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Review paper

The clinical development of the bryostatins

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The bryostatins are a group of novel macrocyclic lactones derived from the marine bryozoan, *Bugula neritina*. *In vitro* evidence indicates that their main mechanism of action is modulation of protein kinase C (PKC) activity. Phase I studies suggested significant antineoplastic activity against several tumor types and defined the main dose-limiting toxicity as myalgia. Bryostatin-1 has subsequently been investigated extensively in phase II clinical trials as a single agent, although trial design has been hampered by lack of human pharmacokinetic data. Results have been generally disappointing but *in vitro* and animal data suggests an important role for bryostatin-1 in combination with cytotoxic agents. Preliminary results of phase I studies support these observations but further work needs to be done to define the future role of the bryostatins in the clinic. [© 2002 Lippincott Williams & Wilkins.]

Key words: Bryostatin, protein kinase C, signal transduction.

Introduction

The bryostatins are a family of at least 20 novel naturally occurring macrocyclic lactones derived from the marine bryozoan *Bugula neritina*. They have been shown to have promising antineoplastic and immunomodulatory activity in preclinical models. In this review we will briefly discuss the *in vitro* and animal data on the potential mechanisms of action of the bryostatins. We will then focus on the published data now rapidly emerging from phase I and II clinical trials concerning the use of bryostatin-1 either as a single agent or in combination with conventional chemotherapeutic agents.

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Discovery and isolation

The prototypic member, bryostatin-1 (see Figure 1), was first isolated from a crude extract of Californian *B. neritina* in 1982 by Pettit *et al.*[1] using the murine P388 leukemia model as a bioassay. Subsequent work has isolated bryostatins from *B. neritina* collected at multiple locations worldwide; the yield and type of bryostatin obtained depending on the geographical site, season and depth of collection. The complexity of the macrocyclic lactone structure has meant that no commercially viable chemical synthesis pathway has been developed (see Mutter and Wills[2] for a review of current approaches) and the bryostatin used in the laboratory and clinical settings is at present still extracted from natural sources. However, *B. neritina* is now cultivated on a commercial basis.[3]

Biological activity (see Table 1)

Interaction with protein kinase C (PKC)

Bryostatin is a partial agonist of the PKC family. This consists of 12 isoenzymes with serine threonine kinase activity that play crucial roles in cellular signaling, influencing proliferation and differentiation by phosphorylation of downstream effector proteins.[4] It is known that the binding of bryostatin-1 to PKC results in its transient activation, autophosphorylation and translocation to the cell membrane. Bryostatin-bound PKC is then down-regulated by ubiquitination and degradation in proteasomes.[5,6]

PKCs are also the targets for the classical group of tumor promoters, phorbol esters that bind to the same site on the enzymes as bryostatins. The reasons for the differences in actions between these two groups of compounds are unclear, although the prolonged activation of PKCs induced by phorbol

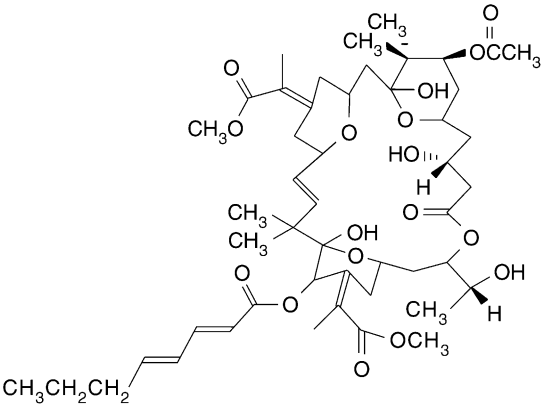

Figure 1. Bryostatin-1.

