Progress in the clinical development of new marine-derived anticancer compounds

Jose Jimeno[a], J. A. López-Martín[a], A. Ruiz-Casado[a], M. A. Izquierdo[a], P. J. Scheuer[b] and K. Rinehart[c]

Naturally derived anticancer agents continue to be instrumental in the systemic therapeutic intervention against solid tumors and hematological malignancies. Such compounds now have a relevant role in contemporary models of combination with targeted agents, thus providing a rationale to consider nature as a valid tool to discover new innovative anticancer agents. The marine ecosystem has increasingly been the focus of interest for new discoveries in the field that are expected to be of significant therapeutic impact in cancer patients. A critical review of the integrated data generated in our marine-derived anticancer program seems to confirm such expentancies. ET-743 (Yondelis) represents the first new agent developed against advanced pretreated soft tissue sarcoma in the past 25 years, and also harbors activity in women bearing pretreated ovarian cancer and a solid potential in combination therapy. The lack of cumulative toxicities makes this compound suitable for long-lasting therapies, reversible transaminitis being the most prevalent toxicity. Aplidin has shown a positive therapeutic index in phase I trials and phase II studies are ongoing. In contrast to the lack of bone marrow toxicity, a set of translational results anticipates a potential in leukemia. Kahalalide F has also successfully completed the phase I program in solid tumors with evidence of activity in resistant tumors and phase II studies are under way. Finally, the mechanistic data generated in parallel with the clinical program confirms the potential of the marine ecosystem in the discovery of new agents acting against new cellular targets of relevance in cancer cell biology. *Anti-Cancer Drugs* 15:321–329

Anti-Cancer Drugs 2004, **15**:321–329

Keywords: aplidin, ET-743, kahalalide F, marine drugs

[a]PharmaMar R & D, Madrid, Spain, [b]Chemistry Department, University of Hawaii, HI, USA and [c]School of Chemical Sciences, University of Illinois, IL, USA.

This paper is dedicated to the memory of P. J. Scheuer (Heilbronn, Germany 1915–Hawaii 2003) a pioneer in marine drug research and discoverer of kahalalide F.

Correspondence to J. Jimeno, Pharma Mar R & D, Avda de los Reyes 1, Pl La Mina-norte, 28770 Colmenar Viejo, Madrid, Spain.
Tel: +34 91 846 60 37; fax: +34 91 823 45 05;
e-mail: jjimeno@pharmamar.com

Received 5 November 2003 **Revised form accepted** 10 December 2003

Introduction

A vast number of anticancer agents still commonly used in the clinical setting are naturally derived drugs. Such cytotoxics are considered instrumental in the systemic pharmacological intervention of the disease.

The confirmation of the biological impact of the self-sufficiency of growth signaling led to the identification and development of a number of compounds that hit a defined and relevant target(s) in cancer. The high prevalence of polyclonality in cancer indicates the need to establish a multiple pharmacological approach based on well-designed/rational combinations of cytotoxics and 'targeted' agents [1].

The marine ecosystem is considered to be a productive tool to discover new anticancer entities. The discovery, development and marketing approvals of (cytosine arabinoside) ARA-C and its fluorinated derivative gemcitabine represents an available proof-of-concept of this approach [2]; ARA-C is a synthetic derivative of a series of C-nucleotides discovered in the Caribbean sponge *Tethya crypta* [2].

Didemnin B is a reference in the clinical development of marine anticancer compounds. This compound reached phase II studies; however, in spite of the activity noted in heavily pretreated patients with non-Hodgkin's lymphoma [3], its low therapeutic index precluded further developmental efforts.

In the past years the interest to focus on marine anticancer entities has been revamped and comprehensive reviews of the topic have been published elsewhere [4–7].

This paper seeks to integrate the progress made and the perspectives in the clinical development of three innovative marine anticancer compounds; ET-743, aplidin and kahalalide F that have proved to harbor a positive therapeutic index in patients with advanced pretreated tumors.

0959-4973 © 2004 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000125049.43188.95

Ecteinascidin-743 (ET-743; Yondelis, Trabectedin)

An extensive review of the mechanistic, preclinical and early clinical data of ET-743 (Fig. 1) has been published in this journal recently [8].

The safety data generated at the early stages of the phase II program with the RD of 1.5 mg/m^2 given as a 24-h i.v. infusion was of great concern due to the incidence of multiorgan toxicities and drug-induced toxic deaths.

The identification of the baseline hepatobiliary function and the impact of the intercycle peaks of bilirrubin and alkaline phosphatase as indicators of high risk for severe toxicities led to the implementation of a set of guidelines in terms of the selection of patients—normal hepatobiliary function required to treat a patient with full doses of ET-743 and the rules for dose reduction [9]; such an amendment has had a major impact on the therapeutic index of ET-743 [10]. Conceptually, the drug-induced transaminitis is not considered to be a clinical limiting factor due to the pattern of reversibility; in spite of a 20% incidence of grade 4 neutropenia, the incidence of febrile events is just 4% without systematic use of hematological growth factors in such studies. In contrast to classical cytotoxic therapies, ET-743 does not induce mucositis, cardio- or neurotoxicity, diarrhea, or alopecia. Mature data demonstrates a lack of cumulative toxicities, thus making ET-743 suitable for chronic administration (Table 1).

Only 4% of treatment dropouts are related to drug-induced toxicities. The toxic death rate is 1.1%, but two out three of such post-amendment events were the result of serious violations of the guidelines of patient selection and drug reduction, thus demonstrating the need to be adherent to such guidelines to warrant the therapeutic index of ET-743.

The therapeutic index of the proposed RD is also sustained by the dose intensity of 91% achieved in this cohort of mostly heavily pretreated patients.

