factor nuclear factor Y (NF-Y). This effect was observed at clinically achievable concentrations [41]. Furthermore, ET-743 appeared to inhibit the activated, trichostatin-induced expression of *MDR1* in tumor cells, but not the constitutive expression in normal cells [41,42], suggesting that in the clinic ET-743 may selectively inhibit activation of MDR1 expression in tumor cells without affecting constitutive expression in normal cells. However, the relationship between ET-743 activity and MDR1, which encodes P-glycoprotein (P-gp), seems to be complex, as another study reported that P-gp was overexpressed in ovarian carcinoma cells made resistant to ET-743 and that this overexpression could contribute to resistance to ET-743 [43]. Kanzaki *et al.* further investigated the role of MDR1 and P-gp, and they found no overexpression of P-gp in HCT-116 colon carcinoma cells that acquired resistance to ET-743 [44]. Furthermore, multidrug-resistant cells with overexpression of P-gp were not resistant to ET-743, and pretreatment of those cells with ET-743 enhanced the cytotoxicity and the cellular accumulation of doxorubicin. These results suggested that ET-743 resistance is at least not fully accounted for by P-gp expression and that ET-743 could potentiate the activity of other anticancer agents by downregulation of P-gp [44]. Fontaniere *et al.* reported that resistance to ET-743 in neuroblastoma and medulloblastoma xenografts was not related to *MDR* overexpression [45] and also in chondrosarcoma cells that were made resistant to ET-743 P-gp and multidrug resistance-related protein (MRP) could not be detected [46]. This is consistent with recent results from Kim *et al.* who could not find a correlation between ET-743 cytotoxicity in osteosarcoma and expression of MRP [47]. Other experiments investigated the role of the breast cancer resistance protein (BCRP) in resistance to ET-743. However, it was shown that ET-743 was not a substrate for BCRP [48].

Synold *et al.* described the effect of ET-743 on the orphan nuclear receptor SXR. SXR was found to be involved in the expression of CYP3A4 and CYP2C8, and the gene *MDR1* [49]. Therefore, SXR is involved in both the metabolism and the efflux of drugs. For example, paclitaxel induced the SXR activity that resulted in an increased expression of CYP3A4, CYP2C8 and P-gp, inducing the overall clearance of paclitaxel. ET-743 appeared to inhibit paclitaxel-induced SXR activation and to repress *MDR1* transcription through inhibition of SXR, which matches the results of Kanzaki *et al.* [44]. This effect was observed at nanomolar concentrations [49], which is consistent with the concentrations reported for inhibition of trichostatin-induced *MDR1* transcription and for the antineoplastic effects of ET-743 [40,41,50].

Disorganization of the microtubule network

Microtubules are tubulin polymers involved in different functions, including the determination of cell morphology and chromosome segregation during mitosis. Interference with the mechanism of microtubule polymerization could consequently prevent cell division. Several compounds have been described that exert their antitumor activity by preventing the microtubule assembly by different mechanisms [51]. ET-743 affects the microtubules by causing disorganization in the network, without acting directly on tubulin. This mechanism of action of ET-743 is different from those previously reported for other microtubule inhibitors [51]. Whether this effect is essential for the antitumor activity of ET-743 is unclear as the concentrations tested (40 nM to 4 μM) were well above the concentrations required for activity.

Topoisomerase (Topo) I

Martinez *et al.* and Takebayashi *et al.* independently described that Topo I, but not Topo II was a possible target for ET-743. However, relatively high drug concentrations of 4–10 μM were required to demonstrate this effect, which are not achievable in patients [27,52,53]. Therefore, it was most likely that inhibition of Topo is only an auxiliary effect to the primary mode of action of ET-743 [27]. A study comparing normal cells with cells without expression of Topo I revealed no difference in sensitivity to ET-743 between those cells, indicating that Topo I is not required for the cytotoxic activity of ET-743 [54]. This was confirmed by Erba *et al.*, who found that ET-743 at concentrations in the clinically relevant range of 20 nM did not interact with Topo I [55]. Furthermore, the deletion of the topoisomerase gene in yeast cells did not alter the cytotoxic effect of ET-743 [55]. Taken together, the present studies indicate that Topo I is not an essential target for the antitumor activity of ET-743.

Perturbation of the cell cycle

ET-743 was shown to cause perturbation of the cell cycle with a delay of cells progressing from the G_1 to the G_2 phase, an inhibition of DNA synthesis and causing an accumulation in the G_2/M phase [55]. Cells in the G_1 phase appeared to be most sensitive to ET-743 [55]. This effect of ET-743 was not related to the tumor suppresser gene, p53, as no difference in sensitivity could be observed between cells with and without p53-expression [55]. Recent experiments by Simoens *et al.* demonstrated that increasing concentrations and/or incubation times increased the percentage of cells in a blockade in the G_2/M phase of the cell cycle [56]. These results support the results of Erba *et al.* [55]. In addition, one of the tested lung cancer cell lines (A549) also showed a delay in the S phase after treatment with higher concentrations ET-743 [56].

Interference with DNA repair pathways

Erba *et al.*. were the first to describe that cells deficient in transcription coupled nucleotide excision repair (NER) mechanisms are less sensitive to ET-743 [55]. This was further confirmed with the work of Takebayashi *et al.* [57] and Damia *et al.* [58]. The NER system is involved in the repair of lesions caused by, for example, ultraviolet light or the bulky DNA adducts caused by carcinogens, and anticancer agents such as alkylating drugs and cisplatin. Therefore, these cytotoxic drugs are usually more effective in NER-deficient cells as compared to NER-proficient cells. However, when ET-743 has bound to DNA, NER recognizes the complex and appears to cause cell death instead of cell repair [57]. This effect was seen at clinically relevant concentrations, i.e. 1–10 nM. Based on the proposed mechanism, ET-743 is expected to be active against, for example, cisplatin-resistant cells, which have an enhanced NER system [58]. *In vitro* experiments have been performed to evaluate the antitumor effect of the combination of ET-743 and cisplatin [69]. This effect was either additive or synergistic in several tumor types, including sarcomas, non-small cell lung cancer, melanoma and ovarian cancer [69]. To gain further insight into this mechanism, a bacterial nuclease system (UvrABC) was used to investigate how the NER system recognizes and repairs ET-743 DNA adducts [59]. Adducts at favored and non-favored binding sites were compared, and it appeared that preferred binding sites are repaired less efficiently than the non-preferred adducts. It was proposed that the inefficient repair is related to the repair-dependent cytotoxicity described by Damia and Takebayashi [59].

DNA-dependent protein kinase (DNA-PK), which is involved in the DNA double-strand-break repair pathway, was also investigated for its role in the mechanism of action of ET-743 [58]. DNA-PK-deficient and -proficient cell lines were compared for their sensitivity to ET-743. Although no double-strand breaks could be observed in cells treated with cytotoxic concentrations of ET-743, cells lacking DNA-PK were more sensitive to ET-743 than DNA-PK-proficient cells [58].

Recently, the difference between ET-743-sensitive and -resistant chondrosarcoma cells was investigated, to gain further insight in its mechanism of action. It appeared that the two cell types differed in their migratory ability. The ET-743 resistant cells were found to be less invasive than the sensitive cells which was suggested to reflect an inability of the ET-743 resistant cells to digest extracellular matrix [46].

Preclinical studies

Antitumor activity

In vitro

Early *in vitro* studies demonstrated activity of ET-743 against a broad class of solid tumor cell lines, including

melanoma and non-small cell lung cells [60]. The National Cancer Institute (NCI) human *in vitro* cell line panel showed potencies ranging from 1 pM to 10 nM against melanoma, non-small cell lung, ovarian, renal, prostate and breast cancer cell lines [60].

Izbicka *et al.* evaluated the antiproliferative activity of ET-743 in fresh human tumor specimens isolated directly from patients [50]. The drug appeared to inhibit colony-forming units in a broad spectrum of tumors, including breast, non-small cell lung, ovary and melanoma, sarcoma, and renal tumors. The comparison between a 1-h and a 14-day continuous exposure to ET-743 indicated that a prolonged exposure markedly increased the antitumor activity [50]. These findings were confirmed by Ghielmini *et al.*, who demonstrated that a 1-h incubation period was less active than a 24-h continuous exposure or a repeated 1-h exposure on 5 consecutive days [61]. More recently, ET-743 was shown to induce apoptosis in mesenchymal chondrosarcomas in the nanomolar range and to inhibit growth of these cells as well as osteosarcoma cell lines with the same potency [46]. Shtil *et al.* demonstrated cytotoxic activity of ET-743 at sub- to low nanomolar concentrations against a panel neuroblastoma and rhabdomyosarcoma cell lines [62]. The potency of ET-743 against eight human soft tissue sarcoma cell lines was compared with that of clinically used anticancer drugs, such as methotrexate, doxorubicin, etoposide and paclitaxel. ET-743 appeared to be more potent than any of these agents with IC_{50} values in picomolar range [63]. Sarcoma cell lines appeared to be more sensitive to ET-743 than colon cancer cell lines and a breast cancer cell line [63]. In human osteosarcoma cell lines ET-743 showed activity in picomolar concentrations and was more potent than trimetrexate, doxorubicin and methotrexate [47].

