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Review paper

Ecteinascidin 743: a novel anticancer drug with a unique mechanism of action

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Ecteinascidin 743 (Et743) is an interesting compound in phase II/III clinical trials. Its chemistry is complex, its mechanism of action is original and it is active in human cancers, such as sarcomas refractory to conventional chemotherapy. The present review describes the discovery of the drug, its specific interactions with DNA and its reversible alkylation mechanism with guanine N^2 in the DNA minor groove. Et743 is a selective transcription inhibitor, which has the unique characteristic of poisoning transcription-coupled nucleotide excision repair. Understanding the molecular pharmacology of Et743 should help in deciding which patients should receive Et743 treatments and which agents should be most useful in association. [© 2002 Lippincott Williams & Wilkins.]

Key words: Cancer chemotherapy, DNA alkylation, DNA binding, DNA repair, minor groove binder, transcription.

Introduction

Ecteinascidins are a series of related compounds isolated in the laboratory of Dr Ken Rinehart, a professor of chemistry at the University of Illinois, Urbana-Champaign, from a marine tunicate, *Ecteinascidia turbinate* ('Caribbean sea squirt'), which grows preferentially in the roots of mangroves. Extracts from this tunicate were found to have dramatic anticancer activity, even at minuscule doses, in 1969. Small amounts of Ecteinascidins precluded their isolation for almost 20 years. Once isolated, Ecteinascidins were licensed to PharmaMar SA, and recently PharmaMar has licensed ecteinascidin 743 (Et743) to Johnson and Johnson/Ortho Biotech for development of the drug in the US. Et743 is presently in phase II/III clinical development.

Derivatives from natural products represent the majority of drugs used presently in cancer chemotherapy. In a survey by the National Cancer Institute (NCI) in 1995, it was estimated that approximately 4% of extracts from marine species (mainly animals) contained antitumor compounds, which is approximately in the same range as for terrestrial species (mainly plants). Until the mid-1960s, investigation of nature products from marine organisms was essentially non-existent. However, there are currently at least 10 compounds in various stages of clinical development. These compounds were discovered using anticancer screens, which defined their potential anticancer activity before elucidating their mechanism of action. This approach is currently less favored than the screening of chemical libraries against established targets. However, one value of this anticancer screening approach is that it offers the potential of discovering novel targets. In addition to Et743, the other marine compounds in current evaluation are: didemnin B (aplidin) from another tunicate, *Tridedimnum solidum* (protein synthesis inhibitor), bryostatin from the bryozoan *Bugula neritina* (protein kinase C inhibitor), Dolostatin from the sea hare *Dolabella auricularia*, the sarcodictyins from the Mediterranean coral *Sarcodictyon roseum* (tubulin polymerizing agent), eleutherobin from corals (*Eleutherobia* spp) (tubulin polymerizing agent) found off the coast of Australia, and halomon from the red alga *Portiera hornemannii*. The first clinically used marine compound was cytosine arabinoside (isolated in 1959 from the sea sponge *Cryptothethya crypta*). Cytosine arabinoside remains one of the most commonly used drugs for the treatment of leukemia.

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Chemical structure

The structure of the Ecteinascidins (Figure 1) was first reported in 1987 by Rinehart *et al.*[1] Crystallographic studies established unambiguously the remarkable structure of Et743 and suggested the binding of the three fused tetrahydroisoquinoline rings (units A and B in Figure 1) in the DNA minor groove.[2] Because Et743 is more abundant than its *N*-demethyl derivative Et729 (Figure 1), Et743 was selected for clinical development by the NCI in 1993. Ecteinascidins are structurally related to the other tetrahydroisoquinolines saframycin antibiotics (Figure 1) isolated from cultured *Streptomyces* species. Compounds related to saframycins with the same bis(tetrahydroisoquinoline) carbon and nitrogen framework (rings A and B in Figure 1) have been found from other microorganisms and also from a marine sponge.[3] The third unit (unit C) attached to unit B by the 10-membered sulfide-containing lactone is a quite distinctive feature of the Ecteinascidins, both structurally and biosynthetically (see Figure 1). Thus, the potent anticancer activity of Ecteinascidins may, at least in part, be related to the unit C since saframycin A, which lacks the C unit has lower antitumor activity than Ecteinascidins.[3]

