ET-743: A Novel Agent with Activity in Soft Tissue Sarcomas

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LEARNING OBJECTIVES
After completing this course, the reader will be able to:
1. Describe the original mechanism of action of ET-743.
2. Explain the management of patients treated with ET-743, including what biological exams are needed because of toxicity and what is the optimal schedule of administration.
3. Choose the best histological subtype of sarcoma for treatment with ET-743 and describe the clinical aim of treatment.
4. Discuss putative combinations of ET-743 with other therapies.

ABSTRACT
Ecteinascidin-743 (ET-743) is a natural product derived from the marine tunicate Ectenascidia turbinata. ET-743 binds in the minor groove of DNA, blocks transcription factors activity, and traps protein from the nucleotide excision repair system, thus blocking cells in G2-M phase. ET-743 demonstrated cytotoxic activity at very low concentrations against sarcoma cell lines in preclinical studies. In several phase II clinical studies in patients with advanced sarcoma failing conventional doxorubicin- and ifosfamide-based chemotherapy, ET-743 delivered by continuous intravenous 24-hour infusion at a dose of 1,500 μg/m² every 21 days yielded 8% overall response and 30%–40% stabilization rates for a clinical benefit rate close to 40%. Interestingly, long-term stabilizations over more than 3 years have been described. In vivo, ET-743 has a specific toxicity profile, the major toxicity of this product being hepatic, through biliary duct destruction, and hematologic. ET-743 has also been evaluated in first-line treatment for these patients. Finally, due to its original mode of action and the lack of cross-resistance with other chemotherapy agents, ET-743 was tested in a preclinical model in combination with other drugs. Synergy was reported in vitro with doxorubicin and cisplatin; phase I combination studies are in progress. The Oncologist 2005;10:827–832

INTRODUCTION
Despite an optimal loco-regional treatment, 35%–50% of patients with sarcoma will develop metastasis. Systemic chemotherapy is then the standard treatment; the active drugs are doxorubicin and ifosfamide and, to a lesser extent, dacarbazine [1, 2]. Initial response rates to doxorubicin-containing regimens are close to 10%–30%, with few long-term survivors. In second line, after failure of doxoru-
ET-743 binds in the minor groove of DNA and alkylates N2 of guanines located either in the 5'-PuGC-3' or 5'PyrGG-3' sequence [3], which bends DNA toward the major groove [4]. Other alkylations are formed, but they are reversible and less stable [4]. Two of the three subunits of the drug bind to DNA while the third does not have contact with DNA and protrudes out from the minor groove, interfering with several DNA-binding factors. ET-743 strongly inhibits the binding of NF-Y [5], a factor that activates the CCAAT element present in 25% of eukaryotic promoters, including many promoters that regulate genes controlling the cell cycle. In vivo studies showed that the HSP70 promoter containing two CCAAT boxes activated by NF-Y is rapidly inhibited by ET-743 whereas other promoters lacking CCAAT boxes were not affected [6]. Importantly, the MDR1 (multidrug resistance) gene encoding for the P glycoprotein (P-gp) is also under dependence of a promoter containing CCAAT boxes [7]. This may explain why ET-743 is efficient against cells over-expressing MDR1 and why it does not select for the emergence of a P-gp phenotype in ET-743–resistant cell lines or exhibit cross-resistance with other cytotoxic agents [8]. Actually, ET-743 is a general inhibitor of cancer-activated transcription but not of “uninduced” (i.e., basal) transcription: Induction of the Sp1-regulated p21 gene by Trichostatin A (TSA), a promoter activator, was blocked by ET-743 at concentrations that had minimal effect on uninduced constitutive expression, and microarray analysis of cells treated with TSA and/or ET-743 indicated that activation of TSA-responsive promoters was blocked by ET-743 with little effect on nonresponsive promoters [9]. In addition, ET-743 at higher concentrations can alter the interaction between several DNA-binding proteins and DNA. (Two subunits of NF-Y exhibit homology with histones 2A and 2B.) ET-743 may target topoisomerase I, resulting in DNA breaks [10], although this may not be relevant in vivo because this effect is observed at high concentration and the drug remains active in cells deficient for topoisomerase I [11, 12].

