Mar. Drugs **2004**, *2*, 14-29

Marine Drugs

ISSN 1660-3397

www.mdpi.net/marinedrugs/

Review

New Marine Derived Anticancer Therapeutics — A Journey from the Sea to Clinical Trials†

"Nature herself must be our advisor; the path she chalks must be our walk. For as long as we confer with our own eyes, and make our ascent from lesser things to higher, we shall be at length received into her closest-secrets."

William Harvey
De Generatione Animalium, 1651.

J. Jimeno [1,*], G. Faircloth [2], JM Fernández Sousa-Faro [1], P. Scheuer [3] and K. Rinehart [4]

[1] PharmaMar R & D, Avda. de los Reyes 1, PI La Mina-norte, 28770-Colmenar Viejo, Madrid, Spain. Tel. +34 91 823 45 37; F. +34 91 846 60 01
[2] PharmaMar R & D, Cambridge, MA, USA
[3] Chemistry Dept., University of Hawaii, USA
[4] School of Chemical Sciences, University of Illinois, USA
*Author to whom correspondence should be addressed; E-mail: jjimeno@pharmamar.com

† *" This paper is dedicated to the memory of P J. Scheuer (1916-2003) a pioneer in marine drug research and discoverer of Kahalalide F and to the 2.184 cancer patients that have given their informed consent to participate in our marine derived clinical anticancer program".*

Received: 12 November 2003 / Accepted: 23 January 2004 / Published: 25 February 2004

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 implementation of scuba diving tools and the development of sophisticated instruments 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 our discovery and developmental program, three innovative compounds with novel mechanisms of action: ET-743, Aplidin^R and Kahalalide F, have been shown to display a positive therapeutic index and activity in resistant solid tumors that supports the ongoing clinical phase III/II trials. ET-743 represents the first active agent against sarcomas developed in the past 25 years and has demonstrated a therapeutic potential in pretreated ovarian cancer. Several chemical entities are under advanced preclinical testing and additional candidates for clinical development are emerging, including compounds hitting a specific target. Moreover, the development of a given marine candidate implies the collaboration of an interdisciplinary team special focused on supply, formulation, pharmacogenetics and preclinical toxicology.

Keywords: Cancer Treatment, Marine Drugs, ET-743, Aplidin^R, Kahalalide F.

Introduction

Nature has played an instrumental role in providing effective therapeutic entities. In fact, terrestrially derived remedies have generally preceded the progress of medicine in the context of the evolution of humankind [1]. Serendipity sequentially linked to a scientifically oriented approach, pioneered by Paracelsus some centuries ago [2] have led to significant discoveries in therapeutic research. In this context, it is important to consider that the major antiinfective, anticancer, analgesics and immunosuppressive compounds are of natural origin.

By contrast, the historical relationship between humankind and the sea is usually appreciated as the basis for travelling, trading and as a nutritional source. We live in a planet of oceans. The marine ecosystem covers more than 70% of the earth's surface but represents 95% of the biosphere [3]. The first living organisms appeared in the sea more than 3500 million years ago [4, 5] and evolutionary development has equipped many marine organisms with the appropriate mechanisms to survive in a hostile milieu in terms of extreme temperatures, changes in salinity and pressure, as well as overcoming the effects of mutation, bacteria and viral pathogens. Marine organisms have developed exquisitely complex biological mechanisms showing cross phylum activity with terrestrial organisms. Self non-self discrimination processes active in colonial tunicates may represent the evolutionary precursor(s) to major histocompatibility complex genes [6] and sea star factor and rabbit lymphokines show cross activity suggesting a phylogenetic link for lymphokines [7]. These biological capabilities are expressed in their ability to synthesize and release potent

chemical weapons that are active *per se*, excluding the need for bio-activation; those chemical entities are called secondary metabolites; such a conceptual view might be questioned, in our opinion, since such chemical entities should have played or still be playing relevant biological roles as autocrine cell regulators, regulators of the differentiation process. Another important differential factor deals with biodiversity. All but 2 of the 28 major animal phyla are represented in aquatic environments and some of them are exclusive of the marine ecosystem, such as Ctenophore, Echinodermata, Porifera, Phoronidea, Brachiopoda and *Chaetognata* [5]. In terms of evolution and biodiversity, the sea appears to be superior to the terrestrial ecosystem ─ one has to consider that the most important biological explosion took place in the marine ecosystem during the Cambrian period 600 million years ago [4] and marine species comprise approximately a half of the total biodiversity, thus offering a vast source from which to discover useful therapeutics.

Historical Perspective and Current Status

Due to technical barriers there has been a lack of extensive marine folk medicine in the western world. Chinese pharmacopoeia recommends seaweed-based recipes for a number of disorders such as pain, abscesses, menstrual difficulties and cancer [8]. Seaweed remedies are also used by the San Blas Indians in Panama [8] and Romans attributed medicinal effects to some marine animals [8]. Marine based chamanic medicine includes representative examples of antiinfective [8] entities.

The progress made in the "pharmacological exploration" of the seas relates to the development and implementation of scuba diving techniques and submersibles. During the last 25 years natural products derived from marine organisms have been the focus of many investigations: i.e. in a recent review, a high number of marine derived chemical entities with biological activity in different therapeutic settings are discussed [9].