Table 1. Possible modes of action of the bryostatins

PKC modulation
PKD interaction
Apoptosis modulation
Interaction with MDR-1
Neutrophil/monocyte activation
T cell activation
Stimulation of normal hematopoiesis
Radioprotection

esters may be key. Several experimental observations also suggest that differential activation of PKC isoforms is important although contradictory results have been obtained.[7] Indeed, it may be that the PKCδ isoform plays an important role, as its activity is not affected by high intracellular concentrations of bryostatin-1 although it is activated and subsequently down-regulated by phorbol esters.[8] There is also evidence however to suggest that PKC modulation may not be the only pathway by which bryostatins are cytotoxic, at least in some cell lines. Work using B16/F10 melanoma cells has shown that 26 epi-bryostatin-1, a stereoisomer of bryostatin-1 with markedly decreased PKC affinity, inhibited cell growth at the same potency as its parent compound.[9] Protein kinase D has also been implicated in mediating some of the actions of bryostatin.[10]

In conclusion, whilst it is clear that the bryostatins bind to and modulate the activity of PKC, it has not yet been shown that this is the only or indeed the primary antineoplastic mechanism of these agents.

Bryostatin and apoptosis

Many studies suggest a role for PKC activation in apoptosis. They are, however, inconsistent, with modulation of apoptosis appearing to be cell type and environment dependent (see Hofmann[4] for a detailed review). Consistent with this body of evidence, bryostatin-1 has been shown to affect apoptosis through effects on the level of phosphorylation of *bcl-2* (an apoptosis suppressor) mediated by PKC.[11] Results from *in vitro* studies are again somewhat contradictory. For instance, bryostatin-1 increased levels of active phosphorylated *bcl-2* in the early pre-B acute lymphoblastic leukemia cell line, Reh, so inducing resistance to drug-induced apoptosis[12] in one study. In another, it was shown to down-regulate *bcl-2* and increase sensitivity to microtubule inhibitor-induced apoptosis in the same cell line.[13] Work in other leukemia cell lines has shown that bryostatin not only increases the rate of apoptosis by increasing the Bax:*bcl-2* ratio[14] but also sensitizes cells to apoptosis induced by paclitaxel and 2-chlorodeoxyadenosine.[14,15]

These contrasting results suggest that the modulation of PKC activity by bryostatin-1 may have divergent effects on apoptosis dependent on the cell line and its environment as well as the triggers inducing apoptosis. Whether this is the main pathway by which bryostatins induce their antineoplastic effects is still unclear.

Modulation of P-glycoprotein (mdr-1)

Bryostatin-1 influences the function of P-glycoprotein in several cancer cell lines leading to reversal of multidrug resistance. Work in prostatic carcinoma demonstrated down-regulation of *mdr-1* with enhancement of doxorubicin-induced cytotoxicity.[16] A similar result was seen in a diffuse large cell lymphoma cell line exposed to vincristine.[17] On the other hand, bryostatin-1 decreased *mdr-1* phosphorylation in the breast cancer cell line MCF-7 but had no effect on *mdr-1* function or multidrug resistance.[18] Whilst these results suggest a possible further downstream mechanism for the action of bryostatin, data regarding PKC activity and multidrug resistance remain complex.[4]

Modulation of immune system

Bryostatin-1 has been shown to modulate the activity of several cell types. It activates neutrophils[5] and monocytes[19] at subnanomolar concentrations, resulting in the production and secretion of interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor (TNF)-α.

Of note, bryostatin-activated neutrophils have been shown to inhibit the growth of the erythroleukemic cell line K562 in a dose-dependent manner *in vitro*.[20] The addition of TNF-α to bryostatin-1 results in an increased inhibition of the growth of leukemia cell lines *in vitro* suggesting the activation of normal polymorphonuclear cells may be significant *in vivo*.[21]

The activation of T lymphocytes by bryostatin-1 may be even more significant for subsequent clinical developments. Bryostatin-1 in combination with a calcium ionophore, e.g. ionomycin, activates PKC and increases intracellular calcium which together mimic the intracellular signals triggered by major histocompatibility complex/ antigen binding to the T cell receptor. This results in prolonged T cell activation with the release of IL-2 and interferon (IFN)-γ and the exocytosis of cytolytic granules.[22] It has also been shown that bryostatin-1 induces a 4-fold increase in IL-2 receptor-expressing T cells *in vivo* in humans[23] and enhances lymphokine-activated killer cell activity on incubation of peripheral blood mononuclear cells with IL-2.[24]

