



## TRABECTEDIN: A NOVEL MOLECULAR THERAPEUTIC IN CANCER

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## ABSTRACT

Nature has been instrumental as a source for therapeutics. Despite the fact that we live in an oceanic planet, a number of technical factors have historically hampered the evolution of a marine-based chamanic medicine. With the advancements in the development and discovery of new techniques and tools for the isolation and elucidation of structures of natural products from marine organisms, major advances have been made in the discovery of marine derived therapeutics. The availability of ARA-C, a nucleoside analog that is a basic component in the treatment of acute myeloid leukemia, and its fluorinated analog Gemcitabine, an important therapeutic tool in the treatment of pancreatic cancer and in non small cell lung cancer, is a solid proof and validation of the potential of this approach. As a result of discovery and developmental program, an innovative compound with novel mechanisms of action: ET-743 has been shown to display a positive therapeutic index and activity in resistant solid tumors as well as sarcomas and pretreated ovarian cancer.

**Keywords:** Trabectedin, sarcoma, ET-743, Yondelis

## INTRODUCTION

Nature has always been a highly productive source for the development of anticancer therapies. Renewed interest in the potential of this tool has recently been sparked by the realization that the marine ecosystem can be used for the discovery and development of new compounds with clinical potential in advanced resistant tumors. Trabectedin (ET- 743, Yondelis®) and PM00104 (Zalypsis®) are marine derived compounds that have antitumor activity. Trabectedin (ET-743, Yondelis), derived from the marine tunicate *Ecteinascidia turbinata*, was approved for clinical use in 2007. It binds to the DNA minor groove leading to interferences with the intracellular transcription pathways and DNA-repair proteins. *In vitro* antitumor activity was demonstrated against various cancer cell lines and soft tissue sarcoma cell lines. In phase I studies tumor responses were observed also in osteosarcomas and different soft tissue sarcoma subtypes. The most common toxicities were

myelosuppression and transient elevation of liver function tests, which could be reduced by dexamethasone premedication. Combinations with other agents are currently studied with promising results. In summary Trabectedin is an active new chemotherapeutic agent that has demonstrated its role in the treatments for patients with sarcomas<sup>1</sup>.

**Trabectedin: Molecular mechanism****ET-743: Mechanisms of Action**

Trabectedin Ecteinascidin-743 (Ecteinascidin-743, Yondelis™, Pharma Mar/Johnson & Johnson) is a tetrahydroisoquinoline alkaloid derived from a Caribbean tunicate *Ecteinascidia turbinata*<sup>1</sup>. ET-743 is a chemotherapeutic agent (Alkylating drugs ) that bind to DNA and disrupt its function. It has shown *in vivo* activity in nude mice harbouring human resistant xenografted tumors<sup>2</sup>. (Figures 1 and 2).



Fig. 1: Ecteinascidia turbinata, the sea squirt, growing in its natural habitat

Ecteinascidin 743 is a substance isolated from a small marine animal called sea squirt living on mangrove roots in the Caribbean Sea. ET-743 has a unique method of action that differs from other alkylating agents. It binds the DNA helix along the minor groove and causes the DNA structure to be bent<sup>3</sup>. ET-743 attaches to specific sequences of

DNA that contain guanine, one of the building blocks of DNA<sup>4</sup>. This blocks the cell from completing the process of cell division<sup>5</sup>, disrupts the organization and assembly of the cells skeleton<sup>6</sup>, and inhibits the function of topoisomerase I, an enzyme important in the process of DNA replication<sup>7</sup>.