Representative examples of marine derived therapeutics include Manoalide, a non steroidal sesterpenoid, a novel marine compound that might be considered as a prototype with therapeutic potential as anti-inflammatory and as analgesic. Manoalide is the first inhibitor of phospholipase A2 and is being explored in a number of disorders [10]. Discodermolide is a novel polyhydroxylated lactone with potent immunosuppressive activity being, *in vivo*, 100-1000 times more potent than cyclosporine-A and that also harbours cytotoxic activity [11]. The available data with the antimalarial compound axinositrile 3 indicates activity in resistant strains and anticipates an important potential in the clinical setting [12]. Also, new polyesters with antifungal activity have been discovered from the marine dinoflagellate *Gambierdiscus toxicus* [13]. These chemical entities are the most potent antifungal agents discovered, with *in vitro* potency 2000 fold higher than anfotericin-B and with moderate toxicity against mammalian cells. Such data gives evidence of a positive therapeutic index and indicates a favourable tolerability profile in the clinical setting. In addition, a number of approaches incorporating targeted based discovery models are yielding to the identification of novel entities with therapeutic potential in a number of diseases; for instance Conotoxins are selective peptide antagonists/agonists of ionic channels and G-protein-receptors that have shown relevant activity in chronic pain [14] and potential in other conditions.

Marine Derived Anticancer Agents

Nature has been a relevant resource for the discovery of anticancer entities. Today, more than 60% of the anticancer drugs commercially available are of naturally origin [15]; naturally derived antiproliferative drugs such as doxorubicin, daunomicin, bleomycin, mytomicin C, vincristine and vinblastine play an important role in curative cancer chemotherapy in a number of solid tumors and haematological malignancies and the most important recent incorporations to the clinical armamentarium in oncology, taxanes and campthothecins, are also naturally derived compounds.

The relevance of the sea as a tool to discover novel anticancer compounds was validated by the discovery, development and marketing approval of 1-beta-D-arabinofuranosylcytosine (ARA-C) [16]. ARA-C is a basic component in the curative setting of acute myeloid leukaemia [17]. Moreover, the search for novel deoxicytidine analogues led to the identification and development of 2,2'-difluoro-deoxycytidine, gemcitabine. Clinical data has demonstrated its important role in palliative therapy for pancreatic and non-small cell lung cancers [18,19].

The available results clearly anticipated the potential of the marine ecosystem in cancer therapy. During the last decade about 2500 new metabolites with antiproliferative activity have been reported; a recent review discussed 68 new marine derived anticancer chemical entities, most of them with undetermined modes of action [20].

The novelty of most of these chemical entities is supported by our data which shows that more than 60% of our discoveries in the anticancer setting harbour a negative COMPARE analysis; such findings suggest that most of those compounds identified might act through a new/innovative mechanism of action. The identification of new targets for therapeutic intervention in cancer is instrumental to improve the natural history of cancer patients. The clinical results generated with a number of marine derived compounds such as the dolastatins, didemnin B and Bryostatin, have been recently reviewed [21]. This manuscript critically summarizes the developmental status of three of our innovative marine derived anticancer agents: ET-743 (Yondelis), Aplidin^{R} and Kahalalide, that have proven to have a positive therapeutic index in pretreated cancer patients.

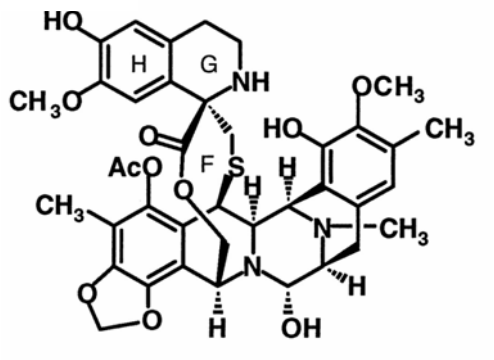
ET-743 (Trabectedin, Yondelis^{TM})

Our first compound ET-743 (Figure 1) is a tetrahydroisoquinoline alkaloid produced by the colonial tunicate *Ecteinascidia turbinate* [22]. ET-743 is a novel DNA interactive agent that has shown *in vivo* activity in nude mice harbouring human resistant xenografted tumors [23].

Recent mechanistic data demonstrates that ET-743 induces a broad inhibition of activated transcription with no effect on the constitutive transcription [24,25,26]: ET-743 inhibits the activation of the multidrug resistant pathway [27] that is considered to be the main mechanism of primary and acquired resistance of cancer cells to natural drugs such doxorubicin and taxanes. ET-743 is the only known anticancer entity for which there is an inverse correlation between the DNA repair efficiency and the sensitivity/resistance pattern [28,29]; such evidence offered a rationale to

implement combinations studies with platin salts [30] and to seek for correlations in patients between the DNA efficiency and the response to ET-743 [31].

Figure 1. Chemical Structure of ET-743



Ecteinascidin-743

An extensive phase I program assessing different schedules of administration was completed [32, 33,34,35]: The dose limiting toxicities were bone marrow toxicity and fatigue. As predicted in the preclinical toxicology, transaminitis is noted in the majority of the patients but such a drug induced effect is transient/reversible and non cumulative and therefore does not represent a limiting factor for long-term therapy. Consistent evidence of antitumor activity in patients bearing resistant disease was reported in the phase I program. In fact, objective remissions in breast cancer, melanoma and mesothelioma were observed together with a consistent evidence of antitumor activity in patients with advanced resistant sarcoma. Such evidence was the starting point for a fast track pivotal phase II program in patients with advanced soft tissue sarcoma resistant or relapsed to conventional therapies. Long-term results from such studies have clearly confirmed a significant therapeutic impact in this disease setting [36,37,38,39,40]. In these studies ET-743 has been given as a 24 hours intravenous infusion every 3 weeks at a dose of 1.5 mg/m^2. Moreover, a study performed with the same schedule in patients with advanced breast cancer resistant or relapsed to anthracyclines and taxanes has also provided evidence of activity [41]. The pharmacological profile of ET-743 has been extensively defined during the phase I and early phase II program: at the recommended dose for the protracted 24 hours infusion schedule a three compartment model was characterized: the slow terminal phase (median beta 73 hours) observed support further development incorporating a 3 hours outpatient schedule [35]. The identification of the biliary function as a parameter to predict the onset of severe toxicities has been instrumental to identify good risk patients as well as a guideline to treat patients with full doses of ET-743 or to proceed with dose reductions [42] with the protracted infusion schedule. The mature safety data generated with this schedule demonstrates a lack of cumulative toxicities that allows chronic therapy; In contrast with conventional (i.e. terrestrial derived) cytotoxics, ET-743 does not induce hair loss, mucositis, neurotoxicity or diarrhoea [43]. The pharmacokinetic profile of ET-743 provided a

