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A clinical armamentarium of marine-derived anti-cancer compounds

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The sea, covering 70% of the Earth's surface, offers a considerably broader spectrum of biological diversity than *terra firma*. Containing approximately 75% of all living organisms, the marine environment offers a rich source of natural products with potential therapeutic application. Marine organisms have evolved the enzymatic capability to produce potent chemical entities that make them promising sources of innovative cytotoxic compounds. Prominent in the identification and development of novel anti-cancer agents from marine sources is the Spanish biotechnology company, PharmaMar, which currently has a large number of oncology products in late preclinical and clinical development. These include: ecteinascidin-743 (ET-743), a marine-derived antitumor agent isolated from the Caribbean tunicate, *Ecteinascidia turbinata*; aplidine (Aplidin), a cyclopeptide cytotoxic agent derived from the Mediterranean tunicate, *Aplidium albicans*; kahalalide F, a depsipeptide isolated from the Hawaiian mollusc, *Elysia rufescens*; and ES-285, a molecule isolated from the mollusc, *Spisula polynyma*. Many of these innovative compounds have novel mechanisms of anti-tumor action that have yet to be fully elucidated. [© 2002 Lippincott Williams & Wilkins.]

Key words: marine, ecteinascidin-743, aplidine, kahalalide F, ES-285, anti-cancer.

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Introduction

Potent cytotoxic agents have a well-established role in the treatment of cancer. Many of the anti-cancer agents currently in use are of natural origin (e.g. vinca alkaloids, taxanes and anthracyclines), derived from terrestrial plants and micro-organisms, or are natural product derivatives.[1,2] Despite the diverse range of anti-cancer compounds currently available, problems of drug toxicity, poor efficacy and cross-resistance create the need for the development and introduction of novel cytotoxic agents with innovative mechanisms of action. The sea, which covers approximately 70% of the Earth's surface, represents a largely untapped natural source of such compounds, with the marine ecosystem accounting for more than 95% of the total biosphere. Following in the wake of the explosion in the number of biological species that occurred some 540 million years ago during the Cambrian period, marine organisms have evolved and developed the enzymatic capability to produce chemical entities that might serve, not only as defense systems against micro- and macro-predators, but also as regulators of biological function.[3,4] These compounds offer a good starting point in the quest for innovative marine-derived anti-cancer drugs.

The Spanish biopharmaceutical company, PharmaMar, founded in 1986, is at the forefront in the discovery, screening and development of new anti-cancer agents from marine sources. In its search for oncology products, the company has focused exclusively on the sea, in recognition of the greater potential for activity with marine than with terrestrial sources. Thus, whereas an estimated 1 in 10 000 compounds of terrestrial origin screened for anti-tumor activity yield a candidate for drug development, the corresponding figure for marine sources is closer to 1 in 100.

On the basis of its screening program, PharmaMar has developed an extensive marine library of more than 22 000 organisms. Within this library, a number of organisms have been identified as important sources of novel anti-cancer agents (Table 1). More than 110 chemical compounds have been identified as having in-vitro cytotoxic activity against a

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Table 1. Marine-derived anti-cancer compounds

Organism	Group	Metabolite	Sea location
Ecteinascidia turbinata	Tunicate	ET-743	Caribbean
Aplidium albicans	Tunicate	Aplidine	Mediterranean
Elysia rufescens	Mollusc	Kahalalide F	Hawaii
Spisula polynyma	Mollusc	ES-285	Japan
Micromonospora marina	Actinomycete	Thiocoraline	Mozambique Strait
Bugula neritina	Bryozoan	Bryostatin 1	Gulf of California
Portieria hornemannii	Red alga	Halomon	Philippines
Aplysia kurodai	Sea hare	Aplyronine A	Japan
Dolabella auricularia	Sea hare	Dolastatin 10	Indian Ocean
Crambe crambe	Sponge	Crambescidin-816	Mediterranean
Halichondria okadai	Sponge	Halichondrin B	Okinawa
Lissodendoryx spp.	Sponge	Isohomohalichondrin B	New Zealand
Mycale spp.	Sponge	Mycaperoxide B	Thailand
Tridemnum soldium	Tunicate	Didemnin B	Caribbean

variety of human cancer cell lines. The majority of these chemical entities are negative for the National Cancer Institute (NCI) COMPARE analysis,[5] confirming their novel characteristics, in fact compounds with unique profiles of growth inhibition, suggesting modes of action unrelated to those of known classes of chemotherapeutic agent, have been identified through this innovative tool.

