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ET-743

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Contents

Abstract

▲ ET-743 is a novel antineoplastic DNA-binding agent derived from the marine tunicate *Ecteinascidia turbinata*. It has significant cytotoxic activity against soft tissue sarcomas (STS). It also has *in vitro* activity against melanoma, breast, ovarian, colon, renal, non-small cell lung and prostate carcinomas.

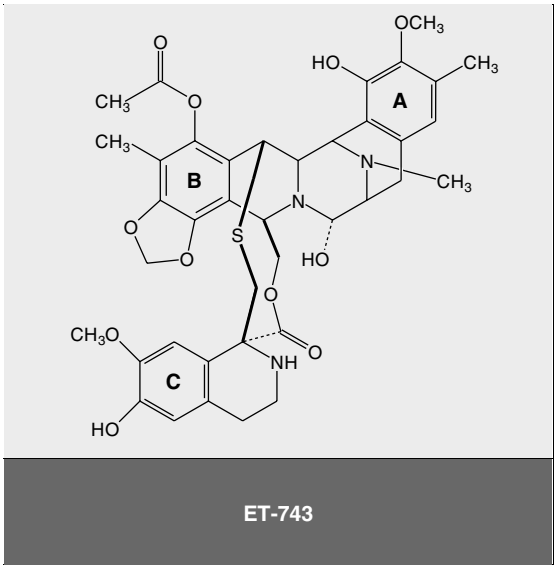
▲ The drug has unique mechanism of action which includes *in vitro* inhibition of transcription-dependent nucleotide excision repair pathways and inhibition of cell cycle progression leading to p53-independent apoptosis. It also selectively inhibits transcriptional activation of multidrug-resistance (MDR1) gene in human sarcoma cells *in vivo*.

▲ The efficacy of ET-743 has been investigated in patients with advanced STS in three multicentre phase II clinical trials. Patients receiving ET-743 as second- or third-line treatment had partial tumour response rates of 6 to 8%. Patients receiving ET-743 as first-line chemotherapy had a partial response rate of 18%. Forty-two to 50% of all patients in these trials achieved stable disease. All responses were durable up to 14 months.

▲ A pooled analysis of the three multicentre phase II trials showed the following: median overall survival time of 10.2 months, 1-year survival rate of 40% and 6-month progression-free rate of 27.2%.

▲ ET-743 is generally well tolerated. The most common adverse events in clinical trials were non-cumulative haematological and hepatic toxicities. Transient and reversible elevation of hepatic transaminases, nausea, vomiting and asthenia were common but seldom severe and never treatment-limiting. Mucositis, alopecia and cardiac or neurotoxicities were not observed.

Features and properties of ET-743 (ecteinascidin-743, NSC 648766)	
Indication	
Advanced soft tissue sarcoma	
Development status	
Advanced phase II clinical trials	
Mechanism of action	
Antineoplastic agent; binds to DNA in the minor groove and bends it towards the major groove	Inhibition of transcription-dependent nucleotide excision repair pathways; inhibition of cell cycle progression leading to p53-independent apoptosis
Dosage and administration	
Usual dosage in clinical trials	1500 μg/m^2
Route of administration	24-hour continuous intravenous infusion
Frequency of administration	Every 3 weeks
Pharmacokinetic profile (after single dose of 1500 μg/m^2)	
Peak plasma concentration	1.8 μg/L
Area under the plasma concentration-time curve	55 μg•h/L
Volume of distribution	>1200L
Metabolism	Hepatic (predominantly via cytochrome P450 enzymes)
Elimination half-life	45.6h
Adverse events	
Most frequent	Neutropenia, reversible elevation of alkaline phosphatase and hepatic transaminases, nausea, vomiting and asthenia
Treatment-limiting	Neutropenia, thrombocytopenia, anaemia and febrile neutropenia
Serious events (<2%)	Long-lasting pancytopenia, renal and hepatic failure and rhabdomyolysis



ET-743

Soft tissue sarcomas (STS) are one of the most difficult neoplasms to treat. Almost half of patients with STS succumb to their disease, usually with distant metastases and with median survival of 8 to 12 months from the time metastases are recognised.[1-3] Surgery alone is curative only in the early stages and for low-grade tumours.[1] The majority of chemotherapeutic agents have only marginal activity against STS,[1] with the most active agents (doxorubicin and ifosfamide) having objective response rates of approximately 20% as first-line treatment.[2-8] Their use is, however, limited by serious and potentially life-threatening toxicities[8] (although the recent development of pegylated liposomal doxorubicin offers a much improved safety profile[9]) and frequent development of tumour cell resistance.

