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Review paper

Pharmaceutical development of anticancer agents derived from marine sources

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The marine ecosystem is more and more acknowledged as a source of potential anticancer agents. After the identification of a potential substance several hurdles have to be overcome before a marine candidate can enter the clinic. Amongst these are the establishment of a method which ensures sufficient supply and, which is the focus of this review, the development of a clinically useful pharmaceutical formulation. General issues with respect to the pharmaceutical development of marine anticancer agents will be discussed, which will be illustrated by highlighting aspects of the pharmaceutical development and clinical use of some representative compounds. [© 2000 Lippincott Williams & Wilkins.]

Key words: Anticancer agent, bryostatins, didemnins, dolastatins, ecteinascidins, marine, pharmaceutical formulation.

Introduction

Cancer is still one of the major causes of death in Europe and North America. As for many types of the disseminated disease no curative therapy is available, the discovery and development of new, active chemotherapeutic agents is highly needed.[1] To date, nature counts as the primary source of antitumor agents. Approximately 65% of the anticancer drugs commercially available in Europe and the US are of natural origin, either microbially derived (e.g. dactinomycin, doxorubicin) or extracted from plant sources (e.g. the taxanes paclitaxel and docetaxel).[2] At present, there is a growing interest for potential anticancer agents derived from the marine ecosystem.[3–9] Water, comprising approximately 70% of the Earth's surface and 95% of the biosphere, was the cradle for the first living organisms approximately 700 million years ago.[1,2,7] In the course of evolution, many organisms have been equipped with survival mechanisms, e.g. to withstand attacks by predators. Often these organisms have the capability to produce toxic compounds. There are more than a million species of marine invertebrates and more than 25 000 known species of fishes, illustrating the enormous source for potentially useful bioactive (e.g. antineoplastic) agents.[3] In this review, general issues with respect to the pharmaceutical development of marine anticancer agents will be discussed. This will be illustrated by highlighting aspects of the pharmaceutical development and clinical use of some representative compounds. Reference is made to our own research programs and current literature available in this field.

Marine anticancer agents

The initial evidence of the sea's potential in cancer chemotherapeutics dates from the early 1950's as the C-nucleosides spongouridine and spongothymidine were isolated from the Caribbean sponge *Cryptotheca crypta*.[1,2] The synthetic analog arabinosyl cytosine (Ara-C) displayed *in vitro* and *in vivo* activity in leukemic models and is currently an essential component of curative treatments of acute leukemias. A fluorinated derivative of Ara-C, gemcitabine, is currently used in the treatment of different solid tumors. However, the systematic investigation of marine environments only began in the mid-1970s, enhanced by new diving technologies which enabled deep-water acquisition.[2] From 1960 to 1982, approximately

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16 000 marine organism-derived extracts were screened for antitumor activity and thousands of metabolites, often belonging to totally novel chemical classes unknown from terrestrial sources, have been isolated. It can be concluded that the seas offer an unlimited resource and range of diverse and often highly complex secondary metabolites, which exhibit a variety of biological properties including cytotoxicity, antiviral and antifungal activity. Although only a small fraction of the marine ecosystem has been explored yet, several potential anticancer agents have been isolated and identified.[9] Interestingly, *in vitro* screening programs have shown that many of the marine candidates act through novel mechanisms of action.[7] Table 1 gives an overview of anticancer compounds derived from marine organisms and which are currently in preclinical or clinical development programs. Thus far, five compounds from marine origin have entered the clinic. Also, synthetic analogs of cryptophycin (LY355703) and dolastatin 15 (LU103793) are currently under early clinical investigation.[10] Furthermore, kahalalide F, a promising anticancer agent derived from the Hawaiian mollusk *Elysia rufescens* with highly selective antitumor activity against androgen-independent prostate cancer cells, is planned to enter phase I clinical studies by the end of the year 2000.[6,11,12] Several compounds are at a preclinical stage (e.g. toxicology, formulation studies), including thiocoraline, ES-285 and isohomohalichondrin B, all substances with potent *in vitro* and/or *in vivo* activity against various cancer cell lines.[1,7,13] After its identification as a potential anti-cancer agent, several hurdles have to be taken before a marine candidate can enter the clinic. Amongst these are the establishment of a method which ensures sufficient supply and, which will be the focus of this review, the development of a clinically useful pharmaceutical formulation.[7]

Supply issues

Supply is a very important factor in the development of marine candidates.[1,2,7,8,9] Large-scale harvesting of marine organisms, such as sponges, is neither practical nor ecologically acceptable. However, a sufficient and guaranteed supply of drug substance is a prerequisite for (pre)clinical evaluation and ultimately commercial development. Total synthesis has, in principle, the potential for supplying sufficient quantities of natural product. However, the complex molecular structures (e.g. stereochemical issues, macrocycle formations) of many of the marine anticancer agents present problems but also considerable challenges to the organic chemists.[14,15] Total synthesis methods have been developed for some of the marine compounds on a bench scale, but are too labor-intensive for economically viable production. For instance, the total synthesis of halichondrin B and bryostatin 7 requires 120 and 80 steps, respectively.[14] The exception to this rule is aplidine, for which recently a commercially feasible total synthesis route has been developed. After using natural and hemisynthetic (through a one-step synthesis from didemnin A) substance in the initial toxicology and phase I trials, further clinical evaluation will be performed with the synthetic compound. Also, synthesis provides the possibility of the preparation of

Table 1. Anticancer agents derived from marine sources and their current development status

Compound	Source	Current status
1. Didemnin B	tunicate (*Tridemnum solidum*)	phase II (closed)
2. Bryostatin 1	bryozoan (*Bugula neritina*)	phase II
3. Dolastatin 10	sea hare (*Dolabella auricularia*)	phase II
4. Ecteinascidin-743	tunicate (*Ecteinascidia turbinata*)	phase II
5. LY355703	derivative of cryptophycin	phase II
6. LU103793	derivative of dolastatin 15; see 3.	phase II
7. Aplidine	tunicate (*Aplidium albicans*)	phase I
8. Kahalalide F	mollusc (*Elysia rufescens*)	preclinical (approved for phase I)
9. ES-285	clam (*Spisula polynyma*)	preclinical
10. Thiocoraline	actinomycete (*Micromonospora marina*)	preclinical
11. Halichondrin B	sponge (*Halichondria okadai*)	preclinical
12. Isohomohalichondrin B	sponge (*Lissodendoryx* sp.)	preclinical
13. Icadamide B	sponge	preclinical
14. Halomon	red alga (*Portieria hornemannii*)	preclinical
15. Aplyronine A	sea hare (*Aplysia kurodai*)	preclinical
16. Mycaperoxide B	sponge (*Mycale* sp.)	preclinical
17. Bengamide B	sponge (*Jaspidae* sp.)	preclinical
18. Crambescidin-816	sponge (*Crambe crambe*)	preclinical

non-natural analogs, e.g. to improve solubility or stability of the parent compound.[15] Examples are the development of the water-soluble cryptophycin analog LY355703 and the dolastatin 15 analog LU103793 (see 'Dolastatins'). Currently, however, isolation from the natural source still provides the method of production of most of the marine anticancer agents. Large-scale harvesting of marine organisms is not feasible due to the limited wild stock in view of the huge amounts of organisms necessary to obtain sufficient active material. For example, if halichondrin B or ishomohalichondrin B would make it to the market, the annual amount of drug substance necessary will be approximately 5 kg (based upon preclinical activity data), corresponding to a quantity of at least 5000 tonnes of sponge. On the basis of extensive environmental surveys, however, the total biomass of *Lissodendoryx* sponge on Earth was estimated at only 289 ± 90 tonnes.[9] Therefore, a lot of effort is put into the research and development of methodologies for the controlled growth and processing of marine organisms. At present, so-called mariculture procedures for the production of ecteinascin-743 and bryostatin 1 are in full operation.[2,7,8] Furthermore, thiocoraline and cryptophycin 1 are obtained by controlled fermentation routes.[9] The initial stock of bryostatin 1 necessary for preclinical and early clinical studies was acquired by collecting 13 000 kg of *Bugula neritina* off the coast of southern California. This amount of wild material provided 18 g of bryostatin 1. At present, the substance is isolated from organisms grown in a controlled environment in maricultures. An efficient supercritical fluid extraction technique was developed which enabled the extraction and partial purification of bryostatins from both wild and aquacultured organisms. The cultured Bryozoan species give a yield of bryostatin 1 comparable to yields from wild material (7–8 μg/g of bryostatin 1), sufficient to provide for adequate supply of bryostatin 1 in case the drug makes it to the commercial market.[2,8] For the tunicate *Ecteinascidia turbinata*, the natural source of the ecteinascidins, it was shown that colonies of this organism could be grown in aquaria equipped with a natural photoperiod, artificial seawater, filters, controlled temperature, aeration and salinity. This provided the knowledge for growing the organism in aquacultures, currently the source of ecteinascidin-743 raw drug substance.[1]