In vitro activity of ET-743 has also been studied in combination with other anticancer agents in order to design rationally clinical studies with combination regimens. The first combination studies were conducted by Eckhardt *et al.* who found at least additive activity with vinorelbine and paclitaxel against CALU-3 lung adenocarcinomas and with paclitaxel against MCF-7 breast adenocarcinomas [64]. Takahashi *et al.* [65,66] studied the benefit of the combination of ET-743 with doxorubicin, trimetrexate and paclitaxel in fibrosarcoma (HT-1080) and liposarcoma (HS-18) cell lines. This study confirmed the activity of ET-743 alone in these cell lines and showed that addition of doxorubicin resulted in synergism. The sequence ET-743 followed by doxorubicin was somewhat more effective [66]. Evidence was obtained that the observed synergy could be mediated through induced apoptosis as both the HT-1080 and the HT-18 cell lines showed a greater percentage of apoptotic cells after treatment with the combination of drugs than with either ET-743 or doxorubicin alone [66]. ET-743 and paclitaxel result in strong cytotoxic synergism when paclitaxel is administered before ET-743. However, the reverse sequence or concomitant administration caused a less than additive cytotoxicity. When exposed to ET-743 followed by trimetrexate, there was a synergistic effect in the HS-18 cell line, whereas the HT-1080 cells. Concomitant administration of the two agents also resulted in an antagonistic effect [66].

Riccardi *et al.* also studied the combination of ET-743 with doxorubicin and observed synergism of the combination [67]. However, in contrast to the findings of Takahashi *et al.* the synergism was shown to be independent of the sequence of the administration. Of particular interest is the earlier finding of antagonism between ET-743 and doxorubicin in human rhabdomyosarcoma cell lines by Moore *et al.* [68]. D'Incalci *et al.* have observed beneficial effects of the combination of ET-743 with cisplatin [69]. Synergistic effects were seen in multiple solid cancer cell lines and in ovarian and sarcoma xenografts in nude mice. A phase I clinical study has been designed to investigate the combination in patients [69].

In vivo

Rinehart *et al.* demonstrated *in vivo* antitumor activity in murine tumors against P388 leukemia and B16 melanoma [60]. Furthermore, in human xenograft studies with nude mice bearing human tumors, ET-743 was very effective (10 of 10 tumor free) against early stage mammary carcinoma (MX-1) and produced significant remissions (none of 10) in advanced stage MX-1. Furthermore, i.v. administration was more effective than the intraperitoneal route [60].

Other *in vivo* studies were performed with human ovarian carcinoma xenografts in nude mice [70]. At the maximum tolerated dose (MTD) of 0.2 mg/kg the response to ET-743 varied between complete long-lasting regression (HOC22-S) and moderate activity (HOC18). Partial regression at half MTD was also observed in the HOC22-S xenografts. A tumor (MNB-PTX-1) which was highly resistant to chemotherapy (cisplatin, doxorubicin, cyclophosphamide and paclitaxel) was also resistant to ET-743 [70]. Activity in HOC18 and HOC22 xenografts was confirmed by Hendriks *et al.*, who also demonstrated activity of ET-743 in melanoma and non-small cell lung xenografts [71]. Apart from HOC18, ET-743 induced complete remissions in these xenografts at the cost of significant toxicity. Two administration schedules were evaluated in this study: an intermittent schedule (on days 0, 4, 8) and a fractionated schedule (days 0–2, 13–15). It appeared that the intermittent schedule was more effective in terms of extent of tumor regression, growth delay and numbers of complete remissions. Although the same total amount was given in both treatment

schedules, higher doses given less frequently were more effective than more injections at a lower dose [71]. Recently, tumor growth inhibition by ET-743 was demonstrated in mice bearing osteosarcoma xenografts. In this study, ET-743 was more effective than trimetrexate with leucovorin and trastuzumab [72].

Fontaniere *et al.* reported the results of activity studies with mice bearing advanced stage pediatric tumors, neuroblastomas and medulloblastomas [45]. Moderate activity was seen in neuroblastoma xenografts, but significant response was achieved in medulloblastomas [45].

The combination of ET-743 with dexamethasone pretreatment was studied in B16 tumor cells and B16 and osteosarcoma xenografts and it appeared that the antitumor activity of ET-743 was increased with this combination as compared to ET-743 alone [73]. This was rather unexpected as dexamethasone is an inducer of the supposedly primary metabolizing enzyme of ET-743, cytochrome P450 3A4 (CYP3A4) and the results were explained by a reduced expression of this enzyme [73]. Although further research is warranted with this combination, the results could match the findings of Synold *et al.* [49] who showed that ET-743 represses the CYP3A4 activating effect of SXR. Another explanation for the increased activity in the presence of dexamethasone could be that one of the metabolites that are formed through CYP3A4 is (at least in part) responsible for the antitumor activity.

Toxicology

In vitro

Ghielmini *et al.* examined the toxicity of various ET-743 treatment schedules on hemopoietic cells and compared it with the cytotoxic effects on a variety of human cancer cells, to characterize the '*in vitro* therapeutic index' [61]. Prolonged (24 h) or repeated (daily × 5) exposures were more myelotoxic than a single 1-h exposure and therefore it was suggested that ET-743 myelotoxicity is area under the curve (AUC) dependent. However, due to the higher sensitivity to prolonged exposure of several tumor cell lines, prolonged treatment yielded a more favorable *in vitro* therapeutic index [61].

Gomez *et al.* investigated the effect of ET-743 on murine hemopoietic stem cells [74]. Bone marrow cells were incubated with ET-743 for 24 h and their capacity to form to mature blood cells was compared with untreated cells. The effects on the stem cells was moderate suggesting that long-term myelosuppression after ET-743 would not be expected [74].

As anticancer drugs interacting with the microtubule network are known for their neurotoxicity, an *in vitro* study was carried out to assess the potential neurotoxicity of ET-743. At a concentration range of 10^{-18} to 10^{-4} M, no acute neurotoxic effects were observed [15,75]. Other *in vitro* studies were initiated to assess the cardiotoxicity of ET-743; however, this effect was not observed [15]. Toxicology studies with a panel of non-tumor cell lines also indicated that cardiotoxicity of minor importance with ET-743 [76]. These experiments further demonstrated considerable effects on liver and myelogenous cells, while kidney and skeletal muscle cells were less sensitive to ET-743 [76].

In vivo

The *in vivo* toxicology studies involved administration of ET-743 to mice, rats, dogs and monkeys and both single-dose schedules (daily × 1) and multiple-dose (daily × 5) schedules were evaluated [15,77,78]. It appeared that the main toxicities were significant hematologic and hepatic effects, and these were mostly reversible. Hematological toxicity was characterized by a decrease in blood cell counts, whereas hepatic toxicity was observed as an increase in liver enzymes and cholangitis [15]. Female rats were the most sensitive animals, followed by male rats, dogs, mice and monkeys. In general, the toxicity profiles were similar between the two dose schedules, indicating that the qualitative toxicological profile was not dependent on the treatment schedule. However, the incidence and severity of the pathological effects were significantly reduced with the multiple dose schedule, suggesting a relation to peak blood concentrations (C_{max}). At high concentrations of ET-743, leukopenia, anemia and moderate thrombocytopenia were seen, but all these effects resolved after 3–4 weeks. Additional sequential studies (3 cycles) were carried out in mice and rats to assess cumulative and reversible toxicity. Hematological toxicity was reversible in both mice and rats, whereas liver toxicity did not resolve in rats [15].

Metabolism

Knowledge on the metabolic pathway of drugs is of great importance to explain toxicological and anticancer effects as well as drug–drug interactions. However, data on the metabolism of ET-743 are scarce and although several experiments have been performed, its metabolic fate remains to be elucidated. Due to the relatively high potency of the drug, metabolite concentrations *in vivo* will be in the picomolar range, which hampers the identification of the metabolic products. *In vitro* incubation experiments with ET-743 and rat and human hepatic microsomes have shown a time-dependent decrease of the drug concentration, although metabolic products could not be identified [79]. These experiments have also generated indications that demethylation may occur, because formaldehyde was formed during the incubation experiments. Nevertheless, *N*-desmethyl ET-743 (ET-729) could not be detected [79]. This is supported by the

findings of Rosing *et al.* that there was no ET-729 present in plasma of treated patients, which allowed this compound to be used as internal standard for the bio-analytical assay [80]. ET-743 metabolic clearance after incubation with male rat liver microsomes appeared to be substantially higher than with female microsomal preparations, which is probably caused by the male predominance of the responsible enzyme (CYP3A2) in this species. These differences in metabolism may contribute to the higher sensitivity of female rats for ET-743, as was seen in *in vivo* toxicity studies [80]. It is unlikely that this difference in rate of metabolism between male and female rats will also be observed in humans because cytochrome P450 enzymes do not exhibit such gender differences.

Sparidans *et al.* have investigated the metabolism of ET-743 both *in vitro* and *in vivo* [81]. ET-743 was incubated with human microsomes and human plasma and uridine 5-diphosphoglucuronyl transferase (UDPGT). Several metabolic and degradation products were formed and could be structurally identified using a previously developed LC-MS/MS bio-analytical method with solid-phase extraction as a sample pretreatment procedure. The experiments indicated a major breakdown of the ET-743 molecule. However, attempts to identify these metabolites in plasma, bile and urine of treated patients have failed [81]. In the compassionate use program with ET-743, a patient showing Gilbert's syndrome was treated. Patients showing this syndrome have a reduced activity of hepatic bilirubin UDPGT and therefore an impaired ability to form glucuronidated metabolites [82,83]. With this patient, however, no elevations in AUC or elimination half-life could be observed as compared to other patients. Furthermore, the patient did not experience toxicity. These results indicate that glucuronidation is probably not a significant route of detoxification for ET-743 [81].