ET-743 is the prototype of a novel class of antineoplastic DNA-binding agent with unique mechanisms of action. It is derived from the marine tunicate *Ecteinascidia turbinata*. Recently, the efficacy and tolerability of ET-743 have been evaluated in several phase II multicentre trials primarily in patients with advanced STS, most of whom had previously received chemotherapy.

1. Pharmacodynamic Profile

ET-743 is a chemotherapeutic agent that binds to the N^2 position of guanine[10,11] in the minor groove of the DNA double helix to bend the DNA molecule towards the major groove.[12,13] This is a unique feature distinguishing ET-743 from all currently available DNA-binding agents which cause structural perturbation of the DNA molecule by bending it towards the site of their interaction with DNA rather than away from it.

- ET-743 consists of three fused tetrahydroisoquinoline rings.[14] Two of these rings (subunits A and B) provide the framework for covalent interaction with the minor groove of the DNA double helix, while the third ring (subunit C) protrudes from the DNA duplex[11] and interacts with adjacent nuclear proteins accounting for the cytotoxicity of ET-743.[15]

- DNA repair capability appears to be a prerequisite for ET-743-mediated cytotoxicity.[16-18] The transcription-coupled nucleotide excision repair (TC-NER) involves recognition of DNA damage and recruitment of various nucleases at the site of DNA damage. At micromolar concentrations, ET-743 traps these nucleases in a malfunctioning nucleases-ET-743-DNA adduct complex, thereby inducing irreparable single-strand breaks in DNA.[16,17,19] Mammalian cell lines deficient in TC-NER showed resistance to ET-743.[17,18]

- *In vitro* exposure to clinically relevant (i.e. low nanomolar) concentrations of ET-743 induced strong perturbation of the cell cycle with delay of cell progression from G_1 to G_2 phase, an inhibition of DNA synthesis and a cell cycle arrest in G_2 phase in human colon carcinoma cell lines resulting in p53-independent apoptosis.[19-21] Furthermore, there is evidence that nanomolar concentrations of ET-743 cause promoter-selective inhibition of transcriptional activation of genes involved in cell proliferation (i.e. c-jun, c-fos).[22]

- ET-743 has consistently demonstrated *in vitro* activity against a wide range of solid tumour cell lines, including STS, melanoma, breast, ovarian, colon, renal, non-small cell lung and prostate car-

cinomas,[23-31] and human tumour explants.[32] These results have been supported by *in vivo* xenograft studies.[33-35]

• An *in vitro* study of cytotoxic effects in eight human STS cell lines showed that ET-743 is far more potent (up to 6 log units) than the standard anticancer agents (i.e. doxorubicin, methotrexate, etoposide, paclitaxel) with a 50% inhibitory concentration in the picomolar range. Cytotoxicity of ET-743 was concentration- and exposure time-dependent but independent of cell status regarding tumour suppression proteins p53 and pRb. In the same study, STS cell lines were also 1 to 4 log units more sensitive to ET-743 than colon adenocarcinoma and breast carcinoma cell lines.[36]

• The induction of MDR1 gene expression in tumour cells represents a major obstacle to successful chemotherapy.[37,38] DNA-damaging drugs (e.g. doxorubicin) can cause rapid induction of MDR1 gene expression in human sarcoma cells *in vivo*.[39] Addition of ET-743 to STS cell lines for 24 hours before doxorubicin prevents MDR1 gene expression and results in a synergistic cytotoxic effect.[40] To date, ET-743 is the only cytotoxic drug found to selectively inhibit activation of the MDR1 gene transcription.[20]

2. Pharmacokinetic Profile

The pharmacokinetics of ET-743 have been investigated in patients with solid tumours in five phase I clinical trials using different intravenous infusion schedules (administered over 1, 3, 24 or 72 hours, or administered over 1 hour on 5 consecutive days; all regimens repeated every 3 weeks) and for doses ranging from 6 to 1800 μg/m^2.[41-46]

• For 3- and 24-hour infusion schedules of ET-743, peak plasma drug concentration (C_{max}) and area under the plasma concentration-time curve (AUC_∞) were proportional to the administered dose.[44,46] Non-linearity of pharmacokinetics was found when the drug was administered over 1 and 72 hours.[45,47,48]

