Natural products in anticancer therapy

Adriana B da Rocha, Rafael M Lopes and Gilberto Schwartsmann*

Many pharmaceutical agents have been discovered by screening natural products from plants, animals, marine organisms and microorganisms. Vincristine, irinotecan, etoposide and paclitaxel are examples of plant-derived compounds that are being employed in cancer treatment, and dactinomycin, bleomycin and doxorubicin are anticancer agents derived from microbial sources. Citarabine is an example of an anticancer agent originating from a marine source. Other agents originating from marine sources are bryostatin-1, aplidine, dolastatin 10 and ET-743, which have recently entered phase I and II clinical trials.

Addresses
Comprehensive Cancer Center, The Lutheran University of Brazil, Rua Miguel Tostes, 101 Bairro São Luís, Canoas, RS 92420-280, Brazil
*e-mail: gschwart.ez@terra.com.br

Current Opinion in Pharmacology 2001, 1:364–369

1471-4892/01/$ – see front matter

Introduction

The role of natural products as a source for remedies has been recognized since ancient times [1,2]. Despite major scientific and technological progress in combinatorial chemistry, drugs derived from natural product still make an enormous contribution to drug discovery today. Table 1 shows some examples of agents derived from natural sources that are currently used in clinical practice [3–5].

Nature is an attractive source of new therapeutic candidate compounds as a tremendous chemical diversity is found in millions of species of plants, animals, marine organisms and microorganisms. For many living organisms, this chemical diversity reflects the impact of evolution in the selection and conservation of self-defense mechanisms that represent the strategies employed to repel or destroy predators.

The development of novel agents from natural sources presents obstacles that are not usually met when one deals with synthetic compounds. For instance, there may be difficulties in accessing the source of the samples, obtaining appropriate amounts of the sample, identification and isolation of the active compound in the sample, and problems in synthesizing the necessary amounts of the compound of interest. These problems became evident when the tubulin-interacting agent paclitaxel was introduced in clinical use. Initial antitumor activity was observed in various types of cancer, including ovarian and breast adenocarcinoma. Paclitaxel was originally isolated from the bark of the yew tree *Taxus brevifolia* [6], a finite source of the compound. It took some years to develop a semi-synthetic analog (docetaxel) which is derived from a renewable source, the leaves of *Taxus baccata* [7]. Currently, total synthesis has been achieved for both agents and drug supply is no longer a problem.

An analysis of the number of chemotherapeutic agents and their sources indicates that over 60% of approved drugs are derived from natural compounds [2]. In recent years, advances in deep-sea collection and acquaculture technology have led to a significant number of compounds derived from marine organisms entering preclinical and early clinical evaluation as anticancer candidates [8,9]. A number of natural product derived anticancer compounds in late preclinical development and in early clinical trials are briefly discussed in this review.

Plant-derived agents

Plant species with a capacity to defend themselves from potential predators and to inhibit other plants competing for space have been selected for ('natural selection') [10]. In order to survive, plants have developed sophisticated mechanisms including an elaborate chemical arsenal of toxic substances, such as terpenes and alkaloids, that inhibit the growth of other plants and make them unattractive to predators. An interesting example is tannin production by certain species of trees. When a predator starts eating a tree in a grove, that tree releases ethylene into the air. This signals to other trees in the grove to increase leaf tannin production, making themselves poisonous and unpalatable to the predator animal. Some plants are also able to produce phenol and tannin when attacked by caterpillars, using a similar signaling process.

Several plant-derived compounds are currently successfully employed in cancer treatment (Table 2). One of the most significant examples is the vinca alkaloid family isolated from the periwinkle *Catharanthus roseus*, which is found in the rain forests of Madagascar [11]. The introduction of the vinca alkaloid vincristine was responsible for an increase in the cure rates for Hodgkin's disease and some forms of leukemia [12]. Vincristine inhibits microtubule assembly, inducing tubulin self-association into coiled spiral aggregates ([11]; see also the review by KW Wood, WD Cornwell and JR Jackson, this issue, pp 370–377).