Bio-analysis

ET-743 is administered to patients in $\mu g/m^2$ dosages, resulting in low plasma concentrations (pg/ml to ng/ml). This demands special requirements in terms of sensitivity for the bio-analytical method, for the pharmacokinetic research during the phase I studies. Initially, a reversed-phase high-performance liquid chromatography method with ultraviolet detection was developed with solid-phase extraction (SPE) as sample pretreatment [84]. Propyl-*p*-hydroxybenzoate was used as internal standard and the assay was linear over a concentration range of 1–50 ng/ml with an adequate accuracy and precision and a lower limit of quantitation was 1 ng/ml using 500 µl plasma [84]. However, with the 1-h infusion at a starting dose of $50\ \mu g/m^2$, only the plasma concentration at the end of the infusion could be quantified [85]. In addition, no drug could be detected at the starting dosages of the 24-h infusion and the daily × 5 infusion [86]. Therefore, an analytical method with a much higher sensitivity was required for the bio-analysis of ET-743. This was accomplished by combining high-performance liquid chromatography with an electrospray ionization sample inlet and two quadruple mass analyzers (LC/ESI-MS/MS) [80]. The sample pretreatment procedure remained identical and ET-729 could be used as internal standard as it appeared that no ET-729 was present as a metabolic product in human plasma. The assay was linear at a concentration range of 0.01–2.5 ng/ml with an acceptable accuracy and precision. The method permitted an LLQ of 0.01 ng/ml using 500 µl plasma which was suitable for the pharmacokinetic monitoring of the clinical studies [80].

Another bio-analytical method was developed by Ryan *et al.* [87] and involved LC-ESI-MS. Quinocarcinol octylamide, which is structurally related to ET-743, was used as an internal standard and SPE was used for sample preparation. This method also provided a means for sensitive quantification of ET-743 with the LLQ being 25 pg/ml with acceptable accuracy and precision, using a sample volume of 1 ml [87].

Clinical studies

Phase I

A phase I clinical program was conducted with ET-743 both in Europe and the US. The preclinical studies indicated that schedule dependence might exist for both antitumor activity and toxicity. Furthermore, the preclinical toxicity data indicated that the incidence and severity of the hepatic and hematological side effects in mice, rats and dogs reduced with a multiple, divided dose schedule [15]. In addition, the MTD in dogs was 30% higher in a fractionated schedule (daily × 5) as compared to a single dose, suggesting that toxicity might be C_{max} related [15]. Therefore multiple infusion schedules, including fractionated and prolonged schedules were selected for studies in cancer patients. Initially, four phase I dose-finding studies have been conducted, in which five different treatment regimens were evaluated: a 1- and 3-h infusion, every 3 weeks; a 24-h infusion every 3 weeks; a 1-h infusion on 5 consecutive days (daily × 5), every 3 weeks and a 72-h continuous infusion, every 3 weeks [85–88]. In addition to the treatment regimen, the four phase I studies were similar in terms of their design [85–88]. In addition, a phase I study investigating a weekly schedule [93] and a phase I trial in pediatric patients [94] have been performed.

1- and 3-h infusion

The first study involved a regimen where ET-743 was initially administered as a 1-h infusion, every 3 weeks, with a starting dose of $50\ \mu g/m^2$ [85,89,90]. Nine dose levels were evaluated and at $1100\ \mu g/m^2$ the MTD was

reached and 1000 μg/m^2 was considered a safe dose for further studies with this schedule. The dose-limiting toxicities (DLTs) were grade 4 thrombocytopenia and grade 3 fatigue. However, as parallel phase I studies with other treatment regimens DLTs were observed at considerably higher dosages than with the 1-h infusion [88,93]. Therefore, the infusion duration was prolonged from 1 to 3 h in order to improve the dose intensity and safety profile as the longer infusion duration reduced the maximally achieved plasma concentrations. The starting dose for the 3-h infusion was 1000 μg/m^2 and dose escalation continued up to 1800 μg/m^2, which was considered the MTD. With this regimen, the DLTs were pancytopenia and fatigue. An early onset, transient and non-cumulative transaminitis was also noted in most of the patients. The recommended phase II dose was 1650 μg/m^2 administered as a 3-h infusion [85,90].

Non-compartmental pharmacokinetic analyses indicated that dose-dependent pharmacokinetics might exist with the 1-h schedule, as total clearance decreased with increasing dose level [85]. However, only the three highest dose levels could be evaluated for this schedule as data at the other dose levels were obtained with the less sensitive LC-UV bio-analytical method, yielding incomplete pharmacokinetic profiles. There was no evidence for non-linearity in the pharmacokinetics with the 3-h infusion. Body surface area appeared to correlate significantly to clearance with the 1-h schedule, but not with the 3-h schedule. No other clinically relevant correlations between pharmacokinetic parameters and patient characteristics were reported.

Pharmacokinetic–pharmacodynamic analyses with the 3-h infusion data revealed that the percentages decrease in absolute neutrophil count, white blood cells and platelets were significantly related to AUC and C_{max}, and also to dose (μg) [85]. Due to the substantial variability, discrimination between these predictors of toxicity could not be made. Hepatic toxicity increased with dose, AUC and C_{max}. Patients treated with the 3-h regimen that experienced grade 3 or 4 elevations in AST and AP, showed significantly higher values of C_{max} than patients with milder toxicity [94]. A pretreated patient with a metastatic leiomyosarcoma had a complete remission with a time to progression of 32 weeks after treatment with 1500 μg/m^2 ET-743 in 8 cycles [90].

24-h infusion

The feasibility of ET-743 administration was also evaluated as a 24-h infusion, repeated every 3 weeks [86,91]. The starting dose was 50 μg/m^2 and 52 patients were treated at nine dose levels ranging from 50 to 1800 μg/m^2 in a total of 158 courses. Hematological toxicity, i.e. neutropenia and thrombocytopenia, was dose-limiting and the dose of 1800 μg/m^2 was considered the MTD. Also, at the recommended phase II dose of 1500 μg/m^2 neutropenia was the most commonly observed toxicity, thrombocytopenia was less frequent. The nadir occurred around day 13 with a median duration of grade 3 or 4 toxicity was 7 days. At day 22, recovery to normal values was usually reached. The most important non-hematological side effect were AST and ALT elevations, which were transient and non-cumulative as was described for the shorter infusions. At the recommended dose (1500 μg/m^2), grade 3 or 4 elevations was observed in 68% of the patients. Major elevations usually started at day 2 with a peak around day 5 and were resolved to baseline by day 15. At two lower dose levels (900 and 1200 μg/m^2), the severity of this toxicity decreased in successive treatment cycles. Hepatic toxicity was not considered a DLT because it was reversible before day 28. Patients with abnormalities in baseline AP levels (i.e. above 1 × the upper limit of normal) appeared to be at higher risk for transaminase elevations. The severity of transaminitis correlated with the occurrence of hematological toxicity and it was therefore suggested that the early peak in transaminases could serve as a predictor for severe hematological toxicity [91]. Other toxicities observed were serum bilirubin and AP elevations, asthenia, and nausea and vomiting. The latter could be treated with standard 5-HT$_3$ antagonists [91].

Three patients achieved a partial response after treatment with this schedule [91]. A patient with advanced breast cancer had a partial response after course 1 at 1800 μg/m^2, lasting for 3.3 months. In a patient with a bulky osteosarcoma treated at 1500 μg/m^2 the partial response in the lung metastases lasted for 2.8 months. A third patient with metastatic hepatic and cutaneous liposarcoma experienced a partial response lasting for 15 months. Four additional patients with progressing soft tissue sarcomas achieved disease stabilization lasting longer than 3 months [91]. On the basis of these positive bit preliminary results, several patients who were ineligible for phase II studies were treated on a named-patient basis compassionate use program [92]. Thus, 17 additional patients were treated with 1500 μg/m^2 and this resulted in two partial responses with patients with soft tissue sarcomas, a partial response with a patient with osteosarcoma, two minor responses and six patients with disease stabilization [92].

The pharmacokinetics of ET-743 were linear at the dose range tested (50–1800 μg/m^2) [86]. Considerable interpatient variability was observed for all pharmacokinetic parameters (e.g. coefficient of variation of 45% for AUC, see Table 3); however, the variability could not be explained by patient characteristics. Evaluation of the pharmacokinetic parameters of the first, second and fifth treatment cycle could not identify significant differences,

indicating that prior exposure to ET-743 does not effect the pharmacokinetic profile in the subsequent cycles. Patients with grade 3 or 4 elevations in AST and ALT, showed significantly higher AUC values than patients with milder toxicity. Hematological toxicity, defined as the percentage decrease in platelets, WBC and ANC was best correlated to ET-743 AUC [86].

Daily × 5 schedule

The starting dose in this study was 6 μg/m^2/day, administered on 5 consecutive days, every 3 weeks [86]. With this schedule, 42 patients were treated with 118 courses at dose levels ranging from 6 to 380 μg/m^2/day. The dose of 380 μg/m^2/day was considered the MTD as one patient developed grade 4 neutropenia and thrombocytopenia after the first course of treatment and two other patients experienced grade 4 neutropenia lasting 5 days in course 2. At a dose of 325 μg/m^2/day, no DLTs were seen in 42 courses administered to 13 patients and therefore this dose level was considered the recommended phase II dose [86].

Neutropenia was the most commonly observed hematological toxicity with this schedule. The onset was generally on day 7 and the median time to nadir was 14 days with recovery to pretreatment values around day 22. The effects on platelets and red blood cells were mild to moderate. Non-hematological toxicity included hepatic toxicity characterized by elevations in AST and/or ALT levels, although this was never dose-limiting. Generally, transaminitis occurred between day 3 and 8, peak levels of AST and ALT were seen between day 5 and 8 and were recovered to pretreatment values by day 28. The severity or the duration of the hepatic toxicity did not increase in subsequent treatment courses. Hepatic toxicity coincided with significant fatigue. Other toxicities included discomfort and inflammation at the site of injection, and nausea and vomiting [88].