## Ecteinascidin 743

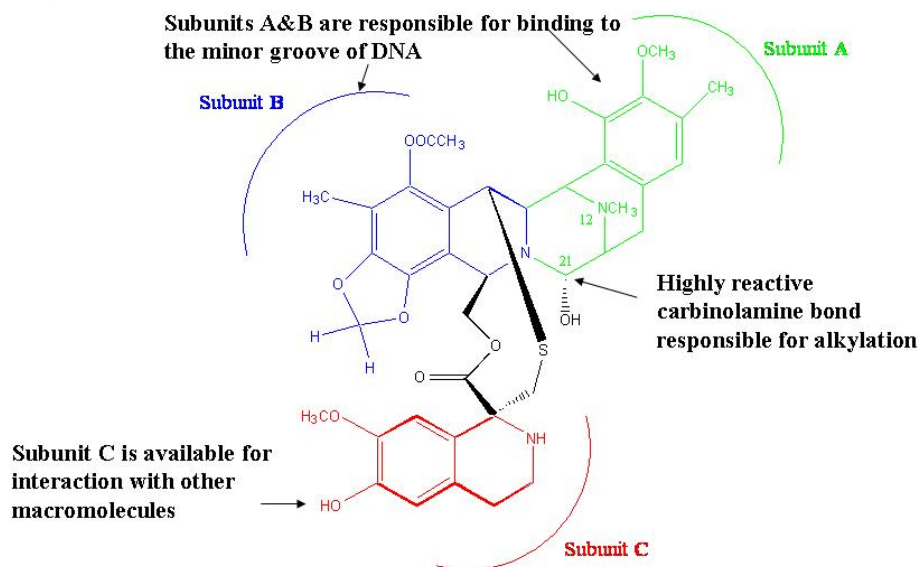


Fig. 2: Chemical structure of ET-743

There are 3 subunits of the molecule labeled A, B, and C important for how the drug works. Subunits A and B are responsible for binding to DNA. The binding of ET-743 to DNA changes the normal structure of the molecule, causing it to bend. In addition, the carbinolamine group functions to alkylate the DNA, which is the substitution of one chemical group for an active hydrogen atom in the DNA. When such a substitution occurs, DNA strands are cross-linked and can not replicate, thus causing the cell to die. Subunit C interacts with other molecules, such as transcription factors. ET-743 also interferes with the normal binding of proteins to DNA that function to activate genes<sup>8</sup>. The MDR1 gene is one of the genes that ET-743 can inhibit the activation. When MDR1 is activated, it expresses a protein that pumps some types of chemotherapeutic drugs out of cancer cells, decreasing the ability of these drugs to kill the cancer cell. When the MDR1 pump is present in high amounts it leads to resistance to chemotherapy<sup>9</sup>, and therefore ET-743 is choice of molecule which prevents the development of chemotherapy resistance because it causes less MDR1 protein to be made. This is supported by experiments that showed that ET-743 can increase the levels of doxorubicin and vincristine in cells with over-expression of MDR1 and by the ability of ET-743 to decrease the amount of MDR1 in a cell line<sup>10</sup>. However, in another study MDR1 was suggested to be one of the ways in which cancer cells become resistant to ET-743<sup>11</sup>. Further testing has found that not all ET-743 resistant cell lines have increased levels of MDR1 and not all cell lines with increased levels of MDR1 are resistant to ET-743. A chondrosarcoma cell line made resistant to ET-743 was found to have changes in its skeleton structure and to make less collagen compared to the parent cell line<sup>12</sup>.

Surprisingly, tumor cell lines that do not have the ability to repair damage to DNA are resistant to the effects of ET-743. Researchers have proposed that the binding of ET-743 in normal cells traps proteins responsible for this process. The structure formed not only inhibits the normal repair process but without the trapped proteins, ET-743 is less toxic to the cell<sup>13,14</sup>. Micro array studies, a method to determine differences in gene expression between two DNA samples, have shown that following treatment with ET-743, cells will have changes in genes involved with DNA damage response, transcription and signal transduction<sup>15</sup>. Furthermore, ecteinascidin 743 might induce a decrease in the speed of cell cycle multiplication and eventually a cell cycle arrest, thereby preventing cancer cells to grow or at least slowing down their growth.

Although much is known, there is not a complete understanding at this time of all the effects of ET-743 and how it causes cancer cells to die.

### Treatment

Surgery is currently the main choice of therapy for early stage soft tissue sarcomas. For larger sarcomas, and where it is thought there is a possibility of cancer cells being left behind, after a surgical intervention, radiotherapy (using high-dose x-rays or other high-energy rays to kill cancer cells) is usually used as well as surgery. Once the tumour has progressed beyond possible surgery, chemotherapy (using drugs to kill cancer cells) remains the treatment of choice.