ET-743 activity may also involve the DNA repair machinery. Defects in the mismatch repair pathway, usually associated with increased resistance to methylating agents and cisplatin, do not affect the cytotoxic activity of ET-743 [11]. DNA-dependent protein kinase may repair ET-743–induced damages because it is active in cells deficient in this enzyme [11]. Whereas all known DNA-interacting cytotoxic drugs are either more or equally active in nucleotide excision repair (NER)–deficient cells, ET-743 exerts decreased cytotoxic activity in NER-deficient cell lines [11, 13, 14]. Indeed, ET-743 interacts with the transcription-coupled NER machinery to induce lethal DNA strand breaks. The preferred binding sequences for ET-743 are less efficiently excised and trap DNA-NER proteins, forming cytotoxic complexes similar to a poisoned topoisomerase I- or topoisomerase II-DNA complex. In the absence of an intact NER nuclease complex, this toxic lesion does not occur, and the ET-743–DNA adducts, though not repaired by the NER pathway, are less toxic to cells [15]. Moreover, translesion synthesis and homologous recombinations lead to ET-743 resistance of NER-deficient cells [16]. p53 may activate apoptosis after ET-743–induced DNA damage because an increase of p53 is observed in cell lines expressing wild-type p53. However, p53 status does not appear to correlate to sensitivity to ET-743 [17]. Finally, the telomerase activity decreases the efficiency of ET-743, [18].

ET-743 blocks cells in G2-M phase. Indeed, cell lines exposed to ET-743 for 1 hour progress through S phase more slowly than control cells and then accumulate in the G2-M phase. The sensitivity to ET-743 of G1 synchronized cells was much higher than that of cells synchronized in S phase and even higher than that of cells synchronized in G2-M [13, 17]. Expression microarray experiments were used to identify genes involved in sensitivity or resistance to ET-743. A first study determined a set of 70 genes whose expression was modulated in drug-resistant cells [19]. Another study with a cDNA microarray containing 6,700 cancer-related genes showed upregulation of 86 genes and downregulation of 244 genes in response to ET-743 [20]. Immunohistochemistry revealed marked differences in the cytoskeleton architecture between ET-743–sensitive and –resistant cells, and collagen I seems to be an important protein [21].

ET-743, then, exerts its cytotoxic role through an original mode of action involving DNA repair machinery. Its potent cytotoxic activity on sarcoma cell lines prompted investigation of its activity in the clinic.

**Toxicity in Clinical Trials**

The most prominent toxicities observed in the different phase II studies were grade 3 or 4 transaminase increase (26%–59%, [22–26]) and neutropenia (33%–52%, [22–
Regarding liver toxicity, studies in rats showed a predominant biliary toxicity: Twenty-four hours after ET-743, liver degeneration and patchy focal necrosis of bile duct epithelial cells were observed and associated with mild inflammation followed by fibrosis.

Sporadic and focal zones of hepatic necrosis and hemorrhage were observed although the majority of hepatocytes appeared normal. Pathological alterations persisted up to 3 months [27]. In humans, patients with any baseline liver-function test exceeding the upper limit of the normal ranges have a significantly greater incidence of severe hepatic toxicity [28]. Levels of plasma liver enzymes (e.g., transaminases, bilirubin, alkaline phosphatases, and 5'-nucleotidase) should be checked before each course. Dexamethasone may decrease this toxicity [28]. Pretreatment, but not concomitant treatment, of rats with high dose of dexamethasone 24 hours before ET-743 improved or prevented ET-743–induced liver damages [29], probably through the induction of the cytochrome p450. Beta-naphthoflavone [30] and indole-3-carbinol [31] may protect liver as well. Granulocytic (colony-forming units granulocyte-macrophage [CFU-GM]) and megakaryocytic progenitors are sensitive to the drug [32], but the dose of ET-743 that inhibits 90% of CFU-GM only inhibited 45% survival of stem cells, resulting in the lack of long-term myelosuppression [33]. A specific toxicity for monocytes/macrophages may account for the anti-inflammatory properties of ET-743 [34].