Antitumor activity was observed in three patients at the recommended dose of 325 μg/m^2/day. A patient with a papillary serous carcinoma of the peritoneum demonstrated no evidence of disease up to 10 months after treatment with 6 courses. Furthermore, radiological methods could not detect any disease progression 33 months after start of therapy. Another, heavily pretreated patient with an advanced epithelial ovarian carcinoma had a 32% reduction in retroperitoneal metastases and a 25% reduction in a para-aortic lymph node, lasting for 6 months. A 41% reduction in lung metastases and a 27% overall tumor response was noted in a patient metastatic uterine leiomyosarcoma. Furthermore, a patient who had progression of a renal carcinoma before ET-743 showed stable disease for 10 months after treatment at 96 μg/m^2/day [88].

Pharmacokinetic sampling was performed in all patients on treatment day 1 and day 5 of the first course. Parameters that were determined with the non-compartmental approach for the recommended phase II dose are listed in Table 2. Total clearance of ET-743 was not significantly correlated to dose, suggesting that the pharmacokinetics at this treatment are dose independent. Nevertheless, the relationships between dose and C_{max} or AUC on either day 1 or 5 were not evidently linear. All patients had measurable ET-743 plasma concentrations before treatment on day 5, resulting from accumulation due to the long terminal half-life. No relationships were evident between pharmacokinetic parameters and pretreatment values of AST, ALT, AP, total bilirubin or creatinine clearance. Furthermore, CL was not related to BSA. Investigation of the pharmacokinetic–pharmacodynamic relations for this schedule, revealed that the percentage increase in AST during the first course was related to the AUC on day 1 and day 5. Furthermore, treatment with a higher ET-743 dose or a higher AUC (either day 1 or day 5) increased the risk for developing grade 3 transaminitis. There was no clear relation between the percentages decrease in ANC or platelets and AUC or C_{max} on either day 1 or 5, but patients with grade 3–4 hematological toxicity received in general a higher dose or showed a higher C_{max} [88].

Table 2 Dose-limiting toxicities and pharmacokinetic–pharmacodynamic relationships

Treatment	DLT	Pharmacokinetic–pharmacodynamic relationships		References
		Hematological toxicity	Hepatic toxicity	
1-h infusion	thrombocytopenia; fatigue	NA	NA	93
3-h infusion	pancytopenia; fatigue	% decrease PLT, WBC, ANC positively correlated with AUC, C_{max} and dose (μg)	in general: toxicity ↑ with dose ↑; grade 3/4 rise in AP and AST at significantly higher AUC values than grade 0–2	93
24-h infusion	thrombocytopenia neutropenia	% decrease PLT, WBC and ANC positively correlated with AUC	grade 3,4 rise in AST and ALT at significantly higher AUC values than grade 0–2	90,95
Daily × 5 infusion	neutropenia; thrombocytopenia	grade 3–4 toxicity resulted from higher dose or higher C_{max} value	% increase in AST levels positively correlated to AUC day 1 and 5; toxicity ↑ with dose ↑	94
72-h infusion	grade 4 transaminitis; rhabdomyolysis	% decrease WBC significantly correlated to dose, C_{max} and AUC	% increase AST significantly correlated to AUC	89

DLT: dose-limiting toxicity; NA: not applicable; PLT: platelets; WBC: white blood cell count; ANC: absolute neutrophil count; AUC: area under the plasma concentration versus time curve.

Table 3 Pharmacokinetic parameters of ET-743 during four phase I studies, presented as mean (SD)

Treatment	Linearity	Dose level ($\mu g/m^2$)	CL (l/h)	$t_{1/2}$ (h^{-1})	AUC_{0-inf} (h·ng/ml)	C_{max} (ng/ml)	V_{ss} (l)	References
1-h infusion	CL ↓ with ↑dose	1000	32 (6.1)	33 (14)	36 (6.4)	17 (5.2)	910 (720)	85
3-h infusion	dose-independent kinetics	1650	87 (30)	26 (7)	38 (10)	8.6 (2.5)	1400 (490)	85
24-h infusion	dose-independent kinetics	1500	59 (31)	89 (41)	55 (25)	1.8 (1.1)	3900 (1900)	86,91
Daily × 5 infusion (day 5)	dose-independent kinetics	325	21 (8.1)[a]	28 (13)	5.8 (1.7)[c]	3.4 (1.7)	808 (383)	88
72-h infusion	disproportionate increase of AUC after 1050 $\mu g/m^2$	1050	28 (23)[a]	69 (57)	37 (27)	0.32 (0.15)	1941 (1102)[b]	87

[a]Units of this parameter are $l/h/m^2$.
[b]Units of this parameter are l/m^2.
[c]AUC from 0 to 24 h.
CL: clearance; $t_{1/2}$: terminal half-life; AUC_{0-inf}: area under the plasma concentration time curve with extrapolation to infinity; C_{max}: maximal plasma concentration; V_{ss}: apparent volume of distribution at steady state.

72-h infusion

The fourth phase I study applied the administration of a 72-h continuous infusion to investigate the possibility of enhancing the therapeutic index of ET-743 by prolonging the duration of the exposure, based on results of the preclinical studies [87]. The starting dose was 600 $\mu g/m^2$ (200 $\mu g/m^2$/day), which was the highest dose with the 24-h infusion study that did not produce any toxicity greater than grade 2. In total, 21 patients were entered into the study and were given four different dose levels: 600, 900, 1050 and 1200 $\mu g/m^2$.

At a dose of 1200 $\mu g/m^2$, two of nine patients experienced DLTs in the first cycle of therapy, being reversible grade 4 transaminitis. As opposed to the other phase I studies, in this trial grade 4 transaminitis was considered a DLT regardless of the reversibility pattern. A third patient at this dose level experienced grade 4 rhabdomyolisis during the second treatment course, as well as renal failure requiring hemodialysis, grade 4 neutropenia and thrombocytopenia. The study protocol restricted determination of DLTs to the first course. Nevertheless, the 1200 $\mu g/m^2$ dose level was designated the apparent MTD, because of two DLTs occurring during cycle 1, and severe toxicity in a third patient during cycle 2. Subsequently, a dose level of 1050 $\mu g/m^2$ was evaluated in 6 patients and this was established as the recommended phase II dose for the 72-h schedule. Hematological toxicity was not considered clinically relevant (i.e. grade 1 or above) at doses below 1200 $\mu g/m^2$. Hepatic toxicity, however, defined as AST and ALT elevations was observed at all dose levels at its frequency and severity increased in a dose-dependent manner. At the recommended phase II dose of 1050 $\mu g/m^2$, 50% of the cycles were complicated with a grade 3 transaminitis. The time course of the enzyme elevations was predictable, beginning 4–5 days after start of the infusion, peaking on days 7–9 and resolving by day 21. The hepatic toxicity was not cumulative, as patients treated with multiple cycles did not experience worsening of the transaminitis with each successive cycle. Evidence of antitumor activity was seen in two patients with this schedule. A patient with metastatic malignant mesothelioma had a 41% reduction of measurable disease in the metastases with significant symptomatic improvement that lasted for 5 months. A patient with choroidal melanoma showed stable disease in the liver, although new mesenteric nodules were observed [87].

Non-compartmental pharmacokinetic analyses of the plasma concentration time data of the 72-h infusion study, revealed a departure from linear pharmacokinetics at the highest administered dose level (i.e. 1200 $\mu g/m^2$), indicated by a more than proportionate increase in AUC at this level. Pharmacokinetic parameters of this study are listed in Table 2. During the 72-h infusion, steady-state conditions were never achieved. Evaluation of the relationships between laboratory values and the pharmacokinetic parameters showed a weak correlation between the terminal half-life of the drug and total bilirubin (Pearson's correlation coefficient $r = -0.529$, $p = 0.017$). The maximum percentage increase in AST was related to the AUC. Furthermore, the percentage decrease in white blood cells was significantly correlated to dose, C_{max} and AUC [87].

A summary of the DLTs and the most prominent pharmacokinetic–pharmacodynamic relationships for the different phase I studies is presented in Table 2. As can be seen, in all studies hematological effects were dose limiting, except for the 72-h infusion where hepatic toxicity was more important [87]. Hepatic toxicity was already observed at the starting dose of 600 $\mu g/m^2$ with this schedule. However, this dose did not produce any clinically relevant hepatic toxicity when given over 24 h [91]. These results suggest that schedule dependency in toxicity exists. With the 72-h infusion higher AUCs were reached than with the 3- and 24-h schedule at comparable dosages [85–87]. With the shorter infusion schedules (i.e. 1-, 3- and 24-h) much higher C_{max} values were established with hematological toxicity as the DLT [85]. Hepatic toxicity and not myelosuppression was considered a DLT in the 72-h infusion, and it therefore seems likely that hematological toxicity may be related to C_{max} [85,87].

Pharmacokinetic parameters at the recommended dosages of the four phase I studies are summarized in

Table 3. Linear pharmacokinetics were observed at all schedules except the 1- and 72-h infusion [87]. As a possible explanation for the more than proportionate increase in AUC with the 72-h schedule, it was suggested that liver toxicity caused by ET-743 occurs early enough during the infusion to reduce the metabolic capacity of the drug [87]. As metabolism is the major pathway of elimination of ET-743 [81] this could result in decreased clearance at higher dosages [87]. However, this could not explain the observed dose-dependent pharmacokinetics with the 1-h infusion [85]. Saturation of the metabolic enzymes seems a more appropriate explanation as relatively high plasma concentrations are reached with this schedule [85]. In all studies, large values of volume of distribution were reported, indicating that ET-743 is extensively distributed in the body. This is followed by a slow redistribution and elimination, which is reflected by the long terminal half-life. Pharmacokinetic parameters showed considerable interpatient variability with all treatment regimens. However, this variability could not be readily explained by patient characteristics [85–88].