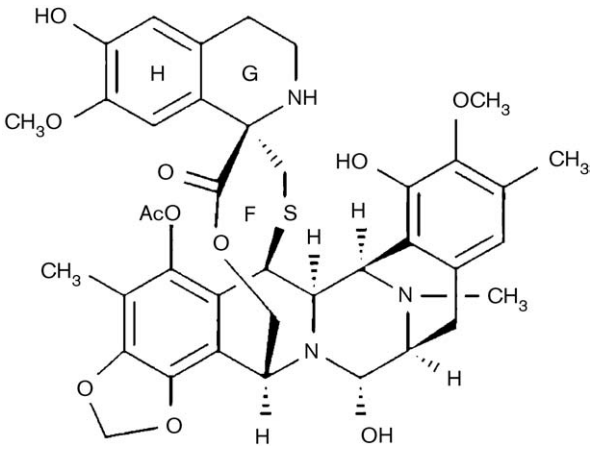
Progress in the understanding of the pharmacodynamics of the drug-induced hepatobiliary toxicity has been made in experimental models [11]. This data must be interpreted with caution due to the metabolic interspecies differences noted. The sequence of events observed in this *in vivo* model seems to be consistent with the prognostic impact of the drug-induced biliary events. This experimental study also demonstrates that the ET-743-related hepatotoxicity can be the subject of pharmacological modulation, at least in animal models.

A recent pharmacokinetic (PK)/pharmacodynamic (PD) analysis conducted in patients treated with the RD of ET-743 in protracted infusion has confirmed the correlation between an area under the curve (AUC) above the threshold of 70 ng·h/m (8) and the onset of severe toxicities [12]. Those cases that experienced grade 4 NCI-CTC toxicities had a median AUC on cycle 1 = 76 ng·h/ml.

Long-term results generated with ET-743 in a large phase II cohort of patients with advanced pretreated soft tissue sarcoma (STS) are now available [13–17] (Table 2).

The results demonstrate that ET-743 is able to induce long-lasting objective remissions, median duration of 11 months, and tumor control in a clinically meaningful proportion of patients. The minor responses lasted a median of 8 months. The progression-free survival at 6 months ranks ET-743 in the category of active drugs in sarcoma [18]. The overall median survival achieved of 10.3 months strongly indicates, with the caveat of the

Fig. 1



Ecteinascidin-743 (trabectidin, Yondelis).

Table 1 Safety profile of ET-743[a] in adult patients with advanced solid tumors exposed to 6 or more cycles

Parameter	Grade				
	0	1	2	3	4
Fatigue	47	35	17	1	–
Vomiting	85	10	4	–	–
Diarrhea	97	3	1	–	–
Mucositis	97	3	1	–	–
Transaminitis	24	35	24	15	1
Bilirrubin	92	6	1	1	–
Alkaline phosphatase	72	27	1	–	–
Neutropenia	35	20	19	19	6
Febrile neutropenia	100	–	–	–	–
Platelets	89	5	3	3	1
Anemia	53	26	19	2	–

[a]ET-743 1.5 mg/m^2 given as a 24-h i.v. infusion q3–4 weeks [62 patients out of 266 (23.1%) treated with 6 or more cycles].
Figures expressed in percent.

Table 2 Long-term results in the phase II pivotal program with ET-743[a] in advanced pretreated STS

Parameter	Tumor burden (%)[b]	Full cohort ($n=183$ patients)	Tumor shrinkage (%)
Partial remission	+87	14 (8%) 95% CI 4–12.5	−67 (52–99)
Minor response	+87	14 (8%) 95% CI 4–12.5	−35 (30–49)
Stable disease	+56	13 (10%) 95% CI 4–12	–
Tumor control		41 (22%) 95% CI 17–29	–

[a]ET-743 1.5 mg/m^2 24-h i.v. infusion q3–4 weeks.
[b]Tumor burden within the 3 months period pre-study entry.

phase II nature of the data, an improvement of survival in this cohort of patients harboring progressive pretreated STS that included 30% of cases with primary resistant disease to both doxorubicin and ifosfamide.

ET-743 was the first cytotoxic tested in a cohort of c-*kit*$^+$ patients with advanced gastrointestinal stromal sarcoma, but no responses were reported [19]. A phase II study with protracted 24-h infusion of ET-743 in a group of heavily pretreated patients with bone sarcoma has reported three minor responses in 25 patients treated [11].

An active program is seeking to define the therapeutic profile of an outpatient schedule; in this set of studies ET-743 is given as a 3-h i.v. infusion every 3 weeks. The initial proposed RD of 1.65 mg/m^2 [20] has been proven to be unfeasible in phase II patients, mainly due to the emergence of severe fatigue as well as a result of the guidelines of dose reduction in place after the amendment. Current studies have established a positive therapeutic index when a dose of 1.3 mg/m^2 of ET-743 is given as a 3-h infusion [21].

A pooled analysis of the 20 heavily pretreated adults and pediatric Ewing sarcoma patients treated with this schedule of ET-743, RD in children = 1.1 mg/m^2, suggests a therapeutic potential of ET-743 with one complete response in a pediatric Ewing sarcoma that lasted 12 months and two partial remissions of 11 months of duration [22,23].

Preliminary data in a small cohort of patients with advanced STS treated with this dose and schedule yielded a 7% response rate and a progression-free survival at 6 months of 21% [24,25].

Also, this dose and schedule is being actively investigated in patients with advanced pretreated ovarian cancer. Mature results from such a trial [26] demonstrate that ET-743 is an active agent in this disease setting. Ten partial remissions in 40 women harboring disease resistant or relapsed to platin and taxanes have been reported, resulting in a 25% response rate, the median duration of response is 6+ months and the response rate in the relapsed population is 47%. The safety profile of ET-743 in this patient population is appropriate with lack of grade 4 transaminitis and severe asthenia. This study has established the foundation to implement a phase III study in second line versus the standard of care; such a study is now under implementation.

Major progress in the knowledge of the mechanism of action of the drug has resulted in solid paradigms to rationally design ET-743-containing combinations. An elegant study has confirmed the downregulation of the P-glycoprotein (P-gp)/MDR1 path by ET-743 [27] and lack of cross-resistance to ET-743 in MDR$^+$ cell lines. No P-gp overexpression has been noted in a number of ET-743-resistant cell lines. Extensive *in vivo* results [28,29] provided a solid basis to combine ET-743 with anthracyclines and taxanes.

The availability of these combinations is instrumental to further develop ET-743 in minimally pretreated sarcoma, breast and ovarian cancer.