rational to develop short/outpatient schedules. A phase II trial with ET-743 given as a 3 h infusion every 3 weeks at a dose of 1.3 mg/m^2 in patients with advanced pretreated ovarian cancer has confirmed a high therapeutic potential in this tumor type, with a 25% response rate in women resistant or relapsed to Platin-taxane chemotherapy and a 47 % response rate in the relapsed cohort [44]. A phase III study vs conventional therapy in second line therapy is now being designed. ET-743 has also proved to be feasible in paediatric patients and activity in heavily pretreated patients with advanced Ewing sarcoma is being reported [45]. A dose dense sub toxic weekly schedule [46] is being investigated in different tumor types.

The available data as single agent anticipates a lack of overlapping toxicities between ET-743 and conventional therapies. The data generated with the combination of Cisplatin and ET-743 demonstrates a positive therapeutic index and activity in patients with resistant tumors [47]. Additional combination studies with ET and doxorubicin, taxanes and with other platin salts are ongoing; such results are expected to establish the basis to develop ET-743 in settings with available therapies that require the phase III trials.

Aplidin^R (APL)

Aplidin^R (Figure 2) is a new cyclic depsipeptide [48] incorporated to clinical development back in 1999. This compound induces cytotoxicity in a non-MDR/p53 dependent manner, blocks the cell cycle progression at G1 and decreases the secretion of the Vascular Endothelial Growth factor (VEGF) and the expression of the VEGF-r1 receptor [49,50]. The phase I program has investigated the feasibility of dose dense schedules and have confirmed a positive therapeutic index in patients harbouring pretreated solid tumors and lymphoma [51,52,53,54,55].

The dose limiting toxicity with the protracted schedule is muscular with a remarkable lack of haematological toxicity in spite of the cytotoxicity noted at low concentrations in leukemic blasts explanted from patients [56,57]. Consistent evidence of activity has been noted in pretreated neuroendocrine tumors [58] and other tumor types. Phase II studies are now ongoing with an every other week schedule giving APL as a protracted or 3 hours intravenous infusion at a dose of 5 mg/m^2.

Kahalalide F (KF)

Kahalalide F (Figure 3) is one of a family of dehydroaminobutyric containing cyclic peptides isolated from the Hawaiian mollusc E. Rufencens [59,60]. This mollusc is able to sequester chloroplasts from an alga to participate in the synthesis of secondary metabolites. Kahalalide-F is an US-NCI COMPARE negative compound that seems to have the lysosomes as the cellular target [61, 62]. The evidence of *in vivo* activity in experimental human cancer models of androgen independent prostate cancer and other solid tumors established a rational to implement a clinical program with this innovative compound [63].

Figure 2. Chemical Structure of Aplidin^{R}

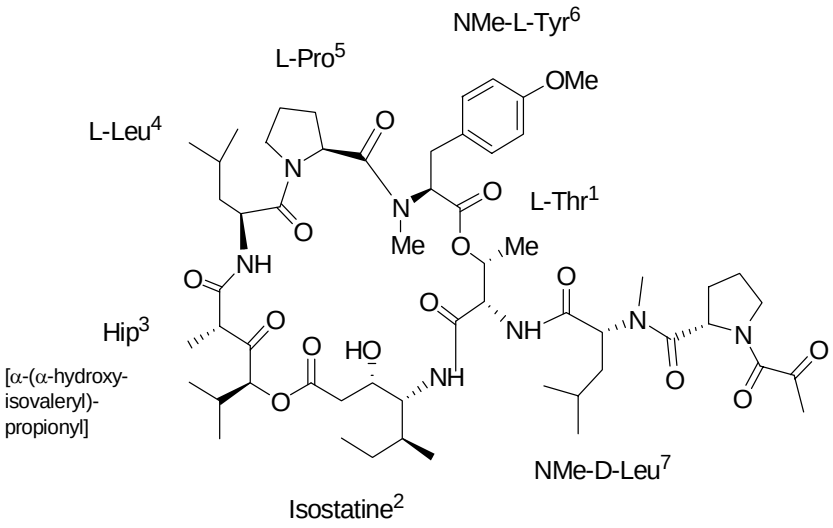
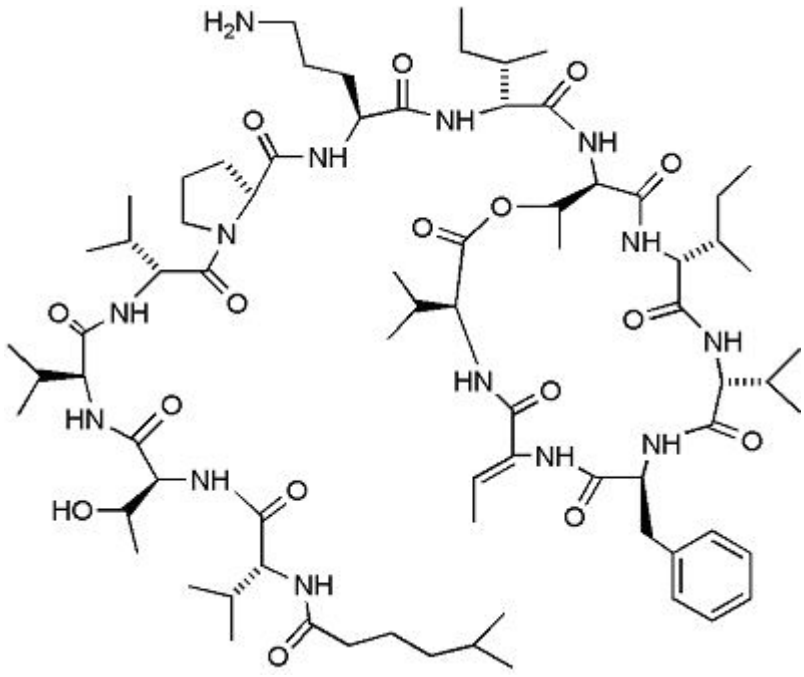