The phase I program was further extended with a dose-escalation study evaluating the feasibility of ET-743 administration as a 3-h, weekly × 3 infusion, every 4 weeks, in order to improve the therapeutic index [93]. Thus far, 20 patients were treated at doses ranging from 300 to 650 μg/m^2/week. The MTD was established at 650 μg/m^2 with 580 μg/m^2 as the recommended dose. The DLTs were grade 3 neutropenia lasting longer than 3 weeks in one patient, and grade 4 neutropenia and grade 3 transaminitis and hyperbilirubinemia in another patient. No other clinically significant toxicities were reported and two pretreated liposarcoma patients experienced a minor response. Linear pharmacokinetics were observed. The erythromycin breath test was also incorporated in this study to quantify the hepatic activity of CYP3A4 [93]. However, in 15 patients tested no correlation was observed between the test parameters and ET-743 clearance [93].

Recently, the results of a phase I study with children were presented [94]. Children with refractory tumors received ET-743 as a 3-h infusion every 3 weeks. The starting dose was 1100 μg/m^2, which was 80% of the adult MTD with this schedule. Thirteen patients were accrued (median age 9.9 years) and were treated with 1100 or 1300 μg/m^2 ET-743. The dose of 1300 μg/m^2 was considered the MTD with grade 4 transaminitis and grade 4 reversible hypokalemia as DLTs [94]. Dexamethasone was used as premedication as studies with adult patients indicated that this could reduce the risk of severe toxicities. The recommended dose for phase II studies was 1100 μg/m^2 and was well tolerated in combination with 2.5 mg/m^2 dexamethasone every 12 h for 4 days, starting 1 day prior to ET-743. Pharmacokinetic analyses confirmed the long terminal half-life and the large volume of distribution as was seen with adults [94]. The AUC at a dose of 1300 μg/m^2 was much higher in children than in adults [94] and the C_{max} at this dose was slightly higher. In view of the DLTs found in children this indicates that children are less sensitive to hematological toxicity for a given AUC or C_{max} [94].

Phase II

An extensive phase II program was started and currently ongoing with ET-743 in which the antitumor activity is being assessed in a large number of patients with a variety tumor types, including sarcoma, breast tumors, endometrial cancer and ovarian cancer. In the phase II studies, ET-743 is administered as a 3- and 24-h continuous i.v. infusion, and thus far the results have only been reported in abstracts. Complete results of the finalized studies are still to be published.

The toxicities observed were comparable to those observed with the phase I studies and included grade 3–4 neutropenia, thrombocytopenia, anemia and reversible transaminitis. Furthermore, grade 1–3 nausea, vomiting and fatigue were observed in the patients [95,96].

A relatively high incidence of severe events including toxic deaths was noted at the beginning of the phase II studies with the 24-h infusion. An early report of a phase II trial in patients with advanced soft tissue sarcomas described that there were 3 toxicity related deaths in the 59 patients treated with 1500 μg/m^2 ET-743 as a 24-h infusion [109]. In two patients this was due to neutropenic fever, renal insufficiency and liver failure, the third patient experienced a neutropenic sepsis with an elevation of creatine kinase [109]. Later, a fourth patient died due to multi-organ toxicity after treatment [97]. A statistical analysis was performed to identify prognostic factors for the onset of this multi-organ toxicity [108]. It appeared that intercycle elevations of alkaline phosphatase and AST as well as an elevated bilirubin or alkaline phosphatase level at baseline increased the risk of multi-organ toxicity in subsequent cycles. Based on these findings the study protocol was adjusted to requiring normal bilirubin and alkaline phosphatase at study entry and a dose reduction to 1200 μg/m^2 in case of intercycle elevations of these enzymes [108,109]. This amendment significantly improved the safety of the treatment [100]. A later publication described the treatment of 198 patients on a compassionate use basis. Patients were given 1500 μg/m^2 ET-743 as a 24-h infusion every 3 weeks. The tolerability of treatment was comparable to that in protocol patients, the toxicities being grade 3–4 neutropenia and thrombocytopenia, asthenia and transaminitis, and a relative dose intensity of 81% was reached. A probably treatment-related death occurred due to liver

failure [99]. Two patients in another phase II study developed severe toxicity on the second cycle, characterized by rhabdomyolysis, renal failure, and grade 4 neutropenia and thrombocytopenia [101].

The recommended phase II dosage that was established in the phase I studies for the 3-h infusion (1650 μg/m^2) [84] did not seem generally feasible in the phase II population. In one study, the initial dose of 1650 μg/m^2 had to be reduced to 1500 μg/m^2 due to grade 4 asthenia in all of the 21 patients treated [95]. Reduced doses of 1500 and 1300 μg/m^2 were applied in two other phase II studies [96,98]. In one of these studies, two drug-related deaths were reported. These were due to early-onset myelosuppression and long-lasting thrombocytopenia [96].

The main focus in the phase II studies with ET-743 was on its activity against sarcomas. When given as a second line therapy to patients with soft tissue sarcomas, the objective response rate to this treatment was 11.4% with a median time to progression and median overall survival of 3 and 7 months, respectively [97], indicating modest activity in second line of advanced soft tissue sarcomas. Preliminary results of another multicenter phase II study in 48 evaluable patients with pretreated soft tissue sarcomas showed three partial responses, six minor responses and 17 stable disease with a median duration of 7.6, 3.8 and 5.1 months, respectively. The time to progression and median overall survival were 2.6 and 10.7 months [101]. Demetri *et al.* reported objective responses in six out of 34 soft tissue sarcomas as first line therapy and in three out of 36 soft tissue sarcomas with prior treatment [102]. Responses were noted in leiomyosarcomas, liposarcomas and synovial sarcoma and were durable up to 14 months. Progression-free survival and the overall survival rates at 1-year were 18 and 49%, respectively [102]. In a compassionate use study with the 24-h schedule objective responses were accomplished in five out of 42 patients, including one complete response [99]. Tumor types included osteosarcomas, leiomyosarcoma, fibrosarcoma and synovial sarcoma [99]. Another phase II study evaluated the activity after a 3-h infusion and objective responses were observed in soft tissue sarcomas (three out of 25), Ewing sarcomas (two out of eight) and rhabdomyosarcoma (one out of six) [96]. Progression-free survival with soft tissue sarcoma was 51 and 21% at 3 and 6 months, respectively [96].

A phase II study with 24 patients with heavily pretreated osteosarcomas did not show any objective response to treatment with 1500 μg/m^2 ET-743 given as a 24-h infusion [103]. Furthermore, Dileo *et al.* did not report any response to treatment with ET-743 (1500 or 1300 μg/m^2 over 3 h) in nine osteosarcoma patients [96]. Two patients in a compassionate use study with this tumor type, however, did show a response when treated with 1500 μg/m^2 ET-743 as a 24-h infusion [99]. Yovine *et al.* also reported response in two out of three patients treated at this dose and schedule [101].

Sessa *et al.* reported an overall response rate of 32% as the preliminary results of an ongoing phase II trial with patients with ovarian cancer, which had been pretreated with platinum and taxane therapy. Twenty-nine patients with refractory ($n = 15$) or relapsed disease ($n = 14$) entered the study [95]. Objective responses were observed in both refractory (one complete) and relapsed (seven partial) patients [95].

To assess the pharmacokinetic profile of ET-743 in a large number of patients, limited pharmacokinetic sampling is performed during all phase II studies. A limited sampling strategy has been developed and validated using the phase I data for application with the 24-h infusion studies [105]. Results of preliminary population pharmacokinetic modeling of the 24-h data showed that the pharmacokinetic profile of ET-743 was best described using a 3-compartment model [106]. The estimated clearance (44 l/h) was comparable to that reported in the phase I studies (59 l/h) [86,106]. Preliminary analyses using non-compartmental methods suggested a correlation between age and total plasma clearance, clearance being lower in patients older than 50 years [107].

Discussion

ET-743 is one of the first marine-derived compounds currently in clinical development for the treatment of cancer. Although the precise mechanism through which ET-743 exerts its antitumor activity remains to be fully elucidated, the unique mechanism by which ET-743 interacts with DNA is different from that observed with other alkylating agents. This could explain the activity of ET-743 in preclinical models and clinical trials against tumor types that were not sensitive to established DNA-interacting anticancer drugs.

The recommended phase II dose for the 3-h infusion established in the phase I study did not seem feasible. Currently the actual dose given to patients is 1300 μg/m^2 and at this dose the safety seems manageable [95]. For the 24-h infusion dose reductions are required in patients with high baseline bilirubin levels and patients experiencing bilirubin or alkaline phosphatase elevations between the treatment cycles. Bone marrow suppression was dose limiting in most phase I trials but liver toxicity characterized by an elevation in transaminase levels is also of concern. However, other clinically relevant toxicities such as mucositis, alopecia, diarrhea and neurotoxicity were not observed after ET-743 treatment.

Preclinical studies indicated that a prolonged administration of the drug would be beneficial for the antitumor

activity. Therefore, phase I clinical studies were initiated to explore the potential benefit of prolonged administration in a 24- and 72-h infusion. However, as ET-743 displays such a long terminal half-life, a long duration of infusion such as a 72-h infusion does not seem necessary and would be unpractical in the clinic. In the phase II studies the 3- and 24-h infusion schedules are being compared for their antitumor activity. However, a comparison between these two schedules in terms of activity cannot yet be made as the results of the studies have only been published as preliminary reports.