ET-743 is the only drug known that requires intact nucleotide excision repair machinery to induce cytotoxicity in human cancer cell lines [30]. Such interesting correlation established a rationale [31] and anticipated a potential for combination with platin salts. The combination of ET-743 and cisplatin has proven to be synergistic in *in vivo* models [32] and able to revert the *in vivo* primary resistance of human tumors to either ET-743 or cisplatin. Also, the safety profile of ET-743 as a single agent did anticipate a lack of overlapping toxicities with cisplatin, but also with carboplatin and oxaliplatin. As a result, a phase I program is seeking to define the therapeutic index of those potentially relevant combinations.

Mature results from a phase I program combining ET-743 and cisplatin have been recently presented [33]. This study incorporates therapeutic fixed doses of cisplatin, 40 mg/m^2 i.v. on days 1 and 8, with escalating doses of ET-743 also given weekly every 3–4 weeks. The safety data demonstrates the feasibility to escalate ET-743 up to 700 μg/m^2/day; the dose-limiting toxicities (DLTs) were long-lasting uncomplicated neutropenia and hyperbilirrubinemia. In fact, the frequent treatment delays and the lack of recovery of the bone marrow function indicates that this combination should be given every 4 weeks. The proposed RD of the combination in pretreated patients is cisplatin 40 mg/m^2 on days 1 and 8 plus ET-743 500 μg/m^2 on days 1 and 6. The feasibility of the dose level at which ET-743 is given at a dose of 700 μg/m^2/day is being investigated in minimally pretreated patients. Activity in different tumor types has been reported including early, but encouraging, evidence of antitumor activity in refractory ovarian cancer, with three confirmed partial

remissions in 10 patients treated. Pharmacokinetic interactions in plasma have been ruled out. This combination is expected to provide a solid basis to develop ET-743-containing therapy in different tumor types.

In summary, major progress in the clinical development of ET-743 has been made in the past 2–3 years. ET-743 appears to be the first active drug in STS developed in the past 25 years with a safety profile suitable for chronic administration. An outpatient schedule, 3-h infusion is being actively investigated: the available data demonstrating a solid potential in pretreated ovarian cancer, and promising results in Ewing and soft tissue sarcoma. The advanced results from two potentially relevant ET-743-containing combinations have been recently presented. A phase I trial seeking to characterize the feasibility of ET-743 given in sequence with doxorubicin has been conducted in advanced sarcoma patients untreated for metastatic disease [34]: in this study a fixed dose of doxorubicin, 60 mg/m^2, is combined with escalated doses of ET-743. The MTD of the combination has been reported at a dose of ET-743 = 800 μg/m^2, the DLTs being grade 4 neutropenia and asthenia. A comparison of the plasma disposition of both drugs excludes any significant PK interaction between the two drugs. Four confirmed partial remissions (25.5%) and two stable diseases lasting more than 6 months have been noted among the 17 patients entered. An evaluation of the antitumor activity in an expanded cohort of patients is ongoing. The feasibility of the combination of paclitaxel and ET-743 is being defined in a phase I study in which paclitaxel is given as a 1-h i.v. infusion on day 1 followed by ET-743 given as a 3-h infusion on day 2; treatment is recycled every other week [35]. No DLTs have been noted so far at dose levels of paclitaxel and ET-743 of 120 mg/m^2 and 580 μg/m^2, respectively. Either ET-743 or paclitaxel is unlikely to be a significant inhibitor or inducer of CYP3A4; one confirmed PR in a patient with advanced resistant liposarcoma has been observed among the 12 evaluable cases.

The early results generated from a phase I trial combining ET-743 and capecitabine indicate that this combination is well tolerated and demonstrate antitumor activity; dose escalation is actively ongoing [36].

The current clinical plan is being conducted by a joint partnership agreement with Johnson & Johnson Ortho Biotech.

Aplidine

The didemnins family has been one of the references in the discovery of marine anticancer chemical entities. Didemnin B was the first marine anticancer compound incorporated into clinical trials in the early 1980s. Dose escalation of this compound was hampered by severe emesis [37]. As a result, the first set of phase II studies included a suboptimal dose and no responses were noted in different tumor types. Later on, a phase I trial with appropriate antiemetic protection allowed dose escalation that defined neuromuscular toxicity as the DLT [38]. Phase II studies with a higher RD were implemented and activity in heavily pretreated patients with advanced low-grade non-Hodgkin's lymphoma was reported [39].

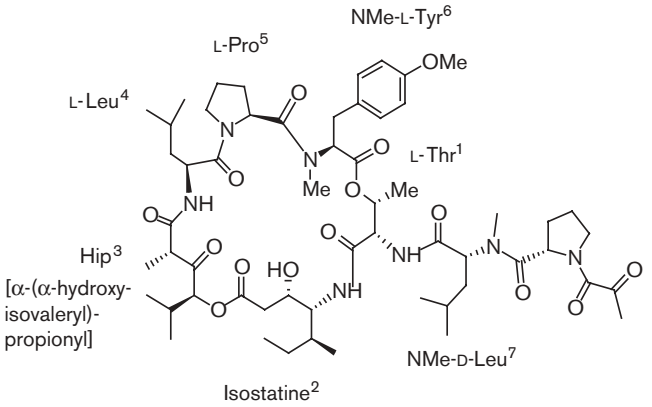
However, the emergence of cardiotoxicity and severe constitutional toxicities precluded the administration of multiple cycles and establishing a negative therapeutic index in patients. The evidence generated with this innovative entity was considered to be the basis to continue seeking for second-generation compounds that might harbor a better therapeutic profile.

As a result of such efforts, a dehydro derivative of the parent compound emerged as a rational alternative based on the evidence of a superior therapeutic index in experimental models [40]. Aplidine (APL; Fig. 2) is a cyclic depsipeptide discovered in the Mediterranean tunicate *Aplidium albicans*. Its antitumor activity seems to be the result of a constellation of molecular events that lead to G_1 block [41] with no effect in macromolecular synthesis. A very interesting study has recently demonstrated the pharmacodynamic effects of therapeutic concentrations in the reduction of vascular endothelial growth factor (VEGF) secretion and downregulation of its receptor VEGFR-1 [42] in MOLT-4 human leukemia cells. In contrast, additional *in vitro* studies in human acute lymphoblastic leukemia blasts failed to correlate the pharmacodynamic effects on VEGF with its cytotoxicity. Several pathways have been proposed to be involved in the drug-related apoptotic effects, induction of early oxidative stress with JNK and p378 activation [43,44].