The ability to activate consistently T cells which can then be expanded *in vitro* by culturing with low dose IL-2 led to the development of bryostatin/ ionomycin (B/I) as a tool for adoptive immunotherapy. It has been shown in many animal models that sensitized T cells extracted from lymph nodes draining malignant tumors and then expanded *in vitro* after activation with B/I and IL-2 cause tumor-specific regression when they are reinfused which is not seen with T cells incubated with IL-2 alone.[25–28] This activity is dependent on the presence of $CD8^+$ T cells[28] and it has been demonstrated that functional memory cells can persist long-term *in vivo* protecting from rechallenge with the same tumor cell line.[29] B/I is now under evaluation as a method of pharmacologically activating T lymphocytes in phase I studies.

Bryostatin-1 also stimulates normal hematopoiesis probably by promoting the release of cytokines such as granulocyte macrophage colony stimulating factor (GM-CSF) and IL-3 from both T lymphocytes and bone marrow stromal cells.[30,31] This effect occurs at the level of committed hematopoietic progenitors as bryostatin-1 has been shown to inhibit proliferation of multipotent $CD34^+$ bone marrow cells.[32] Whilst bryostatin-1 stimulates normal hematopoiesis, it inhibits clonogenic leukemic cells at the same concentrations and limits the self-renewal capacity of leukemic myeloblasts *in vitro*.[33] Importantly, it induces macrophage-like differentiation in a chronic myeloid leukemia cell line[34] and has been shown to differentiate B cell chronic lymphocytic leukemia cells to a non-proliferative hairy cell stage.[35] These opposing effects on normal and abnormal hematopoiesis suggest that bryostatin-1 may have a valuable role to play in the management of leukemias and myelodysplastic syndromes in the future.

Radioprotection

Bryostatin-1 has been shown to potentiate the radioprotective effects *in vitro* of GM-CSF towards normal hematopoietic precursors as well as enhance T cell survival during radiotherapy.[36] It also has *in vivo* radioprotective effects in lethally irradiated mice.[37] Notably, however, it has been shown to increase the sensitivity of human myeloid leukemia cells to low radiation doses.[38]

Conclusions

Whilst modulation of PKC appears to be the most likely mechanism of action of the bryostatins, results from *in vitro* studies are often complex to interpret and at worst directly contradictory. It is also notable that no consistent changes have been demonstrated in the membrane translocation of any one isoenzyme. The pattern of PKC isoenzyme expression may explain some of this complexity as it varies significantly between cell lines and even within clones of the same cell line.[39,40] Therefore the actions of non-specific PKC modulators such as the bryostatins may vary dependent on this pattern. How PKC modulation is antineoplastic is less clear, although there are several possible mechanisms as discussed above. The possibility that other important and, as yet, unelucidated pathways mediate the action of bryostatins should be borne in mind given the activity of a bryostatin analog with markedly decreased PKC affinity.[9]

Preclinical activity and pharmacokinetics

Bryostatin-1 exhibits antitumor activity against a wide variety of human and murine cell lines *in vitro* and in murine models as a single agent (for a summary, see Pettit *et al.*[7]). Whilst it has been suggested that *in vivo* activity may be attributable to bryostatin's immunomodulatory function, there is good correlation between *in vitro* and *in vivo* activity suggesting that this antiproliferative activity involves a direct antitumor mechanism.

More recent research has focused on combination therapy with cytotoxic agents. Pretreatment with bryostatin increases cytotoxicity of cytosine arabinoside (ara-C) in HL-60 leukemia cells[41] and cisplatin in human cervical carcinoma cells.[42] The sequential addition of bryostatin-1 to the purine analogs 2-chlorodeoxyadenosine (2-CdA) or fludarabine results in greater cytotoxicity than either agent used alone or administered concurrently in drug-resistant chronic lymphocytic leukemia cells in xenograft models.[43,44] The addition of bryostatin to vincristine has also been demonstrated to cure mice bearing xenografts of neoplastic B cells derived from human Waldenstroms macroglobulinemia.[45]

In lymphoma xenograft models, the addition of bryostatin-1 potentiated the action of conventional CHOP chemotherapy[46] and was also synergistic with auristatin PE,[47] a novel tubulin polymerization inhibitor.