Another example of a highly active agent derived from a natural product is etoposide, which has produced high cure rates in testicular cancer when used in combination with bleomycin (also derived from a natural product) and cisplatin [13]. Etoposide is a epipodophyllotoxin, derived from the mandrake plant *Podophyllum peltatum* and the wild chervil *Podophyllum emodi* [14]. It has also significant activity against small-cell lung carcinoma [15–17]. Etoposide is a topoisomerase II inhibitor, stabilizing enzyme–DNA cleavable complexes leading to DNA breaks [18].

The taxanes paclitaxel and docetaxel, mentioned briefly in the introduction, show impressive antitumor activity

Table 1

Drugs developed from natural sources.

Drug	Medical use	Mechanism of action	Source
Aspirin	Analgesic, anti-inflammatory, antipyretic	Inhibition of COX	Plant
Atropine	Pupil dilator	Antagonist of ACh at muscarinic receptors at post-ganglionic parasympathetic neuroeffector sites	Plant
Caffeine	Stimulant	Adenosine receptor antagonist	Plant
Codeine	Analgesic, antitussive	Opioid receptor agonist	Plant
Digoxin	For atrial fibrillation and CHF	Inhibition of the Na^+/K^+ ATPase membrane pump	Plant
Eugenol	Toothache	Reduces excitability of sensory nerves (increased K^+ efflux and reduced Ca^{2+} influx)	Plant
Morphine	Analgesic	Opioid receptor agonist	Plant
Pilocarpine	Glaucoma	Muscarinic receptor agonist	Plant
Quinine	Malaria prophylaxis	Inhibition of protein synthesis in the malaria parasite	Plant
Taxol	Anticancer agent	Antimitotic agent (binds to and stabilizes microtubules)	Plant
Penicillin	Antibiotic	Inhibition of synthesis of cell wall peptidoglycan	Microbe
Tetracyclin	Antibiotic	Inhibition of protein synthesis by binding to the ribosome 30S subunit.	Microbe
Cyclosporin A	Immunosuppressant	Inhibition of clonal proliferation of T lymphocytes (via inhibition of lymphokine production)	Microbe
Aurantosides	Antifungal	Inhibition of tubulin polymerization	Marine organism
Spongistatin 1	Antifungal	Inhibition of tubulin polymerization	Marine organism
Manoalide	Analgesic, anti-inflammatory	Inhibition of phospholipase A_2	Marine organism

ACh, acetylcholine; CHF, congestive heart failure; COX, cyclooxygenase.

against breast, ovarian and other tumor types in the clinic. Paclitaxel stabilizes microtubules, leading to mitotic arrest [6]. In addition, the camptothecin derivatives irinotecan and topotecan, have shown significant antitumor activity against colorectal and ovarian cancer respectively [19,20]. These compounds were initially obtained from the bark and wood of Nyssacea *Camptotheca accuminata* and act by inhibiting topoisomerase I [21]. The taxanes and the camptothecins are presently approved for human use in various countries.

Flavopiridol is one of the most exciting plant-based agents currently in development, representing the first cyclin-dependent kinase inhibitor to enter the clinic [22•]. Flavopiridol is a synthetic flavone derived from the plant alkaloid rohitukine, which was isolated from the leaves and stems of *Amoora rohituka* and later from *Dysoxylum binectariferum* (Maliaceae) [22•,23–26]. The mechanism of action of flavopiridol involves interfering with the phosphorylation of cyclin-dependent kinases, hampering their activation and blocking cell-cycle progression at growth phase 1 (G_1) or G_2 [22•,25]. In phase I clinical trials with flavopiridol [27,28], secretory diarrhea was found to be the dose-limiting toxicity, and encouraging response rates were noted in a variety of solid and hematological malignancies. These results led to the initiation of phase II trials in patients with colorectal, prostate, renal cell and non-small-cell lung carcinoma, as well non-Hodgkin's lymphoma and chronic lymphocytic leukemia. Based on *in vitro* synergy of flavopiridol with several conventional cytotoxic agents [29], phase I combination studies to evaluate flavopiridol with paclitaxel or cisplatin against advanced solid tumors are also ongoing.