The phase I program was activated back in 1998 and included a set of dose-escalation studies incorporating different i.v. dose-dense schedules [45–49].

All studies have been completed and the correlative maximal tolerated doses (MTDs) and RDs established (Table 3). The DLTs reported included muscular toxicity, asthenia, skin rash and diarrhea with a remarkable absence of bone marrow toxicity. The drug-induced muscular toxicity is characterized by muscular pain and muscular weakness with late increases in creatine kinase. The optical microscopy characterizes such events as type II diffuse muscular atrophy. Early evidence from the phase I program demonstrates that L-carnitine can prevent or accelerate the recovery of the muscular toxicities; in fact, co-therapy with L-carnitine has allowed

Fig. 2



Aplidin.

Table 3 APL: summary of phase I program [42–46]

Parameter	24-h c.i.v.i. days 1, 8, 15 q28d	3 h i.v. d 1, 15 q28d	1-h i.v. d 1, 8, 15 q28d)	24-h c.i.v.i. d 1, 15 q28d	24-h c.i.v.i. d 1, 15 q 28d + L-carnitine	1 h daily × 5 q21d
No. of patients	35	27	49	47	20	36
MTD	4500	6000	3600	6000	8000	1500/day
DLT	muscular G4; liver G3	liver G3; renal G4; muscular G4	muscular G3 and G4	muscular G3	asthenia G3	skin G3; diarrhea G3
RD	3750	5000	3200	5000	7000	1200/day
Dose intensity at the RD (μg/m^2/week)	2812.5	2500	2400	2500	3500	2250

further dose escalation in one of the phase I trials [50], thus offering the possibility to investigate a dose–response relationship in phase II trials.

The toxicity profile of the cohort of patients exposed at the RD levels is summarized in (Table 4). A sensitive analytical method [51] has allowed the generation of pharmacokinetic results in phase I patients. The PKs of APL are compatible with linearity up to the RD level and follows a tri-compartmental model; the distribution is extensive with a median terminal half-life of 34 h. Therapeutic blood levels are reachable well below the RD level, thus indicating a positive therapeutic profile.

Objective evidence of antitumor activity and clinical benefit has been observed across the phase I studies in different tumor types with a clustering of antitumor activity in neuroendocrine tumors such as medullary thyroid cancer and bronchial carcinoids.

An extensive phase II program is now ongoing and early results are expected to be released soon. In such studies, APL is being given as a 3-h i.v. infusion every other week at a dose of 5 mg/m^2.

Table 4 APL toxicity data (%) at the RD[a]

Parameter	Grade			
	1	2	3	4
Neutropenia	17	2		–
Hemoglobin	47	34	4	–
Neutrophils	6	6	2	–
Lymphocytes	6	42.5	13	–
Muscle weakness	10	4	3	–
Myalgia	32	9	3	–
Fatigue	19.5	52	17	1
Pyrexia	18	1	–	1

[a]RD cohorts from the phase I program: 77 patients, 181 cycles.

The mechanistic and early clinical data were the basis to further investigate the cytotoxic effects of APL against leukemic blasts explanted from acute lymphoblastic leukemia and acute myeloid leukemia patients [52,53]. Solid evidence of cytotoxicity with early and massive apoptotic effects has been noted at *in vitro* concentrations of 5 nM and below, and that are achievable systemically in patients much below of the RD; only high supraphamacological concentrations were noted to be cytotoxic against normal bone marrow progenitors.

Such results are indicative of a selective effect against malignant cells and offer a rationale to develop this

innovative compound in hematological malignancies, particularly when considering the lack of hematotoxicity in patients [54].

Those translational studies are also producing early evidence of *in vitro* synergy of APL in combination with ARA-C in leukemia cell lines, and lack of evidence of cross-resistance between APL and most of the cytotoxics available [55]. The investigation of the mechanisms involved in the primary of the acquired resistance to APL suggests the relevance of the intracellular levels of glutation in the inhibition of the drug-related pharmacodynamic effects [41].

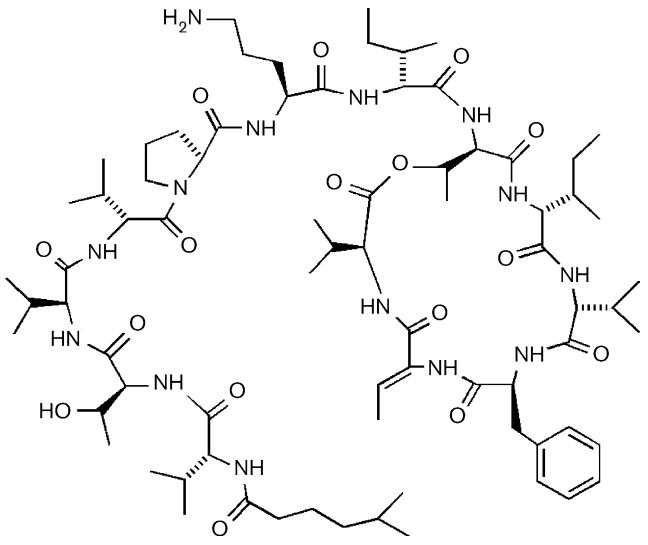
In summary, APL has shown evidence of a positive therapeutic index in adult, pretreated cancer patients. The lack of bone marrow toxicity is a clinically relevant finding, especially considering the antileukemic activity noted in experimental models. The safety data also anticipates the possibility of delivering multiple cycles to pretreated patients. The potential of APL in neuroendocrine tumors and in other malignancies is expected to be characterized shortly in the ongoing phase II program.

Kahalalide F

Kahalalide F (KF) (Fig. 3) is a dehydroamino-butyric acid-containing peptide isolated from the Hawaiian mollusc *Elysia rufescens* [56].

This compound harbors a US-NCI COMPARE negative profile that anticipates a novel mode of action. In fact, is has been observed that KF induces disturbances in lysosomal function that might lead to intracellular acidition and cell death [57].

Fig. 3



Kahalalide F.