Pharmaceutical development

An active drug substance is very rarely administered as the pure chemical compound itself. It is almost always transformed or formulated into a well-defined pharmaceutical product, which is suitable for the intended use in humans.[16] Formulations are often mixtures containing the active compound together with excipients like bulking agents, stabilizers, buffering agents and solubilizers. Anticancer drug formulations for experimental use are generally intended for either i.v. bolus injection or i.v. infusion to obtain absolute bioavailability, circumvent possible disturbance of or degradation in the gastrointestinal tract and to be able to adjust or stop administration of the drug in case of acute toxicity. Consequently, development of a pharmaceutical formulation of novel anticancer agents is focused on issues associated with the design of sterile and stable injectable products. The various steps in the development of an investigational anticancer agent and the pharmaceutical formulation issues are illustrated in Figure 1. Guidelines for the formulation of investigational cytotoxic agents have been drawn up by the Cancer Research Campaign (CRC)/European Organization for Research and Treatment of Cancer (EORTC)/National Cancer Institute (NCI) Joint Formulation Working Party.[17] Also, guidelines for development pharmaceutics have been issued by the regulatory authorities.[18,19] From Figure 1 it can be seen that a broad knowledge of analytical techniques, pharmaceutical sciences, regulatory guidelines [e.g. Good Manufacturing Practices (GMP)] and clinical practice is required to successfully conquer a formulation challenge. Common characteristics of marine compounds of importance in the formulation development are the often complex chemical structures, the high potency (often active in the μg range), low aqueous solubility and limited stability in aqueous solution. Furthermore, besides these common features, each compound has its own specific properties.

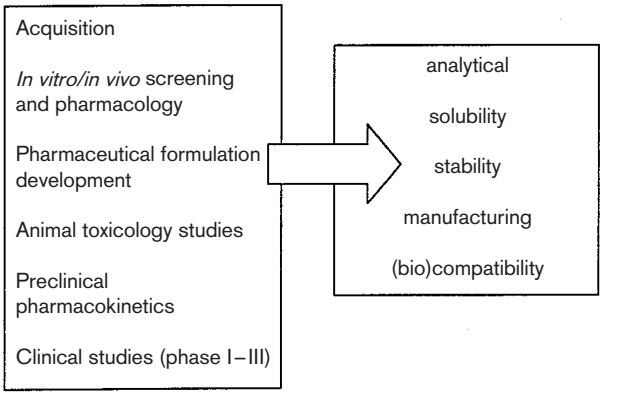

Figure 1. Development route of an investigational anticancer drug and pharmaceutical formulation issues.

Analytical issues

For each investigational drug a set of analytical techniques and methods has to be developed and validated to enable characterization and quality control of raw drug substance and the final pharmaceutical product. Structural characterization is carried out by using techniques like nuclear magnetic resonance (NMR) spectroscopy, mass spectroscopy (MS) and infrared spectroscopy (IR). Analytical characterization and purity determinations are performed by, for example, ultraviolet/visible (UV/Vis) spectrophotometry, liquid or gas chromatographic methods (HPLC, GC) coupled to various detection techniques (e.g. UV, MS, refractive index). Additionally, methods for quantification of residual water (e.g. Karl–Fischer titration) and for the assessment of the physical state of raw substance or pharmaceutical product [e.g. differential scanning calorimetry (DSC)] are frequently used. Furthermore, several techniques are employed to assess the pharmaceutical dosage form, e.g. methods for sterility testing, endotoxin content [Limulus amoebocyte lysate test (LAL)] and determination of particulates. Crucial, however, is the development of a stability-indicating assay which can discriminate between the parent compound and degradation products. The method should also be capable of separating the parent compound from contaminants like synthetic intermediates, starting materials, related substances and excipients used in the pharmaceutical product. Requirements and specifications for analytical methods, raw drug substance and final product have been laid down in several regulatory guidelines.[20–23] Preliminary specifications of the raw drug substance and pharmaceutical product are generally accepted by the regulatory authorities at the time of the Investigational New Drug (IND) application, with the exception of critical items like sterility and pyrogenicity tests.[24,25] Evolution to the final, established specifications at the time of filing of the New Drug Application (NDA) is allowed in the course of the clinical development program. Excipients used in the pharmaceutical development and final product need to comply to pharmacopeial standards [e.g. European Pharmacopeia (EP) or US Pharmacopeia (USP)]. As many marine compounds are still derived from their natural sources, the possibility of batch-to-batch variation in, for example, content and purity exists. This variation is minimized by production and extraction of the source organism and active substance under controlled, well-defined conditions (e.g. mariculture) using validated methods, according to GMP guidelines. From a pharmaceutical point of view, however, quality control of each lot of raw drug substance against a well-defined reference standard is required.[16,17] As already mentioned, great attention has to be paid to the structural and analytical characterization of the marine drug substance. Levels of related substances or other contaminants (e.g. residual solvents used during the extraction procedure) in the raw drug substance need to be assessed and specifications have to be drawn up. Inherent to the complex chemical structures of many of the marine compounds, the development of a stability-indicating assay can result in serious challenges. This is illustrated by the development of a stability-indicating HPLC–UV assay of aplidine, a compound belonging to the didemnin family (see 'Didemnins').[26] The development of this method was complicated by the existence of multiple conformations of aplidine, which was confirmed by NMR analysis. The initial assays, both normal phase, isocratic and gradient HPLC methods, showed two peaks in the chromatograms referring to the equilibrium between *cis* and *trans* isomers of the pyruvoyl–proline amide bond present in the molecule. Although quantification of aplidine concentrations on the total area of both isomer peaks was satisfactory in terms of linearity, accuracy and reproducibility, the stability-indicating capacity of the assay was not. It was shown that upon stress-testing degradation peaks co-eluted with the aplidine–isomer complex. Finally, by adjusting the analytical column temperature, a stability-indicating assay for aplidine was obtained, with a good selectivity for impurities and degradation products.

Solubility issues

A number of solubilization approaches are available for parenteral dosage form development.[16,27] In summary, these consist of pH adjustment in case of an ionizable functional group, use of co-solvents and/or surfactants, use of complexing agents (e.g. cyclodextrins) and dispersed systems (e.g. emulsions, liposomes, nanoparticles). The required solubility for the investigational drug formulation depends on the starting dose level in the phase I clinical dose-finding study and the estimated human maximal tolerated dose (MTD). The human starting dose is calculated from the toxicological dose in mice or rats using a surface area conversion formula.[28] This calculation provides a rough estimate of the human dose from which an indication of the required dosage unit content(s) can be extrapolated. As marine anticancer agents are usually very potent, the starting dose is often very low. Table 2 gives a summary of the pharmaceutical products made out of marine anticancer agents applied in clinical trials. As can be seen, the poor

solubility of most compounds necessitated the use of co-solvents and/or surfactants. Also, the *in vivo* antitumor efficacy of cryptophycins was recently assessed in rats using a co-solvent/surfactant vehicle composed of polyethylene glycol 300, Cremophor EL and normal saline (2/8/90% v/v/v).[10] Besides solubilization, co-solvents and surfactants are capable of preventing sorption of drug substance to surfaces, which is an issue in view of the lipophilicity and the low concentrated (due to the high potency of the compounds) solutions of many of the marine compounds. Drawbacks of the use of these solubilizing excipients include the potential formulation-related toxicities (e.g. trombophlebitis, hemolysis, hypersensitivity reactions) which can occur upon i.v. administration to the patient.[27] Based on current practice, the initial formulation approach for investigational anticancer drugs which lack sufficient aqueous solubility often is solubilization in co-solvent/surfactant systems. Other formulation techniques will generally be used for, if required, second-generation pharmaceutical formulations. Of the antineoplastic agents currently licensed in the US approximately 25% are formulated using a co-solvent and/or surfactant system (e.g. docetaxel, solubilized in polysorbate 80; paclitaxel, solubilized in Cremophor EL/ethanol).[29]