• A study in 25 patients with solid tumours who received a single 24-hour intravenous infusion of ET-743 1500 μg/m^2 reported mean (± SD) C_{max} and AUC_∞ values of 1.8 ± 1.1 μg/L and 55 ± 25 μg•h/L, respectively.[44]

• ET-743 showed considerable interpatient variability of AUC_∞ and of total body clearance (CL) at all dose levels. After administration of a single 1500 μg/m^2 dose of ET-743 over 24 hours, coefficient of variation (r) for AUC_∞ was 45% and mean CL ranged from 28 to 90 L/h.[44] However, the intrapatient variability of AUC_∞ between the first and second course of therapy was relatively small (r = 28%).[44]

• ET-743 is extensively (>95%) bound to plasma proteins and has a high degree of tissue binding.[47] The drug has a large volume of distribution at steady state (V_{ss}), which has been estimated at >1000 L/m^2[43,47] or >1200 L.[44]

• There is evidence from rat and human liver microsomal studies that ET-743 undergoes extensive oxidative metabolism by cytochrome P450 (CYP) 3A4.[49,50] It also appears to undergo metabolism by CYP 2C9/10, 2E1 and 2D6, but not by 2C8.[48] Less than 2% of the administered dose is excreted unchanged in the urine.[47,48] Biliary excretion of intact drug also appears to be limited.[47] The identification of metabolites of ET-743 in humans is ongoing.[51]

• The pharmacokinetic profile of ET-743 after a 24-hour infusion is best described by a 2-compartment model with median distribution and elimination half-lives of 0.5 and 45.6 hours, respectively.[48] Other data also indicate that ET-743 has a terminal half-life of >40 hours at all clinically relevant doses.[44]

• The dose, C_{max} and AUC_∞ of ET-743 were found to be significantly correlated with percentage reduction in white cell count and absolute neutrophil count, whereas elevation of transaminases (ALT, AST) and percentage decrease in platelet count showed positive correlation only with AUC_∞ values.[44,48]

• For the 24-hour infusion schedule over the dose range of 50 to 1800 μg/m^2 no significant relationship was observed between pharmacokinetic pa-

rameters tested (C_{max}, AUC_∞, V_{ss}, CL, $t_{1/2}$) and demographic factors (age, sex, bodyweight, body surface area),[44] renal function (serum creatinine, creatinine clearance),[44] total protein levels[44] or the tumour type.[47] The presence of liver metastases did not alter the CL of ET-743.[44]

• The risk of dose-limiting toxicity can be significantly reduced while maintaining the clinical response rate by keeping the AUC_∞ of ET-743 below the threshold value of 70 μg • h/L[47,48] (as identified by multivariate analysis of phase I trials).

3. Therapeutic Trials

The efficacy of ET-743 in patients with advanced STS has been investigated in three non-randomised multicentre phase II clinical trials[52-54] using a protocol (i.e. 1500 μg/m^2 administered every 3 weeks by 24-hour continuous intravenous infusion) derived from phase I clinical trials. The majority of patients had received previous chemotherapy (e.g. anthracyclines) but the efficacy of ET-743 as first-line treatment has also been evaluated in two of these trials.[52,53] The main histological subtypes of STS encountered in pretreated patients were leiomyosarcoma (41%), liposarcoma (14%) and synoviosarcoma (11%).[55]

The most recent results from the three phase II clinical trials are currently available as abstracts[52-54,56,57] and as a pooled analysis.[55] Partial responses, stable disease and disease progression, where specified,[53,57] were defined according to WHO[58] or WHO and RECIST[59] criteria.

• In one phase II clinical trial conducted in patients with advanced STS receiving ET-743 as second- or third-line treatment, three of 36 (8%) patients had a partial tumour response. The number of patients whose disease was stabilised or had progressed was not reported in this abstract.[52]

• In the other two studies, partial tumour responses were achieved in 8 of 99 (8%)[53] and 3 of 52 (6%)[54] of pretreated patients receiving ET-743. Minor responses (25 to 50% reduction in tumour size) were observed in additional four (8%) patients in the latter study.[54] Disease was stabilised in 50[53] and 42%[54] of patients, while the progression of STS was reported in 33%[53] and 44%[54] of patients, in each study respectively.