Figure 3. Chemical Structure of Kahalalide F



A phase I trial investigating the feasibility of a weekly schedule with KF given as a 1 hour intravenous infusion in patients with advanced pretreated solid tumors has been completed [64].

The dose limiting toxicity is acute transaminitis that precluded the administration of the compound in a weekly fashion, with a remarkable absence of bone marrow suppression, alopecia and other organ toxicities; such early data suggests lack of cumulative toxicities that may allow chronic therapy. The pharmacokinetic profile demonstrates a short terminal half-life, a finding supporting additional studies with longer infusional schedules. Evidence of activity in pretreated patients with melanoma, colorectal cancer and hepatocellular carcinoma has been reported in this study. A phase II trial in patients with advanced liver cancer is now ongoing and further studies in different tumor types shall be opened shortly. A second phase I trial with a daily times five schedule in patients with androgen independent prostate cancer is ongoing and expanding the cohort of patients at the recommended dose [65].

Development of Marine Derived Therapeutics

The Challenge

It is important to emphasize the complexity of the different steps and areas that are involved during the development of a given marine derived candidate. Table 1 lists a number of issues that have to be considered from the earliest stages of development when a marine chemical entity is selected for clinical development.

Table 1

MARINE DERIVED THERAPEUTICS
POTENTIAL LIMITING FACTORS FOR DEVELOPMENT

- Supply (sustainable, industrially feasible)
- Formulation (suitable for clinical use)
- Analytical method and preclinical PKs
- Pharmacogenetics (metabolic pathway)
- Therapeutic index
- Toxicities (Xeno)

A critical step is the incorporation of a sustainable supply, in order to ensure a sequential pathway of preclinical-clinical investigations. A number of approaches are listed in Table 2. The complexity of the chemical structures generally seen in marine derived compounds can limit the

development of synthesis processes. Therefore, major advances in the aquaculture of marine micro organisms and synthesis of complex molecules are needed to facilitate the incorporation of additional candidates to the development track. Within our project, a multidisciplinary team has used the availability of mariculture plus semi-synthesis [66] for ET-743 and fully synthetic processes [67,68] for APL and KF, respectively. Additional factors such as the identification of a feasible clinical formulation, investigation of the metabolic pathways and preclinical evaluation of new toxicological models have to be considered instrumental, clearly implying the need of an interdisciplinary team involving experts from many different areas of research.

Table 2

MARINE DERIVED THERAPEUTICS SUPPLY

- Controlled and sustainable use of natural resources
- Mariculture: favouring (by farming) the growth of the organism in its natural milieu
- Aquaculture: culture of the organism under artificial conditions
- Hemisynthesis: use of a parent / related compound as the starting point followed by a short / industrially effective synthetic process
- Synthesis
- Fermentation
- Genetic intervention.

Conclusions

Conceptually, it is clear that the marine ecosystem offers a huge potential in the naturally based pharmacopoeia of this century. However, an unfavourable balance between discovery and the very small number of candidates incorporated for clinical evaluation exists. So it appears that a better and more pragmatic approach is urgently needed in order to translate innovative discoveries into active clinical therapeutics. The available data demonstrates that the marine ecosystem is not only productive to discover anticancer entities but it is also a tool to identify new cellular targets for therapeutic intervention (Table 3).

New agents include ES-285, an aminoalcohol isolated in the mollusc *Spisula polynyma* [69]. The preclinical results generated [70] were the basis of the design of a phase I program initiated recently.

Table 3

Marine Anticancer Drugs: New Cellular Targets for Therapeutic Intervention

COMPOUND	SOURCE	CHEMICAL CLASS	PROPOSED CELLULAR TARGET(S)
Yondelis	Tunicate	Isoquinolone	•G-selective DNA minor Groove Binder •Inhibition of Transcription
Aplidin	Tunicate	Cyclic Peptide	• VEGF interacting agent •Protein synthesis Inhibition
Kahalalide F	Green Algae	Cyclic Peptide	• Lysosomes

Nevertheless, the efforts being made in different projects involving marine derived therapeutic research are continuously opening new avenues [9]. Also the in depth characterization of the molecular pharmacodynamic changes induced by these entities in human cancer cells can also generate information to understand the genetic basis behind the response/resistance to these compounds [71] projecting the possibility to developed customized therapies according to a given molecular signature. With this in mind, important therapeutic contributions coming from the marine ecosystem are expected to emerge in the near future.