To identify the most promising compounds for clinical development, a number of selection criteria were formulated by PharmaMar. These included: an innovative mechanism of action, lack of cross-resistance with conventional anti-cancer drugs, in-vivo activity in human solid-tumor models, evidence of a positive therapeutic index, and the feasibility of producing the compound in sufficient quantity for large clinical trials. The supply of the identified chemical entity has to be sustainable and industrially feasible, because the large-scale harvesting of marine organisms is neither physically feasible nor environmentally acceptable.

Using these selection criteria, an extensive portfolio of promising new marine-derived anti-cancer compounds has been assembled by PharmaMar. This includes several crambescidins, variolins, trunkamides, lamellarins and isohomohalichondrins that are currently in early preclinical development, the compounds ES-285 and thiocoraline, which are in advanced preclinical development, and, most importantly, the compounds ecteinascidin-743 (ET-743), aplidine (Aplidin) and kahalalide F, which are in various stages of clinical development.

Ecteinascidin-743

ET-743 is a tetrahydroisoquinoline isolated from the Caribbean tunicate, *Ecteinascidia turbinata* (Figure 1).[6] It has a novel mechanism of action that has yet to be fully elucidated.[7] It has been shown to bind to the minor groove of DNA and bend the macromolecule towards its major groove,[8] to inhibit promoter-specific gene transcription,[9]

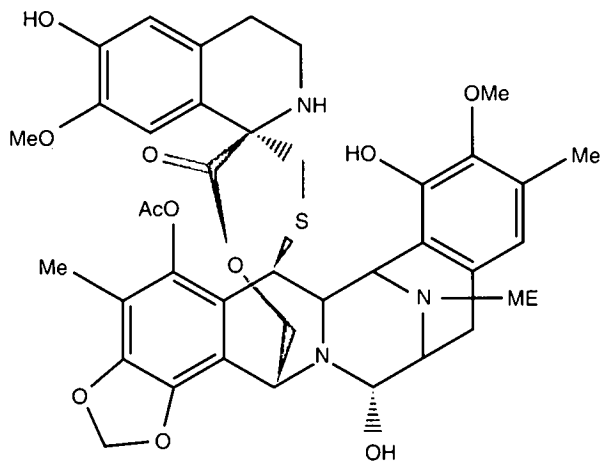

Figure 1. Chemical structure of ET-743.

to interact with DNA repair machinery to induce lethal DNA strand breaks[10,11], and to cause prolonged cell-cycle block in the G_2/M phase.[7] Consequently, ET-743 inhibits DNA synthesis and cell proliferation. Furthermore, it demonstrates in-vitro cytotoxic activity against several human cancer cell lines[7,9], and in-vivo anti-tumor activity in human tumor xenograft models.[12,13]

Phase II trials have demonstrated that ET-743 induces tumor control when used as first- or second-line treatment of advanced soft-tissue sarcomas,[14–16] and a future role can be envisaged for it, either as single-agent or combination chemotherapy, in this indication. Preliminary results of Phase II studies of ET-743 in advanced breast cancer[17] and ovarian cancer[18] also point to activity against these tumor types.

New schedules of administration of ET-743 are being incorporated into the clinical development program. A short treatment schedule using 3 h infusions every 3 weeks, to facilitate out-patient administration, is under active investi-

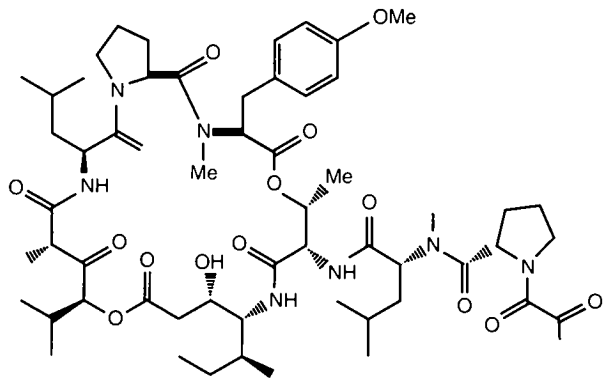

Figure 2. Chemical structure of aplidine.

gation; a weekly 580 mg/m^2 dose regimen has compared favorably with a once-every-3-weeks schedule, showing anti-tumor activity in heavily pretreated liposarcoma and ovarian cancer, and no grade 3–4 toxicities.[19] Phase III trials of ET-743 are planned and further opportunities for the use of the agent as a component of combination and sequential therapies are being investigated. Research demonstrating the interaction of ET-743 with DNA repair mechanisms[9,11,20] provides a strong rationale for combining ET-743 with platinum salts. For example, initial findings from studies of a human ovarian carcinoma cell (1A9 tumor) model indicate that the tumor is resistant to ET-743 alone and cisplatin alone, but sensitive to a combination of these two compounds. Phase I trials investigating the effects of combinations of ET-743 with cisplatin, carboplatin and doxorubicin are currently in progress or in preparation.