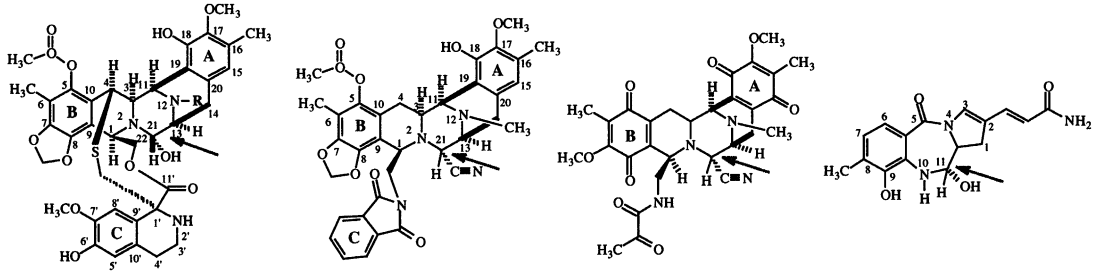
Recently, Corey *et al.* achieved the total synthesis of Et743 in 44 steps with a low yield of 0.75%.[4] These synthetic studies led to the identification of a novel synthetic derivative, phthalascidin (Pt650) (Figure 1), with a different unit C.[5] Phthalascidin has an antiproliferative activity comparable to that Et743.[5] The total synthesis route is not applicable for making Et743 for clinical use and the drug is presently prepared by semi-synthesis from precursors obtained from extracts obtained by aquaculture.[1]

DNA binding

The DNA interactions of Et743 exhibit the following unique characteristics: (i) alkylation of guanine at the exocyclic N^2 position in the minor groove, (ii) sequence-specific minor groove binding, (iii) reversibility of the guanine N^2 adducts, (iv) sharp bending toward the major groove opposite to the adduct and (v) protrusion of the C-ring in the minor groove with potential binding to selective chromatin proteins.

Guanine N^2 alkylation is due to the presence of a reactive carbinolamine, which is also found in phthalascidin, saframycins and anthramycin (arrows in Figure 1). The formation of a covalent bond between guanine N^2 and the carbinolamine center was first shown for anthramycin.[6–8] The formation of guanine N^2 adducts of saframycin was first reported by Lown *et al.* who also noted that these adducts were reversible upon DNA denaturation by heat.[9] For Et743, the formation of a guanine N^2 adduct was first suggested from crystallographic and modeling studies,[2] and was demonstrated by band shift assays.[10] The chemical reaction leading to the guanine N^2 alkylation was proposed to result from an intramolecular acid-catalyzed dehydration of the carbinolamine moiety, resulting in the formation of an electrophilic iminium, which is the DNA-reactive intermediate (Figure 2A).[10,11] This reaction is comparable to the formation of a reactive imine proposed in the case of anthramycin[6,12] and for the activation of saframycin.[13]

The sequence selectivity of the Et743–guanine N^2 adducts was first analyzed by footprinting (demonstrating a 3–5 bp protection) and by band shift assays with short oligodeoxynucleotides.[10] The sequence selectivity was found to be determined primarily by



Ecteinascidins
Et743: R = CH_3 Et729: R = H
(NSC 648766)
(MW = 797)

Phthalascidin (Pt650)
(NSC 701549)
(MW = 655)

Saframycin
(MW = 563)

Anthramycin
(MW = 315)

Figure 1. Structure of Ecteinascidins and carbinolamine derivatives. The arrows point to the carbinolamine center that forms covalent adducts with the exocyclic N^2 residue of guanine in the DNA minor groove.