References

1. Monnier, C. *Les plantes Medicinales. Vertus et Traditions*; Privat Eds. Toulouse **2002**.
2. Sweldlow, J. *Nature and Medicine*; National Geographic Mil Eds.: Paris, **2000**.
3. Ellis, R. *Aquagenesis. The origin and evolution of life in the sea*; Viking Eds.: New York, **2001**.
4. Macdougall, J.D. *A short history of Planet Earth*; John Wiley Eds.: New York, **1996**; p. 5.
5. Argulis, L., Schwartz, K. *Five Kingdoms, an illustrated guide to the phyla of life on Earth*; W.H. Freeman & Company: New York, **1982**; pp. 16-17.
6. Scotfield, V.I.; Schumpberger, J.M.; West, L.A.; Weissman, L. Protocordate allorecognition is controlled by aq MHC-like gene. *Nature* **1982**, *295*, 499-502.
7. Pendergast, R.A.; Lutty, G.A.; Scott, L. Direct inflammation: the phylogeny of lymphokines. *Dev. Comp. Inmunol*. **1983**, *7*, 629-632.

8. Ruggieri, G.D. Drugs from the sea. *Science* **1976**, *194*, 491-496.
9. Haefner, B. Drugs from the deep. Marine natural products as drug candidates. Drug Discov. Today **2003**, *8*, 536-544.
10. Sorriente A. Manoalide. *Current Med.Chemistry* **1999**, *6*, 415-431.
11. Longley, R.; Cardigan, D.; Harmody, D.; Gunasereka, M.; Gunasereka, S. Discodermolide - A new marine derived Inmunosuppressive compound. *Transplantation* **1991**, *52*, pp. 650-661.
12. Angerhofer, C.; Pezzuto, J.; Konig, G.; Wright, A.; Sticcher O. Antimalarial activity of Sestiperquenes from the marine sponge Acanthella klelthra. *J. Nat. Prod*. **1992**, *55*, 1787-1789.
13. Nagai, H., Murata, M., Torigoe, K., Sakate, M., Yasumoto, T. Gambieric acids, New potent antifungal substances with unprecedent polyether structures from a marine dinoflagelate Gambiercus toxicus. *J. Org. Chem.* **1992**, *57*, 5448-5453.
14. Alonso, D.; Khalil, Z.; Satkunanthan, N.; Livett, B.G. Drugs from the Sea: Conotoxins as Drug Leads for Neuropathic Pain and Other Neurological Conditions. *Min. Rev. Med. Chem.* **2003**, *3*, 785-787.
15. Craigg, G.M.; Newman, D.J.; Weiss, R.B. Coral reefs, forests and thermal vents: The worldwide exploration of nature for novel antitumor agents. *Semin. Oncol.* **1997**, *24*, 156-163.
16. Bergmann, W.; Feeney, R.J. Contributions to the study of marine products. The nucleosides of sponges III. Spongothymidine and Spongouridine *J. Org. Chem.* 1985, *20*, 1501-1507.
17. Wolf, S.N.; Marion, J.; Stein, R.S. High dose cytosine arabinoside and daunorubicin as consolidation therapy for acute non lymphoblastic leukemia. *Blood.* **1985**, *65*, 1407-1411.
18. Moore M. Activity of gemcitabine in patients with advanced pancreatic carcinoma: a review. *Cancer.* **1996,** 78(3), 633-637.
19. Guchelaar H.J., Richel Dj., Van Knapen A. Clinical, toxicological and pharmacological aspects of gemcitabine. *Cancer. Treat. Rep*. **1996,** 22, 15-31.
20. Mayer AMS, Gustaveson KR. Marine pharmacology in 2000: antitumor and cytotoxic compounds. *Int J Cancer*. **2003,** 105, 291-299.
21 Amador M, Jimeno J., Paz Ares L., Cortés Funes H., Hidalgo M. Progress in the development and acquisition of anticancer agents from marine sources. *Ann Oncol*. **2003,** 14(11): 1607-1615.
22 Rinehart K.L., Holt T.G., Fregau N.L. Ecteinascidins 729, 743, 745, 759^a, 759b and 770. Potent antitumor compounds from the Caribbean tunicate Ecteinascidia Turbinata. *J Org Chem*. **1990**, 55(15), 4515-4516.
23 Jimeno J.M., Fairlocth G., Cameron L. Cameron L., Vega E., Meely K., Rinehart K., Fernandez-Sousa, JM. Progress in the acquisition of new marine derived anticancer compounds: development of ecteinascidin-743 (ET-743). *Drugs of the Future*. **1996,** 21(11), 1155-1165.
24 Minuzzo M, Marchini S, Broggini M, Faircloth G, D'incalci M And Mantovani R: Interference of transcriptional activation by the anti-neoplastic drug ET-743. *Proc.Natl.Acad.Sci.USA.* **2000,** 97: 6780-6784.

25 Jin S, Gorfajn B, Faircloth G And Scotto K: Ecteinascidin 743, a transcription-targeted chemotherapeutic that inhibits MDR1 activation. *Proc.Natl.Acad.Sci.USA.* **2000,** 97, 6775-6779.

26 Friedman D, Hu Z, Kolb EA, Gorfajn B And Scotto KW: Ecteinascidin-743 inhibits activated but not constitutive transcription. *Cancer Res*. **2002**, 62, 3377-3381.

27 Kanzaki A, Takebayashi Y, Ren XQ, Akiyama S., Bates S., Pommier Y., : Overcoming multidrug drug resistance in P-glycoprotein/MDR1-overexpressing cell lines by ecteinascidin 743. *Mol Cancer Ther.* **2002**, 1, 1327-1334.