A number of additional plant-derived agents are currently under investigation (Table 2). Homoharringtonine, for instance, is an alkaloid isolated from the Chinese tree *Cephalotaxus harringtonia* (Cephalotaxacea) [30], and has shown efficacy against various leukemias [31]. The principal mechanism of action of homoharringtonine is the inhibition of protein synthesis, blocking cell-cycle progression [32]. 4-Ipomeanol is a pneumotoxic furan derivative isolated from the sweet potato *Ipomoeca batatas* (Convolvulaceae) [33] and has been under clinical evaluation as a lung-cancer-specific antineoplastic agent [34]. This compound is converted into DNA-binding metabolites upon metabolic activation by cytochrome P450 enzymes that are present in cells of the lung [33]. Finally, β-lapachone is a DNA topoisomerase I inhibitor that induces cell-cycle delay at G_1 or S (synthesis) phase before inducing either apoptotic or necrotic cell death in a variety of human carcinoma cells, including ovary, colon, lung, prostate and breast [35].

Table 2

Plant-derived anticancer agents.

Compound	Cancer use	Status
Vincristine	Leukemia, lymphoma, breast, lung, pediatric solid cancers and others	Phase III/IV
Vinblastine	Breast, lymphoma, germ-cell and renal cancer	Phase III/IV
Paclitaxel	Ovary, breast, lung, bladder, and head and neck cancer	Phase III/IV
Docetaxel	Breast and lung cancer	Phase III
Topotecan	Ovarian, lung and pediatric cancer	Phase II/III
Irinotecan	Colorectal and lung cancer	Phase II/III
Flavopiridol	Experimental	Phase I/II
Acronyciline	Experimental	Phase II/III
Bruceantin	Experimental	Preclinical/ phase I
Thalicarpin	Experimental	Preclinical/ phase I

Table 3

Microbe-derived anticancer agents.

Compound	Cancer use	Status
Actinomycin	Sarcoma and germ-cell tumors	Phase III/IV
Bleomycin	Germ-cell, cervix, and head and neck cancer	Phase III/IV
Daunomycin	Leukemia	Phase III/IV
Doxorubicin	Lymphoma, breast, ovary, lung and sarcomas	Phase III/IV
Epirubicin	Breast cancer	Phase III/IV
Idarubicin	Breast cancer and leukemia	Phase III/IV
Mitomycin C	Gastric, colorectal, anal and lung cancer	Phase III/IV
Streptozocin	Gastric and endocrine tumors	Phase III/IV
Wortmannin	Experimental	Preclinical
Rapamicin*	Experimental	Preclinical
Geldanamycin	Experimental	Preclinical

*Rapamicin is also a potent immunosupressant.

Microbe-derived agents

Antitumor antibiotics are among the most important cancer chemotherapeutic agents, and include members of the anthracycline, bleomycin, actinomycin, mitomycin and aureolic acid families [36]. Clinically useful agents from these families are the daunomycin-related agents (daunomycin itself, doxorubicin, idarubicin and epirubicin), the peptolides (exemplified by dactinomycin), the mitosanes (such as mitomycin C) and the glycosylated anthracenone mithramycin. The anthracyclines are among the most used antitumor antibiotics in the clinic and exert antitumor activity mainly by inhibiting topoisomerase II [37].

Many pharmaceutical agents have been discovered by screening natural products from a wide range of microorganisms. For example, cyclosporin A (CyA) and FK506 (Prograf, tacrolimus) were discovered at Sandoz and Fujisawa Pharmaceuticals, respectively, in screens for immunosupressive agents that would block a mixed lymphocyte response. These two immunosuppressant drugs have had a dramatic impact on clinical medicine and are widely used to prevent and treat graft rejection and graft-versus-host disease following both solid-organ transplants and bone-marrow transplants.