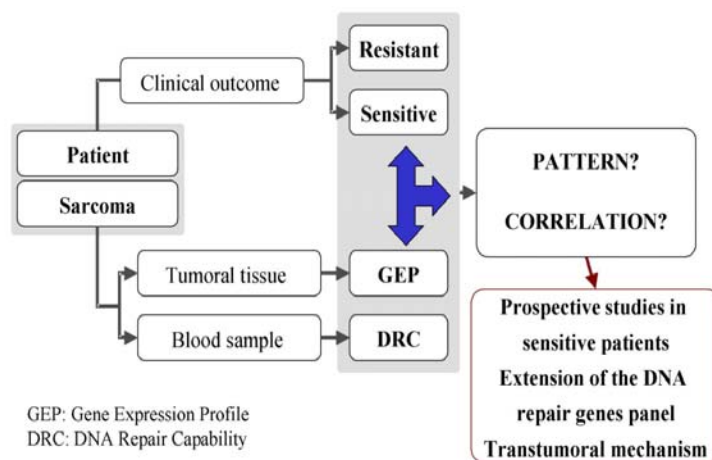
Yondelis™ is active in patients with advanced soft tissue sarcoma that is resistant or has relapsed after conventional therapies, with evidence of long-lasting objective responses and tumor control in 22% of cases<sup>16,17,18</sup>. No significant differences were observed in response to Yondelis™ between chemosensitive and chemoresistant patients. This finding, together with the large differences in survival time observed between responders and non-responders to Yondelis™, may indicate a differential molecular signature that correlates with clinical outcome to Yondelis™, at least in sarcoma patients. Such clinical evidence provided a rationale to look for a potential molecular predictive marker for response to Yondelis™<sup>19</sup>.

The impact of DNA repair capacity on treatment outcome with Yondelis™ is not limited to sarcoma but is applicable to other tumors as well. For this reason, pharmacogenomic studies in other tumors that are highly sensitive to Yondelis™, such as relapsed ovarian cancer<sup>20</sup>, are strongly encouraged.

### Clinical aspects

The clinical development program supporting the efficacy of trabectedin in the treatment of STS

includes one ongoing pivotal, randomised, unblinded, dose-finding, Phase-II study (ET743-ST5-201) and 3 completed Phase II, non-controlled studies, ET-B-005-98, ET-B-008-98, and ET-B-017-99. The original Phase II studies included a variety of different histological types of STS, whereas the randomised, pivotal Phase II study was restricted to patients with L-sarcoma (liposarcoma and leiomyosarcoma).



**Fig. 3: Strategy of the proposed pharmacogenomic program of Yondelis™ in sarcoma patients: Gene expression profiles of tumor samples from sarcoma patients treated with Yondelis will be retrospectively analyzed and correlated with their clinical outcome. The DNA repair capability will be analyzed in blood samples of the same patients in order to be used as surrogate marker of response. The putative correlation found between GEP and clinical outcome will be prospectively analyzed in sarcoma patients and further studied in other tumors.**

#### Chemical, pharmaceutical and biological aspects

Yondelis is a powder for concentrate for solution for infusion containing 0.25 mg and 1 mg of

trabectedin per vial. Each vial is reconstituted with 5 ml and 20 ml of sterile water for injections,

respectively. The reconstituted solution is a clear, colourless or slightly yellowish solution, essentially free of visible particles. The solution obtained has a concentration of 0.05 mg/ml and is for single-use only. Yondelis is supplied in either 10 ml or 25 ml Type I colorless glass vial with a grey bromobutyl rubber stopper with fluoropolymer coating. The stopper is sealed to the vial with an aluminum flip-off seal. The excipients used in the preparation of Yondelis are sucrose, potassium dihydrogen phosphate,

Phosphoric acid and potassium hydroxide (for pH-adjustment), and water for injections.

#### Active Substance

The active substance trabectedin is a chemical entity initially obtained by isolation from the

marine tunicate *Ecteinascidia turbinata* by extraction and purification. A synthetic process was

Subsequently developed and the active substance is since then produced synthetically. The synthetic route starts from the secondary metabolite safracin-B, isolated from fermentation media of *Pseudomonas fluorescens*. The Active Substance Master File (ASMF) procedure was followed for the active substance.

Trabectedin is a white to off-white powder, soluble in polar organic solvents. Trabectedin is insoluble in hydrocarbons. In aqueous

media, trabectedin is practically insoluble in water but solubility increases at acidic pH.