28 Damia G; Silvestri S; Carrassa L; Filiberti L; Faircloth G T; Liberi G; Foiani M; D'Incalci M: Unique pattern of ET-743 activity in different cellular systems with defined deficiencies in DNA-repair pathways. *Int J Cancer.* **2001,** 92, 583-588.

29 Takebayashi Y; Pourquier P; Zimonjic D B; Nakayama K; Emmert S; Ueda T; Urasaki Y; Kanzaki A; Akiyama S I; Popescu N; Kraemer K H; Pommier Y: Antiproliferative activity of ecteinascidin 743 is dependent upon transcription-coupled nucleotide-excision repair. *Nat Med.* **2001,** 7, 961-966.

30 D'incalci M, Colombo T, Ubezio P., Nicoletti I., Giavazzi R., Erba E., Ferrarese L., Meco D., Riccardi R., Sessa C., Cavallini E., Jimeno J., Faircloth G: The combination of yondelis and cisplatin is synergistic against human tumor xenografts. Eur J Cancer. **2003,** 39, 1920-1926.

31 Sarries C., Haura E., Roig B. Pharmacogenomic strategies for developing customized chemotherapy in non-small cell lung cancer. *Pharmacogenomics.* **2002**, 3(6), 763-780.

32 Taamma A, Misset JL, Riofrio M, Guzman C, Brain E, Lopez Lazaro L, Rosing H, Jimeno J, Cvitkovic E.: Phase I and pharmacokinetic study of ecteinascidin-743, a new marine compound, administered as a 24-hour continuous infusion in patients with solid tumors. *J Clin Oncol.* **2001,** 19, 1256-1265.

33 Ryan DP, Supko JG, Eder JP, Seiden M, Demetri G, Lynch TJ, Fischman AJ, Davis J, Jimeno J, Clark JW: Phase I and pharmacokinetic study of ecteinascidin 743 administered as a 72-hour continuous intravenous infusion in patients with solid malignancies. *Clin Cancer Res.* **2001,** 7, 231-242.

34 Villalona-Calero MA, Eckhardt SG, Weiss G, Hidalgo M, Beijnen JH, van Kesteren C, Rosing H, Campbell E, Kraynak M, Lopez-Lazaro L, Guzman C, Von Hoff DD, Jimeno J, Rowinsky EK.: A phase I and pharmacokinetic study of ecteinascidin-743 on a daily x 5 schedule in patients with solid malignancies. *Clin Cancer Res.* **2002,** 8, 75-85.

35 Twelves C., Hoekman K., Bowman A., Vermorken J.B., Anthoney A., Smyth J., Van Kesteren C., Beijnen J.H., Uiters J., Wanders J., Gomez J., Guzmán C., Jimeno J., Hanauske A..: Phase I and pharmacokinetic study of Yondelis (Ecteinascidin 743; ET-743) administered as an infusion over 1h or 3h every 21 days in patients with solid tumors. *Eur J Cancer.* **2003,** 39, 1842-1851.

36 Delaloge S, Yovine A, Taamma A, Riofrio M, Brain E, Raymond E, Cottu P, Goldwasser F, Jimeno J, Misset JL, Marty M, Cvitkovic E. Ecteinascidin-743: a marine-derived compound in

advanced, pretreated sarcoma patients-preliminary evidence of activity. *J Clin Oncol*. **2001**, 19*(5)*, 1248-1255.

37 Demetri G., Manola J., Harmon D., Maki R., Seiden R., Supko J., Ryan D., Puchlaski T., Goss G., Merriam P., Waxman A., Slater S., Potter A., Quigley T., Lopez T., Sancho MA., Guzman C., Jimeno J., Garcia Carbonero R. Ecteinascidin-743 (ET-743) induces durable responses and promising 1-year survival rates in soft tissue sarcomas (STS): Final results of Phase II and Pharmacokinetic studies in the U.S.A American Society of Clinical Oncology, San Francisco, CA, May 12 – 15,.**2001.** Procc. 352a. Abstract 1406.

38 Le Cesne A., Blay J.,. Judson I, Van Oosterom A, Verweij J, Radford J, Lorigan P, Rodenhuis S, Donato De Paola E, Van Glabbeke M., Jimeno J., Nielsen O. ET-743 is an active drug in adult soft-tissue sarcoma: a STBSG-EORTC Phase II trial. American Society of Clinical Oncology, 37th Annual Meeting . San Francisco, CA, May 12 – 15, **2001.** Procc. 353a. Abstract 1407.

39 Yovine A., Riofrio M., Brain E., Blay JY., Kahatt C., Delaloge S., Beautier L., Cottu P., Jimeno J., Cvitkovic E., Misset JL. Ecteinascidin-743 given as a 24 hour (H) intravenous continuos infusion (IVCI) every 3 weeks: results of a Phase II trial in patients (PTS) with pretreated soft tissue sarcomas (PSTS). American Society of Clinical Oncology. 37th Annual Meeting . San Francisco, CA, May 12 – 15, **2001**. Proc. 363a. Abstract 1449.

40 Jimeno J., Maki R., Casali P., Faircloth G., Martinez N., Nieto A., Cañigueral S. Therapeutic impact of ET-743 (Yondelis®, Trabectidin) a new marine derived compound in Sarcoma. Current Opinion in Orthopedics. Submitted September **2003**.

41 Zelek L., Yovine A., Brain E., Jimeno J., Taamma A., Martin C., Spielmann M., Civtkovic E., Misset JL. "Ecteinascidin 743 in pretreated/advanced metastatic breast cancer patients: Preliminary results with the 24 hours infusion schedule". Procc. ASCO New Orleans **2000**, pp 149, abst 592.