Stability issues

Pharmaceutical stability refers to storage life or utility time, i.e. the period of time a raw drug substance, excipient, final product, reconstituted product or infusion solution remains within the limits of the specifications of its characteristics under certain conditions (e.g. temperature, humidity, light). Stability can be subdivided in chemical (i.e. incessant, irreversible degradation of the parent molecule) and physical (e.g. precipitation, collapse of lyophilized product) stability. Besides the low aqueous solubility as discussed in the previous section, a common characteristic of many anticancer agents is the limited chemical stability in solution. This is illustrated by the fact that 45% of the dosage forms of cytotoxic agents marketed in the US are lyophilized products.[29] Lyophilization or freeze-drying is a processing method which removes water or organic solvent (e.g. *tert*-butanol) from a solution containing the (unstable) compound. The obtained dry powder or cake generally provides a high chemical stability. Three out of five marine compounds currently in clinical trials are formulated as lyophilized products which require reconstitution in an appropriate dissolution vehicle at the time of use (Table 2). The aim of the pharmaceutical development of a drug candidate is constitution of a product which has a sufficient shelf-life (more than 1 year) at a practically convenient storage condition [room temperature (20–25°C) or refrigerated condition (2–8°C)]. During the investigational status of a new compound other storage conditions can be accepted but not with large-scale, commercial distribution. Insight into stability parameters is generally obtained in the preformulation stage by accelerated stability testing, often at elevated temperature conditions. Real-time stability testing of the pharmaceutical product at the designated long-term storage condition (temperature, relative humidity) is required but can still be performed during clinical evaluation of the investigational anticancer drug. Also, to anticipate fluctuations which can occur during, for example, shipment of product, stability testing of the final product at defined intermediate and/or accelerated storage conditions must be carried out.[30] For this testing, conditions are dependent on the designated long-term storage condition and the permeability of the container. Besides determination of the stability as a function of temperature and humidity, photostability testing is required by separate testing.[31] Similar requirements apply to the drug substance. Stability of the active compound in infusion solutions can vary with the dilution solution used and the final infusion concentration. Therefore, stability should be assessed for commonly applied infusion solutions (e.g. 0.9% w/v sodium chloride, normal saline; 5% w/v dextrose) over the concentration range and under the

Table 2. Pharmaceutical dosage forms of marine anticancer agents

Compound	Dosage form	Reconstitution solution
1. Didemnin B	solution in co-solvent/surfactant system	NA
2. Bryostatin 1	solution in co-solvent system; lyophilized solid	co-solvent/surfactant system
3. Dolastatin 10/LU103793	sterile aqueous solution	NA
4. Ecteinascidin-743	lyophilized solid	Wfl
5. Aplidine	lyophilized solid	co-solvent/surfactant system

NA, not applicable.

conditions (temperature, light) intended in the clinical setting. Besides stability of the infusion solution during the actual administration period, additional time required for practical issues like preparation, logistics (e.g. transport from the hospital pharmacy to the patient, final installation at the patient site) should be taken into consideration.

Manufacturing issues

Pharmaceutical manufacturing of investigational anticancer drugs to be used in clinical trials, although generally performed on a small scale, has to comply with the principles of GMP.[18] This implies selection, definition and validation of the manufacturing process and its subsequent steps. In case of the production of a sterile dosage form for i.v. use, the selection of the manufacturing process is, for example, related to the stability of the active substance. For instance, heat lability precludes the use of terminal sterilization of the product by moist heat, the sterilization method of first choice.[32] Sterile filtration in combination with aseptic processing generally offers a solution for heat-labile formulations (e.g. ecteinascidin-743, aplidine). An aseptic manufacturing process, however, requires high-standard environmental conditions of the production facilities (e.g. with respect to the level of particulates and microorganisms) and well-trained personnel. Production materials (e.g. filters) and equipment selected have to be validated for their suitability in the manufacturing of a specific pharmaceutic. Definition of critical steps in the manufacturing process (e.g. solubilization time, use of overages, freeze-drying parameters) at the development stage is of crucial importance in view of the future upscaling of the pharmaceutical production.

Compatibility issues

During the formulation studies compatibility of the drug product with the primary packaging material (e.g. container, closure) must be assessed. Furthermore, compatibility testing is important in order to establish the optimal administration parameters of the pharmaceutical formulation. On the basis of these tests, the optimal i.v. administration devices (container and tubing, if necessary equipped with in-line filter) are selected. As already mentioned in the 'Solubility issues' section, many marine compounds are prone to sorption processes as a consequence of their lipophilicity. Furthermore, due to their high potency, infusion solutions contain low concentrations of drug substance which might result in relevant losses in case of adsorption to the surface or absorption into the matrix of the infusion container, tubing or in-line filter. *In vitro* real-time infusion simulations can give insight into the percentage of the intended dose which will be administered to the patient using a specific infusion device, infusion concentration and infusion duration.[33] Also, if the pharmaceutical formulation contains co-solvents or surfactants, compatibility with i.v. administration devices is often limited due to leaching of components (e.g. plasticizing agents) from the material under influence of these excipients. For example, the non-ionic surfactant Cremophor EL is notorious for the leaching of the plasticizer diethylhexyl phthalate (DEHP) from polyvinyl chloride (PVC) administration sets.[33] Furthermore, *in vitro* biocompatibility tests can give insight into, for example, the intravascular precipitation and hemolysis potential of a pharmaceutical formulation, which can be expected upon i.v. administration of solutions containing drugs with low aqueous solubility and co-solvents and/or surfactants.[34] Formulation- and administration-related adverse effects can thus be anticipated preclinically and patient discomfort can be minimized by, for example, adjustment of administration parameters.

In the subsequent sections, the pharmaceutical aspects of the development and clinical use of the marine compounds most advanced in clinical trials will be discussed.

Didemnins

Origin/chemistry/activity

Two representatives of the didemnin family have entered clinical studies thus far, i.e. didemnin B and its dehydroderivative, aplidine. The didemnins represent a class of marine compounds which were first isolated in 1978 by Rinehart *et al.* from the tunicate (ascidian or sea squirt) *Tridemnum solidum* of the family *Didemnidae*, a marine invertebrate which was collected throughout the Carribean in waters off the coasts of Colombia, Honduras, Mexico, Belize and Panama.[35] It was found that the crude extract from these organisms inhibited the growth of herpes simplex virus type 1 grown in CV-1 cells (monkey kidney tissue) and displayed cytotoxic activity against the cells themselves. Originally, three active compounds were isolated from the extract which were assigned didemnin A, B and C. Structural elucidation by mass spectrometric techniques and degradative studies showed that all three didemnins contain the same cyclic depsipeptide, containing hydroxyisovalerylproprionate and a stereoisomer of the highly unusual amino acid statine, and differ only in the substituent

attached to the *N*-methylleucine amino acid (Figure 2).[36–38] Didemnin A, the most abundant component of the tunicate extract, was found to be the parent compound of didemnin B and C. Although all three didemnins were biologically active, didemnin B displayed the highest *in vitro* and *in vivo* antiviral, immunosuppressive, and antitumor potency and was selected for further development by the NCI. Thus far, in total nine didemnins have been derived from the *Tridemnum* tunicate (didemnin A–E, G, X and Y). Furthermore, a dehydro-derivative of didemnin B, dehydrodidemnin B or aplidine, was isolated from the Mediterranean tunicate *Aplidium albicans* (Figure 2). Didemnin B showed only marginal *in vivo* activity and its development was continued on the basis of being a natural COMPARE negative product. However, aplidine was clearly active *in vitro* and in xenograft-based models against a wide variety of tumors.[39] Also, screening of aplidine using an *in vitro* cardiotoxicity model, a dose-limiting side effect seen in clinical studies with didemnin B, revealed a possibly higher therapeutic index.[40] Aplidine was the second representative of the didemnin family selected for clinical evaluation and has recently entered phase I clinical trials (Pharma Mar SA, Tres Cantos, Madrid, Spain). Didemnin B exerts its antitumor activity through interference with protein synthesis through GTP-dependent inhibition of the elongation factor 1α, a protein translation component.[41] Aplidine has cytotoxic activity on many tumor cell lines and interferes with the G_1 phase of the cell cycle progression. In addition, it has been shown to change the expression of important genes like c-*fms*, *ETR*-1 and, specifically, to decrease the expression of the *ftl*-1 receptor for the vascular endothelial growth factor.[42]