#### Drug Product

The aim of the pharmaceutical development was to obtain a formulation of trabectedin, which could be administered by intravenous infusion after appropriate dilution. The intended route of administration precluded the use of several excipients, such as stabilizers and non-aqueous solvents. Therefore, a simple aqueous solution was considered the most appropriate dosage form. Initially a formulation based in mannitol and phosphate buffer solution was designed. Further pharmaceutical development led to an improved sucrose-based formulation using synthetic trabectedin in phosphate. Buffer that was selected for scale up and commercialization, that allows the storage of the product at 2- 8°C.

#### Manufacture of the Product

The manufacturing process for trabectedin powder for concentrate for solution for infusion is carried out under aseptic conditions in restricted areas and comprises: (1) sterilization and depyrogenation of primary packaging materials and equipment that comes into contact with the product (2) preparation of the bulk solution (3) sterilising filtrations (4) filling (5) lyophilization and stoppering (6) final packaging.

#### Stability of the Product

Stability data was provided on batches manufactured at both sites and stored at 5°C ± 3°C (long term conditions) for either 18 or 24 months, and under accelerated conditions (25°C ± 2°C / 60% RH ± 5% RH) for 6 months.

Trabectedin was tested for antitumour activity in a number of sarcoma xenografts of murine and human origin (Table 1).

**Table 1: Anti tumor effect in sarcoma xenografts of murine and human origin**

Tumor origin	Cell line	Route and schedule	Dose(mg/m <sup>2</sup> /day)	Tumor growth inhibition (%)	References
Mouse fibrosarcoma	UV2237	IV qdx1	0.6	63	24
Mouse ovarian reticulosarcoma	M 5076	IV q7dx2	0.45	64	34
Human chondrosarcoma	CHSA	IV qdx2	0.3	76	27
Human osteosarcoma	OSA-FH	IV qdx5	0.12	67	-
Human rhabdomyosarcoma	TE-671	IV qdx1	0.6	33	34

*In vivo*, trabectedin had significant antitumour effects in five sarcoma xenograft models of murine or human origin, causing 33-76% tumour growth inhibition at dose levels between 0.12-0.6 mg/m<sup>2</sup>/treatment.

### Toxicology

Myelosuppression and hepatotoxicity were identified as the primary toxicity for trabectedin. Findings observed included hematopoietic toxicity (severe leucopenia, anemia, and lymphoid and bone marrow depletion) as well as increases in liver function tests, hepatocellular degeneration, intestinal epithelial necrosis, and severe local reactions at the injection site. Trabectedin is genotoxic both *in vitro* and *in vivo*. Fertility studies with trabectedin were not performed but limited histopathological changes were observed in the gonads in the repeat dose toxicity studies. Considering the nature of the compound (Cytotoxic and mutagenic), it is likely to affect the reproductive capacity. Trabectedin, like other cytotoxic agents, is a highly toxic drug requiring careful monitoring of the target organs of toxicity such as the liver, blood forming organs and gastro-intestinal tract. Trabectedin was a strong irritant causing severe injection site inflammation, necrosis and fibrosis in all IV toxicity studies, irrespective of species, as well as in a conventional rabbit test for local tolerance using single and repeated IV and perivenous dose administration

The early studies of ET-743 were complicated by severe toxicities including neutropenia and transaminitis, but in addition cases of rhabdomyolysis. Several of episodes of rhabdomyolysis were seen in conjunction with sepsis resulting in death.