42 Gómez J., López-Lázaro L., Guzmán C., González A., Misset J.L., Twelves C., Bowman A., Hoekman K., Villalona M., Ryan D., Paz-ares L., Jimeno J. "Identification of biochemical parameters that predict the onset of severe toxicities in patients treated with ET-743". ASCO New Orleans **2000**, Procc. Abst 727:187ª.

43 Lopez Martin J.A., Nieto A., Demetri G., Misset JL., Ray-Coquard I., Guzman C., Sancho MA., Parra MC., Ibarra I., Millan S., Martín C., Ruiz A., De Alvaro J., Lopez-Lazaro L., Gomez J., Jimeno J. .: Safety profile of ecteinascidin-743 (ET-743) in phase II clinical trials (CT)in adult patients with solid tumors. Proc Am Soc Clin Oncol. **2002,** 21: 96a.

44 Colombo N., Capri G., Bauer J., Noberasco C., De Braud F., Grasselli G., Jimeno J., Ruiz A., Corradino I., Marsoni S., Sessa C. Phase II and Pharmacokinetics study of 3 hour infusion of ET743 in ovarian cancer patients failing platinum-taxanes. ASCO 38th Annual Meeting, Orlando, Florida, May 18-21, **2002,** Proc. p.221a, Vol., 21, Abstract 880.

45 Casali P., Casanova N., Dileo P., Ferrari S., Bacci G., Picci P., Marsoni S., Jimeno J., Riccardi R., Gianni L. Activity of Ecteinascidin 743 (ET-trabectedin 743) 3 h infusion in adult and

childhood small round cell sarcomas. ASCO 39th Annual Meeting, Chicago IL, May 31-June 3, **2003**,Proc Vol. 22 Abs. 962 pp 240.

46 Forouzesh B., Hidalgo M., Denis L., Schwart G., Hammond L., Monroe P., Guzman C., Lopez-Lazaro L., Supko J., Jimeno J., Rowinsky E. phase I and pharmacokinetic study of the marine-derived DNA minor groove binder ET-743 on a weekly x3 every-4-week schedule in patients with advanced solid malignancies. Proc of AACR-NCI-EORTC International Conference on Molecul targets and cancer therapeutics: Discovery, Biology and Clinical applications. Miami Beach, October 29-November 2, **2001,** 42.

47 Grasselli G., Malossi A., Marsoni S., Colombo N., Perez C., Zucchetti M., D'incalci M., Lopez Martin JA., Jimeno J, Capri G., Raspagliesi F., Curigliano G., Gianni L., Sessa C. Phase I and pharmacokinetic (PK) study of ecteinascidin-743 (ET, Trabectedin) and cisplatin (P) combination in pre-treated patients (pts) with selected advanced solid tumors. Proc Am Soc Clin Oncol **2003** 22: N°542.

48 Rinehart K.L., Gloer J.B., Cook J.C., Mizsak S.A., Scohill T.A., "Structures of the Didemnins, Antiviral and Cytotoxic Depsipeptides from a Caribbean Tunicate". J Am Chem Soc., **1981**, 103, 1857-1859.

49 Erba E., Bassano L., Di Liberti G., Muradore I., Chiorino G., Ubezio P., Vignati S., Codegoni A., Desiderio A., Faircloth G., Jimeno J., D'Incalci M. Cell Cycle phase perturbations and apoptosis in tumour cells induced by aplidine. *Br J Cancer.* **2002**, 86:1510-17.

50. Broggini M, Marchini S., Borsott P., Galliera E., Erba E., Taraboletti G., Giavazzi R., Jimeno J., Faircloth G., D'incalci M. Aplidine, a new anticancer agent of marine origin, inhibits vascular endothelial growth factor (VEGF) secretion and blocks VEGF-VEGFR-1 (flt-1) autocrine loop in human leukemia cells MOLT-4. *Leukemia,* **2003**, 17, 52-9.

51 Anthoney A., Paz-Ares L., Twelves C., Cortes-Funes H., Kaye S., Pronk L., Celli N., Lopez-Lazaro L., Guzman C., Jimeno J. Phase I and Pharmacokinetic (PK) Study of APL (APL) Using a 24-Hour, Weekly Schedule. Proc Am Soc Clin Oncol. **2000**; abstract #734

52 Ciruelos E.M., Twelves C., Dominguez M.J., Mckay H., Anthony, A. Castellanos D., Bezares S., Ruiz A., Lopez-Lazaro L., Jimeno J., Celli C., Cortes-Funes H., Paz-Ares L. Phase I clinical and pharmacokinetic study of the marine compound APL (APL) administered as a 3 hour infusion every 2 weeks. Proc Am Soc Clin Oncol. **2002**, abstract # 422.

53 Bowman A., Izquierdo M.A., Jodrell D., Martinez M., Cicchella B., Jimeno J., Guzman C., Germa-Lluch J., Celli N., Smyth J. Phase I Clinical and Pharmacokinetic (PK) Study of the Marine Compound APL (APL), administered as a 1 Hour Weekly Infusion. Proc Am Soc Clin Oncol. **2001**, abstract #476.