**ET-743 as Monotherapy in Patients with Sarcoma**

Different preclinical and phase I studies determined the schedule of administration: The recommended schedule for ET-743 is delivered by continuous intravenous 24-hour infusion at the dose of 1,500 μg/m² every 3 weeks, until progression or toxicity. Table 1 gives a synthetic overview of the management of ET-743 in sarcoma.

A first multicentric phase II study enrolled 54 heavily pretreated patients. Before ET-743, 48% had received one or two drugs and 52% three or more; 41% had leiomyosarcoma (eight of 22 of uterine origin). Two partial responses (PRs) were observed, for an overall response (OR) rate of 4% (95% confidence interval [CI], 0.5%–12.8%). Four

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**Table 1. Summary for ecteinascidin-743 (ET-743) use**

| Indication | Sarcoma after failure of anthracyclin-based chemotherapy
| Best results with synovial sarcoma, leiomyosarcoma, and liposarcoma |
| Schedule | Continuous intravenous 24-hour infusion at the dose of 1,500 μg/m² every 3 weeks
| 20 mg intravenous dexamethasone 30 minutes before ET-743 |
| Pretherapeutic exams | Neutrophils ≥1,500/mm³ and platelets ≥100,000/mm³
| Normal creatinin
| Normal bilirubin
| Normal alkaline phosphatases: if elevation, normal 5’-nucleotidase is needed
| Transaminases <2.5 ULN |
| Surveillance | Hemogram and ionogram each week
| Transaminases, bilirubin, and alkaline phosphatases twice a week |
| Dose adaptation according to toxicity | Toxicity
| Bilirubin: grade ≥1:
| decrease dose of one level |
| Transaminasitis: grade 3–4, and normalization at grade 0–1 the first day of the next cycle:
| decrease dose of one level |
| Alkaline phosphatases:
| Grade ≥1:
| decrease dose of one level |
| Persistence or reappearance of a grade 1 toxicity: the same dose
| Appearance of a grade ≥2 after first reduction of dose: decrease dose of one level |
| Other toxicity: grade 3–4:
| decrease of one level |
| Dose adaptation
| First reduction: 1,200 μg/m²
| Second reduction: 1,000 μg/m² |
| Evaluation | CT scan every three cycles
| Complete response: continue for two more cycles
| Partial response or disease stabilization: continue treatment
| PD: stop treatment |
| Results | Overall response: 4%–8%
| Clinical benefit (overall response + disease stabilization): 29%–54%
| Overall survival: 9.2–12.8 months
| 6-month progression-free survival: 24%–29% |

Abbreviations: CT, computed tomography; PD, progressive disease; ULN, upper limit of normal.
minor responses and nine disease stabilizations (SDs) lasting more than 6 months were observed. Twenty-four percent of patients were free from progression at 6 months. The median survival was 12.8 months, with 30% of patients alive at 2 years. Two treatment-related deaths occurred [35].

The second study included 36 patients and reported one complete response (CR) and two PRs for a response rate of 8% (95% CI, 2%–23%), with a clinical benefit of 14%. Prolonged responses were observed (up to 20 months), and the 1-year overall survival was 53% (95% CI, 39%–73%), an unusual observation in this population of patients [22].