ET-743 has demonstrated cytotoxic activity in tumor types such as breast cancer [104] and ovarian cancer [95]. Furthermore, activity was seen in soft tissue sarcomas, a tumor type for which limited adequate chemotherapy is currently available. So far, the response rate in first-line treatment with these tumors is 18%, which is comparable to the results obtained with ifosfamide and doxorubicin in this tumor type [16]. However, stabilization of disease was observed more frequently (42–50%) lasting up to 14 months in some patients [16]. This could be a possible advantage of ET-743.

Future studies with ET-743 could focus on the combination with licensed chemotherapeutics, such as paclitaxel and cisplatin. The rationale for these combinations is supported by mechanistic data: ET-743 appeared to repress the increased SXR activity caused by paclitaxel, which would ultimately lead to a reduction in metabolism and efflux of paclitaxel. Furthermore, ET-743 was active in cells with an enhanced NER system, such as cisplatin-resistant cells. Preclinical studies with these combinations have shown synergistic effects. In addition, these combinations seem feasible as ET-743 lacks important toxicities observed with these agents, such as neurotoxicity and nephrotoxicity.

The usefulness of the clinical response to determine the outcome of trials in metastatic soft tissue sarcomas has been discussed by Le Cesne *et al.* [110]. In a randomized trial in this tumor type comparing standard doxorubicin and ifosfamide doses with an intensified regimen they could not demonstrate a significant difference between the two treatment arms in observed partial and complete responses. However, progression-free survival was higher with the intensified treatment, which obviously not correlated with the observed clinical responses [110]. Recently, van Glabbeke *et al.* advocated the use of the progression-free rate at 3 or 6 months in standard phase II trials with non-cytotoxic agents in soft tissue sarcomas [111]. They suggested that the progression-free rate could be a useful endpoint for phase II studies, which aim at screening potentially active compounds in order to justify further investigation of the new treatment. Subsequent phase III trials are also required with ET-743 as a single agent or in combination in less pretreated patients (i.e. first line) to confirm the actual therapeutic benefit of the new treatment. A combined analysis of three multicenter phase II trials with ET-743 demonstrated a progression-free rate at 6 months of 27.2% [16], which seems favorable [111].

Further trials planned are expected to provide additional information on the benefit of ET-743 for patients with soft tissue sarcomas in terms of survival, time to progression and quality of life.

References

1 Adams J, Elliott PJ. New agents in cancer clinical trials. *Oncogene* 2000; **19**:6687–6692.
2 Schwartsmann G, da Rocha AB, Berlinck RGS, Jimeno J. Marine organisms as a source of new anticancer agents. *Lancet Oncol* 2001; **2**:221–225.
3 Da Rocha AB, Lopes RM, Schwartsmann G. Natural products in anticancer therapy. *Curr Opin Pharmacol* 2001; **1**:364–369.
4 Cragg GM, Newman DJ, Weiss RB. Coral reefs, forests, and thermal vents: the worldwide exploration of nature for novel antitumor agents. *Semin Oncol* 1997; **24**:156–163.
5 D'Incalci MD. Some hope from marine natural products. *Ann Oncol* 1998; **9**:937–938.
6 Scheuer PJ. From the rainforest to the reef. *Med Res Rev* 1994; **14**: 487–503.
7 Pettit GR, Day JF. Antineoplastic components of marine animals. *Nature* 1970; **277**:962–963.
8 Jimeno J, Faircloth G, Guzmán C, Vega E, Fernández Sousa JM. Marine derived therapeutics. *Conference Lecture: Medicines from Nature: Scientific, Legal and Ethical Aspects.* The Royal Academy of Medicine, London, 9–10 June 1998.
9 Schellens JHM, Rademaker JL, Horenblas S, *et al.* Phase I and pharmacokinetic study of kahalalide F in patients with advanced androgen refractory prostate cancer. *Proc AACR-NCI-EORTC* 2001: #207.
10 Li LH, Timmins LG, Wallace TL, Joullié. Mechanism of action of didemnin B, a depsipeptide from the sea. *Cancer Lett* 1984; **23**:279–288.
11 Depenbrock H, Peter R, Faircloth GT, Manzanares I, Jimeno J, Hanauske AR. *In vitro* activity of aplidine, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumour cells and haematopoietic precursor cells. *Br J Cancer* 1998; **78**:739–744.
12 Propper DJ, Macaulay V, O'Byrne KJ, *et al.* A phase II study of bryostatin 1 in metastatic malignant melanoma. *Br J Cancer* 1998; **78**:1337–1341.
13 Rinehart KL. Antitumor compounds from tunicates. *Med Res Rev* 2000; **20**:1–27.
14 Pommier Y, Kohlhagen G, Bailly C, Waring M, Mazumder A, Kohn KW. DNA sequence- and structure-selective alkylation of guanine N^2 in the DNA minor groove by ecteinascidin 743, a potent antitumor compound from the Caribbean tunicate *Ecteinascidia turbinata*. *Biochemistry* 1996; **35**:13303–13309.
15 Jimeno JM, Faircloth G, Cameron L, *et al.* Progress in the acquisition of new marine-derived anticancer compounds: development of ecteinascidin-743 (ET-743). *Drugs of the Future* 1996; **21**:1155–1165.
16 Cvetkovic RS, Figgitt DP, Plosker GL. Ecteinascidin-743. *Drugs* 2002; **62**:1185–1192.
17 Rinehart KL, Holt TG, Fregeau NL, *et al.* Potent antitumor agents from the Caribbean tunicate *Ecteinascidia turbinata*. *J Org Chem* 1990; **55**:4512–4515.
18 Sakai R, Rinehart KL, Guan Y, Wang AHJ. Additional antitumor ecteinascidins from a Caribbean tunicate: crystal structures and activities *in vivo*. *Proc Natl Acad Sci USA* 1992; **89**:11456–11460.
19 Guan Y, Sakai R, Rinehart KL, Wang AHJ. Molecular and crystal structures of ecteinascidins: potent antitumor compounds from the Caribbean tunicate *Ecteinascidia turbinata*. *J Biomol Struct Dyn* 1993; **10**: 793–818.
20 Wright AE, Forleo DA, Gunawardana GP, Gunasekera SP, Koehn FE, McConnell OJ. Antitumor tetrahydroisoquinoline alkaloids from the colonial ascidian *Ecteinascidia turbinata*. *J Org Chem* 1990; **55**: 4508–4512.