54 Armand J.P., Ady-Vago N., Faivre S., Chieze S., Baudin E., Ribrag V., Lecot F., Iglesias L., López-Lázaro L., Guzmán C., Jimeno J., Ducreux M., Le Chevalier T., Raymond E. Phase I and Pharmacokinetic Study of APL Given as a 24-Hour Continuous Infusion Every Other Week

(q2w) in Patients (pts) with Solid Tumor (ST) and Lymphoma (NHL). Proc Am Soc Clin Oncol. **2001**, abstract #477

55 Maroun J.A., Goel R., Stewart D.J., Tomiak E., Belanger K., Soulieres D., Charpentier D., Seymour L., Matthews S., Jimeno J., Guzman C. Phase I Study of APL in a 5 Day Bolus Q 3 Weeks in Patients with Solid Tumors and Lymphomas. Proc Am Soc Clin Oncol **2001**; abstract #2082

56 Bresters D., Broekhuizen A., Faircloth G., Jimeno J., Kaspers G. In vitro cytotoxicity of Aplidine and cross-resistance with other cytotoxic drugs in childhood leukemic and normal bone marrow and blood samples; a rational basis for clinical development. *Leukemia*. **2003**, 1-6.

57 Erba E., Serafini M., Gaipa G., Tognon G., Marchini S., Muradore I., Celli N., Rotilio D., Broggini M., Jimeno J., Faircloth G., Biondi A., D'incalci M. The effect of Aplidine in acute lymphoblastic leukaemia cells. *Br J Cancer Suppl*. **2003,** 89, 763-773.

58 Raymond E, Paz-Ares L, Izquierdo MA, Pardo B, Ciruelos E, Baudin E, Domínguez MJ, Martinez M, Jimeno J, Lopez-Martin JA. Activity of APL, a new marine compound, against medullary thyroid carcinoma (MTC): Phase I trials as screening tools for rare tumors. *Ann Oncol.* **2002**; 13 (Suppl. 5): page 22.

59 Hamann MT, Otto CS, Scheuer PJ, "Kahalalides: Bioactive peptides from a marine mollusk *Elysia rufescens* and its algal diet *Bryopsis* sp.", *J. Org. Chem*, **1996,** 61, 6594-660.

60 Hamann MT, Scheuer PJ, "Kahalalide F: A bioactive depsipeptide from the sacoglossan mollusk *Elysia rufescens* and the green alga *Bryopsis* sp.", J Am Chem Soc. **1993**,115, 5825-5826.

61 García-Rocha M, Bonay P, Avila J, "The antitumor compound Kahalalide F acts on cell lysosomes", *Cancer Lett*, **1996**, 99, 43-50.

62 Suarez Y., Gonzalez L., Cuadrado A., Berciano M., Lafarga M., Muñoz A. Kahalalide F, a new marine derived compound induces oncosis in human prostate and breast cancer cells. *Mol. Cancer Therapeutics*, **2003**, 863-872.

63 Supko J.G, Lu H., Jimeno J.M., Grant W., Faircloth G.T. "Preclinical pharmacology studies with the marine natural product Kahalalide F". AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, November 16-19, **1999**. Washington D.C. Abstract no. 315.

64 Ciruelos E., Trigo JM., Pardo B., Paz-Ares L., Estaun N., Cuadra C., Domínguez MJ., Marin A., Ruiz A., Jimeno J., Izquierdo MA. A phase I clinical and pharmacokinetic (PK) study with KahalalideF (KF) in patients (pts) with advanced solid tumours (AST) with a continuous weekly (W) 1-hour iv infusion schedule. 14th NCI-EORTC-AACR Congress, Frankfurt, Germany. European Journal of Cancer, 19-22 November **2002,** Vol 38, Supplement 7, pp S33, Abstract 95.

65 Schellens J.H.M., Rademakerlakhai J.M., Horenblas S., Meinhardt W., Stokvis E., M.De Reijke T., Jimeno J., Lopez-Lazaro L., Lopez-Martin J.A., Beijnen J.H. Phase I and pharmacokinetic study of kahalalide F in patients with advanced androgen resistant prostate

cancer ASCO 38th Annual Meeting. Orlando, Florida, May 18-21, **2002**, Proc.113a, Vol., 21, Abstract 451.

66 Cuevas C; Perez M; Martin M J; Chicharro J L; Fernandez-Rivas C; Flores M; Francesch A; Gallego P; Zarzuelo M; de La Calle F; Garcia J; Polanco C; Rodriguez I; Manzanares I. Síntesis of Ecteinascidin ET-743 and phthalascidin PT-650 from syano safracin B. *Org. Lett.* **2000**, 2, 2245-2548.

67 Joug G., Gonzalez L., Albericio F, Lloyd-Williams P, Giralt E. Total síntesis of dehydro didemnin B. Use of uranium and phosphonium salt coupling reagents in peptide synthesis in solution. *J. Org. Chem*. **1997**, 62, 354-366.

68 Lopez Macia A., Jiménez JC, Royo M., Giralt E., Albericio F. Synthesis and structure determination of Kahalalide F. *Am. Chem Society*. **2001**, 123, 11398-11401.

69 Cuadros R., Montejo De Garcini E., Wandosell F., Faircloth G., Fernández Sousa JM, Avila J. The marine compound Spisulosine, and inhibitor of cell proliferation promotes the disassembly of actin stress fibers. *Cancer Lett*. **2000**, 152. 23-29.

70 Jimeno J., García-Grávalos D., Ávila J., Smith B., Grant W. And Faircloth G.T. ES-285, a marine natural product with activity against solid tumors. AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics. Washington D.C., November 16-19, **1999**, Abstract no. 314.

71 Sanchez Beato M., Moneo V, Martínez N, Fernández I, Carnero A, Piris MA, Jimeno J. Molecular pharmacodynamic (MPD) effects induced by ET-743 (Yondelis) in human sarcoma cells (HSC). Procc International Conference on Applied Genomics – 9th ESCAP/16th ISDQP Meeting, Amsterdam, The Netherlands. October 1-4, **2003,** Abstract C-14 p. 65.