Aplidine (Aplidin)

Aplidine is a cyclopeptide cytotoxic agent (Figure 2) derived from the Mediterranean tunicate, *Aplidium albicans*. Its mode of anti-tumor action is believed to involve down-regulation of the *flt-1 receptor* gene for vascular endothelial growth factor (involved in angiogenesis and tumor vascularization), induction of apoptosis in cancer cells, and arrest of the cell cycle in the G_1 phase.[21,22] The drug shows potent in-vitro activity against a range of human tumor cell lines, including ovarian, colorectal and non-small-cell lung cancers,[23] in addition to in-vivo activity against prostate, bladder and gastric tumors.[24]

Phase I trials with aplidine have been completed in more than 200 patients recruited into five studies in Europe and Canada. The efficacy and safety of various intravenous infusion schedules (24 h/once weekly, 3 h/every other week, 1 h/once weekly, 24 h/every other week, and 1 h/once daily for five consecutive days) were assessed. The dose-limiting toxicity was primarily muscular, characterized by muscle pain and weakness, although this toxicity was responsive to

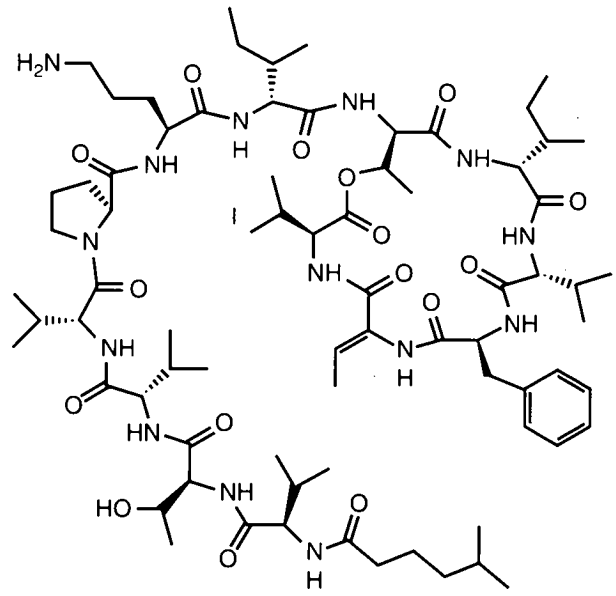

Figure 3. Chemical structure of kahalalide F.

dose reduction or therapeutic intervention with L-carnitine. No evidence of rhabdomyolysis was noted on optical or electron microscopy. Use of L-carnitine enabled the dose of aplidine to be increased by 40%, allowing further exploration of the dose–response relationship. At the recommended dose of aplidine (5 mg/m^2 as a 3 h or 24 h intravenous infusion every other week), the most frequent toxicity was fatigue (generally grade 1–2), affecting 33% of patients; haematological toxicity was not observed in the Phase I studies.[25] The clinical benefit of aplidine treatment was apparent in a variety of tumor types, including renal, bronchial carcinoid and medullary thyroid carcinomas, and non-Hodgkin lymphoma.[25] Its lack of in-vitro cross-resistance with many anti-cancer drugs[26] warrants consideration of Phase I trials of aplidine in combination with other anti-cancer drugs. Phase II studies assessing the dose–response relationship (aplidine 5 mg/m^2 compared with 7 mg/m^2, with L-carnitine protection) in renal and colorectal cancer are in progress, and a further Phase II study incorporating an outpatient infusion schedule is planned.

Kahalalide F

Kahalalide F, a depsipeptide isolated from the Hawaiian mollusc, *Elysia rufescens* (Figure 3), is an anti-tumor compound that is negative for the NCI COMPARE analysis. Although the mechanism of action of kahalalide F has not been fully elucidated, it is known to target lysosomes[27] and it is, therefore, selective for those tumor cells (e.g. prostate cancer cells) with high lysosomal activity. This mechanism, which appears unique among current anti-cancer agents, may lead to intracellular acidification and apoptosis.