The immunosuppressant rapamycin (sirolimus) was originally discovered at Wyeth-Ayerst Pharmaceuticals in a screen for antifungal agents, and was later found to have potent immunosuppressive activity. In addition rapamycin, wortmannin and geldanamycin (also natural products) have been found to have antiproliferative actions and may therefore find clinical use as novel chemotherapeutic agents (Table 3; [38]). Rapamycin and its analogs are products of *Streptomyces hygroscopicus* and inhibit signaling pathways required for T-cell activation and proliferation. Rapamycin blocks progression of the cell cycle at middle-to-late G_1 phase in T cells and B cells, and osteosarcoma and rhabdomyosarcoma cell lines, among others [39]. Geldanamycin is a benzoquinone ansamycin natural fermentation product that was originally thought to be a direct protein tyrosine kinase inhibitor. However, subsequent studies have revealed that geldanamycin binds to, and inhibits the 90 kDa heat-shock protein HSP 90 [40]. Wortmannin is a product of the fungus *Talaromyces wortmanni* and inhibits signal transduction pathways by forming a covalent complex with an active-site residue of phosphoinositide 3 kinase (PI3K), inhibiting PI3K activity [41].

Thus, toxins that originally evolved to kill competing micoorganisms can have a variety of physiological effects in animals. In several cases, the targets of these compounds are components of signal transduction cascades that are conserved in many species, and that have been considered novel targets for anticancer drug discovery [42••].

Marine-organism-derived agents

Marine organisms are a rich source for natural products and many compounds that are derived from these organisms have generated interest both as challenging problems for structure elucidation and synthesis and for their cytotoxicities (Table 4). The new classes of anticancer drugs that have been isolated from marine organisms have been shown to possess cytotoxic activity against multiple tumor types [8,9]. Most of these compounds were identified during the 1980s following great improvements in deep-sea sample collection and in technologies that allowed the production of drugs on a large scale through aquaculture and synthesis [43]. Overall, more than 3000 new substances have been identified from marine organisms during the

Table 4

Marine-organism-derived anticancer agents.

Compound	Cancer use	Mechanism of action	Status
Citarabine	Leukemia, lymphoma	Inhibition of DNA synthesis	Phase III/IV
Bryostatin 1	Experimental	Activation of PKC	Phase I/II
Dolastatin 10	Experimental	Inhibition of microtubules and pro-apoptotic effects	Phase II
Ecteinascidin 743	Experimental	Alkylation of DNA	Phase II
Aplidine	Experimental	Inhibition of cell-cycle progression	Phase I/II
Halicondrin B	Experimental	Interaction with tubulin	Preclinical
Discodermolide	Experimental	Stabilization of tubulin	Preclinical
Cryptophycin	Experimental	Hyperphosphorylation of Bcl-2	Preclinical

PKC, protein kinase C.

past three decades, demonstrating the great potential of the sea as a source of novel chemical classes [44,45].

The first anticancer product derived from marine sources to enter clinical trials was didemnin B, a cyclic depsipeptide isolated from the tunicate *Tridemnum solidum* [45]. Preliminary results from these clinical trials showed a partial activity against non-Hodgkin's lymphoma [46]; however, the observation of severe neuromuscular and cardiac toxicity led to the discontinuation of clinical trials [47,48]. Didemnin can inhibit protein synthesis and induce G_1 cell-cycle arrest [49]. Aplidine is a related depsipeptide that appears to be more active than didemnin B in preclinical models, and so far does not seem to produce similar life-threatening neuromuscular toxicity [49]. Preclinical studies indicate that aplidine is active against several tumor types through blockade of cell-cycle progression at G_1 [49].

Numerous ecteinascidins have been isolated from the marine tunicate *Ecteinascidia turbinata*. Preclinical studies have shown that one of these ecteinascidins (ET-743) is toxic to most tumor cell lines in nanomolar and sub-nanomolar concentrations [45]. This compound was selected for clinical trials, and antitumor effects have been observed in phase I trials with concentrations of less than 2mg/m^2 body surface [50,51]. ET-743 is a tetrahydroisoquinilone alkaloid that acts by selective alkylation of guanine residues in the DNA minor groove. It therefore differs from the other DNA-alkylanting agents so far introduced in the clinic [52,53], and also interacts with nuclear proteins [54]. ET-743 is currently in phase II clinical trials in Europe and the United States [50,51].