### Pre-clinical studies of ET-743 in Sarcomas

ET-743 was tested in many bony and soft tissue sarcoma cell lines before clinical testing<sup>21,22</sup>, which is unusual in drug development. Li and colleagues tested ET-743 in human fibrosarcoma, mesenchymal chondrosarcoma, liposarcoma, hemangiopericytoma, mixed mesodermal and malignant fibrous histiocytoma (MFH) cell lines. In this study the fibrosarcoma and MFH cell lines were the most sensitive to the drug, which is of interest as fibrosarcomas are clinically not very sensitive to chemotherapy. ET-743 was found to be more effective than other agents used in sarcoma therapy such as methotrexate, doxorubicin, etoposide and paclitaxel. The presence of commonly altered proteins that may decrease response to chemotherapy such as p53, pRb, and overexpression of MDM2 were not found to affect the therapeutic benefit of ET-743. In addition, ET-743 did not affect the levels of proteins that can increase cell survival. This same group studied the effects of ET-743 in conjunction with doxorubicin, trimetrexate, or paclitaxel on fibrosarcoma, liposarcoma and breast cancer cell lines<sup>23,24</sup>. They determined that the combination of ET-743 with doxorubicin lead to enhanced activity. The sequencing of drugs was also important with greater activity seen when ET-743 was given before doxorubicin or paclitaxel. The combination of ET-743 with trimetrexate lead to decreased activity compared to the effects of either drug alone. Additional studies testing the combination of doxorubicin with ET-743 in rhabdomyosarcoma and fibrosarcoma cell lines found the combination to be more effective than either agent alone<sup>25</sup>. In particular, the combination of the drugs when given simultaneously was shown to have significant activity in a fibrosarcoma cell line resistant to doxorubicin. In addition, ET-743 has been studied in combination with cisplatin, a drug commonly used in osteosarcomas<sup>26</sup>. In a variety of cell lines, including a rhabdomyosarcoma cell line, the combination of ET-743 with cisplatin was more effective than either agent alone. Of particular interest to this audience is the work by Scotlandi and colleagues that evaluated ET-743 in Ewing's sarcoma and osteosarcoma cell lines. ET-743 was found to have effects against bone sarcoma cell lines as well as some cell lines made resistant to methotrexate, cisplatin, or adriamycin. *In vitro* treatment of Ewing's sarcoma cell lines to ET-743 for 24-72 hours caused cell death. Lastly, the combination of ET-743 with a novel antiangiogenic agent lead to increased death of tumor cells and fewer blood vessels in a chondrosarcoma model<sup>27</sup>.

### Phase I Clinical Trials

The primary purpose of a Phase I clinical trial is to determine the maximum tolerated dose that can be given safely without severe side effects. Phase I studies of ET-743 have tested a variety of schedules. The first phase I study assessed evaluated ET-743 as a 24-hour continuous infusion repeated every 21 days<sup>28</sup>. Doses from 50 to 1800 micrograms/m<sup>2</sup> were tested. The most common side effects that limited an increase in dose were low neutrophil and platelet counts. In addition, the majority of patients were noted to have elevations of their liver function tests, and patients with underlying liver abnormalities were found to be more likely to have severe side effects. The maximum tolerated dose was 1500 micrograms/m<sup>2</sup> for patients that had received prior chemotherapy. Three patients had tumor shrinkage, including patients with osteosarcoma and liposarcoma, and four patients with progressing soft tissue sarcomas at the time of study entry had stable disease for 3 or more months. Activity in sarcomas using this schedule was reported separately including 12 patients from the phase I trial above as well as an additional 17 patients treated on a compassionate use program<sup>29</sup>. This report included 25 soft tissue, 3 osteogenic and 1 Ewing's sarcoma patients treated at 1200, 1500 or 1800 micrograms/m<sup>2</sup>. The majority of patients had large tumor volumes and they were considered resistant to chemotherapy, including anthracyclines, standard first line therapy for soft tissue sarcoma (STS) in the adjuvant and metastatic disease setting. Two patients each with soft tissue sarcoma and osteosarcoma had significant tumor shrinkage with an additional 12 patients having disease stabilization for 2.8-15 months. Median duration of response was 10.5 months with median duration of disease stabilization being 5.2 months.

### Phase II Clinical Trials

Phase II clinical trials test a new drug or combination of drugs and look to see what the efficacy of the drug is in a specific disease setting. The 24-hour infusion schedule has been evaluated in STS in patients that have metastatic disease. For chemotherapy naïve patients, objective response rates were noted in 14% with an additional 14% with stable disease<sup>30</sup>. The 12-month progression free survival in these patients was 18% and 49% of patients were alive with disease at one year<sup>31</sup>.