Kahalalide F also inhibits both erbB2 transmembrane tyrosine kinase activity and transforming growth factor-α expression.[28] Further evidence of the novel mechanism of anti-tumor action of kahalalide F is provided by its lack of cross-resistance with many established anti-cancer agents.[29]

Kahalalide F displays selectivity for hormone-independent prostate-derived cell lines *in vitro* and is active against corresponding human tumor xenografts *in vivo*.[28] A Phase I dose-escalation study has been implemented with the agent (1 h infusion daily for five consecutive days every 3 weeks) in patients with hormone-resistant prostate cancer.[30] The target plasma drug concentration has been attained with no severe toxicity, and early results are indicative of clinical benefit. A second Phase I trial investigating a low-dose schedule (1 h infusion weekly) in patients with solid tumors is in progress.

ES-285

ES-285, a new chemical entity that is obtained from the North Atlantic mollusc, *Spisula polynyma*, inhibits the activity of Rho (a GTP-binding protein), causing disruption of actin stress fibers and subsequent apoptosis (J Avila, personal communication). The anti-tumor activity and therapeutic potential of Rho-interactive compounds have been reviewed recently.[31] The in-vivo anti-tumor activity of ES-285 has been demonstrated in a variety of human tumors, including hepatoma, renal and prostate tumor models.[32] ES-285 is in late-stage preclinical development and Phase I trials are expected to commence this year.

Conclusions

The continuing PharmaMar exploration program has shown the potential use of marine ecosystems as a source of new anti-cancer compounds. These naturally occurring drugs may have an important part to play in the varied pharmacological approaches to cancer treatment. In addition, further investigation of the novel mechanisms of action of these compounds has identified new cellular targets for therapeutic intervention. Further research and development of these marine-derived compounds requires the collaboration of a diverse range of disciplines, including marine biology, biochemistry, toxicology, pharmacology and oncology.

References

1. Cragg GM, Newman DJ, Snader KM, *et al.* Natural products in drug discovery and development. *J Nat Prod* 1997; **60**: 52–60.
2. Cragg GM, Newman DJ. Discovery and development of antineoplastic agents from natural sources. *Cancer Invest* 1999; **17**: 153–63.
3. Ellis R. *Aquagenesis. The origin and evolution of life in the sea.* New York: Viking Penguin, Penguin Putnam Inc., 2001.
4. Gould SJ. *The book of life: an illustrated history of the evolution of life on Earth.* New York: WW Norton & Co. Inc., 2001.
5. Paull KD, Shoemaker RH, Hodes L, *et al.* Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. *J Natl Cancer Inst* 1989; **81**: 1088–92.
6. Guan Y, Sakai R, Rinehart K, *et al.* Molecular and crystal structures of ecteinascidins: potent antitumor compounds from the Caribbean tunicate Ecteinascidia turbinata. *J Biomol Struct Dyn* 1993; **10**: 793–818.
7. Erba E, Bergamaschi D, Bassano L, *et al.* Ecteinascidin-743 (ET-743), a natural marine compound, with a unique mechanism of action. *Eur J Cancer* 2001; **37**: 97–105.
8. Zewail-Foote M, Hurley LH. Ecteinascidin-743: a minor groove alkylator that bends DNA towards the major groove. *J Med Chem* 1999; **42**: 2493–7.
9. Jin S, Gorfajn B, Faircloth G, *et al.* Ecteinascidin 743, a transcription-targeted chemotherapeutic that inhibits MDR1 activation. *Proc Natl Acad Sci USA* 2000; **97**: 6775–9.
10. Damia G, Silvester S, Carrassa L, *et al.* Unique pattern of ET-743 activity in different cellular systems with defined deficiencies in DNA-repair pathways. *Int J Cancer* 2001; **92**: 583–8.
11. Takebayashi Y, Pourquier P, Zimonjic DB, *et al.* Antiproliferative activity of ecteinascidin 743 is dependent upon transcription-coupled nucleotide-excision repair. *Nature Med* 2001; 7: 961–6.
12. Hendricks HR, Fiebig HH, Giavazzi R, *et al.* ET-743: a marine natural product with high antitumor activity in human tumour xenograft models. *Ann Oncol* 1996; 7(suppl 1): 33, abstract P109.
13. Valoti G, Nicoletti MI, Pellegrino A, *et al.* Ecteinascidin-743, a new marine natural product with potent antitumor activity on human ovarian carcinoma xenografts. *Clin Cancer Res* 1998; **4**: 1977–83.
14. Demetri GD, Manola J, Harmon D, *et al.* 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 USA [abstract]. *Proc Am Soc Clin Oncol* 2001; **20**: 352.
15. Yovine A, Riofrio M, Brain E, *et al.* Ecteinascidin-743 given as a 24-hour intravenous continuous infusion every 3 weeks: results of a Phase II trial in patients with pretreated soft tissue sarcomas [abstract]. *Proc Am Soc Clin Oncol* 2001; **20**: 363.
16. Le Cesne A, Blay J, Judson I, *et al.* ET-743 is an active drug in adult soft-tissue sarcoma: a STBSG–EORTC Phase II trial. *Proc Am Soc Clin Oncol* 2001; **20**: 353.
17. Zelek L, Yovine A, Brain E, *et al.* Preliminary results of Phase II study of ecteinascidin-743 with the 24-hour continuous infusion Q3 weeks schedule in pretreated advanced/metastatic breast cancer patients. *Proceedings of the 11th NCI–EORTC–AACR Symposium*, Amsterdam, 7–10 November 2001; p. 85, abstract 212.
18. Curigliano G, Bauer J, Capri G, *et al.* Ecteinascidin-743 in ovarian cancer: activity in xenografts and preliminary results of an ongoing study in patients failing platinum-taxanes. *Proceedings of the 11th NCI–EORTC–AACR International Conference*, Miami Beach, Florida, 29 October–2 November 2001; p. 78, abstract 380.
19. Forouzesh B, Hidalgo M, Denis L, *et al.* Phase I and pharmacokinetic study of ET-743, a minor groove DNA binder, administered weekly to patients with advanced cancer. *Eur J Cancer* 2001; **37**(suppl 6): 32, abstract 106.
20. Minuzzo M, Marchini S, Broggini M, *et al.* Interference of transcriptional activation by the antineoplastic drug ecteinascidin-743. *Proc Natl Assoc Sci USA* 2000; **97**: 6780–4.
21. Erba E, Ronzoni S, Bergamaschi D, *et al.* Mechanism of antileukemic activity of aplidin [abstract]. *Proc Am Assoc Cancer Res* 1999; **40**: 14.