The cytotoxicity induced by KF appears to be independent of the expression level of the multidrug resistance MDR1 and of the tyrosine kinase HER2/*neu*, but only slightly affected by the BCL-2 protein levels [58]. This interesting study has also detected losses of the mitochondrial membrane potential and of lysosomal integrity after *in vitro* exposure to KF. Cell kinetic analysis reveals a sub-G_1 selective block [59]. In preclinical models, KF displayed a selective *in vitro* and *in vivo* cytotoxicity profile in androgen-independent prostate cancer and other solid tumors, with lack of complete cross-resistance with conventional anticancer agents [60,61].

The lack of non-reversible toxicities in animal models [58] supported the implementation of a clinical plan with this innovative compound; therefore, a phase I program incorporating dose-dense schedules was opened back in 2000.

Such dose-finding/feasibility trials have included a disease-oriented trial in patients with advanced androgen-independent prostate adenocarcinoma that are treated with an i.v. 1-h infusion given daily for 5 consecutive days every 4 weeks and a second study investigating an i.v. 1-h infusion given weekly to patients with advanced solid tumors not amenable to conventional therapy [62,63].

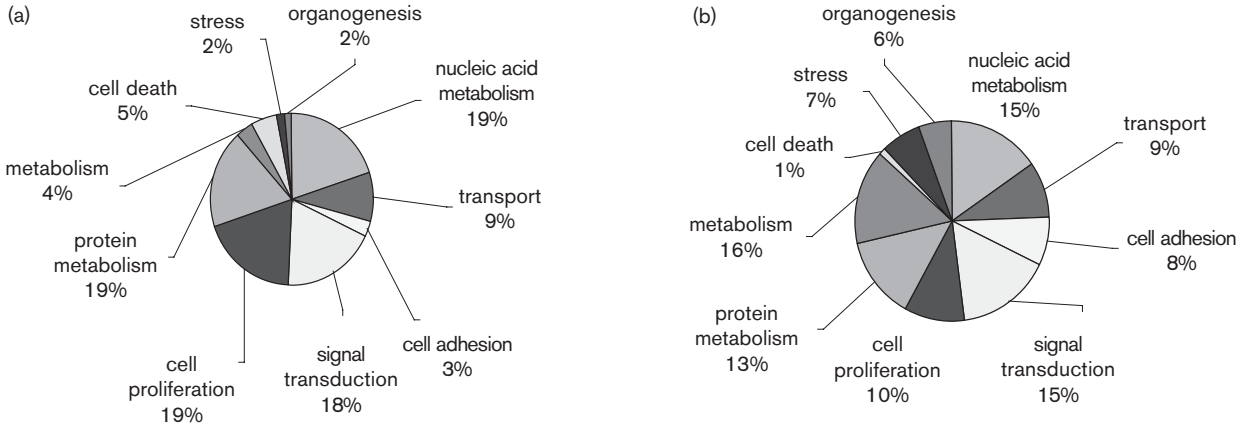
The tolerability of KF in these studies demonstrates feasibility in advanced pretreated patients, the DLTs being acute transaminitis and hypersensitivity reactions. In contrast to standard cytotoxics, KF lacks bone marrow toxicity, mucositis, neurotoxicity and alopecia.

The phase I program in prostate cancer is now accruing an extended cohort of patients at the RD and advanced results are expected to be available soon. The phase I program with the weekly schedule has been now completed. The pharmacokinetic profile of KF fits with linear C_{max} and AUCs up to the RD level, the plasma levels reaching well above the *in vitro* cytotoxic concentrations.

Objective responses and clinical benefit have been reported in advanced pretreated colorectal cancer, melanoma and hepatocellular carcinoma; a proportion of these patients have received multiple cycles of KF, thus indicating a lack of cumulative toxicities.

A phase II trial in patients with advanced hepatocellular carcinoma is now ongoing as a high priority program and further studies shall be activated in the near future.

Fig. 4



Genes commonly deregulated in two sensitive human sarcoma cell lines explanted from chemonaive patients and exposed *in vitro* to ET-743. (a) Functions of downregulated genes. (b) Functions of upregulated genes.

In summary, the data available demonstrates that use of this innovative compound is feasible in adult patients bearing advanced pretreated solid tumors. A dose-dense subtoxic weekly schedule has shown early evidence of antitumor activity in different disease settings. KF appears to be a feasible compound for long-lasting treatment.

Discussion

Our marine-derived anticancer program is confirming the potential of the marine ecosystem as a tool to discover new anticancer entities. Beyond the discovery of new chemical entities, our marine research and development program is also identifying new mechanistic paradigms that might have an impact in the current clinical armamentarium [64].

ET-743 emerges as the first active agent developed in sarcomas in the last 25 years, with very promising results as single agent in pretreated ovarian cancer as well. A number of ET-743-based combinations are now under active development to form the foundation of further studies in specific tumor types.

APL and KF have generated early evidence of antitumor activity that requires confirmation in phase II studies. Naturally derived anticancer agents are still a valid option in the era of molecular targets [1] and the marine ecosystem has been shown to be a highly productive tool. The in-depth knowledge of the pathways linked to the antitumor activity of these marine drugs and the analysis of their molecular pharmacodynamics can generate valuable information of the genes involved in the sensitivity/resistance to such agents [65] (Fig. 4). Such an approach might lead to the identification of patterns allowing customized therapies.

Table 5 Marine-derived therapeutics: potential limiting factors for development

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

The development of marine anticancer agents is linked to a number of limiting factors (Table 5) that might have a negative impact in clinical trials. Such an impact, as demonstrated in our marine-derived anticancer project, can be minimized by a close collaboration between marine biologists, medicinal chemists, preclinical toxicologists, pharmacologists and medical oncologists.

References

1 Chabner B. Cytotoxic agents in the era of molecular targets and genomics. *The Oncologist* 2002; **7(suppl 3)**.
2 Bergmann W, Feeney R. Contributions to the study of marine products. The nucleosides of sponges. III. Spongothymidine and spongouridine. *J Org Chem* 1955, **20**:1501–1507.
3 Kucuk O, Young ML, Habermann TM, Wolf BC, Jimeno J, Cassileth PA. Phase II trial of didemnin B in previously treated non-Hodgkin's lymphoma: an Eastern Cooperative Oncology Group (ECOG) Study. *Am J Clin Oncol* 2000; **23**:273–277.
4 Schwartsmann G, Brondani DA, Rocha A, Berlinck RGS, Jimeno J. Marine organisms as a source of new anticancer agents. *Lancet Oncol* 2001; **2**:221–225.
5 Amador ML, Jimeno J, Hidalgo M. Progress in the development and acquisition of anticancer agents from marine sources. *Ann Oncol* 2003; **14**: 1607–1615.