Bryostatin-1 has also been shown to enhance the cytotoxicity of paclitaxel *in vitro* and in murine models. However, unlike most other cytotoxic agents, paclitaxel needs to be administered before bryostatin. This may be due to the inhibition of $p34^{cdc2}$ kinase by bryostatin-1 as the function of this enzyme appears crucial for the efficacy of paclitaxel.[48] These data suggest that there is significant augmentation of the anti-tumor effects of cytotoxic chemotherapy by bryostatin-1 but that this may be dependent on the precise scheduling of the drugs.

Using i.v. administered [C26-^{3}H]bryostatin-1 in mice, the pharmacokinetics of the drug have been studied. The plasma disappearance curve fits a two-compartment model with urinary excretion being the main elimination pathway initially, followed by fecal excretion. Bryostatin-1 is widely distributed but concentrated in lungs, liver, gastrointestinal tract and fatty tissue.[49] Unfortunately, pharmacokinetic studies have not yet been performed satisfactorily in humans.

Following treatment with bryostatin-1, rats exhibited lethargy, unsteadiness and hematuria in toxicology tests. Significant decreases in platelet and lymphocyte counts were also noted.

Other bryostatins

Much less research has been performed with other bryostatins. In one direct comparison, Bryostatins-1, -5 and -8 were shown to have similar abilities to inhibit the growth of the K1735-M2 murine melanoma cell line *in vivo*, but bryostatins 5 and 8 caused significantly less weight loss.[50] PKC isoenzyme binding patterns were similar for bryostatin-1 and -10 analogs in one *in vitro* study[51]. Bryostatins-4, -7 and -10 have all been shown to have biological activity *in vitro* (see Petit *et al.*[7] for a summary). None of these agents have yet entered clinical trials.

Clinical trials

Since the publication of the first phase I trial in humans in 1993, 55 clinical trials using bryostatin-1 in a wide variety of malignancies have been registered with the National Cancer Institute. Of these, 15 were still recruiting in January 2002.

Phase I trials

The first single-agent phase I trial[52] involved a schedule in which bryostatin-1 was infused i.v. over 1 h every 2 weeks. It identified a maximum tolerated dose (MTD) of 35 μg/m^2 with further dose escalation being limited by myalgia. Hematological toxicity was only documented at 65 μg/m^2. No anti-tumor activity was seen in 19 patients. Philip *et al.*[53] reported an MTD of 25 μg/m^2 when bryostatin-1 was infused over 1 h weekly for 3 weeks out of every 4 weeks. Myalgia was again dose limiting and they reported two responses in patients with metastatic melanoma. In both studies, a formulation of 60% ethanol/40% normal saline was used as bryostatin-1 is insoluble in aqueous solution. This formulation was associated with a high incidence of thrombophlebitis at all dose levels. In an attempt to reduce this, Philip *et al.* examined a PET formulation (60% polyethylene glycol/30% ethanol/10% Tween 80), which reduced the incidence of thrombophlebitis, but was complicated by an acute adverse reaction characterized by dyspnea, hypotension, flushing and bradycardia when infused over 1 h.

A further phase I trial investigated the bryostatin PET formulation infused over 24 h with normal saline weekly for 8 weeks.[24] This successfully overcame the hypersensitivity reaction and again a MTD of 25 μg/m^2/week was identified. All patients still experienced at least grade I phlebitis when bryostatin was administered peripherally. Two partial responses and two minor responses were seen in patients with ovarian carcinoma and low-grade non-Hodgkin's lymphoma (NHL).