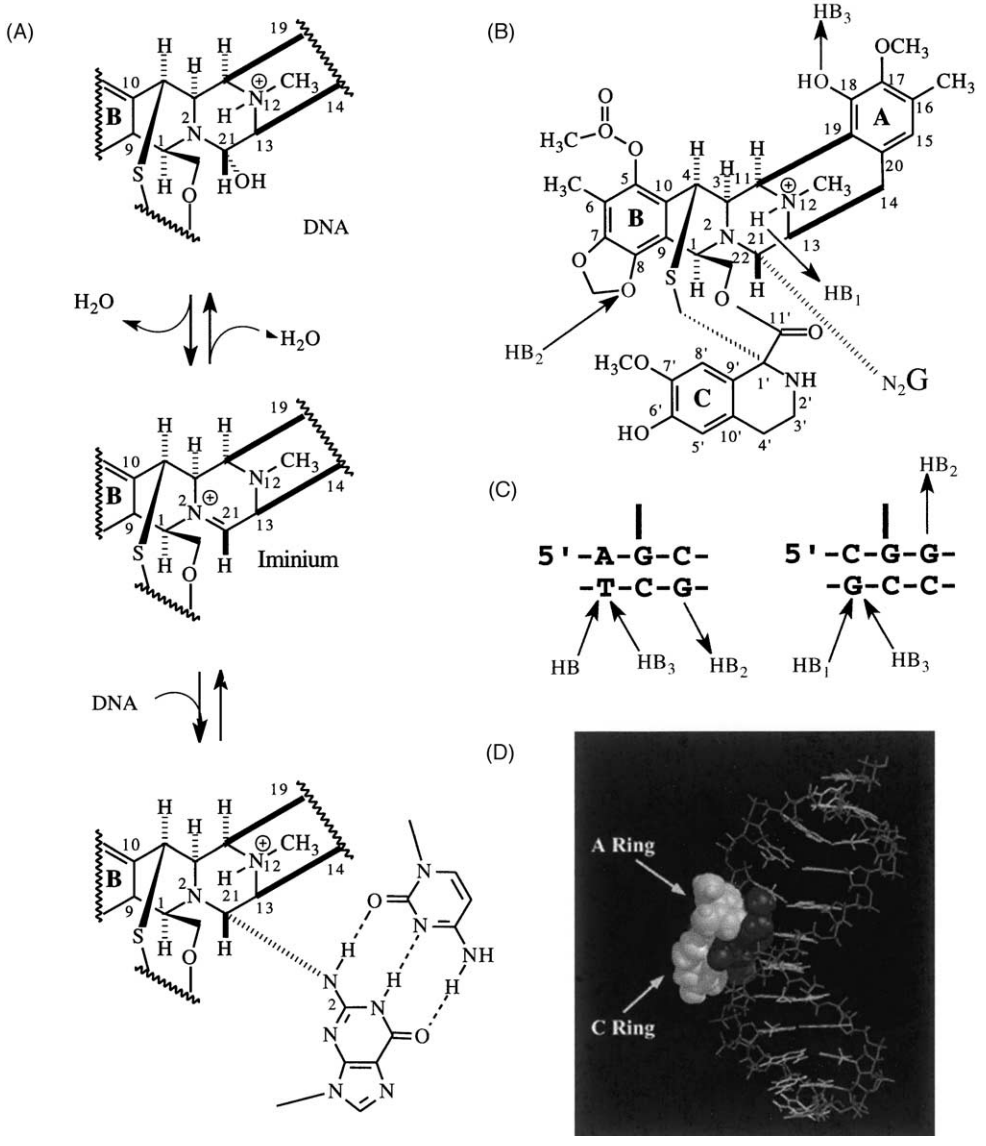


Figure 2. DNA binding of Et743. (A) Acid catalyzed activation of Et743 leading to the formation of an iminium intermediate that reacts with guanine N^2 in the DNA minor groove. (B) Hydrogen bond network between Et743 and the DNA. Outward arrows indicate hydrogen bond donors and inward arrows indicate hydrogen bond acceptors. (C) The two most preferred sequences for Et743 alkylation and the network of hydrogen bonds with the DNA bases flanking the alkylated guanine (vertical bar). (D) A representation of the Et743 bond in the DNA minor groove with the ring C protruding outside the minor groove, while the DNA is bent toward the major groove. (From Zewail-Foote *et al.*[33])

three consecutive nucleotides: the central alkylated guanine, and the nucleotides immediately 5′ and 3′ from the central guanine.[10] NMR studies provided a rationale for the sequence selectivity based on the formation of a network of four hydrogen bonds with these 3 bp (Figure 2).[14] In contrast to anthramycin,[10] the preferred sequences were 5′-Pu-G*-C and 5′-Py-G*-G, and the least preferred sequences were 5′-N-G*-(A/T) (where N is any of the four possible nucleotides).[10,14] The requirement for a G or C 3′ from the covalently linked G (Figure 2C) can be explained by the importance of a hydrogen bond between the oxygen at position 8 of Et743 and the guanine N^2 3′ from the adducted G on either strand (HB2 in Figure 2B and C).[14]

The reversibility of the guanine N^2 adducts upon DNA denaturation was first reported by Pommier *et al.*[10] Similarly, for anthramycin both the formation and reversal rates were found to be acid catalyzed,[6] and reversibility of the adducts is also the case for

saframycin A.[9] A recent study demonstrated that the Et743 adducts reverse under non-denaturing conditions and that the rate of reversion is greatest at non-preferred sequences.[15] The current concept is that Et743 can migrate from its less favored sequences [5′-N-G*-(A/T)] toward its most favored target sequences [5′-Pu-G*-C or 5′-Py-G*-G]. The rate of reversibility arises from differences in the stability of the Et743–DNA adducts, and particularly from the hydrogen bond, which is present at the most favored sequences (HB2 in Figure 2; between the lactone oxygen 8 of Et743 and the guanine N^2 immediately 3′ from the adducted guanine either on the adducted or non-adducted strand).[15]

The bending of the DNA toward the major groove immediately opposite from the adduct was determined by electrophoretic mobility shifts of ligated oligomers containing site-directed adducts[16] and by molecular modeling.[17] This bending (Figure 2D) is a unique feature among DNA-interacting agents. The protrusion of the ring C in the minor groove also differentiates Et743 from other guanine N^2 alkylating agents such as saframycin A and even more from anthramycin, which have markedly smaller groups in the region corresponding to the ring C of Et743. Both the protrusion of the ring C in the minor groove and the sharp bend toward the major groove may contribute to the selectivity of Et743 for certain DNA-binding proteins and DNA repair complexes. Alternatively, transcription factors that stabilize major groove bending of the DNA (such as the zinc-binding Sp1) may enhance the binding of Et743,[18] which might stabilize both the Sp1–DNA and the Et743–DNA complexes.