The dolastatins are a class of peptides that were originally derived from a mollusk from the Indian Ocean, the sea hare *Dolabella auricularia*. These peptides have cytotoxic activity and of the various compounds of this class, Dolastatin 10 and Dolastatin 15, have received the greatest clinical interest [55]. Indeed, Dolastatin 10 has entered Phase I and Phase II trials, after showing significant antitumor activity in preclinical models [55]. Its mechanism of action involves inhibition of microtubule assembly, which causes cell-cycle arrest in metaphase [56,57]. The toxicity profile for Dolastatin 10 includes bone marrow toxicity and local reaction at the injection site. Also, a mild peripheral neuropaty was observed in some patients. Despite its remarkable activity in *in vitro* studies, Dolastatin has failed to show significant antitumor effects in Phase I and II trials conducted for several types of solid tumors [58–61].

The bryostatins comprise a structurally and functionally novel group of 20 macrocyclic lactones originally isolated from *Bugula neritina* and other marine bryozoa. These macrocyclic compounds have shown significant activity against murine P388 (a lymphocytic leukemia cell line) [62], and have subsequently been identified as important leads in cancer chemotherapy. Bryostatin 1 has recently entered phase II clinical trials for the treatment of melanoma, non-Hodgkin's lymphoma, renal cancer and colorectal cancer [63–66] and continues to be evaluated in phase I clinical trials, alone and in combination with other drugs, for the treatment of other types of cancer. Bryostatin 1 has been found to promote the normal growth of bone marrow progenitor cells, to provide *in vivo* protection against normally lethal doses of ionizing radiation, and to serve as an immune stimulant, enhancing the normal production of interleukin 2 and interferons [67].

Conclusions

The introduction of active agents derived from nature into the cancer armamentarium has changed the natural history of many types of human cancer. Experimental agents derived from natural products are offering us a great opportunity to evaluate not only totally new chemical classes of anticancer agents, but also novel and potentially relevant mechanisms of action.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z: Medicinal plants in therapy. *Bull World Health Organ* 1985, 63:965-981.

2. Cragg GM, Newman DJ, Snader KM: Natural products in drug discovery and development. *J Nat Prod* 1997, 60:52-60.

3. Balandrin MF, Kinghorn AD, Farnsworth NR: Plant-derived natural products in drug discovery and development: an overview. In *Human Medicinal Agents from Plants.* Edited by Kinghorn AD, Balandrin MF. North Carolina, USA: Oxford University Press USA; 1993:2-12. [American Chemical Society Symposium Series.]

4. Farnsworth NR: The role of ethnopharmacology in drug development. *Ciba Found Symp* 1990, 154:2-11.

5. Cragg GM, Newman DJ: Discovery and development of antineoplastic agents from natural sources. *Cancer Invest* 1999, 17:153-163.

6. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from ***Taxus brevifolia***. *J Am Chem Soc* 1971, 93:2325-2327.

7. Cortes JE, Pazdur R: Docetaxel. *J Clin Oncol* 1995, 13:2643-2655.

8. Schwartsmann G: Marine organisms and other novel natural sources of new anticancer drugs. *Ann Oncol* 2000, 11:235-243.

9. Schwartsmann G, Rocha AB, Berlinck R, Jimeno J: Marine organisms as a source of new anticancer agents. *Lancet Oncol* 2001, 2:221-225.

10. Mans DRA, Rocha AB, Schwartsmann G: Anti-cancer drug discovery and development in Brazil: targeted plant collection as a rational strategy to acquire candidate anti-cancer compounds. *Oncologist* 2000, 5:185-199.

11. Noble RL: The discovery of the vinca alkaloids – chemotherapeutic agents against cancer. *Biochem Cell Biol* 1990, 68:1344-1351.

12. DeVita VT Jr, Serpick AA, Carbone PO: Combination chemotherapy in the treatment of advanced Hodgkin's disease. *Ann Intern Med* 1970, 73:881-895.

13. Williams SD, Birch R, Einhorn LH, Irwin L, Greco FA, Loehrer PJ: Treatment of disseminated germ-cell tumors with cisplatin, bleomycin, and either vinblastine or etoposide. *New Engl J Med* 1987, 316:1435-1440.

14. Stähelin H: Actvity of a new glycosidic lignan derivative (VP-16-213) related to podophyllotoxin in experimental tumors. *Eur J Cancer* 1973, 9:215-221.