21 Reid JM, Walker DL, Ames MM. Preclinical pharmacology of ecteinascidin 729, a marine natural product with potent antitumor activity. *Cancer Chemother Pharmacol* 1996; **38**:329–334.
22 Corey EJ, Gin DY, Kania RS. Enantioselective total synthesis of ecteinascidin 743. *J Am Chem Soc* 1996; **118**:9202–9203.
23 Martinez EJ, Corey EJ. A new, more efficient, and effective process for the synthesis of a key pentacyclic intermediate for production of ecteinascidin and phthalascidin antitumor agents. *Org Lett* 2000; **2**:993–996.
24 Cuevas C, Pérez M, Martin MJ, *et al.* Synthesis of ecteinascidin ET-743 and phthalascidin Pt-650 from cyanosafracin B. *Org Lett* 2000; **2**:2545–2548.
25 Saito N, Tachi M, Seki R, Kamayachi H, Kubo A. A practical synthesis of the ABC ring model of ecteinascidins. *Chem Pharm Bull* 2000; **48**: 1549–1557.
26 Zhou B, Guo J, Danishefsky SJ. Studies directed to the total synthesis of ET-743 and analogues thereof: an expeditious route to the ABFGH subunit. *Org Lett* 2002; **4**:43–46.
27 Martinez EJ, Owa T, Schreiber SL, Corey EJ. Phthalascidin, a synthetic antitumor agent with potency and mode of action comparable to ecteinascidin 743. *Proc Natl Acad Sci USA* 1999; **96**:3496–3501.
28 Martinez EJ, Corey EJ, Owa T. Antitumor activity- and gene expression-based profiling of ecteinascidin Et 743 and phthalascidin Pt 650. *Chem Biol* 2001; **8**:1151–1160.
29 Moore BM, Seaman FC, Wheelhouse RT, Hurley LH. Mechanism for the catalytic activation of ecteinascidin 743 and its subsequent alkylation of guanine N^2. *J Am Chem Soc* 1998; **120**:2490–2491.
30 Nuijen B, Bouma M, Manada C, *et al.* Pharmaceutical development of anticancer agents derived from marine sources. *Anticancer Drugs* 2000; **11**:793–811.
31 Marco E, García-Nieto R, Mendieta J, Manzanares I, Cuevas C, Gago F. A 3*(ET743)–DNA complex that both resembles an RNA–DNA hybrid and mimics zinc finger-induced DNA structural distortions. *J Med Chem* 2002; **45**:871–880.
32 Moore BM, Seaman FC, Hurley LH. NMR-based model of an ecteinascidin 743–DNA adduct. *J Am Chem Soc* 1997; **119**:5475–5476.
33 Seaman FC, Hurley H. Molecular basis for the DNA sequence selectivity of ecteinascidin 736 and 743: evidence for the dominant role of direct readout via hydrogen bonding. *J Am Chem Soc* 1998; **120**:13028–13041.
34 García-Nieto R, Manzanares I, Cuevas C, Gago F. Bending of DNA upon binding of Ecteinascidin 743 and phthalascidin 650 studies by unrestrained molecular dynamics simulations. *J Am Chem Soc* 2000; **122**:7172–7182.
35 García-Nieto R, Manzanares I, Cuevas C, Gago F. Increased DNA binding specificity for antitumor Ecteinascidin 743 through protein–DNA interactions? *J Med Chem* 2000; **43**:4367–4369.
36 Zewail-Foote M, Hurley LH. Differential rates of reversibility of ecteinascidin 743–DNA covalent adducts from different sequences lead to migration to favored bonding sites. *J Am Chem Soc* 2001; **123**:6485–6495.
37 Hurley LH, Zewail-Foote M. The antitumor agent ecteinascidin 743: characterization of its covalent DNA adducts and chemical stability. *Adv Exp Med Biol* 2001; **500**:289–299.
38 Zewail-Foote M, Hurley LH. Ecteinascidin 743: a minor groove alkylator that bends DNA toward the major groove. *J Med Chem* 1999; **42**: 2493–2497.
39 Bonfanti M, La Valle E, Fernandez Sousa Faro J, *et al.* Effect of ecteinascidin-743 on the interaction between DNA binding proteins and DNA. *Anticancer Drug Des* 1999; **14**:179–186.
40 Minuzzo M, Marchini S, Broggini M, Faircloth G, D'Incalci M, Mantovani R. Interference of transcriptional activation by the antineoplastic drug ecteinascidin-743. *Proc Natl Acad Sci* 2000; **97**:6780–6784.
41 Jin S, Gorfajn B, Faircloth G, Scotto KW. Ecteinascidin 743, a transcription-targeted chemotherapeutic that inhibits MDR1 activation. *Proc Natl Acad Sci* 2000; **97**:6775–6779.
42 Markenson D, Hu Z, Scotto KW. ET-743 inhibits SP1-mediated transcriptional activation of the p21 promoter. *Proc Am Ass Cancer Res* 2001; **42**:203.
43 Erba E, Bergamaschi D, Bassano L, *et al.* Isolation and characterisation of an IGROV-1 human ovarian cancer cell line made resistant to ecteinascidin-743 (ET-743). *Br J Cancer* 2000; **82**:1732–1739.
44 Kanzaki A, Takebayashi Y, Fukumoto M, Uchida T, Pommier Y. Activity of Ecteinascidin 743 and synergism with doxorubicin and vincristine in P-glycoprotein/MDR1 over-expression cell lines. *Proc Am Ass Cancer Res* 2001; **42**:811.
45 Fontaniere C, Terrier-Lacombe M, Santos A, *et al.* Antitumor activity of ET-743 (ecteinascidin-743) in neuroblastoma and medulloblastoma xenografts. *Proc Am Ass Cancer Res* 2001; **42**:477.
46 Shao L, Weissbach L, Faircloth GT, Horniceh FJ. *In vitro* invasiveness of chondrosarcoma altered by ecteinascidin-743. *Proc Am Ass Cancer Res* 2002; abstr 2676.
47 Kim H, Healey J, Sowers R, *et al.* Expression of drug resistance related genes and drug cytotoxicity is discordant in human osteosarcoma. *Proc Am Ass Cancer Res* 2002; abstr 5486.
48 Maliepaard M, Van Gastelen M, Faircloth GT, Jimneo JM, Beijnen JH, Schellens JHM. Novel marine derived anticancer agents ET-743, Aplidine, Spisulosine (ES-285) and Kahalalide F are not transported by the Breast Cancer Resistance Protein. *Proc Am Ass Cancer Res* 2001; **42**:4352.
49 Synold TW, Dussault I, Forman BM. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* 2001; **7**:584–590.
50 Izbicka E, Lawrence R, Raymond E, *et al. In vitro* antitumor activity of the novel marine agent, ecteinascidin-743 (ET-743, NSC-648766) against human tumors explanted from patients. *Ann Oncol* 1998; **9**:981–987.
51 García-Rocha M, García-Gravalos MD, Avila J. Characterisation of antimitotic products from marine organisms that disorganise the microtubule network: ecteinascidin 743, isohomohalichondrin-B and LL-15. *Br J Cancer* 1996; **73**:875–883.
52 Takebayashi Y, Pommier Y. DNA minor groove alkylation by ecteinascidin 743 (ET743) induces sequence specific topoisomerase I-mediated DNA damage. *Proc Am Ass Cancer Res* 1999; **40**:108.
53 Takebayashi Y, Pourquier P, Yoshida A, Kohlhagen G, Pommier Y. Poisoning of human DNA topoisomerase I by ecteinascidin 743, an anticancer drug that selectively alkylates DNA in the minor groove. *Proc Natl Acad Sci* 1999; **96**:7196–7201.
54 Takebayashi Y, Goldwasser F, Urasaki Y, Kohlhagen G, Pommier Y. Ecteinascidin 743 induces protein-linked DNA breaks in human colon carcinoma HCT116 cells and is cytotoxic independently of topoisomerase I expression. *Clin Cancer Res* 2001; **7**:185–191.
55 Erba E, Bergamaschi D, Bassano L, *et al.* Ecteinascidin-743 (ET-743), a natural marine compound, with a unique mechanism of action. *Eur J Cancer* 2001; **37**:97–105.
56 Simoens C, Korst AEC, Pattyn GGO, *et al.* Cell cycle effects of ET-743. *Proc Am Ass Cancer Res* 2002; abstr 324.
57 Takebayashi Y, Pouqier P, Zimonjic DB, *et al.* Antiproliferative activity of ecteinascidin 743 is dependent upon transcription-coupled nucleotide-excision repair. *Nat Med* 2001; **7**:961–966.
58 Damia G, Silvestri S, Carrassa L, *et al.* Unique pattern of ET-743 activity in different cellular systems with defined deficiencies in DNA-repair pathways. *Int J Cancer* 2001; **92**:583–588.
59 Zewail-Foote M, Li VS, Kohn H, Bearss D, Guzman M, Hurley LH. The inefficiency of incisions of ecteinascidin 743–DNA adducts by the UvrABC nuclease and the unique structural feature of the DNA adducts can be used to explain the repair-dependent toxicities of this antitumor agent. *Chem Biol* 2001; **8**:1033–1049.
60 Rinehart KL, Gravalos LG, Faircloth G, Jimeno J. ET-743: preclinical antitumor development of a marine derived natural product. *Proc Am Ass Cancer Res* 1995; **36**:2322.
61 Ghielmini M, Colli E, Erba E, *et al. In vitro* schedule-dependency of myelotoxicity and cytotoxicity of ecteinascidin 743 (ET-743). *Ann Oncol* 1998; **9**:989–993.
62 Shtil AA, Kolb EA, Faircloth G, LaQuaglia M, Scotto KW. Ecteinascidin 743, a novel natural cytotoxic compound, is potent for human neuroblastoma and rhabdomyosarcoma cell lines: multiple mechanisms of cell kill. *Proc Am Ass Cancer Res* 2001; **42**:4353.
63 Li WW, Takahashi N, Jhanwar S, *et al.* Sensitivity of soft tissue sarcoma cell lines to chemotherapeutic agents: identification of Ecteinsacidin-743 as a potent cytotoxic agent. *Clin Cancer Res* 2001; **7**:2908–2911.
64 Eckhardt SG, Degen D, Ortiz V, Faircloth GT, Jimeno JM, Von Hoff DD. *In vitro* studies of a novel marine cytotoxic, ecteinascidin (ET-743). *Ann Oncol* 1996; **7**(**suppl 5**):632P.
65 Takahashi N, Li W, Faircloth G, Jimeno J, *et al.* Ecetinascidin-743 (ET-743) and doxorubicin produce synergistic cytotoxic effects in soft tissue sarcoma lines HT-1080 and HS-18. *Proc Am Ass Cancer Res* 2000; abstr 208.
66 Takahashi N, Li WW, Banerjee D, Scotto KW, Bertino JR. Sequence dependent enhancement of cytotoxicity produced by Ecteinascidin 743 (ET-743) with doxorubicin or paclitaxel in soft tissue sarcoma cells. *Clin Cancer Res* 2001; **7**:3251–3257.
67 Riccardi R, Colombo T, Meco D, *et al.* Effective combinations of ET-743 and doxorubicin for tumor growth inhibitions against murine and human sarcomas in athymic mice. *Proc Am Ass Cancer Res* 2001; **42**:1132.

68 Moore R, Revilla M, Jimeno J, Faircloth G, Weitman S. Sequencing evaluation of ET-743 combinations with standard chemotherapy agents against a panel of human tumor cell lines. *Proc NCI-EORTC-AACR* 2000; abstr 504.

69 D'Incalci M, Erba E, Damia G, *et al.* The combination of ET-743 and cisplatin (DDP): from a molecular pharmacology study to a phase I clinical trial. *Proc Am Ass Cancer Res* 2002; abstr 404.

70 Valoti G, Nicoletti MI, Pellegrino A, *et al.* Ecteinascidin-743, a new marine natural product with potent antitumor activity on human ovarian carcinoma xenografts. *Clin Cancer Res* 1998; **4**:1977–1983.

71 Hendriks HR, Fiebig HH, Giavazzi R, Langdon SP, Jimeno JM, Faircloth GT. High antitumour activity of ET743 against human tumour xenografts from melanoma, non-small-cell lung and ovarian cancer. *Ann Oncol* 1999; **10**:1233–1240.

72 Mazza BD, Yang R, Sowers RS, *et al.* The establishment of xenograft models from osteosarcoma samples and their growth inhibition by ET-743. *Proc Am Ass Cancer Res* 2002; abstr 4575.