Cellular pharmacology

Et743 slows cell cycle progression in S phase and arrests cells in G_2[19–21] at pharmacological concentrations. This arrest is accompanied by an increase of p53, which is consistent with DNA damage.[19,21] However, cell lines with p53 deficiency are not resistant to Et743.[19–21] This characteristic is therapeutically important because it suggests that Et743 would be active in p53-deficient tumors, which represent the majority of carcinomas that are presently poorly responsive to cancer chemotherapy. Another interesting feature of Et743 is its greatest activity in cells in G_1.[21]

Apoptosis has been observed in cells treated with Et743. However, this response is generally delayed and probably secondary to the drug-induced cell cycle arrest.[19,21]

In early studies, Et743 was shown to disorganize the microtubule network.[22] Cells exposed to pharmacologically relevant doses of Et743 had a perinuclear distribution of microtubule aggregates, which was reminiscent of the effects of the widely used anticancer agent Taxol. However, in contrast to Taxol, no direct interaction of Et743 with tubulin or GTP could be shown.[22] Thus, the effects on microtubules may be a cellular response to DNA damage via some unknown pathway and not a principal event induced by Et743 itself.

Comparison of the spectrum of antiproliferative activity of Et743 in panels of cell lines using the COMPARE analysis[23] demonstrates that Et743 has a unique spectrum of activity,[19,20] which suggests a unique mechanism of action.[23] This activity profile is correlated with that of phthalascidin (Pt650)[20] and to a lesser extent with other DNA binders (such as morpholinodoxorubicin or actinomycin D).[19] Thus, the cellular pharmacology of Et743 exhibits some unique characteristics relative to the anticancer drugs presently used clinically: (i) activity in cells in G_1 (independent of DNA replication), (ii) activity in p53-deficient cells and (iii) unique profile of activity in the COMPARE analysis.

Molecular pharmacology

The molecular targets of Et743 are being actively investigated. The initial observations that Et743 was a specific DNA binder with unique characteristics (see above) suggested that the Et743–DNA adducts could exert their potent cytotoxicity by interfering with chromatin and DNA processing enzymes. Two other cellular approaches have provided further insight: (i) the generation and molecular analysis of cell lines selected for drug resistance, and (ii) the systematic analysis of cells with known defects in molecular pathways.

The molecular interactions of Et743 with biological targets can be divided into three categories: transcriptional effects, poisoning of nucleotide excision repair (NER) and inhibition of topoisomerases. At the present time, the specificity of Et743 for NER is the most intriguing molecular determinant with respect to potential clinical use. Nevertheless, understanding the full range of molecular events may reveal other important characteristics of E743 and perhaps indicate other pathways involved in Et743 response, as well as additional cellular targets.

Transcription inhibition

Minor groove binders such as distamycins or CC1065[24–26] inhibit transcription factors specific for A/T-rich sequences. This suggested that Et743 could inhibit GC-specific transcription factors such as NF-Y or Sp1.

Et743, at pharmacological concentrations, is a potent transcription inhibitor in a variety of cellular systems.[20,27–30] Notably, this inhibition appears selective for certain genes, which share the presence of preferential Et743-selective binding GC sequences for common transcription-activator binding sites in their promoters: CCAAT box, Sp1 and SXR binding elements.[20,27–30] Studies performed with reporter plasmids transiently transfected in mammalian cells demonstrated that Et743 blocks NF-Y- and SXR-mediated transcription activation.[27–30] However, these studies consistently demonstrated lack of detectable inhibition of NF-Y binding to the CCAAT sequences under these conditions. Also, biochemical experiments (using band shift assays) failed to demonstrate inhibition of NF-Y binding at pharmacological drug concentrations.[31] Thousand-fold higher Et743 concentrations (10–30 μM) are, in fact, required to detect inhibition of NF-Y binding. TBP, E2F and SRF binding require even higher concentrations (50–300 μM), and Et743–DNA adducts have no detectable effect on Sp1, Maf, Myb or Myc binding at 300 μM.[31] The apparent discrepancy between the low potency of Et743 to inhibit the binding of NF-Y and other GC-specific transcription factors in biochemical assays and the high potency of Et743 in mammalian cells suggests that the cellular effects of the drugs are more complex than just an inhibition of the binding of transcription factors to DNA. An alternative possibility is that Et743 stabilizes the binding of transcription factors, possibly because Et743 bends the DNA toward the major groove, which then would stabilize the protein–DNA complexes.[18] Therefore, it is possible that, in cells, Et743 stabilizes and alters, rather than inhibits the binding of transcription factors, such as NF-Y and other GC-specific factors, and that these alterations lead to the selective transcription inhibition.