Due to preclinical evidence suggesting that bryostatin therapy is more effective when administered on consecutive days,[54] Vartersarian *et al.*[55] assessed a 72-h continuous infusion scheduled every 2 weeks. They defined a MTD of 120 μg/m^2 per dose in patients with relapsed CLL or low-grade NHL. Dose

limiting toxicity was again myalgia. Stable disease was achieved in 11 of 29 patients treated. The reason for the 2.4-fold higher dose intensity than that defined in the three other phase I trials is not clear. This may be related to the drug scheduling, but it has been shown that significant adsorption of bryostatin-1 occurs to many plastic surfaces.[56] The use of polypropylene infusion devices has been standard in most studies and although adsorption to this is minimal at 24 h, potentially clinically significant levels of adsorption were noted after 7 days incubation.[57] This may indicate that the dose to which the patients were actually exposed to in this study was less than the calculated administered dose.

Bryostatin-1 has also been evaluated in the pediatric setting.[58] A 1-h infusion weekly was administered to 22 children with a variety of pediatric malignancies. A MTD of 44 $\mu g/m^2$ was defined with the dose limiting toxicities of myalgia and photophobia. The explanation for the higher tolerated dose in children was unclear but toxicity was less in the youngest patients treated, suggesting that it may be an age-related phenomenon.

Phase II trials

A combination of preclinical data and evidence of activity from phase I studies led to the design of a series of phase II trials evaluating bryostatin as a single agent. Eleven have been reported and these are summarized in Table 2. All three infusion regimens have been tested at or above the MTD defined by the phase I studies. Unfortunately, the reported single-agent activity has been almost uniformly disappointing with only two objective responses in 117 melanoma patients[59–61,67] treated, a 7% response rate in renal cell carcinoma[62,65] and no activity in metastatic colorectal carcinoma,[63] sarcoma,[66] advanced head and neck cancer,[66] CLL[64] or relapsed myeloma.[68] Although one study of 18 low-grade NHL patients reported a response rate of 17% with a 72-h infusional regimen,[64] the use of a 24-h infusion failed to demonstrate any activity.[57]

Disease stabilization for several months has however been noted in a small number of patients in all studies; most notably in the two renal cell carcinoma trials[62,65] where 12 of the 60 patients' disease was stable for at least 6 months.

Toxicity

The most significant side effect attributed to bryostatin-1 is myalgia (see Table 3 for CTC grading), which has been reported in 10–87.5%. of patients treated in phase II trials. Myalgia tends to be cumulative with repeated treatments and develops 1–2 days after infusion of bryostatin-1. Initially the calves, thighs and extraocular muscles are affected, but myalgia becomes more generalized with continuing therapy. Notably, the myalgia is often eased by exercise, but returns on resting. For grade I/II myalgia treatment with simple analgesia is often adequate. However, this may not suffice for more severe symptoms and other drugs have been tried such as prednisolone, gabapentin and strong opiates with only partial efficacy. Bryostatin dose reduction or delay can also be used to manage myalgia, but myalgia is often a contributory reason for discontinuing therapy as it can impact significantly on the patients quality of life. The exact etiology of bryostatin-induced myalgia remains uncertain. Creatinine kinase levels, inflammatory markers, urinary myoglobin excretion and electromyograms are normal in patients with severe myalgia.[24,52,53] One study using magnetic resonance spectroscopy demonstrated long-lasting impairment of oxidative metabolism in muscle mitochondria felt to be due to reduced vascular flow and therefore impaired muscle reoxygenation following exercise.[69] A prospective trial of the vasodilator nifedipine, although it abolished the impaired reoxygenation, failed to alter mitochondrial activity or reduce myalgia indicating that the toxicity cannot be explained by vasoconstriction.[70]

Other reported side effects such as frontal headache and odynophagia are probably secondary to myalgia affecting frontalis and muscles of the hypopharynx.