15. Chabner BA: Anticancer drugs. In *Cancer: Principles and Practice,* edn 4. Edited by DeVita VT Jr, Hellman S, Rosenberg AS. Philadelphia: Lippincott; 1991:325-417.

16. Perry MC (Ed): *The chemotherapy sourcebook.* Baltimore: Williams & Wilkins; 1992.

17. Harvey AL: Medicines from nature: are natural products still relevant to drug discovery? *Trends Pharmacol Sci* 1999, 20:196-198.

18. Liu LF: DNA topoisomerase poisons as antitumor drugs. *Annu Rev Biochem* 1989, 58:351-375.

19. Creemers GJ, Bolis G, Gore M, Scarfone G, Lacave AJ, Guastalla JP, Despax R, Favalli G, Kreinberg R, Van Belle S *et al.*: Topotecan, an active drug in the second-line treatment of epithelial ovarian cancer. *J Clin Oncol* 1996, 14:3056-3061.

20. Bertino JR: Irinotecan for colorectal cancer. *Semin Oncol* 1997, 24:S18-S23.

21. Liu LF, Desai SD, Li TK, Mao Y, Sun M, Sim SP: Mechanism of action of camptothecin. *Ann New York Acad Sci* 2000, 922:1-10.

22. • Kelland LR: Flavopiridol, the first cyclin-dependent kinase inhibitor to enter the clinic: current status. *Expert Opin Invest Drugs* 2000, 9:2903-2911.
This review focuses on the clinical development of flavopiridol, the prototype broad-spectrum inhibitor of cyclin-dependent kinases.

23. Harmon AD, Weiss U, Silverton JV: The structure of rohutukine, the main alkaloid of ***Amoora rohituka*** (syn. ***Aphanamixis polystachya***) (Maliaceae). *Tetrahydron* 1979, 20:721-724.

24. Cragg G, Suffness M: Metabolism of plant-derived anticancer agents. *Pharmacol Ther* 1988, 37:425-432.

25. Worland PJ, Kaur G, Stetler-Stevenson M, Sebers S, Sartor O, Sausville EA: Alteration of the phosphorylation state of p32cdc2 kinase by the flavone L86-8275 in breast carcinoma cells. *Biochem Pharmacol* 1993, 46:1831-1836.

26. Losiewicz MD, Carlson BA, Kaur G, Sausville EA, Worland PJ: Potent inhibition of cdc2 kinase activity by the flavonoid L86-8275. *Biochem Biophys Res Commun* 1994, 201:589-595.

27. Senderowicz AM, Headlee D, Stinson SF, Lush RM, Kalil N, Villalba L, Hill K, Steinberg SM, Figg WD, Tompkins A *et al.*: Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms. *J Clin Oncol* 1998; 16:2986-2999.

28. Wright J, Blatner GL, Cheson BD: Clinical trials referral resource. Clinical trials of flavopiridol. *Oncology* 1998, 12:1023-1024.

29. Kaur G, Stetler-Stevenson M, Sebers S, Worland P, Sedlacek H, Myers C, Czech J, Naik R, Sausville E: Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275. *J Natl Cancer Inst* 1992, 84:1736-1740.

30. Powell RG, Weisleder D, Smith CR Jr, Rohwedder WK: Structures of harringtonine, isoharringtonine, and homoharringtonine. *Tetrahydron Lett* 1970; 11:815-818.

31. Kantarjian HM, O'Brien S, Anderlini P, Talpaz M: Treatment of myelogenous leukemia: current status and investigational options. *Blood* 1996, 87:3069-3081.

32. Zhou DC, Zittoun R, Marie JP: Homoharringtonine: an effective new natural product in cancer chemotherapy. *Bull Cancer* 1995, 82:987-995.

33. Rehm S, Devor DE: Acute effects of 4-ipomeanol on experimental lung tumors with bronchiolar or alveolar cell features in Syrian hamsters or C3H/HeNCr mice. *J Cancer Res Clin Oncol* 1993, 120:41-50.

34. Rowinsky EK, Noe DA, Ettinger DS, Christian MC, Lubejko BG, Fishman EK, Sartorius SE, Boyd MR, Donehower RC: Phase I and pharmacological study of the pulmonary cytotoxin 4-ipomeanol on a single dose schedule in lung cancer patients: hepatotoxicity is dose limiting in humans. *Cancer Res* 1993, 53:1794-1801.