Poisoning of transcription-coupled (TC)-NER

Recent investigations of Et743 in cells lacking various proteins required for NER have implicated this pathway as a determinant of drug sensitivity.[21,32,33] NER functions to remove base lesions distorting the DNA backbone.[34] These lesions can be induced by UV irradiation (resulting in 6–4 photoproducts and cyclobutane pyrimidine dimers), carcinogenic agents and chemotherapeutic drugs that alkylate DNA. They are genotoxic and mutagenic, and NER functions to remove these modifications and insures the passage of intact genetic material to daughter cells. Deficiency in proteins comprising the NER pathway results in the heritable disease xeroderma pigmentosum (XP), which is phenotypically characterized by photosensitivity and a greater than 1000-fold increase in skin cancer incidence. The genetic defects responsible are divided into seven complementation groups, XPA–G. NER is further divided into two distinct subpathways, global genome repair (GG-NER) and TC-NER.[35] TC-NER functions to remove lesions blocking the advancement of RNA polymerase II in actively transcribed genes, while GG-NER removes adducts from the rest of the genome. Cockayne syndrome (CS) is another disease associated with a deficiency in NER. CS patients are photosensitive and have neurologic abnormalities, but have no increased incidence of skin cancer. The genetic abnormalities are classified in two known complementation groups, CSA and CSB.

Fibroblast cell lines derived from patients with defects in each of the seven XP complementation groups and the two CS complementation groups are available. Rodent homologs are also available. Cell lines from patients with a defect in the XPC complementation group are deficient in GG-NER, but have normal TC-NER, whereas cell lines from all of the remaining XP complementation groups are characterized by a deficiency in both GG-NER and TC-NER. By contrast, cells from CS patients are selectively defective for TC-NER, but have intact GG-NER.

We found[32] that two Et743-resistant cell lines were deficient for XPG and systematically studied a panel of NER-deficient human cell lines. Whereas NER-deficient XPA and XPD fibroblasts are resistant to Et743 and complementation restores sensitivity, we found that GG-NER-deficient XPC cells are sensitive to Et743 and that complementation does not alter their response. These observations plus the fact that the TC-NER-deficient CS fibroblasts are also resistant to Et743 indicate that sensitivity to Et743 requires an intact and functional TC-NER pathway. The relative resistance of NER-deficient cells has also been reported by d'Incalci *et al.*[21] and Hurley *et*

The hypersensitivity of cells with intact TC-NER to Et743 suggests that the TC-NER is implicated in killing cells (Figure 3). Because TC-NER-proficient cells produce single-strand breaks, while cells with mutated TC-NER proteins make fewer or no breaks, we proposed that the Et743–guanine adducts trap TC-NER at the cleavage step. The two NER endonucleases are XPF/ERCC1 and XPG. XPG cleaves the adducted strand 3′ from the adduct, whereas the XPF/ERCC1 complex cleaves the adducted strand 5′ from the adduct[34] Thus, our current model for the generation of DNA single-strand breaks (summarized in Figure 3) is that the Et743 adducts trap transcription complexes and engage the TC-NER. The Cockayne proteins (CSA and CSB) would be implicated in initiating the TC-NER pathway and the binding of XPA, TFIIH and RPA, and ultimately the activation of the NER endonucleases. The presence of the Et743 adduct, however, would prevent the repair from going to completion and block the religation of the broken DNA. At this time, there is no direct evidence for how and which of the NER nucleases are trapped. However, recent biochemical studies with the bacterial homolog of the mammalian NER, the UvrABC system, are consistent with this model. Zewail-Foote *et al.*[33] showed that the most stable Et743 adducts