Fatigue and lethargy are also commonly reported, although these are generally mild and do not interfere with treatment. Other less common but consistently reported toxicities are low-grade pyrexia, nausea and anorexia. Grade III dyspnea has been reported in two studies.[62,65] Hematological toxicities are unusual, although grade I/II thrombocytopenia and leukopenia were seen in several studies. Biochemical toxicities appear to be limited to mild abnormalities of liver function although one study reported three cases of grade II/III hyponatremia of uncertain etiology.[66]

Human *in vivo* bioactivity and pharmacokinetics

Little convincing evidence of PKC modulation in humans has been published. Jayson *et al.*[24] failed to

Table 2. Summary of the published data on the activity and toxicity of bryostatin-1 as a single agent

Reference	Bryostatin schedule	Tumor type	Patient nos	Partial response	Complete response	Response rate (%)	Rate of myalgia
59	25 μg/m^2 over 1 h weekly 3 weeks out of 4	metastatic malignant melanoma	16	0	0	0	II 50%, III 37.5%
60	25 μg/m^2 over 1 h weekly 3 weeks out of 4	metastatic malignant melanoma	18	0	0	0	II 11%, III 6%
61	25 μg/m^2 over 24 h weekly (A) OR 120 μg/m^2 over 72 h every 2 weeks (B)	metastatic malignant melanoma	17 (A)/17(B)	1/0	0/0	6/0	I/II 33% (A)/I/II 37%, III 24%, IV 6% (B)
62	25 μg/m^2 over 30 min weekly 3 weeks out of 4	metastatic renal cell carcinoma	30	1	1	7	I/II 13%, III 10%
63	25–40 μg/m^2 over 24 h weekly 3 weeks out of 4	metastatic colorectal carcinoma	28	0	0	0	I/II 42%, III/IV 8%
64	120 μg/m^2 over 72 h every 2 weeks	CLL or relapsed NHL	7(CLL)/ 18(NHL)	0/2	0/1	0/17	III 24%
65	35–40 μg/m^2 over 1 h weekly 3 weeks out of 4	metastatic renal cell carcinoma	30	2	0	7	I/II 67%, III 20%
57	25 μg/m^2 over 24 h weekly	NHL	17	0	0	0	I/II 4%, III 6%
66	120 μg/m^2 over 72 h every 2 weeks	sarcoma/head and neck	12/12	0/0	0/0	0/0	III 17%, IV 8%
67	25 μg/m^2 over 24 h weekly (A) OR 120 μg/m2 over 72 h every 2 weeks (B)	metastatic malignant melanoma	12 (A)/37 (B)	0/1	0/0	0/3	II 57%, III 28% (B only), IV 1% (B only)
68	120 μg/m^2 over 72 h every 2 weeks	relapsed myeloma	9	0	0	0	II 56%, III 11%

Table 3. National Cancer Institute Common Toxicity Criteria for myalgia

Grade 0	None
Grade 1	Mild brief pain that does not require analgesic drugs. Patient is fully ambulatory
Grade 2	Moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living
Grade 3	Severe pain: pain or analgesics severely interfering with activities of daily living
Grade 4	Disabling

demonstrate consistent changes in either peripheral blood mononuclear cell (PBMNC) total PKC or activated PKC in four patients assayed. Varterasian *et al.*[55] showed early up-regulation followed by subsequent down-regulation of total cellular PKC activity that began between 2 and 24 h, and persisted for the remainder of a 72-h bryostatin infusion in four patients. A further study[71] that evaluated 1-h, 24-h and split (day 1 and 4) infusions of bryostatin demonstrated heterogeneous activity with all three regimens, although a non-significant trend to decreased PKC activity was seen at 72 h.

Bedikian *et al.* conducted an investigation of PKC isoenzyme levels in cutaneous melanoma deposits[67] excised prior to the first dose of bryostatin and during the last 3 h of a 24-h infusion. They showed an overall decline in PKC levels by Western blotting of whole-cell homogenates with a marked decrease in the PKCε isoform although the mechanism for this was not elucidated. Notably, no analysis has been done of PKC membrane localization in any human study.