6 Schwartsmann G, Brondain DA, Rocha A, Mattei J, Martins Lopes R. Marine derived anticancer agents in clinical trials. *Expert Opin Invest Drugs* 2003; **12**:1367–1383.

7 Haefner B. Drugs from the deep: marine natural products as drug candidates. *Drug Discov Today* 2003; **8**:536–544.

8 Van Kesteren CM, De Vooght MM, Lopez-Lazaro L, Mathôt RAA, Schellens JHM, *et al.* Yondelis® (trabectedin, ET-743): the development of an anticancer agent of marine origin. *Anticancer Drugs* 2003; **14**:487–502.

9 Gomez J, Lopez-Lazaro L, Guzman C, Gonzalez A, Misset JL, Twelves C, *et al.* Identification of biochemical parameters that predict the onset of severe toxicities in patients treated with ET-743. *Proc Am Soc Clin Oncol* 2000; **19**:187 (abstr 727).

10 Lopez Martin JA, Nieto A, Demetri G, Misset JL, Ray-Coquard I, Guzman C, *et al.* Safety profile of Ecteinascidin 743 (ET-743) in phase II clinical trials (CT) in adult patients with solid tumors. *Proc Am Soc Clin Oncol* 2002; **21**:96a (abstr 382).

11 Donald S, Verschoyle R, Greaves P, Grant T, Colombo T, D'Incalci M, *et al.* Complete protection by high-dose dexamethasone against the hepatotoxicity of the novel antitumor drug Yondelis (Trabectedin ET-743) in the rat. *Cancer Res* 2003; **63**:5902–5908.

12 Laverdiere C, Kolb A, Supko J, Gorlick R, Meyers P, Maki R, *et al.* Phase II study of ET-743 in heavily pretreated patients with recurrent osteosarcoma. *Cancer* 2003; **98**:832–840.

13 Lopez-Martin JA, Verweij J, Blay JY, Lecesne A, Judson I, Van Oosterom A, *et al.* An exploratory analysis of tumor growth rate (TGR) variations induced by trabectedin (Ecteinascidin-743, ET-743) in patients with pretreated advanced soft tissue sarcoma. *Proc Am Soc Clin Oncol* 2003; **22**:819 (abstr 3293).

14 Demetri G, Manola J, Harmon D, Maki R, Seiden R, Supko J, *et al.* Ecteinascidin-743 (ET-743) induces durable responses and promising 1-year survival rates in soft tissue sarcomas (STS): final results of phase II and pharmacokinetic studies in the USA. *Proc Am Soc Clin Oncol* 2001; **20**:352a (abstr 1406).

15 Le Cesne A, Blay J, Judson I, Van Oosterom A, Verweij J, Radford J, *et al.* ET-743 is an active drug in adult soft-tissue sarcoma: a STBSG–EORTC phase II trial. *Proc Am Soc Clin Oncol* 2001; **20**:353a (abstr 1407).

16 Yovine A, Riofrio M, Brain E, Blay JY, Kahatt C, Delaloge S, *et al.* Ecteinascidin-743 given as a 24 hour (H) intravenous continuous infusion (IVCI) every 3 weeks: results of a phase II trial in patients (PTS) with pretreated soft tissue sarcomas (PSTS). *Proc Am Soc Clin Oncol* 2001; **20**:363a (abstr 1449).

17 Jimeno J. Status and perspectives of the clinical and translation program of ET-743. In: *Proc 2nd Int Conf on Targeted Therapies in Cancer*, Washington, DC; 2003.

18 Van Glabbeke M, Verweij J, Judson I, Nielsen OS. Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas. *Eur J Cancer* 2002; **38**:543–549.

19 Ryan D, Puchalski T, Supko J, Harmon D, Butkiewicz L, Maki R, *et al.* A phase II and pharmacokinetic study of Ecteinascidin 743, in patients with gastrointestinal stromal tumors. *The Oncologist* 2002; **7**:531–538.

20 Twelves C, Hoekman K, Bowman A, Vermorken JB, Anthoney A, Smyth J, *et al.* Phase I and pharmacokinetic study of Yondelis™ (ET-743) administered as an infusion over 1 hour or 3 hours every 21 days in patients with solid tumors. *Eur J Cancer* 2003; **39**:1842–1851.

21 Ruiz Casado A, Sessa C, Provencio M, Casali P, Paz Ares L, *et al.* Safety profile for Yondelis (ET-743, Trabectedin) 1.3 mg/m^2 over 3 hours. *Proc Eur Cancer Conf* 2003; **12**:S173 (abstr 573).

22 Casanova M, Casali P, Sessa C, Bacci G, Aglietta M, Ferrari A, *et al.* Phase II study of 3-hour infusion ecteinascidin-743 (ET-743) in pretreated adult and pediatric patients with advanced or recurrent sarcomas: preliminary results. In: *Proc SIOP XXXIV Meet*, Porto; 2002, p. 257 (abstr 154).

23 Baruchel S, Blaney S, Hershon L, Krailo M, Seibel N, Guzman C, *et al.* A phase I study of ET-743 in pediatric refractory solid tumors: a Children's Oncology Group Study. *Proc Am Soc Clin Oncol* 2002; **21**:96a.

24 Dileo P, Casali P, Bacci G, Casanova M, Aglietta M, Ferrari S, *et al.* Phase II evaluation of 3 hour infusion of ET743 in patients with recurrent sarcomas. *Proc Am Soc Clin Oncol* 2002; **21**:408a (abstr 1628).

25 George S, Maki R, Harmon D, Supko J, Morgan J, Seiden M, *et al.* Phase II study of ecteinascidin743 (ET743) given by 3 hour IV in patients (pts) with soft tissue sarcomas (STS) failing prior chemotherapies. *Proc Am Soc Clin Oncol* 2002; **21**:408a (abstr 1630).