35. Li YZ, Li CJ, Pinto AV, Pardee AB: Release of mitochondrial cytochrome ***c*** in both apoptosis and necrosis induced by β-lapachone in human carcinoma cells. *Mol Med* 1999, 4:232-239.

36. Cragg GM, Newman DJ, Weiss RB: Coral reefs, forests, and thermal vents: the worldwide exploration of nature for novel antitumor agents. *Semin Oncol* 1997, 24:156-163.

37. Binaschi M, Farinosi R, Borgnetto ME, Capranico G: ***In vivo*** site specificity and human isoenzyme selectivity of two topoisomerase II poisoning anthracyclines. *Cancer Res* 2000, 60:3770-3776.

38. Patrick Y: Major microbial diversity initiative recommended. *Am Soc Microbiol News* 1997, 63:417-421.

39. Alberts MW, Williams RT, Brown EJ, Tanaka A, Hall FL, Schreiber SL: FKBP-Rapamycin inhibits a cyclin-dependent kinase activity and a cyclin D1-Cdk association in early Gl of an osteosarcoma cell line. *J Biol Chem* 1993, 268:22825-22829.

40. Schulte TW, Neckers LM: The benzoquinone ansamycin 17-allylamino-17demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. *Cancer Chemother Pharmacol* 1998, 42:273-279.

41. Cadenas ME, Sandfrison A, Cutler NS, Heitman J: Signal-transduction cascades as targets for therapeutic intervention by natural products. *Trends Biotechnol* 1998, 16:427-433.

42. •• Adjei AA: Signal transduction pathway targets for anticancer drug discovery. *Curr Pharmaceut Design* 2000, 6:361-378.
This article provides an excellent overview of the anticancer agents inhibiting signal transduction pathway molecules that have entered clinical trials.

43. Pomponi AS: The bioprocess-technological potential of the sea. *J Biotechnol* 1999, 70:5-13.

44. Schweitzer J, Handley FG, Edwards J, Harris WF, Grever MR, Schepartz SA, Cragg G, Snader K, Bhat A: Summary of the workshop on drug development, biological diversity, and economic growth. *J Natl Cancer Inst* 1991, 83:1294-1298.

45. Rinehart KL: Antitumor compounds from tunicates. *Med Res Rev* 2000, 20:1-27.

46. Chun HG, Davies B, Hoth D, Suffness M, Plowman J, Flora K, Grieshaber C, Leyland-Jones B: Didemnin B. The first marine compound entering clinical trials as an antineoplastic agent. *Invest New Drugs* 1986, 4:279-284.

47. Shin DM, Holoye PY, Murphy WK, Forman A, Papasozomenos SC, Hong WK, Raber M: Phase I/II clinical trial of didemnin B in non-small cell lung cancer: neuromuscular toxicity is dose-limiting. *Cancer Chemother Pharmacol* 1991, 29:145-149.

48. Urdiales JL, Morata P, Nunez de Castro I, Sanchez-Jimenez F: Antiproliferative effect of dehydrodidemnin B (DDB), a depsipeptide isolated from Mediterranean tunicates. *Cancer Lett* 1996, 102:31-37.

49. Geldof AA, Mastbergen SC, Henrar REC, Faircloth GT: Cytotoxicity and neurocytoxicity of new marine anticancer agents evaluated using ***in vitro*** assays. *Cancer Chemother Pharmacol* 1999, 44:312-318.

50. Demetri G, Garcia-Carbonero R, Harmon D, Seiden M, Jimeno J, Merriam P, Waxman A, Supko J, Quigley MT, Ryan D: Ecteinascidin-743 (ET-743) induces objective responses and disease control in patients with advanced non-osseous sarcomas: results from phase II trials. *Ann Oncol* 2000, 11(Suppl 4):126.

51. Le Cesne A, Judson I, Radford J, Blay JY, Van Oosterom A, Rodenhuis S, Lorigan P, Di Paola ED, Jimeno J, Verweij J: Phase II study of ET-743 in advanced soft-tissue sarcoma (ASTS) in adult: A STBSG-EORTC trial. *Ann Oncol* 2000, 11(Suppl 4):126.