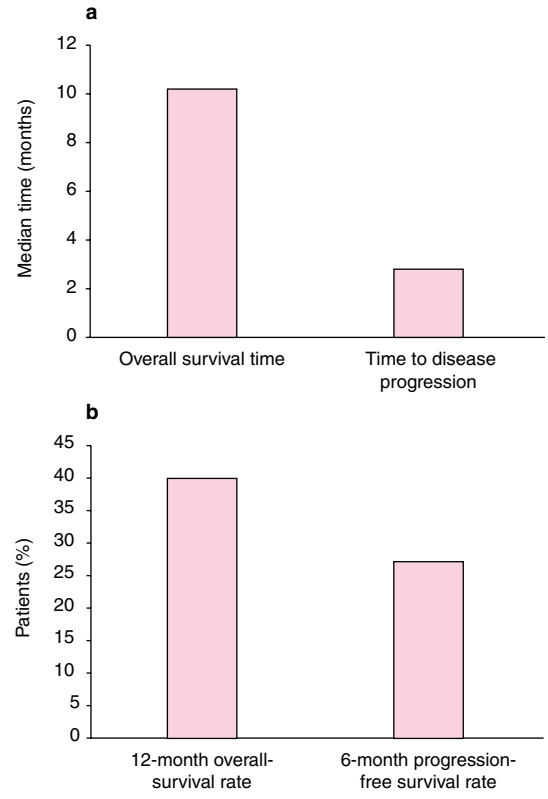


Fig. 1. Effects of ET-743 therapy on (**a**) median time to disease progression and overall survival time and on (**b**) overall survival rate at 12 months and disease progression-free survival rate at 6 months in 127 patients with advanced soft tissue sarcoma. Patients received 1500 μg/m^2 of ET-743 as a 24-hour intravenous infusion every 3 weeks (data from the pooled analysis of three phase II clinical trials).[55]

• In patients who had received prior chemotherapy, 6-month progression-free survival (PFS) rates ranged from 24 to 31%.[53,54] A 1-year PFS rate of 11% was reported in a third study.[52] The overall survival (OS) rates at 1 year for pretreated patients were in the range of 25 to 55%.[52-54]

• In patients with STS receiving ET-743 as first-line chemotherapy, the partial response rate was

18% (6 of 34 patients).[52] At 1 year, PFS and OS rates for first-line treatment with ET-743 were 18 and 49%, respectively.[52] A study conducted in 28 patients with advanced gastrointestinal stromal tumours (a type of STS[60]) reported no objective response to first-line treatment with ET-743.[56]

• All responses have been durable up to 13 months[54] (pretreated patients only) or 14 months[52] (both pretreated and previously untreated patients).

• Results from 127 evaluable patients (median age 51 years and performance status 0 to 1) from the pooled analysis of the three multicentre phase II clinical trials showed the following: median time to disease progression of 2.8 months, median overall survival time of 10.2 months, 1-year OS rate of 40% and 6-month PFS rate of 27.2% (figure 1). Tumour response (partial and minor) or stable disease was reported in 57% of patients (figure 2).[55]

4. Tolerability

• In clinical trials, ET-743 was generally well tolerated with non-cumulative haematological and hepatic toxicities being the most commonly reported adverse events.[52-54]

• The most common adverse event was grade 3 to 4 neutropenia, reported in 28 to approximately 60% of patients with advanced STS receiving ET-743.[52-54] A pooled analysis of these studies reported grade 4 neutropenia in 14%, grade 3 to 4 thrombocytopenia in 12%, grade 3 to 4 anaemia in 18% and grade 1 to 2 hyperbilirubinaemia in 9% of 91 evaluable patients (figure 3).[55] Neutropenia and thrombocytopenia were reported to be dose-limiting.[54]

• Dose-related, asymptomatic and reversible elevation of hepatic transaminases was prevalent (40% grade 3 to 4 ALT elevation)[55] but was not a treatment-limiting toxicity (figure 3).[54] Grade 1 to 2 elevation of alkaline phosphatase (ALP) level was reported in 46% of patients receiving treatment (figure 3).[55]

• Nausea (30% grade 2 to 3), vomiting (7% grade 3 to 4) and asthenia (30% grade 2 to 3) were seldom severe (figure 3.[55] Mucositis, alopecia and cardiac or neurotoxicities were not observed.[54]

• The most severe adverse events associated with ET-743 at the recommended dose included long-lasting pancytopenia, renal and hepatic failure and rhabdomyolysis. These were encountered in 6 of 331 patients (1.81%) and were responsible for three deaths.[61]