26 Colombo N, Sessa C, Perotti A, Curigliano G, Noberasco C, Jimeno J, *et al.* Phase II study of 3-hour infusion ET-743 in patients with refractory/ relapsing ovarian cancer. In: *Proc Chemotherapy Foundation Symp*, New York; 2002, p. 35 (abstr 27).

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

28 Meco D, Colombo T, Ubezio P, Zucchetti M, Zaffaroni M, Riccardi A, *et al.* Effective combination of ET-743 and doxorubicin in sarcoma: preclinical studies. *Cancer Chemother Pharmacol* 2003; **52**:131–138.

29 Takahashi N, Li W, Banerjee D, Guan Y, Wada-Takahashi Y, Brennan MF, *et al.* Sequence-dependent synergistic cytotoxicity of ecteinascidin-743 and paclitaxel in human breast cancer cell lines *in vitro* and *in vivo*. *Cancer Res* 2002; **62**:6909.

30 Damia G, Adimoolan S, Carrassa L, D'Incalci M, Faircloth G, *et al.* Role of nucleotide excision repair pathway in ET-743 mechanism of action. *Proc Am Ass Cancer Res* 2003; **44**:abstr 5464.

31 Furuta T, Ueda T, Aune G, Sarasin A, Kraemer K, Pommier Y. Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. *Cancer Res* 2002; **62**:4899–4902.

32 D'Incalci M, Colombo T, Ubezio P, Nicoletti I, Giavazzi R, Erba E, *et al.* The combination of yondelis and cisplatin is synergistic against human tumour xenografts. *Eur J Cancer* 2003; **39**:1920–1926.

33 Colombo N, Grasselli G, Noberasco C, De Braud F, Malossi A, Perez C, *et al.* A phase I and pharmacokinetic (PK) study of Yondelis and cisplatin in patients with advanced solid tumors including platinum resistant ovarian cancer patients. In: *Proc Chemotherapy Foundation Symp XXI: Innovative Cancer Therapy for Tomorrow*; 2003, p. 89.

34 Gianni L, Grasselli G, De Braud F, Curigliano G, Noberasco C, Marsoni S, *et al.* Definition of the least toxic sequence and optimal therapeutic dose of Yondelis in combination with doxorubicin in patients with untreated metastatic soft tissue sarcomas and advanced pretreated anthracyclines–naïve breast cancer patients. In: *Proc AACR–NCI–EORTC Int Conf on Molecular Targets and Cancer Therapeutics*; 2003, p. 62 (A49).

35 Chu Q, Forouzesh B, Schwartz G, Jones C, Garrison M, Goldston M, *et al.* Clinical and pharmacokinetic phase I study of sequential paclitaxel and ET-743 every 2 weeks in patients with advanced solid tumors. In: *Proc AACR–NCI–EORTC Int Conf: Molecular Targets and Cancer Therapeutics*; 2003, p. 6a (A47).

36 Holden S, Rivera E, Bascher M, Gore L, Raj S, O'Bryant C, *et al.* A phase I study of ecteinascidin 743 and capecitabine in patients with advanced solid malignancies. In: *Proc AACR–NCI–EORTC Int Conf: Molecular Targets and Cancer Therapeutics*; 2003, p. 61 (A45).

37 Razis E, Mittelman A, Coombe N, Puiccio C, Chun H, Ahmed T *et al.* Phase II clinical trial of Didemnin B (DB) in patients with glioblastoma multiforme (GM). *Proc Am Soc Clin Oncol* 1992; **11**:A432.

38 Shin D, Holoye P, Murphy W, Forman A, Papasozomenos S, Hong W, *et al.* Phase I/II clinical trail of Didemnin B in non-small-cell-lung cancer: neuromuscular toxicity is dose-limiting. *Cancer Chemother Pharmacol* 1991; **29**:145–149.

39 Kucuk O, Young ML, Habermann TM, Wolf BC, Jimeno J, Cassileth PA. Phase II trial of didemnin B in previously treated non-Hodgkin's lymphoma: an Eastern Cooperative Oncology Group (ECOG) Study. *Am J Clin Oncol* 2000; **23**:273–277.

40 Faircloth G, Grant W, Nam S, Jimeno J, Manzanares I, Rinehart KL. Preclinical development of Aplidin, a novel marine-derived agent with potent antitumor activity. In: *Proc 10th NCI–EORTC Symp on New Drugs in Cancer Therapy*, Amsterdam; 1998, abstr 129.

41 Erba E, Bassano L, Di Liberti G, Muradore I, Chiorino G, Ubezio P, *et al.* Cell cycle phase perturbations and apoptosis induced by Aplidin. *Br J Cancer* 2002; **86**:1510–1517.

42 Broggini M, Marchini S, Borsott P, Galliera E, Erba E, Taraboletti G *et al.* Aplidin, a new anticancer agent of marine origin, inhibits VEGF secretion and blocks VEGF–VEGF-1 (flt-1) autocrine loop in human leukemic cells molt-41. *Leukemia* 2003; **17**:52–59.

43 García-Fernández LF, Losada A, Alcaide V, Álvarez A, Cuadrado A, González L, *et al.* Aplidin™ induces the mitochondrial apoptotic pathway via oxidative stress-mediated JNK and p38 activation and protein kinase C delta. *Oncogene* 2002; **21**:7533–7544.

44 Cuadrado A, García-Fernández LF, González L, Suárez Y, Losada A, Alcaide V, *et al.* Aplidin™ induces apoptosis in human cancer cells via glutathione depletion and sustained activation of the epidermal growth factor receptor, Src, JNK, and P38 MAPK. *J Biol Chem* 2003; **278**:241–250.

45 Anthoney A, Paz-Ares L, Twelves C, Cortes-Funes H, Kaye S, Pronk L, *et al.* Phase I and pharmacokinetic study of Aplidin using a 24 hour weekly schedule. *Proc Am Soc Clin Oncol* 2000; **19**:189 (abstr 734).