22. Faircloth G, Hanauske A, Depenbrock H, *et al.* Preclinical characterization of aplidin (APD), a new marine anticancer depsipeptide (MADEP) [abstract]. *Proc Am Assoc Cancer Res* 1997; **38**: 103.
23. Depenbrock H, Peter R, Faircloth GT, *et al. In vitro* activity of aplidin, a new marine-derived anti-cancer compound, on freshly explanted clonogenic human tumour cells and haematopoietic precursor cells. *Br J Cancer* 1998; **78**: 739–44.
24. Faircloth G, Grant W, Nam S, *et al.* Schedule dependency of Aplidin, a marine depsipeptide with antitumor activity [abstract]. *Proc Am Assoc Cancer Res* 1999; **40**: 2612.
25. Raymond E, Paz-Ares L, Izquierdo M, *et al.* Phase I trials with Aplidine, a new marine-derived anticancer compound. *Proceedings of the 11th European Cancer Conference*, Lisbon, 21–25 October 2001; p. S32, abstract 107.
26. Kaspers GJ, Brestets D. In vitro sensitivity to aplidine in normal bone marrow and blood in leukemic samples from children [abstract]. *Clin Cancer Res* 2001; 7(suppl): 243.
27. Garcia-Rocha M, Bonay P, Avila J. The antitumoral compound kahalalide F acts on cell lysosomes. *Cancer Lett* 1996; **99**: 43–50.
28. Faircloth G, Grant W, Smith B, *et al.* Preclinical development of kahalalide F, a new marine compound selected for clinical studies. *Proceedings of the American Association for Cancer Research*, San Francisco, 1–5 April 2000.
29. Medina LA, Gomez L, Cerna C, *et al.* Investigation of the effects of kahalalide F (PM92102) against human tumor specimens taken directly from patients. *Proceedings of the American Association for Cancer Research*, New Orleans, March 24–28 2001: 213.
30. Schellens J, Rademaker JL, Horenblas S, *et al.* Phase I and pharmacokinetic study of kahalalide-F in patients with advanced androgen-refractory prostate cancer. *Proceedings of the NCI–EORTC–AACR Conference*, Miami Beach, Florida, 29 October–2 November 2001; p. 42, abstract 207.
31. Prendergast GC. Farnesyltransferase inhibitors: antineoplastic mechanisms and clinical prospects. *Curr Opin Cell Biol* 2000; **12**: 166–73.
32. Jimeno JJ, Garcia-Gravalos D, Avila J, *et al.* ES-285, a marine natural product with activity against solid tumours. *Proceedings of the AACR–NCI–EORTC International Conference*, Washington DC, 16–19 November 1999; abstract 314.