• A multivariate analysis of data from phase I trials identified baseline and drug-induced cholestases as risk factors for severe toxicity. This led to a protocol amendment[61] requiring normal ALP level at study inclusion and a dose reduction of ET-743 to 1200 μg/m^2 in case of either bilirubin or ALP intercycle peak. In subsequent phase II clinical trials the therapeutic index of ET-743 was improved and drug-related mortality rate was reduced from 1.6% (2 of 124 patients) to 0.8% (2 of 255 patients).[55]

5. Dosage and Administration

There are currently no formal dosage recommendations for the use of ET-743 in the treatment of patients with STS. However, in clinical trials the

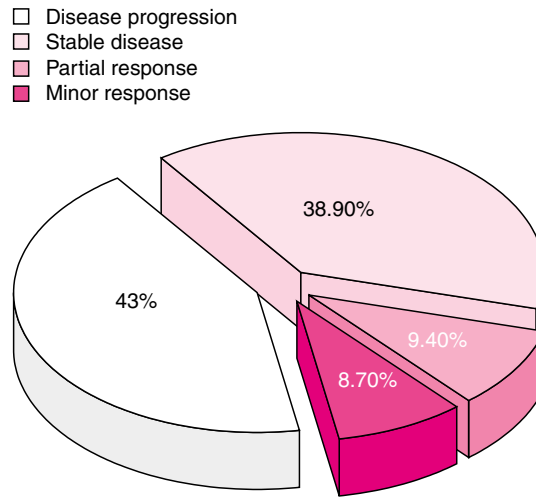


Fig. 2. Tumour response rates in 127 patients with advanced soft tissue sarcoma treated with ET-743. Patients received 1500 μg/m^2 of ET-743 as a 24-hour intravenous infusion every 3 weeks (data from the pooled analysis of three phase II clinical trials).[55]

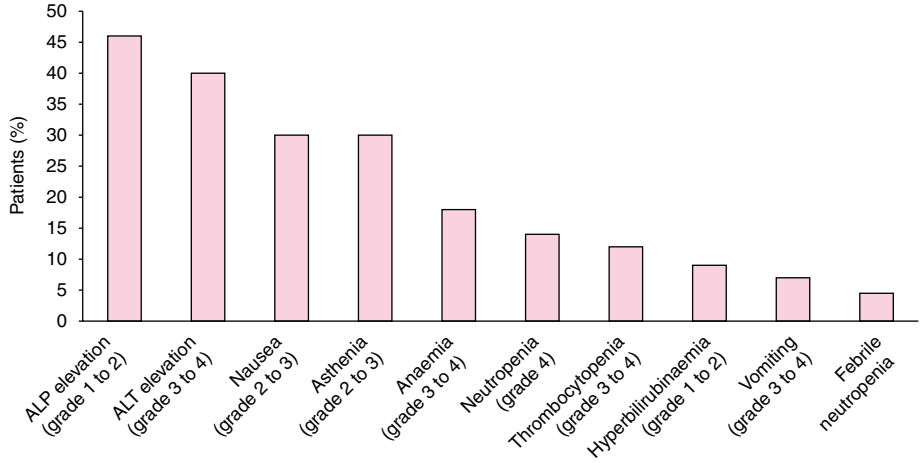


Fig. 3. Tolerability profile of ET-743. Overall incidence of adverse events reported at frequencies >2% in three phase II clinical trials (data from the pooled analysis) in 91 evaluable patients with advanced soft tissue sarcoma receiving ET-743 as a 24-hour intravenous infusion of 1500 μg/m^2 every 3 weeks [the dose of ET-743 was reduced to 1200 μg/m^2 if either bilirubin or alkaline phosphatase (ALP) intercycle peak occurred].[55]

most commonly used dose was 1500 μg/m^2 of ET-743 administered as a continuous intravenous infusion over 24 hours. This regimen was then repeated every three weeks. In case of either bilirubin or ALP intercycle peak during phase II clinical trials, the dose of ET-743 was reduced to 1200 μg/m^2.

6. ET-743: Current Status

Data from phase II multicentre trials indicate that ET-743 is generally well tolerated and effective in either previously treated or untreated patients with advanced STS. A randomised phase III clinical study is planned to assess the survival benefit associated with ET-743 therapy.[52] A dose-fractionation phase I study is currently evaluating the tolerability of ET-743 administered weekly in an effort to improve its therapeutic index.[62]

In addition, the efficacy of ET-743 for the treatment of advanced breast cancer is currently being investigated in a phase II clinical trial.[63,64]

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