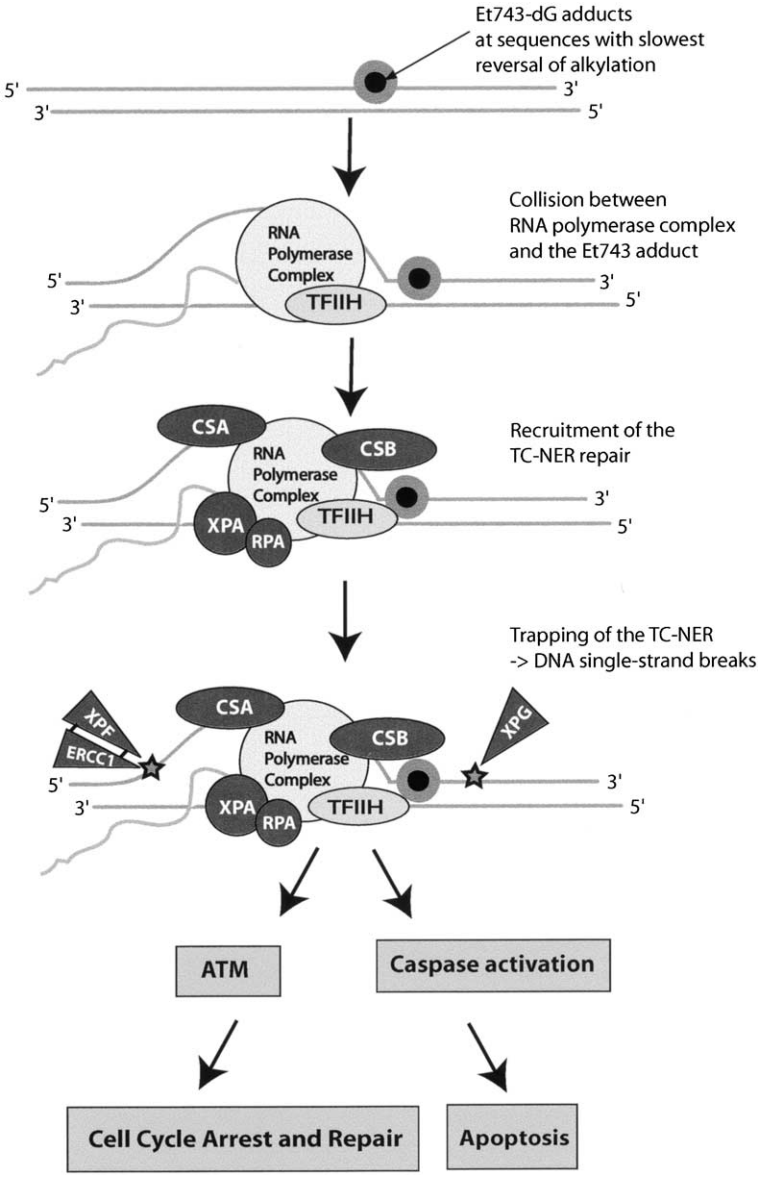


Figure 3. Proposed trapping of TC-NER by the Et743 adduct (gray and black circles) and cellular responses downstream from the DNA single-strand breaks (stars).

trap the UvrBC endonucleases after they have cleaved the DNA. The role of the C-ring, which protrudes from the DNA minor groove and is critical for Et743's biological activity is also intriguing. This portion of the Et743 molecule might bind to and interfere with the function of the NER complexes.

The TC-NER-dependent killing mechanism by the Et743 adducts is reminiscent of the mechanism of action of topoisomerase inhibitors. Topoisomerase inhibitors trap topoisomerase–DNA complexes in cleavage complexes, as Et743 probably does with the TC-NER complexes. Thus, in both cases, the drugs trap enzyme–DNA complexes by forming ternary complexes (drug–enzyme–DNA) and convert these enzymes (topoisomerases for topoisomerase inhibitors and TC-NER in the case of Et743) into cellular poisons.

Topoisomerase I Inhibition and chromatin alterations

While we were purifying nuclear proteins that bind to Et743–DNA adducts, we found topoisomerase I.[36] Et743 adducts can also induce the formation of topoisomerase I cleavage complexes both in cells and with recombinant topoisomerase I[5,36] and Et743 can induce protein-linked DNA breaks, which are a hallmark of topoisomerase I cleavage complexes.[19] Similarly, phthalascidin can trap topoisomerase I.[5] The trapping of topoisomerase I cleavage complexes by the Et743–guanine N^2 adducts is consistent with the effects of benzo[*a*]pyrene diol epoxide adducts, which will also bind to guanine N^2 in the minor groove and trap topoisomerase I both in biochemical systems and with recombinant topoisomerase I.[37] As topoisomerase I poisoning by Et743 is only observed at micromolar concentrations, which are much higher than the pharmacological concentrations required to kill cells, and because mammalian cells[19] and yeast cells[38] deficient for topoisomerase I remain sensitive to Et743, it appears that topoisomerase I poisoning is limited at pharmacological concentrations and is probably not a critical determinant for the antiproliferative activity of Et743 (Table 1).

Cellular and molecular determinants of cellular response downstream from DNA damage

Despite the recent work implicating TC-NER as the principal target of Et743, little is known about the transduction pathways linking the drug-induced DNA lesions discussed in the previous section with the cellular responses. Table 1 summarizes relevant published data using cell lines with known deficiencies along with some observations made in our laboratory as a starting point for elucidating the cellular events downstream of the DNA lesions. These observations can be divided into two distinct categories: (i) cellular responses that result in sensitivity or hypersensitivity to Et743 and (ii) cellular pathways that are not critical for Et743 activity. Figure 3 summarizes the possible pathways downstream from the Et743-induced DNA lesions.