Pharmaceutical formulation

Due to its poor aqueous solubility, didemnin B is solubilized using a co-solvent/surfactant system composed of a mixture of polyoxyethylated castor oil (Cremophor EL), ethanol and normal saline (CES).[43] The pharmaceutical product consists of a sterile solution containing 500 μg/ml of didemnin B in 5/5/90% v/v/v CES, each ampoule containing 1.0 ml of solution and to be stored under refrigeration (2–8°C). The Cremophor EL/ethanol vehicle is, for example, also used in the formulation of paclitaxel, another cytotoxic agent from natural origin with very poor aqueous solubility (Taxol®, 6 mg/ml paclitaxel in 50/50% v/v Cremophor EL/ethanol). Apart from its solubilizing properties, the use of Cremophor EL is associated with the occurrence of severe hypersensitivity reactions.[44,45] Therefore, patients who are treated with Taxol® (up to doses equivalent to 40 ml of Cremophor EL) receive prophylactic medication to suppress anaphylactic reactions. However, during preclinical studies with the didemnin B formulation in dogs, a sensitive species for Cremophor EL-induced anaphylactic reactions, no side effects were reported. Nevertheless, investigators were warned for the occurrence of hypersensitivity reactions upon i.v. administration of didemnin B prior to the start of the clinical stuides. Stability of didemnin B in infusion fluid was determined in normal saline and 5% dextrose at room temperature (20–25°C). Didemnin B exhibited no decomposition after 4 days of storage. The use of Cremophor EL necessitated the use of plasticizer-free containers or bags (e.g. glass) and administration sets. Biocompatibility studies showed that the formulation is compatible and non-hemolytic

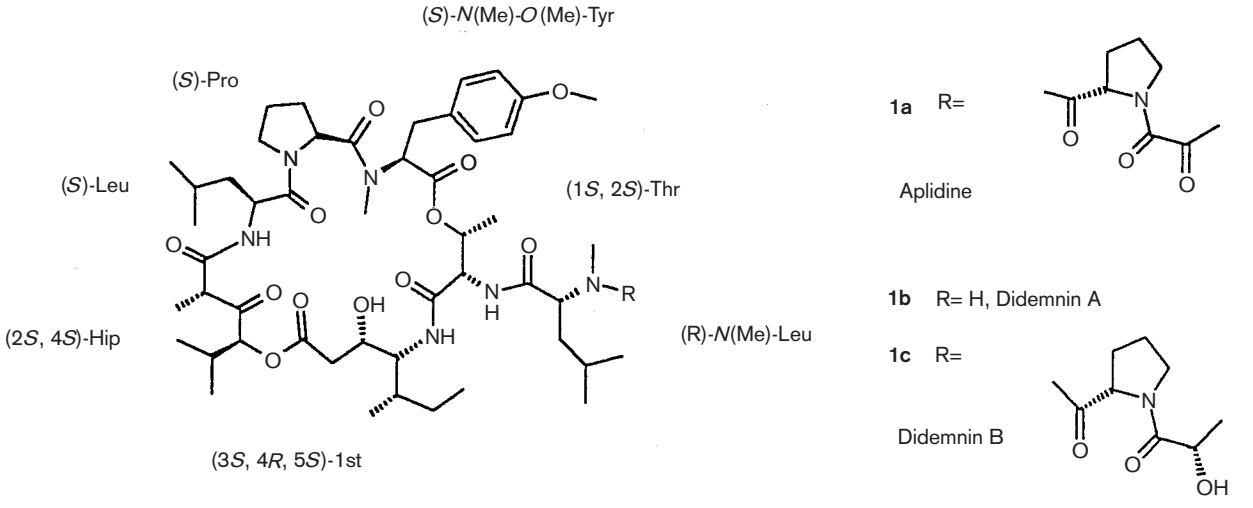

Figure 2. Structural formulas of aplidine, didemnin A and didemnin B.

in human and dog serum, plasma and whole blood tested in a 1:1 v/v ratio.

Like didemnin B, aplidine showed insufficient aqueous solubility with respect to the intended dose levels in phase I clinical studies. Also, aplidine displayed limited chemical stability in aqueous media. A lyophilized formulation was developed containing 500 μg per dose unit and 25 mg mannitol as bulking agent.[26] Freeze-drying was performed from an aqueous mixture containing 40% v/v *tert*-butanol in order to solubilize the active substance. Usually, lyophilization is performed from solutions in water. In case of a drug with poor aqueous solubility, however, the organic solvent *tert*-butanol can be an excellent choice for its combination of solubilizing and freeze-drying properties.[46,47] Ongoing stability studies show a stability of the lyophilized product of at least 1 year at 2–8°C. A co-solvent/surfactant system composed of 15/15/70% v/v/v Cremophor EL/ethanol/water was selected as reconstitution medium, resulting in a solution containing 500 μg/ml of aplidine to be further diluted in normal saline before intravenous administration. Compatibility studies with PVC and PVC-free infusion devices were carried out using an *in vitro* real-time infusion simulation model.[33] A combination of a glass infusion container and silicone tubing was found most optimal for the administration of an aplidine infusion solution at concentrations equal or above 28.8 μg/ml. At lower concentrations, loss of drug substance due to sorption was substantial. Infusion solutions are stable for at least 48 h using the glass/silicone infusion system. Besides considerable leaching of the plasticizer DEHP, as expected due to the presence of the Cremophor EL/ethanol vehicle, extensive sorption of aplidine occurred with the PVC administration sets. Biocompatibility experiments showed that neither hemolysis nor precipitation of drug substance is expected upon i.v. administration of aplidine infusions.

Clinical studies

Didemnin B. In all clinical studies of didemnin B performed thus far, the formulation of 500 μg/ml in Cremophor EL, ethanol and normal saline (5/5/90% v/v/v) was used.[48–70] Dorr *et al.* performed a phase I study in which didemnin B was administered as a 30-min i.v. infusion every 28 days.[48] Besides the dose-limiting toxicity (DLT) consisting of emesis, one patient experienced an allergic reaction (chills, diaphoresis, flushing and hypotension), attributed to the presence of Cremophor EL in the formulation vehicle. With pretreatment of i.v. cimetidine, diphenhydramine and dexamethasone this patient could continue the treatment course. On the basis of suspicion of suboptimal dosing of didemnin B, a repeat phase I study of the single i.v. 30-min infusion was carried out by Shin *et al.*[49] Again, one patient experienced an allergic reaction upon the didemnin B infusion, which could be managed by the addition of methylprednisolone to the antiemetic premedication regimen. DLT was found to be neuromuscular. Formulation-related toxicity was also reported in a phase I study by Stewart *et al.*, who examined the MTD of didemnin B in a daily $\times 5$ schedule.[50] Two out of 35 patients with advanced cancer developed anaphylactic reactions upon a 10-min i.v. administration of didemnin B which could be ceased by prophylactic treatment with cimetidine, diphenhydramine and prednisone. No relationship between the occurrence of anaphylactic reactions and the dose level or number of courses could be found. The authors questioned the necessity of routine prophylaxis for anaphylactic reactions in view of the sporadic character of this toxicity and suggested a close observation of the patient during treatment would suffice. In contrast, Maroun *et al.* advised prophylactic treatment for hypersensitivity reactions based on the results of a phase I trial with a weekly $\times 4$ i.v. infusion in a 6-week cycle.[51] A number of patients treated at dose levels of 0.6–2.1 mg/m^2/week developed hypersensitivity reactions with symptoms like flushing, chills, anxiety, headache and hypotension. As four out of six patients treated at a dose level of 1.5 mg/m^2/week experienced anaphylactic reactions, the protocol had to be amended with respect to the administration instructions for this and higher dose levels. Didemnin B infusion solution was prepared by diluting the appropriate volume of formulation solution in 250 ml of 5% dextrose for infusion instead of 100 ml of normal saline and the infusion duration was prolonged from 10 min to 1 h. Furthermore, patients received pretreatment of H_1 and H_2 receptor blockers (i.v. diphenhydramine and cimetidine). No further hypersensitivity reactions occurred with this administration protocol, and prophylactic H_1 and H_2 receptor blocking agents were recommended for the phase II trials. An overview of the clinical phase II studies performed with didemnin B is given in Table 3. Most of the phase II trials were carried out using the single i.v. 30 min bolus infusion every 28 days at dose levels ranging from 2.6 to 6.3 mg/m^2. Besides severe drug-related toxicities (including nausea, vomiting, anemia, conduction disorders and neuromuscular toxicity), formulation-related toxicity frequently occurred. Table 3 shows the percentages of patients who experienced anaphylactic reactions upon administration of didemnin B formulated in 5/5/90% v/v/v Cremophor EL/ethanol/normal saline during phase II clinical studies.