73 Faircloth GT, Grant W, Jimeno J, *et al.* Dexamethasone potentiates the activity of Ecteinascidin 743 in preclinical melanoma and osteosarcoma models. *Proc Am Ass Cancer Res* 2002; abstr 379.

74 Gomez SG, Bueren JA, Faircloth GT, Jimeno J, Albella B. Toxicity of ET-743, aplidine and kahalalide F on hemopoietic stem cell function. *Proc Am Ass Cancer Res* 2002; abstr 5419.

75 Dorr RT, Shipp NG, Lee KM. Comparison of cytotoxicity in heart cells and tumor cells exposed to DNA intercalating agents *in vitro*. *Anticancer Drugs* 1991; **2**:27–33.

76 Luber-Narod J, Smith B, Grant W, Jimeno J, Lopez-Lazaro L, Faircloth GT. *In vitro* safety toxicology of ecteinascidin 743, a marine natural product with chemotherapeutic potential against solid tumors. *Proc Am Ass Cancer Res* 2001; **42**:1133.

77 Mirsalis JC, Schindler-Horvat JE, Tomaszewski JE, *et al.* Preclinical toxicology studies of ecteinascidin 743. *Proc Am Soc Clin Oncol* 1996; **37**:2556.

78 Tomaszewski JE, Donohue SJ, *et al.* Preclinical toxicity of ecteinascidin 743. *Proc PAMM/EORTC* 1995.

79 Kuffel MJ, Reid JM, Ames MM. Cytochrome P450 catalyzed metabolism of ecteinascidin 743 by rat and human liver microsomes. *Proc Am Ass Cancer Res* 1997; **38**:4003.

80 Rosing H, Hillebrand MJX, Jimeno JM, *et al.* Quantitative determination of ecteinascidin 743 in human plasma by miniaturized high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J Mass Spectr* 1998; **33**:1134–1140.

81 Sparidans RW, Rosing H, Hillebrand MJX, *et al.* Search for metabolites of ecteinascidin-743, a novel marine derived anti-cancer agent. *Anticancer Drugs* 2001; **12**:653–666.

82 Adachi Y, Yamamoto T. Hepatic bilirubin-conjugating enzymes of man in the normal state and in liver disease. *Gastroenterol Jpn* 1982; **17**:235–240.

83 Black M, Billing BH. Hepatic bilirubin UDP-glucuronyl transferase in liver disease and Gilbert's syndrome. *N Engl J Med* 1969; **280**:1266–1271.

84 Rosing H, Hillebrand MJX, Jimeno JM, *et al.* Analysis of ecteinascidin 743, a new potent marine-derived anticancer drug, in human plasma by high-performance liquid chromatography in combination with solid-phase extraction. *J Chrom B* 1998; **710**:183–189.

85 van Kesteren Ch, Twelves C, Bowman A, *et al.* Clinical pharmacology of the novel marine-derived anti-cancer agent Ecteinascidin 743 administered as a 1- and 3-h infusion in a phase I study. *Anticancer Drugs* 2002; **13**: 381–393.

86 van Kesteren Ch, Cvitkovic E, Taamma A, *et al.* Pharmacokinetics and pharmacodynamics of the novel marine-derived anti-cancer agent Ecteinascidin 743 in a phase I dose-finding study. *Clin Cancer Res* 2000; **6**:4725–4732.

87 Ryan DP, Supko JG, Eder JP, *et al.* Phase I and pharmacokinetic study of ecteinascidin 743 administered as a 72-hour continuous intravenous infusion in patients with solid malignancies. *Clin Cancer Res* 2001; **7**: 231–242.

88 Villalona-Calero M, Eckhardt SG, Weiss G, *et al.* A phase I and pharmacokinetic study of Ecteinscidin-743 on a daily × 5 schedule in patients with solid malignancies. *Clin Cancer Res* 2002; **8**:75–85.

89 Bowman A, Twelves C, Hoekman K, *et al.* Phase I clinical and pharmacokinetic (PK) study of ecteinascidin-743 (ET-743) given as a one hour infusion every 21 days. *Ann Oncol* 1998; **9**(**suppl 2**):452.

90 Twelves C, Hoeckman H, Bowman A, *et al.* A phase I and pharmacokinetic study of ET-743 evaluating a 3-hour intravenous infusion in patients with solid tumors. *Clin Cancer Res* 1999; **5**:307.

91 Taamma A, Misset JL, Riofrio M, *et al.* Phase I and pharmacokinetic study of ecteinascidin-743, a new marine compound, administered as a 24-hour continuous infusion in patients with solid tumors. *J Clin Oncol* 2001; **19**:1256–1265.

92 Delaloge S, Yovine A, Taama A, *et al.* Ecteinascidin-743: a marine-derived compound in advanced, pretreated sarcoma patients—preliminary evidence of activity. *J Clin Oncol* 2001; **19**:1248–1255.

93 Forouzesh B, Hidalgo M, Denis L, *et al.* Phase I and pharmacokinetic study of the marine-derived DNA minor groove binder on a weekly × 3 every 4 weeks schedule in patients with advanced solid malignancies. *Proc AACR-NCI-EORTC* 2001; abstr 209.

94 Baruchel S, Blaney S, Hershon L, *et al.* A phase I study of ET-743 in pediatric refractory solid tumors: a Children's Oncology Groups Study. *Proc Am Soc Clin Oncol* 2002; **21**:96a.

95 Sessa N, Colombo N, Bauer J, *et al.* Phase II study of salvage ET-743 given as 3-hr infusion in ovarian cancer patients. *Ann Oncol* 2002; **13**(**suppl 5**): 397a.

96 Dileo P, Casali PG, Bacci G, *et al.* Phase II evaluation of 3-hr infusion ET-743 in patients with recurrent sarcomas. *Proc Am Soc Clin Oncol* 2002; **21**:408a.

97 Le Cesne A, Blay J, Judson I, *et al.* ET-743 is an active drug in soft-tissue sarcoma (sts): a STBSG–EORTC phase II trial. *Proc Am Soc Clin Oncol* 2001; **20**:1407.

98 George S, Maki RG, Harmon D, *et al.* Phase II study of ecteinscidin-743 (ET-743) given by 3-hour IV infusion in patients with soft tissue sarcomas failing prior chemotherapies. *Proc Am Soc Clin Oncol* 2002; **21**:408a.

99 Ruiz-Casado A, Lopez-Martin J, Nieto A, *et al.* Ecteinascidin in heavily pretreated advanced sarcoma patients as a compassionate basis. *Proc Am Soc Clin Oncol* 2002; **21**:408a.

100 Lopez-Martin JA, Nieto A, Demetri GD, *et al.* Safety profile of ecteinscidin-743 (ET-743) in phase II clinical trials in adult patients with solid tumors. *Proc Am Soc Clin Oncol* 2002; **21**:96a.

101 Yovine A, Riofrio M, Brain E, *et al.* Ecteinascidin (ET-743) given as a 24-hour (h) intravenous continuous infusion (ivci) every 3 weeks: results of a phase II trial in patients (pts) with pretreated soft tissue sarcomas (ptsts). *Proc Am Soc Clin Oncol* 2001; **21**:1449.

102 Demetri GD, Seiden M, Garcia-Carbonero R, *et al.* Ecteinascidin (ET-743) shows promising activity in distinct populations of sarcoma patients: summary of 3 U.S.-based phase II trials. *Proc Am Soc Clin Oncol* 2000; **19**:2177.

103 Laverdiere C, Kolb A, Meyers P, *et al.* Phase II study of ET-743 in recurrent osteosarcoma. *Proc Am Soc Clin Oncol* 2002; **21**:96a.

104 Zelek L, Yovine A, Brain E, *et al.* Preliminary results of phase II study of ecteinascidin-743 (ET-743) with the 24 hour (h) continuous infusion (cl) q3week schedule in pretreated advanced/metastatic breast cancer (A/MBC) patients (pts). *Proc NCI-EORTC-AACR* 2000; abstr 212.

105 van Kesteren Ch, Mathôt RAA, López-Lázaro L, *et al.* A comparison of limited sampling strategies for prediction of Ecteinascidin 743 clearance when administered as a 24-hour infusion. *Cancer Chemother Pharmacol* 2001; **48**:459–466.

106 Mathôt R, van Kesteren C, le Cesne A, *et al.* Population pharmacokinetics of the novel marine derived anti-cancer agent ecteinascidin 743 in two phase II studies using non-linear mixed effects modeling (NONMEM). *Proc Am Soc Clin Oncol* 2001; **20**:372.

107 Garcia-Carbonero R, Demetri G, Ryan D, *et al.* Population pharmacokinetics of ecteinascidin-743 in patients with advanced soft tissue sarcoma. *Proc NCI-EORTC-AACR* 2000; abstr 211.

108 Gómez J, López-Lázaro L, Guzman C, *et al.* Identification of safety parameters that predict the onset of severe toxicities in patients treated with ET-743. *Proc Am Soc Clin Oncol* 2000; **19**:187a.

109 Le Cesne A. Phase II study of ET-743 advanced in soft-tissue sarcoma in adult: a STBSG–EORTC phase II trial. *Proc Am Soc Clin Oncol* 2002; **21**:554a.

110 Le Cesne A, Judson J, Crowther D, *et al.* Is obtention of partial response (PR) in from line chemotherapy critical to improve outcome of metastatic soft tissue sarcoma (MSTS) patients (PTS). *Proc Eur Soc Med Oncol* 1998: #556.

111 Van Glabbeke M, Verweij J, Judson I, Nielsen OS. Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas. *Eur J Cancer* 2002; **38**:543–549.