Table 1. Molecular determinants for Et-743 sensitivity

Critical determinants	Phenotype	References
TC-NER (XPA, XPB, XPD, CSA and CSB)	resistance	32,33,38
ATM	sensitivity	38
Minor determinants	**Phenotype**	**References**
Topoisomerase I	small or none	19,36
P-gp	?	38,44
p53	none	19,21
DNA-PK	small or none	38, our unpublished observations
Mismatch repair	none	38
PARP	none	our unpublished observations
XRCC1	none	our unpublished observations
β-Polymerase	none	our unpublished observations

It is important to stress that defects in TC-NER render the cells resistant to Et743 and that, alternatively, we have observed that cells with enhanced NER, such as cisplatin-pretreated cells, are highly sensitive to Et743. Ataxia telangiectasia (ATM) cells have recently been noted to exhibit enhanced sensitivity to Et743,[38] indicating that ATM deficiency impairs the cellular response pathways. ATM is known to be activated by DNA breaks, and to facilitate survival after DNA lesions by promoting cell cycle arrest (checkpoint function) and DNA repair, and possibly regulating apoptosis.[39] The hypersensitivity of ATM cells is consistent with DNA being the primary target for Et743.

A recent report suggested that cells deficient in double-strand break repair are more sensitive to

Et743.[38] DNA-dependent protein kinase (DNA-PK)-deficient cells from human glioblastoma MO59J (DNA-PK$^-$) were found to be more sensitive than MO59K (DNA-PK$^+$) cells. However, since MO59J cells are also deficient for ATM, the difference could be due to ATM. Consistent with this possibility, we have seen little or no difference in Et743 sensitivity in MO59J cells complemented with DNA-PK (unpublished observation). Thus, the most likely response to Et743-induced DNA single-strand breaks is detection and transduction via ATM.

p53 stabilization is consistent with ATM activation. By Western blotting it has been shown that p53 protein levels increase in response to Et743 treatment, which is a response consistent with other DNA-damaging agents.[19] However, cell lines deficient in p53 retain sensitivity to Et743 and thus one can conclude that Et743 cytotoxicity is generally p53 independent.[19–21]

The processing and repair of the transcription-coupled single-strand breaks is not known. These breaks are persistent after drug removal.[19] Recently, we have looked at the importance of the XRCC1-dependent repair pathway, which repairs DNA single-strand breaks in association with several enzymes including poly(ADP-ribose)polymerase (PARP), polynucleotide kinase phosphatase (PNKP), β-polymerase and DNA ligase III.[40,41] However, no hypersensitivity to Et743 could be detected in cell growth assays with PARP-, β-polymerase- or XRCC1-knockout cells (unpublished).

The redundancy of the upstream pathways of the apoptotic response makes it difficult to speculate which pathway triggers programmed cell death in Et743-damaged cells. The timing of apoptosis seems to be slower than that seen with other DNA-damaging agents and this observation may be an important clue as to the identity of upstream events. Nevertheless, further studies are needed to more clearly identify the molecular pathways involved in the cellular response to Et743-induced strand breaks.

Cellular resistance

As indicated above, the two colon carcinoma cell lines that we selected for Et743 resistance had acquired XPG deficiency,[32] as shown by complementation experiments in both cell lines, sequencing of the XPG cDNA and Western blotting in the HCT116/ER5 cells.[32] It is intriguing that both cell lines had XPG deficiency when deficiency in any of the XP factors implicated in TC-NER leads to Et743 resistance.[20,32,38] In fact, XPG deficiency is not uncommon in primary human tumors[42] and it is intriguing why XPG might be inactivated more easily than other NER factors.

In our Et743-resistant cells, we did not observe overexpression of the multidrug resistance transporters and no cross-resistance to doxorubicin, Taxol or VP-16 (etoposide), which are well-characterized P-glycoprotein (P-gp)/MDR1 substrates.[32] Our results apparently contradict a report indicating that ovarian cell lines selected for resistance to Et743 overexpressed P-gp (MDR1), were cross-resistant to doxorubicin and VP-16 (etoposide), and that the cyclosporin analog, SDZ PSC-833, sensitized the resistant cells to Et743.[43] In a set of recent studies, we examined this question in more detail using well-characterized MDR-overexpressing cells and found no cross-resistance to Et743.[44] Furthermore, we confirmed that Et743 down-regulated MDR1 gene expression.[28,44] Therefore, there is no direct evidence that Et743 is a P-gp/MDR1 substrate.