Two phase II trials from the US and France enrolled patients previously treated with single agent doxorubicin and ifosfamide or the combination<sup>32,33</sup>, with a group of patients with extensive pretreatment in the French study. In these two studies objective responses were noted in 8% and 4% of patients respectively, with one complete response in a patient with liposarcoma. Responses were also seen in leiomyosarcoma, with minor responses in endometrial sarcoma and fibrosarcoma. Sixty percent of patients with responsive disease or disease stabilization in the French trial were resistant to anthracyclines and/or ifosfamide. French investigators reported 24% of the patients were without progression of their disease at 6 months, with 30% of patients alive at 2 years. The American trial reported 9% of patients were without progression of disease at one year; however 53% of patients remained alive with disease at one year. Partial and minor responses were reported in uterine leiomyosarcomas, fibrosarcoma, and endometrial uterine sarcoma, with stable disease in renal sarcoma, alveolar sarcoma, and neurogenic sarcoma. Although response rates have been low in these trials, these studies looked at patients that had progressed following standard therapies and were found to have prolonged progression free survivals. This may be due to a change in growth kinetics of tumors once on ET-743<sup>34</sup>. Based on the activity seen in phase I and II trials, an ongoing phase II randomized trial is comparing the 24-hour infusion schedule to a 3-hour weekly infusion every three weeks out of four in patients with metastatic leiomyosarcoma and liposarcoma that have progressed following anthracycline and ifosfamide chemotherapy. A phase II trial in patients with previously treated osteogenic sarcoma using the 24-hour infusion schedule demonstrated some activity<sup>35, 36, 37, 38, 39</sup>. Patients in this study were younger than in most of the other previously reported studies, with the average being 18 years old, but ranging from 12-67. The patients had all received prior standard

therapy for their disease and the majority had also received chemotherapy for recurrent disease. The median number of prior chemotherapies was 5. Treatment was associated with the expected toxicities, but drug levels in patients that had severe toxicity during the first cycle of therapy were found to be increased compared to the levels in patients without these severe side effects. There were no patients with complete or partial responses; however 3 patients had minor responses, with a decrease in tumor size of 49%, 36% and 25%. Overall, in this heavily pretreated group, the median time to disease becoming worse was only 1.3 months. A phase II study found no clinical activity in patients with GIST, with only 2 of 20 patients having stable disease for 4 and 10 months<sup>40</sup>.

Currently ongoing phase II trials are evaluating the efficacy of ET-743 in advanced breast, ovarian, endometrial and prostate cancers. An upcoming study coordinated by the Children's oncology group will be opening, testing ET-743 in recurrent or refractory soft tissue sarcomas and Ewing's family tumors for patients diagnosed before the age of 21.

#### Production (Manufacturer) of Trabectedin

PharmaMar is the world leading biopharmaceutical company of the Zeltia group, committed to advancing the treatment of cancer through the discovery and developments of marine derived medicines. Yondelis® is the first Spanish antitumoral compound, currently marketed in the European Union for the treatment of soft tissue sarcomas in adults after the failure of standard therapy.

YONDELIS® is currently being marketed in the European Union for the treatment of Soft tissue Sarcoma in adults after the failure of standard therapy. PharmaMar has started a phase III multicenter study of Yondelis® as first-line therapy in patients with tumor traslocation, Ewing's sarcoma, or not rhabdomyosarcomatose soft tissue sarcomas and other types of STS. Yondelis® is being studied in solid tumors with high incidence and prevalence in the population, such as prostate, breast and lung cancer.

Yondelis® has been designated orphan drug for the treatment of soft tissue sarcomas and ovarian cancer in the European Union, United States and Switzerland, and for soft tissue sarcomas in Korea. According to the agreement between PharmaMar – a subsidiary of Zeltia, S.A- and Ortho Biotech Products, L.P. – a subsidiary of Johnson & Johnson- , under which Yondelis® is developed, PharmaMar will market Yondelis® in Europe (including Eastern Europe) and Japan, and Ortho Biotech Products, L.P. will market Yondelis® in the rest of the world.

#### CONCLUSION

ET-743 is a unique drug molecule against various soft sarcomas. It was identified as a potential drug by screening sea organisms. Its method of killing cells is complex but clearly different from other known anticancer drugs. We still have more to understand which tumors will benefit from treatment with ET-743. Based on the clinical data from the studies already done, ET-743 has shown that it is active in a group of tumors that do not have many active agents. The information on the activity in soft tissue sarcomas is real and clearly some patients are benefiting from this novel compound. The upcoming phase II clinical study in pediatric patients with sarcomas will extend information and provide further exclusive data as to its role in the treatment of Ewing's sarcoma in particular.

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