52. Erba E, Bergamaschi D, Bassano L, Damia G, Ronzoni S, Faircloth GT, D'Incalci M: Ecteinascidin-743 (ET-743), a natural marine compound, with a unique mechanism of action. *Eur J Cancer* 2001, 37:97-105.

53. Damia G, Silvestri S, Carrassa L, Filiberti L, Faircloth GT, 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.

54. Minuzzo M, Marchini S, Broggini M, Faircloth G, D'Incalci M, Mantovani R: Interference of transcriptional activation by the antineoplastic drug ecteinascidin-743. *Proc Natl Acad Sci USA* 2000, 97:6780-6784.

55. Poncet J: The dolastatins, a family of promising antineoplastic agents. *Curr Pharm Des* 1999, 5:139-162.

56. Bai R, Pettit GR, Hamel E: Dolastatin 10, a powerful cytostatic peptide derived from a marine animal. Inhibition of tubulin polymerization mediated through the vinca alkaloid binding domain. *Biochem Pharmacol* 1990, 39:1941-1949.

57. Pathak S, Multani AS, Ozen M, Richardson MA, Newman RA: Dolastatin 10 induces polyploidy, telomeric associations and apoptosis in a murine melanoma cell line. *Oncol Res* 1998, 5:373-376.

58. Wright JJ, Blatner G, Cheson BD: Clinical trials referral resource. Clinical trials of dolastatin 10. *Oncology* 1999, 13:68-70.

59. Pitot HC, McElroy EA, Reid JM, Windebank AJ, Sloan JA, Erlichman C, Bagniewski PG, Walker DL, Rubin J, Goldberg RM *et al.*: Phase I trial of dolastatin 10 (NSC 376128) in patients with advanced solid tumors. *Clin Cancer Res* 1999, 5:525-531.

60. Madden T, Tran HT, Beck D, Huie R, Newman RA, Pusztai L, Wright JJ, Abbruzzese JL: Novel marine-derived anticancer agents: a phase I clinical, pharmacological, and pharmacodynamic study of dolastatin 10 (NSC 376128) in patients with advanced solid tumors. *Clin Cancer Res* 2000, 6:1293-12301.

61. Krug LM, Miller VA, Kalemkerian GP, Kraut MJ, Ng KK, Heelan RT, Pizzo BA, Perez W, McClean N, Kris MG: Phase II study of dolastatin 10 in patients with advanced non-small-cell lung cancer. *Ann Oncol* 2000, 11:227-228.

62. Pettit GR: The bryostatins. *Fortschr Chem Org Naturst* 1991, 57:153-195.

63. Pagliaro L, Daliani D, Amato R, Tu SM, Jones D, Smith T, Logothetis C, Millikan RA: Phase II trial of bryostatin-1 for patients with metastatic renal cell carcinoma. *Cancer* 2000, 89:615-618.

64. Zonder JA, Shields AF, Zalupski M, Chaplen R, Heilbrun LK, Arlauskas P, Philip PA: A phase II trial of bryostatin 1 in the treatment of metastatic colorectal cancer. *Clin Cancer Res* 2001, 7:38-42.

65. Propper DJ, Macaulay V, Obyrne KJ, Braybrooke JP, Wilner SM, Ganesan TS, Talbot DC, Harris, AL: A phase II study of bryostatin 1 in metastatic malignant melanoma. *Br J Cancer* 1998, 78:1337-1341.

66. Varterasian ML, Mohammad RM, Shurafa MS, Hulburd K, Pemberton PA, Rodriguez DH, Spadoni V, Eilender DS, Murgo A, Wall N *et al.*: Phase II trial of bryostatin 1 in patients with relapsed low-grade non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *Clin Cancer Res* 2000, 6:825-828.

67. Ahmad I, Al-Katib AM, Beck FW, Mohammad RM: Sequential treatment of a resistant chronic lymphocytic leukemia patient with bryostatin 1 followed by 2-chlorodeoxyadenoside: case report. *Clin Cancer Res* 2000, 6:1328-1332.