Drug combinations

A recent study in several human sarcoma lines showed that Et743 is synergistic with both doxorubicin and Taxol,[45] and clinical trials of Et743 combined with doxorubicin or Taxol are being initiated. Down-regulation of the MDR1 gene may explain these findings. At a minimum, the case can be made for using Et743 in combination with chemotherapy agents that are pumped out by the P-gp receptor or simply in tumors that overexpress MDR1 *de novo*. Et743 was also recently reported to inhibit MDR1 transcription by blocking the steroid xenobiotic receptor (SXR), which is known to induce MDR1 transcription by interactions at the promoter level.[30] SXR also induces expression of the cytochrome P-450 enzyme, CYP3A4, in response to Taxol, which in turn facilitates its metabolic inactivation. Interestingly, Et743 was shown to block expression of CYP3A4 as well.[30] Collectively, these two observations provide further insight into the possible mechanism of the observed synergism with Taxol.

The inhibition of CYP3A4[30] by Et743 might also potentiate the activity of other drugs that are metabolized by CYP3A4 (e.g. carbamazepine, diazepam, cyclosporine, verapamil, macrolide antibiotics and HMG-CoA reductase inhibitors).

Combination with cisplatin would appear logical. Cells that are proficient for NER would be sensitive to

Et743, whereas cells that are NER-defective would be sensitive to cisplatin.[46] Also, cisplatin treatment might enhance NER, which would suggest that sequential treatment with cisplatin followed by Et743 would produce a synergistic effect. In one of the phase I clinical studies, one of the patients who responded to Et743 had a malignant mesothelioma refractory to cisplatin.

Clinical trials

Et743 is entering phase III clinical trials. Results of phase I/II studies are summarized briefly in Table 2 and a detailed review has recently been published elsewhere.[47] These clinical trials showed that Et743 has a remarkably distinct antitumor spectrum and can be safely administered at effective doses. The tumors presently evaluated in phase III are primarily soft tissue sarcoma and osteosarcomas, which showed responses in the phase I/II clinical trials. Notably the GIST (gastrointestinal stromal tumors), which have recently been reported to be a potential indication for the Bcr–Abl inhibitor, Gleevec, do not appear to respond to Et743.

The known properties of Et743 allow for the prediction of potential clinical scenarios in which the drug might prove effective. Et743 sensitivity is highly dependent on intact TC-NER, which makes tumors overexpressing proteins of that pathway a logical target. Also, it has been well characterized that one mechanism of cisplatin resistance in ovarian carcinoma patients at time of disease progression is enhanced NER. Consequently, either combination with cisplatin or use in refractory patients can be justified. The data coming out of early phase II is encouraging for two reasons. No severe life threatening toxicities have been noted to date and Et743 is active in a wide spectrum of tumors. These responses should not be downplayed, as most if not all of the patients had been heavily pretreated with other chemotherapy regimens in addition to the fact that Et743 was used as a single agent in each. In future studies these response rates will hopefully be improved as Et743 is used in logical combinations with other agents based on molecular observations made at the bench.

Conclusions

The poisoning of TC-NER by Et743 represents a unique mechanism of action. The advent of techniques to isolate and complement cell lines with well-defined genetic defects has proven critical to the development of our current understanding of Et743 activity. The studies done with Et743 illustrate how antitumor compounds can be screened to obtain a profile of response based on presence or absence of various molecular pathways. As our understanding of pathways governing cellular response to cytotoxic agents increases, similar experiments may prove useful as a relatively simple method to screen compounds with respect to their activity in different pathways, thus amplifying greatly the number of compounds available for investigation. The clinical translation is obvious, as correlation with available and obtainable data on the status of these same pathways in human tumors will lead to a more logical selection for testing of which tumors might show efficacy in *in vivo* studies. Indeed the current studies done with Et743 can be extrapolated in this manner. Because sensitivity requires intact TC-NER, one could speculate tumors overexpressing NER proteins may be a good clinical model in which to test the drug. Similarly, because of its ability to suppress MDR1 expression, use in combination with well-characterized chemotherapy drugs that are substrates for the P-gp receptor might prove useful. With greater understanding of the molecular and

Table 2. Antitumor spectrum and toxicities in phase I and II clinical trials

Phase	Number of studies	Toxicities	Responsive tumors	References
I	4	elevated transaminases, myelosuppression, neutropenia, thrombocytopenia, nausea/vomiting, fatigue	mesothelioma, osteosarcoma, leiomyosarcoma, liposarcoma, ovarian carcinoma	48–52
II	5	elevated transaminases, myelosuppression, neutropenia, thrombocyopenia, nausea/vomiting, fatigue	leiomyosarcoma, liposarcoma, synovial sarcoma, osteosarcoma, breast renal carcinoma	53–56

cellular effects of promising chemotherapy drugs in development will come the ability to provide more tumor selective therapy, more logical combination regimens and dosing schedules, and ultimately better response rates and fewer side effects.

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