Total doses of Cremophor EL administered in the single i.v. bolus every 28 days schedule ranged from approximately 0.4 to 1.0 ml. However, no relationship could be found between the total Cremophor El dose and the occurrence of hypersensitivity reactions (e.g. Weiss *et al.* versus Shin *et al.*, Table 3). As already noted in the phase I clinical studies, the same accounts for the number of treatment courses and incidence of hypersensitivity reactions. However, the dose schedule of didemnin B might be of influence, which is illustrated by a study carried out by Goss *et al.* examining the efficacy of didemnin B in a weekly $\times 4$ (2.3 mg/m^2/week) schedule in patients with non-Hodgkin's lymphoma (NHL).[64] Based on the experiences from the phase I study with this dose scheme, patients received anaphylaxis–prophylaxis (cimetidine and diphenhydramine) prior to a 1-h infusion of didemnin B. Despite this pretreatment 40% of the patients developed hypersensitivity reactions, of which one was life threatening. The efficacy of prophylactic treatment with antihistamine agents in the single dose regimen is illustrated by a study carried out by Taylor *et al.*[68] Patients were heavily pretreated with dexamethasone, diphenhydramine and cimeti-

Table 3. Phase II studies of didemnin B

Author (reference)	Dose schedule	Tumor type	No. of patients	Percent anaphylactic reactions	Response
1. Rossof *et al.*[52]	3.47 mg/m^2 q 21 days	colorectal cancer	15	7	no response
2. Motzer *et al.*[53]	4.2 mg/m^2 q 28 days	advanced renal cell cancer	21	19	1 PR
3. Benvenuto *et al.*[54]	5.6 mg/m^2 q 21–28 days	metastatic breast cancer	16	none reported	no response
4. Jones *et al.*[55]	3.47 mg/m^2 q 28 days	colon	14	none reported	no response
5. Cain *et al.*[56]	2.6 mg/m^2 q 28 days	epithelial ovarian cancer	12	17	no response
6. Taylor *et al.*[57]	3.4 mg/m^2 q 28 days	adenocarcinoma of the kidney	22	9	no response
7. Weiss *et al.*[58]	2.6 or 5.6 mg/m^2 q 28 days	squamous carcinoma of the uterine cervix	27	11	no response
8. Jacobs *et al.*[59]	4.2 mg/m^2 q 28 days	squamous carcinoma of the uterine cervix	21	none reported	no response
9. Malfetano *et al.*[60]	4.2 mg/m^2 q 28 days	epithelial ovarian cancer	16	none reported	no response
10. Sondak *et al.*[61]	4.2 mg/m^2 q 28 days	metastatic malignant melanoma	11	36	no response
11. Weiss *et al.*[62]	4.9 mg/m^2 q 28 days	myeloma	15	9–32	no response
12. Shin *et al.*[63]	6.3 mg/m^2 q 28 days	SCLC	15	none reported	no response
13. Goss *et al.*[64]	2.3 mg/m^2/week $\times$ 4 every 6 weeks	NHL	8	40 (pretreatment with H_1 and H_2 blockers)	no response
14. Williamson *et al.*[65]	3.5 or 6.3 mg/m^2 q 28 days	metastatic prostate cancer	32	3	1 PR
15. Kucuk *et al.*[66]	5.6 or 6.3 mg/m^2 q 28 days	IG/HG NHL or LG NHL	29 (IG/HG) 22 (LG)	2	1 CR, 1 PR (IG/HG) 1 CR, 4 PR (LG)
16. Malfetano *et al.*[67]	6.3 mg/m^2 q 28 days	squamous cell carcinoma of the cervix	26	4	1 CR
17. Taylor *et al.*[68]	6.3 mg/m^2 q 28 days	CNS	39	none reported (pretreatment with H_1 and H_2 blockers)	1 PR
18. Mittelman *et al.*[69]	4.3 mg/m^2 q 28 days	glioblastoma multiforme	13	none reported	no response
19. Hochster *et al.*[70]	4.2 mg/m^2 q 28 days	malignant melanoma	17	37	inconclusive due to high incidence of anaphylactic reactions

SCLC, small cell lung cancer; NHL, non-Hodgkin's lymphoma; CNS, central nervous system; CR, complete remission; PR, partial response; for all studies didemnin B was diluted in 25–150 ml of normal saline and administered as a 30-min i.v. infusion with the exception of 13 in which a 1-h i.v. infusion was used.

dine, and no hypersensitivity reactions were reported in this study. In contrast, anaphylactic reactions occurred at such frequency and severity in a recently published phase II trial by Hochster *et al.* that interpretation of the study outcomes was troubled.[70] In this study patients received no prophylaxis. The authors concluded that didemnin B has not been given an adequate trial of its therapeutic potential and it was suggested to continue testing of the substance, e.g. in a different schedule, using an alternative formulation solvent system or more modern approaches to anaphylactic reactions.

In conclusion, in many of the studies carried out anaphylactic reactions were reported which were attributed to the presence of Cremophor EL in the pharmaceutical formulation of didemnin B. The use of diphenhydramine, an H_1 receptor blocking agent applied in many of the studies in emesis-prophylaxis, might even obscure the real incidence of the hypersensitivity reactions. Severity and occurrence of anaphylactic reactions could not be related to a specific didemnin B dose level or number of treatment courses. A more frequent dose schedule, however, seemed to increase the incidence of anaphylactic reactions. The formulation-related hypersensitivity reactions could be minimized or even ceased with prophylactic H_1 and H_2 receptor blocking agents, together with a glucocorticoid. However, due to the low activity and severe drug-related toxicities further clinical trials with didemnin B were ceased.

Aplidine. Phase I clinical studies with aplidine are currently ongoing in the UK, France, Spain and Canada.[71–73] Concentration-dependent phlebitis was found in a study using a 24-h weekly continuous infusion $\times 3$ followed by 1 week rest.[71] It is not clear whether this side effect can be attributed to the aplidine formulation. None of the studies reported the occurrence of hypersensitivity reactions thus far.[71–73] Aplidine DLT was primarily muscular, manifested by increases in creatine kinase and in some cases rhabdomyolysis and hepatic, characterized by reversible increases in transaminases. Therapeutic activity has been seen in different tumors, including melanoma and NHL.