These results were confirmed by a large phase II study of the Soft Tissue and Bone Sarcoma Group of the European Organization for Research and Treatment of Cancer in 104 pretreated patients. Eight PRs were observed for an OR rate of 8%, and 45 SDs (longer than 6 months in 26% patients) were observed. A clinical benefit was observed for 56% of leiomyosarcoma and 61% of synovial sarcoma patients. The progression-free survival at 6 months was 29% [24]. The median duration of survival of 9.2 months was again unusually high in this cohort of heavily pretreated patients, especially considering the low response rate. The rates of objective regression and stable disease are similar in doxorubicin/ifosfamide chemosensitive and chemoresistant sarcomas. However, in this study, patients exhibiting a prolonged stable disease had an outcome similar to responding patients, supporting the relevance of the progression arrest rate (i.e., objective response + stable disease) as a prognostic parameter instead of objective response [36].

In a single institution experience, 89 patients (82 assessable) with advanced refractory sarcoma (leiomyosarcoma 36%, liposarcoma 18%, and osteosarcoma 16%) were treated with ET-743. The objective response rate was 7% (one CR, five PRs), and the clinical benefit rate at 3 and 6 months was 38% and 23%, respectively [26].

To decrease toxicity, a randomized phase II study of ET-743 given by two different dosing schedules (3-hour infusion weekly × 3 every 4 weeks versus 24-hour infusion every 3 weeks) in patients with leiomyosarcoma or liposarcoma (due to the observed better activity of ET-743 in these subtypes with respect to the other) refractory to conventional doxorubicin and ifosfamide chemotherapy was carried out. Objective responses and stable disease were observed in a subset of refractory patients with both treatment schedules, but response rate and progression-free survival were superior with 24-hour CIV (continuous infusion of vancomycin) treatment [37]. This trial has been extended, and final results are expected at the end of 2005. A comparative phase III trial of ET-743 and ifosfamide is under discussion in patients with an anthracycline-refractory uterine leiomyosarcoma.

After promising results in pretreated patients, ET-743 was tested in the first line of treatment of unresectable advanced sarcoma. Thirty-six patients (35 assessable) were treated with the standard schedule: One CR and five PRs were achieved for an OR rate of 17.1% (95% CI, 6.6%–33.6%). In addition, one patient had a minor response. The estimated 1-year progression-free and overall survival rates were 21% (95% CI, 11%–41%) and 72% (95% CI, 59%–88%), respectively [25]. ET-743 was ineffective in gastrointestinal stromal tumor [23] and in osteosarcoma [38], but responses were reported in Ewing sarcoma [39].

**ET-743 in Combination with Other Drugs**

Because of its original mechanism of action, ET-743 may act synergistically in combination with other cytotoxic agents. Several preclinical or phase I studies explored this possibility. Preclinical studies showed that ET-743 and cisplatin are synergetic, without additive toxicity [40]. Interestingly, ET-743 could be used at lower, relatively nontoxic doses to potentiate cytotoxicity of cisplatin. A phase I study was performed to determine ET-743 and cisplatin doses in combination regimens [41]. The combination with doxorubicin may be effective for tumors displaying low sensitivity to each drug given alone [42]. For tumor cells sensitive to both agents, additive effects are observed, whereas another study showed synergy [43]. The most favorable synergy in vitro was observed using a sequence with ET-743 first, followed by doxorubicin. Based on these findings, phase I studies on the combination of both drugs were initiated. Other combinations were tested with paclitaxel (showing a limited schedule dependent synergy) and with plassminogen-related protein B (an antiangiogenic factor) in preclinical models of chondrosarcoma [44]. No synergy was observed, however, between ET-743 and radiotherapy [45].

**Conclusion**

ET-743 has an original mode of action, involving enzymes of the DNA repair machinery. ET-743 has a demonstrated activity as first or second treatment of advanced sarcomas, after doxorubicin, ifosfamide, and dacarbazine. Some patients achieved very prolonged long-term survival. The utility of combination regimens, as well as the activity of this agent in other tumor types, in particular ovarian carcinoma, is currently under investigation.

**Disclosure of Potential Conflicts of Interest**

The authors indicate no potential conflicts of interest.
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