46 Ciruelos EM, Twelves C, Dominguez MJ, McKay H, Anthony A, Castellanos D, *et al.* Phase I clinical and pharmacokinetic study of the marine compound

APL (APL) administered as a 3 hour infusion every 2 weeks. *Proc Am Soc Clin Oncol* 2002; **21**:abstr 422.
47 Bowman A, Izquierdo MA, Jodrell D, Martinez M, Cicchella B, Jimeno J, *et al.* Phase I clinical and pharmacokinetic (PK) study of the marine compound APL (APL), administered as a 1 hour weekly infusion. *Proc Am Soc Clin Oncol* 2001; **20**:abstr 476.
48 Armand JP, Ady-Vago N, Faivre S, Chieze S, Baudin E, Ribrag V, *et al.* Phase I and pharmacokinetic study of APL given as a 24-hour continuous infusion every other week (q2w) in patients (pts) with solid tumor (ST) and lymphoma (NHL). *Proc Am Soc Clin Oncol* 2001; **20**:abstr 477.
49 Maroun JA, Goel R, Stewart DJ, Tomiak E, Belanger K, Soulieres D, *et al.* Phase I study of APL in a 5 day bolus q 3 weeks in patients with solid tumors and lymphomas. *Proc Am Soc Clin Oncol* 2001; **20**:abstr 2082.
50 Ady-Vago N, Lopez-Lazaro L, Faivre S, Lacroix C, Luber-Narod J, Adams D, *et al.* L-Carnitine as a protector against Aplidin induced skeletal muscle toxicity. *Proc Am Ass Cancer Res* 2001; **20**:545 (abstr 2928).
51 Sparidans RW, Jantien-Kettenes Van Den Bosch J, Van Tellingen O, Nuyen B, Henrar REC, Jimeno J, *et al.* Bioanalysis of Aplidin, a new marine antitumoral depsipepetide, in plasma by high-performance liquid chromatography after derivatization with trans-4′-hydrazino-2-stilbazole. *J Chromatogr B* 1999; **729**:43–53.
52 Bresters D, Broekhuizen AJ, Kaaijk P, Faircloth GT, Jimeno J, Kaspers GJ. *In vitro* cytotoxicity of Aplidin and cross-resistance with other cytotoxic drugs in childhood leukemic and normal bone marrow and blood samples; a rational basis for clinical development. *Leukemia* 2003; **17**:1338–1346.
53 Erba E, Serafini M, Gaipa G, Tognon G, Marchini S, Muradore I, *et al.* The effect of Aplidin in acute lymphoblastic leukaemia cells. *Br J Cancer* 2003; **89**:763–773.
54 Banerjee D, Kaspers G, Erba E, Grossi A, D'Incalci M, Jimeno J, *et al.* Pharmacodynamics of Aplidin^{R} (APL) in experimental models of haematological malignancies (HAMA). *Proc Eur Cancer Conf* 2003; **12**:S192 (abstr 638).
55 Banerjee D, Medina D, Strair R, Li W, Elisseyev Y, Dhorvac R, *et al.* Drug combination studies with aplidin in experimental leukemia and lymphoma models. In: *Proc 5th Int Symp on Leukemia and Lymphoma*, Amsterdam; 2003, p. 686 (abstr 63).
56 Hamann MT, Otto CS, Scheuer PJ. Kahalalides: bioactive peptides from a marine mollusk *Elysia rufescens* and its algal diet *Bryopsis* sp. (1). *J Org Chem* 1996; **61**:6594–6600.
57 Becerro MA, Goetz A, Paul VJ, Scheuer PJ. Chemical defenses of the sarcoglossan mollusk *Elysia rufescens* and its host alga *Bryopsis* sp. *J Chem Ecol* 2001; **27**:2287–2299.
58 Suarez Y, Gonzalez L, Cuadrado A, Berciano M, Lafarga M, Muñoz A. Kahalalide F, a new marine derived compound induces oncosis in human prostate and breast cancer cells. *Mol Cancer Ther* 2003; **2**:863–872.
59 Janmaat ML, Kruyt F, Jimeno JM, Rodriguez JA, Giaccone G. Kahalalide F (KF) induces cell death in HER-2/*neu* overexpression breast cancer cells that is independent of caspase, cathepsin B and cathepsin D activity. In: *Proc. 2nd Int Symp on Signal Transduction Modulators in Cancer Therapy*, Amsterdam; 2003, p. B-02.
60 Medina LA, Gomez LA, Cerna C, Faircloth G, Yochmowitz M, Weitman S. Investigation of the effects of Kahalalide F against human tumor specimens taken directly from patients. *Proc Am Ass Cancer Res* 2001; **42**:abstr 1139.
61 Faircloth G, Smith B, Grant W, Jimeno J, Garcia-Gravalos L, Scotto K, *et al.* Selective antitumor activity of Kahalalide F, a marine-derived cyclic depsipeptide. *Proc Am Ass Cancer Res* 2001; **19**:213 (abstr 1140).
62 Schellens JHM, Rademakerlakhai JM, Horenblas S, Meinhardt W, Stokvis EM, De Reijke T, *et al.* Phase I and pharmacokinetic study of kahalalide F in patients with advanced androgen resistant prostate cancer. *Proc Am Soc Clin Oncol* 2002; **21**:113a (abstr 451).
63 Ciruelos E, Trigo JM, Pardo B, Paz-Ares L, Estaun N, Cuadra C, *et al.* A phase I clinical and pharmacokinetic (PK) study with Kahalalide F (KF) in patients (pts) with advanced solid tumours (AST) with a continuous weekly (W) 1-hour iv infusion schedule. 14th NCI–EORTC–AACR Congress, Frankfurt, Germany. *Eur J Cancer* 2002; **28**(**suppl 7**): S33 (abstr 95).
64 Karamouzis M, Gorgoulis V, Papavassiliou A. Transcription factors and neoplasia: vistas in novel drug design. *Clin Cancer Res* 2002; **8**:949–961.
65 Sanchez Beato M, Moneo V, Martínez N, Fernández I, Carnero A, Piris MA, *et al.* Molecular pharmacodynamic (MPD) effects induced by ET-743 (Yondelis) in human sarcoma cells (HSC). In: *Proc Int Conf on Applied Genomics–9th ESCAP/16th ISDQP Meeting*, Amsterdam; 2003, p. 65 (abstr C-14).