Bryostatins

Origin/chemistry/activity

Bryostatin 1 was isolated by Pettit *et al.* in 1982 from the marine bryozoan *Bugula neritina* of the phylum *Ectoprocta*.[2,8] The invertebrate *B. neritina* is a plate-like creature which forms multicellular aggregates that foul boats and docks, and has a widespread occurrence throughout the oceans. However, only three populations off the coasts of California and Florida are known to produce bryostatins. The bryostatins constitute a class of more than 20 macrolides containing a multiringed macrocyclic lactone structure with varying side chains. Thus far, bryostatin 1 is the only member of this class of compounds which has entered clinical trials (Figure 3). Bryostatin 1 elicits a wide range of activities including hematopoetic and immune stimulation, and induction of differentiation of both myeloid and lymphoid cell lines.[74] The compound has displayed *in vitro* and *in vivo* activity against various murine and human tumor types. Although the exact mechanism of antitumor activity has not been clearly defined yet, the wide variety of biological effects of bryostatin 1 appears to be mediated through modulation of protein kinase C (PKC) enzymes, which are involved in the signal transduction pathways regulating cellular proliferation and differentiation.[75]

Pharmaceutical formulation

Bryostatin 1 is a colorless, odorless, crystalline substance, and is relatively insoluble in water and normal saline, but very soluble in absolute ethanol (above 4 mg/ml). Therefore, bryostatin 1 was initially formulated as a 100 μg/ml solution in absolute

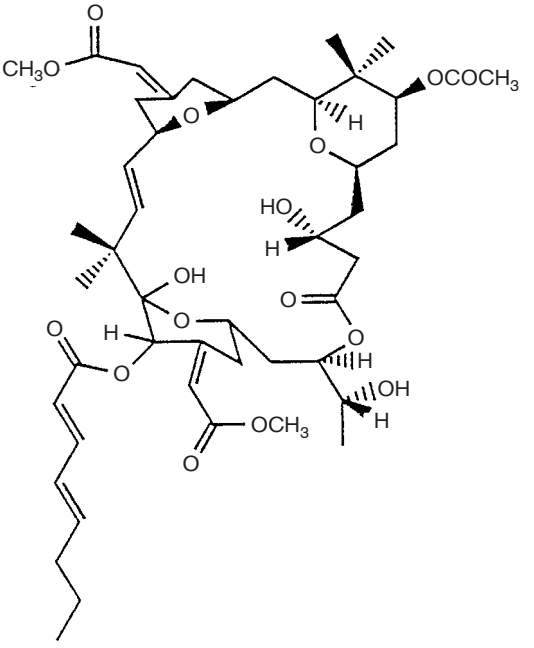

Figure 3. Structural formula of bryostatin 1.

ethanol, filled into clear glass ampoules (1.5 ml/ampoule). The solution did not show any degradation after 3 weeks stability testing at 20 and 50°C under ambient light conditions or after 8 months at −20°C in the dark. As 100% ethanol cannot be administered i.v. to the patient as such, the solution of bryostatin 1 was diluted with normal saline to obtain a 60/40% v/v ethanol/normal saline solution. This infusion solution was found to be stable for 24 h in a polypropylene (PP) syringe.[76] However, due to the high ethanol concentration, the formulation caused serious incidences of ethanol-related phlebitis and cellulitis at the injection site in the initial phase I trials (see 'Clinical studies'). To eliminate these side effects, an alternative formulation was developed by the Pharmaceutical Research Branch of the NCI using a diluent composed of polyethylene glycol 400, ethanol and polysorbate 80 (Tween 80) 60/30/10% v/v (PET).[77] Furthermore, to improve stability, bryostatin 1 was formulated as a lyophilized product containing 100 μg of active substance and 5 mg of povidone as bulking agent. Freeze-drying was performed from a 40% v/v *tert*-butanol solution. The lyophilized product is to be reconstituted with 1 ml of the PET diluent and subsequently further diluted with normal saline. Cheung *et al.* carried out a compatibility and stability study of the bryostatin 1–PET formulation in infusion devices.[77] The bryostatin 1–PET formulation was diluted to 1 and 10 μg/ml in normal saline and benzyl alcohol preserved saline in PP and PVC bags and subsequently stored at room temperature (27°C). A storage period of 28 days was selected in order to generate stability and compatibility data to support the 21-day dose schedule intended for phase I clinical studies. Besides the bryostatin 1 concentration, the benzyl alcohol as well as leachable plasticizer (DEHP) concentrations were monitored over this 4-week period. It was shown that both bryostatin 1 infusion concentration levels were stable and compatible with the PP infusion devices. However, a decrease of bryostatin 1 concentrations was observed for the PVC administration set, along with a decrease in the benzyl alcohol concentration for the preserved saline solution. This effect was larger with the low infusion solution concentration of bryostatin 1. As no decomposition products were observed, loss of bryostatin 1 was explained by sorption to the PVC bags. Furthermore, leakage of the plasticizer DEHP into the infusion solution was shown with the PVC infusion sets. The amount leached was dependent on the drug concentration and is most likely related to the presence of the nonionic surfactant polysorbate 80 in the solution. In conclusion, it was advised to administer the bryostatin 1–PET formulation diluted with normal saline or benzyl alcohol preserved saline using PP infusion bags and to avoid PVC administration sets.

Clinical studies

Table 4 gives an overview of the phase I and phase II clinical studies of bryostatin 1 carried out thus far. As can be seen, the ethanol-based formulation was only applied in the initial two phase I studies.[76,78] Both studies applied an i.v. infusion of bryostatin 1 dissolved in a mixture of 60% ethanol and 40% normal saline. Complicating factor, however, was the high incidence of trombophlebitis and cellulitis caused by the administration of this vehicle. Prendiville *et al.* reported that approximately 60% of the cycles of bryostatin 1 administered were accompanied by cellulitis and thrombophlebitis at the infusion site.[76] These side effects were seen at all dose levels, although slightly more frequently at the higher levels. The occurrence of cellulitis and trombophlebitis was attributed to the high ethanol concentration in the infusion solution, although a drug-related effect as contributory factor could not be excluded. In an attempt to eliminate these side effects, subsequent patients treated at the highest dose levels (50 and 65 $\mu g/m^2$) received an i.v. infusion of normal saline together with the bryostatin 1 treatment in order to flush the bryostatin 1 solution from the treatment vein. These patients did not experience any cellulitis or phlebitis. In the study by Philip *et al.*, phlebitis occurred at an incidence of 50% of the treatment cycles.[78] As in the study by Prendiville *et al.*, there was no relationship with the dose administered and the toxicity could be minimized or avoided by a concomitant infusion of normal saline. It was decided, however, to switch to the newly developed bryostatin 1–PET formulation in order to lower the amount of ethanol administered to the patients. Lyophilized bryostatin 1 was reconstituted to a concentration of 100 μg/ml with PET diluent and subsequently diluted with normal saline to a final infusion concentration of 5 μg/ml. Polypropylene plastic syringes and a polyfin extension set were used in the preparation and administration of bryostatin 1 to avoid sorption. Also, the tubing of the drug-infusion system was primed with a bryostatin 1 solution at a concentration similar to that administered to the patient. The bryostatin 1–PET formulation caused phlebitis in approximately 15% of the patients, which was related to the PET vehicle. Furthermore, several patients experienced an acute hypersensitivity reaction which was characterized by dyspnea, flushing, hypotension and bradycardia. As these symptoms started only minutes after beginning of the 1-h infusion and ceased within 10 min after discontinuation of the

Table 4. Phase I/II studies of bryostatin 1

Author (reference)	Phase I/II	Dose schedule	Formulation	No. of patients	Formulation-related toxicity	Outcome
1. Prendiville *et al.*[76]	I	1 h i.v. infusion 5–65 μg/m^2 every 2 weeks	EtOH	19	about 60% phlebitis	1 h i.v. 35–50 μg/m^2 every 2 weeks; DLT myalgia
2. Philip *et al.*[78]	I	1 h i.v. infusion a. 35 or 50 μg/m^2 every 2 weeks b. 25 μg/m^2 once a week c. 25 μg/m^2 once a week $\times$3	EtOH, PET	35	EtOH: 48% phlebitis PET: 15% phlebitis; 46% anaphylactic reactions	1 h i.v. 25 μg/m^2 once a week $\times$3 every 4 weeks; DLT myalgia; 2 PR, metastic melanoma
3. Jayson *et al.*[79]	I	24 h i.v. infusion 25–50 μg/m^2/week $\times$8	PET	19	44% phlebitis	24 i.v. infusion 25 μg/m^2/week $\times$8; DLT myalgia; 2 PR, ovarian, LG NHL
4. Varterasian *et al.*[80]	I	72 h i.v. infusion 12–180 μg/m^2 every 2 weeks	PET	19	none reported	72 h i.v. infusion 120 μg/m^2 every 2 weeks; DLT myalgia
5. Weitman *et al.*[81]	I	1 h i.v. infusion 20–57 μg/m^2/week $\times$3 every 4 weeks	PET	19	14% anaphylactic reactions	1 h i.v. infusion 44 μg/m^2/week $\times$3 every 4 weeks; DLT myalgia
6. Propper *et al.*[82]	II, metastatic melanoma	1 h i.v. infusion 25 μg/m^2/ week $\times$3 every 4 weeks	PET	15 (children)	31% phlebitis	no response
7. Gonzalez *et al.*[83]	II, metastatic melanoma	1 h i.v. infusion 25 μg/m^2/ week $\times$3 every 4 weeks	PET	17	none reported	no response
8. Varterasian *et al.*[84]	II, LG NHL; CLL	72 h i.v. infusion 120 μg/m^2 every 2 weeks	PET	25	4% cellulitis	1 CR (LG NHL), 2 PR (CLL)

EtOH, ethanol formulation; PET, PET formulation; CLL, chronic lymphocytic leukemia; IG/HG, LG NHL, intermediate/high-grade, low-grade non-Hodgkin's lymphoma; CR, complete remission; PR, partial response; for phase I studies the recommended phase II starting dose is given as outcome.

administration, they were considered as formulation-related toxicities. In all further clinical studies the bryostatin 1–PET formulation was used with minimal side effects, with the exception of a study by Jayson *et al.*[79] In this phase I trial, bryostatin 1 administered as a 24-h continuous infusion caused thrombophlebitis in 44% of the treatment cycles. Concomitant infusion of normal saline diluting the vehicle concentration was applied to minimize the severity of the complication. No hypersensitivity reactions were reported. As the thrombophlebitis was believed to be related to venous stasis during long-term infusion, further studies were carried out using venous access devices for the administration of the drug solution. Varterasian *et al.*, for example, examined both toxicity and activity of a 72-h continuous bryostatin 1 i.v. infusion using a peripherally inserted central catheter, portable infusion pump or other central venous access devices and did not report any cases of thrombophlebitis.[80,84] Besides the study by Philip *et al.*, anaphylactic reactions to the PET vehicle were only seen in a phase I study in children with refractory solid tumors by Weitman *et al.* Mild hypersensitivity reactions (flushing, difficult breathing and hypotension) occurred in three out of 22 patients.[78,81]

In conclusion, the initial bryostatin 1–ethanol formulation was clinically not feasible due to the high incidence of severe cellulitis and thrombophlebitis at the administration site. The switch to lyophilized bryostatin 1 to be reconstituted with PET diluent did not, however, completely exclude formulation-related toxicities. In fact, besides phlebitis, in some of the clinical studies PET vehicle-related anaphylactic reactions were reported. Thrombophlebitis could be avoided by administering bryostatin 1 infusions via a central venous access device. Bryostatin 1 as single-agent therapy has shown limited activity with dose-limiting neuromuscular toxicity (Table 4). (Pre)clinical investigations of combination therapies with other antineoplastic agents (e.g. with paclitaxel, cisplatin) are ongoing, all using the bryostatin 1–PET formulation.[85–87]

Dolastatins

Origin/chemistry/activity

The dolastatins were first identified in the crude extract of the Indian Ocean sea hare *Dolabella auricularia*. It was shown that the extract of this

marine mollusk strongly inhibited proliferation of murine P388 leukemia.[88] Subsequent isolation and characterization revealed the presence of 15 novel cytotoxic peptides, which were designated dolastatin 1–15. Of these, dolastatin 10 and 15 appeared to be the most potent compounds. For dolastatin 10, for example, an IC_{50} for P388 murine leukemia cells of 5×10^{-11} M was determined, illustrating the extreme *in vitro* potency.[89] Although total synthesis of both dolastatin 10 as well as dolastatin 15 was accomplished, the complexity and low yields of the chemical process hindered broad clinical evaluation. Furthermore, both compounds are highly lipophilic and display poor water solubility. Therefore, attempts were made to synthesize a water-soluble analog, resulting in the development of LU103793 (*N*,*N*-dimethyl-L-valyl-L-*N*-methyl-L-valyl-L-prolyl-L-prolinebenzylamide hydrochloride), a compound which retains much of the backbone of dolastatin 15 (Figure 4).[89] Furthermore, an analog of dolastatin 10, auristatin PE, has been synthesized. This structural analog in which the dolaphenine unit is substituted by phenythylamine is currently under preclinical investigation.[85] Dolastatin 10, dolastatin 15, auristatin PE and LU103793 are all linear depsipeptides, and are all potent inhibitors of tubulin polymerization with efficacy against several human cancer cell lines. At present, dolastatin 10 and LU103793 (cemadotin) are in clinical studies.

Pharmaceutical formulation

Although dolastatin 10 is a highly lipophilic compound, aqueous solubility was sufficient to prepare a solution at a concentration required for the early clinical studies. Dolastatin 10 was formulated and supplied by the NCI as a 200 μg/ml solution for injection in 0.01 M potassium phosphate buffer (pH 7), each vial containing 1 ml of drug solution.[90] The product was stable under refrigerated conditions (2–8°C). The buffered solution was administered undiluted to the patient. As dolastatin 15 displayed insufficient aqueous solubility, a water-soluble analog was synthesized to enable clinical evaluation. The pharmaceutical product of LU103973 was supplied by Knoll Pharmaceutical (Parsippany, NY).[89] LU103973 was formulated as a 1 mg/ml solution for injection in ampoules containing 10 ml of solution. Like dolastatin 10, this solution was infused without further dilution.

Clinical studies

Table 5 shows an overview of the clinical trials performed with dolastatin 10. As the drug is formulated in an aqueous solution buffered at approximately physiological pH with a maximal injection volume of 5 ml, no formulation-related toxicities were expected. However, in all phase I clinical studies local irritation or phlebitis at the administration site were reported.[88,90] Pitot *et al.* reported several episodes of local irritation upon peripheral i.v. bolus injection with no relationship to the administered dose of dolastatin 10.[90] To avoid these side effects, Madden *et al.* either flushed the catheter with normal saline after the i.v. bolus injection or applied a central venous access.[88] As extravasation of the drug solution produced severe chronic inflammation and ulceration at the injection site during preclinical studies in dogs, the irritation was probably caused by dolastatin 10 itself. Also, a poorly soluble drug which is injected rapidly into the bloodstream might precipitate and cause irritation at the injection site. No responses were observed in a phase II study carried out by Krug *et al.*, which followed previous disappointing results of phase II studies in patients with prostate, colorectal and hepatobiliary carcinomas, melanoma, and sarcoma, in which also no dolastatin 10 activity was observed.[91] Several clinical studies with dolastatin 10 are still ongoing. Cardiovascular toxicity was found to be dose limiting. No formulation-related toxicity has been reported thus far in several phase I studies with LU103973 examining the toxicity and feasibility of different administration schedules.[92–97]

Ecteinascidins

Origin/chemistry/activity

In 1973, Lichter *et al.* first noted the antitumor activity of extracts from *Ecteinascidia turbinata*, a colony-forming marine tunicate growing preferentially on mangrove roots.[15] The active compounds in the extracts, however, were only elucidated in the late 1980s when adequate separation and characterization assays were developed [e.g. fast atom bombardment (FAB)–MS and NMR techniques].[15] Major antitumor compounds in the *E. turbinata* extract appeared as bis- and tris-tetrahydroisoquinoline alkaloids, comprising a family of 10 related compounds which were assigned as ecteinascidin (ET) followed by a number indicating the ion mass originally observed for the respective compounds. The ETs are structurally related to saframycins, safracins, quinocarcins and naphthyridinomycins. Originally, preclinical studies were initiated with ET-729, which displayed high *in vitro* and *in vivo* potency in various murine and human tumor assays.[1] However, as ET-743 was found to be more abundant in the *E. turbinata* tunicate and

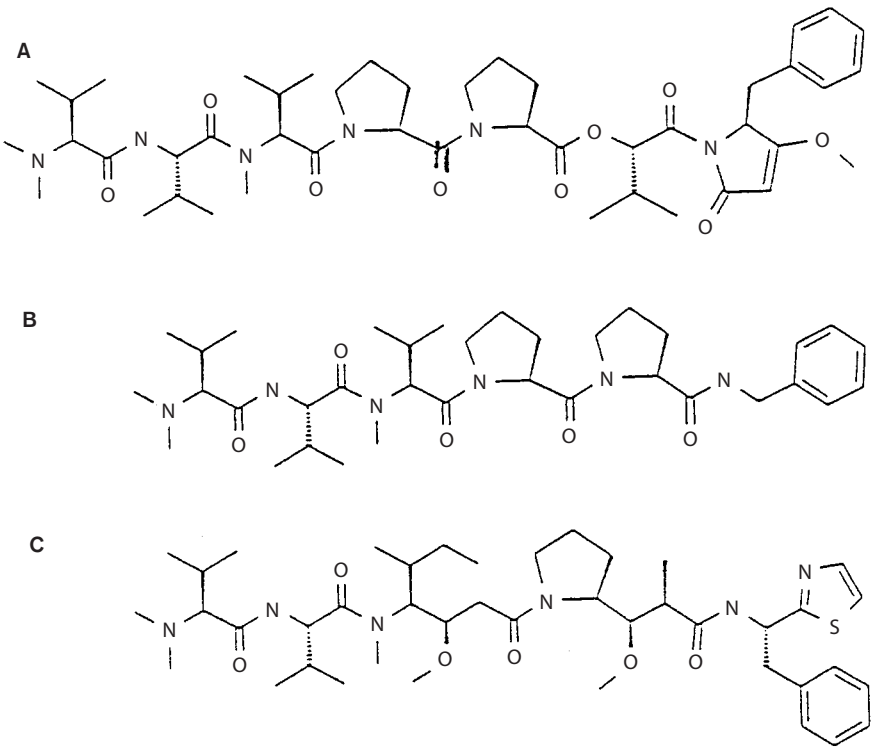

Figure 4. Structural formulas of dolastatin 15, LU103793 and dolastatin 10.

Table 5. Phase I/II studies of dolastatin 10

Author (reference)	Phase I/II	Dose schedule	No. of patients	Outcome
1. Madden *et al.*[88]	I	i.v. bolus injection 65–300 μg/m^2 every 3 weeks	19	300 μg/m^2 every 3 weeks; DLT granulocytopenia
2. Pitot *et al.*[90]	I	i.v. bolus injection 65–450 μg/m^2 every 3 weeks	30	325 μg/m^2 every 3 weeks (heavy prior treatment) or 400 μg/m^2 every 3 weeks (minimal prior treatment); DLT granulocytopenia
3. Krug *et al.*[91]	II, NSCLC	i.v. bolus injection 400 μg/m^2 every 3 weeks	19	no response

NSCLC, non-small cell lung cancer; for phase I studies the recommended phase II starting dose given as outcome.

displayed similar selectivity and potency as ET-729 in comparative studies, further development was focused on this compound. Structurally, ET-743 consists of three tetrahydroisoquinolone subunits (A, B and C) and has a complex stereochemistry, which was ultimately resolved by X-ray crystallography and total synthesis (Figure 5). Rinehart recently reviewed the structural elucidation of the ecteinascidins.[15] As a consequence of rapid loss of water, the initially assessed molecular weight of 743 was found to be actually 761. ET-743 exerts its antitumor activity by an unique mechanism of action, comprising effects on DNA (binding to guanine-rich areas of the minor groove of DNA with concomitant binding to nuclear proteins; reversible binding upon DNA denaturation), interference with microtubules-organization, and blocking cell cycle progression in the late S/G_2 phase.[1,98] ET-743 interferes with transcriptional activation. ET-743 selectively inhibits *in vitro* CCAAT-box binding of NF-Y. Upon heat induction, transcription of NIH3T3 lines with integrated HSP70 promoter dependent upon NF-Y and heat shock factor were blocked by ET-743 at biologically relevant concentrations and in a CCAAT-dependent way. However, another alkylator of the minor groove of the DNA, tallimustine, has no effect and the activity of the CCAAT-less SV40

promoter is not affected by ET-743 indicating that it is not a general *Pol*II inhibitor. Hence, ET-743 is a promoter specific transcriptional interfering agent.[98] In addition, it inhibits the inducible, but not the constitutive expression of P-glycoprotein by the gene *mdr*1.[99]

Pharmaceutical formulation

ET-743, like the didemnins and bryostatin 1, has limited aqueous solubility. However, by pH adjustment adequate concentrations of ET-743 could be reached. Instability of ET-743 in aqueous solution necessitated lyophilization in order to increase the storage stability. Currently, ET-743 is formulated as a lyophilized product containing 250 μg of active substance per dosage unit and 250 mg of mannitol as bulking agent. The product contains 0.05 M phosphate buffer at pH 4 in order to solubilize ET-743.[1] Reconstitution is performed by adding back 5 ml of water for injection (WfI), with subsequent dilution in normal saline before i.v. infusion. Due to instability upon long-term storage at refrigerated and room temperature, the lyophilized product must be stored between -10 and -20°C, protected from light. Attempts are made now to improve the stability of the current formulation. ET-743 infusion solutions are stable for 24 h at ambient temperature (20–25°C) and light.

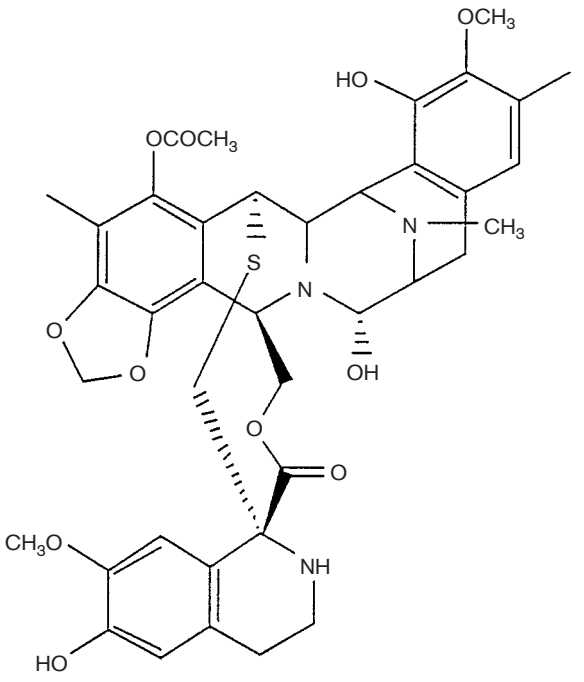

Figure 5. Structural formula of ecteinascidin-743 (ET-743).

Clinical studies

Toxicity of ET-743 was assessed during phase I clinical studies applying several dose schedules (i.e. 1 h infusion daily $\times 5$, 24-h continuous infusion, 72-h continuous infusion, all every 3 weeks).[101–105] DLTs found were neutropenia and thrombocytopenia. As expected from the composition, the pharmaceutical formulation was well tolerated. Besides the occurrence of phlebitis that was minimized by increasing the infusion volume or eliminated by using a central catheter, no clear formulation-related toxicities were noted thus far. ET-743 administered as a 24 h continuous infusion every 3 weeks at 1500 μg/m^2 was selected for the phase II clinical studies. Currently, ET-743 is examined in an extensive phase II program in various tumor types, including soft tissue sarcoma, melanoma, breast and renal carcinoma. Preliminary reports have revealed promising clinical activity of ET-743 with clear evidence of activity in breast cancer and soft tissue sarcoma.[106–109]

Conclusions

In this review aspects of the pharmaceutical development of investigational anticancer drugs from marine origin have been discussed. Furthermore, several pharmaceutical formulations of marine anticancer agents used in clinical practice have been reviewed. It was shown that poor aqueous solubility of the active marine compound often necessitated the use of co-solvent/surfactant systems (e.g. didemnin B, aplidine, bryostatin 1). Furthermore, lack of stability in solution was in some instances reason to formulate a lyophilized product (e.g. aplidine, bryostatin 1, ecteinascidin-743). The presence of co-solvent/surfactants in didemnin B, aplidine and bryostatin 1 infusion solutions induced leakage of plasticizers, thus precluding the use of PVC administration sets. Aplidine as well as bryostatin 1 were shown to sorb to contact surface areas, therefore requiring maximal dilution levels in infusion fluids or specific administration set materials. For didemnin B and bryostatin 1, formulation-related toxicities (hypersensitivity reactions and phlebitis, respectively) were reported in clinical trials which, however, were all manageable by either adjustment of administration parameters or prophylactic treatment.

In conclusion, the search for and development of potential anticancer agents from marine sources is in full swing. The development of a new marine-derived anticancer agent is a team effort involving marine biologists, chemists, and many other specialists. To enable clinical evaluation of the marine candidate, the

availability of a clinically feasible, pharmaceutical formulation is a prerequisite.

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