Pharmacokinetic analysis of bryostatin-1 in humans has also proven difficult as *in vivo* blood concentrations are not detectable by mass spectrometry or high-performance liquid chromatography. An indirect platelet activation assay has been developed,[72] but this was not able to detect bryostatin-1 in a phase Ib study.[71] A competition assay in which bryostatin competes for binding to a rat brain membrane preparation with tritiated phorbol-12,13-dibutyrate has been used in two studies. In pediatric patients it was unable to detect plasma bryostatin at the end of infusion in 11 of 14 patients.[58] In a second study that utilized a 25 μg/m^2 infusion over 1 h, bryostatin-1 was detected in 10 of 14 patients analyzed.[60] Despite bryostatin-1 being undetectable in five of these patients 1 h after discontinuation of the infusion and in eight at 3 h, tentative pharmacokinetic constants have been calculated suggesting a volume of distribution of 2.9 l and an elimination clearance of 32.9 ml/min/m^2. Clearly, lack of adequate pharmacokinetic data means that optimization of human treatment schedules cannot be achieved.

Combination treatments

On account of *in vitro* and animal data presented above, and the lack of overlapping toxicities, bryostatin-1 is a promising agent for investigation in combination with cytotoxic agents. Phase I/II trials of several combination regimens are in progress (see Table 4) and initial results have been published in abstract form.

Bryostatin-1 has been combined with cytosine arabinoside in patients with refractory/relapsed acute leukemia safely with variable effects on blast PKC activity.[73] It has also been used sequentially with the purine analogs 2-chlorodeoxyadenosine and fludarabine in refractory CLL[74] and indolent NHL[75] with clinical responses.

The combination of bryostatin-1 and cisplatin has been assessed in several phase I studies. Rosenthal *et al.*[76] reported one PR in 13 evaluable metastatic melanoma patients. Another study investigated 15–55 μg/m^2 bryostatin-1 infused over 72 h followed by 50 mg/m^2 cisplatin every 3 weeks in patients with metastatic carcinoma of stomach or lung.[77] Three responses were noted in 16 patients. A further study has defined a MTD for bryostatin-1 infused over 1 h every 3 weeks of 80 μg/m^2 given before 50 mg/m^2 cisplatin. Myalgia was the DLT and no unexpected toxicities were reported.[78]

Weekly paclitaxel (80 mg/m^2) followed 24 h later by bryostatin-1 (15–45 μg/m^2) has also been assessed. The pharmacokinetics of paclitaxel were not altered by the addition of bryostatin-1 and all dose levels were well-tolerated with myalgia perhaps surprisingly being limited to grades I and II.[79] The weekly triple drug regimen of paclitaxel, bryostatin and cisplatin has also been investigated. At doses of paclitaxel 90 mg/m^2, bryostatin 50 μg/m^2 and cisplatin 20 mg/m^2, neutropenia was the DLT.[80]

Vincistine and bryostatin-1 have also been investigated in patients with hematological malignancies. In a feasibility study,[64] patients with CLL who progressed on bryostatin-1 were given a vincristine bolus dose immediately after their bryostatin infusion. Vincristine at 2 mg could be administered safely with the only additional side effect being the expected

Table 4. Agents under investigation in combination with bryostatin-1 (see text for references).

Hematological malignancies	Solid tumors
Cytosine arabinoside	Cisplatin
2-Chlorodeoxyadenosine	Paclitaxel
Fludarabine	Gemcitabine
Vincristine	IL-2
Cladribine	Tretinoin
GM-CSF	
Tretinoin	

sensory neuropathy. A subsequent phase I study of this combination demonstrated prolonged stable disease in patients with transplant-failed myeloma and NHL.[81]

Phase I/II studies are ongoing in combination with IL-2 in melanoma and renal cell carcinoma, with gemcitabine in advanced solid malignancies and with GM-CSF in refractory myeloid malignancies (http://www.cancernet.nci.nih.gov/cgi-bin/srchcgi.exe accessed on 7 January 2002).

Adoptive immunotherapy

The results of a phase I trial of B/I activated lymph node lymphocytes has been reported.[82] This assessed the feasibility and toxicity associated with using tumor- or vaccine-draining lymph node cells activated and expanded *ex vivo* and then infused i.v. followed by IL-2 infusion in six patients with advanced solid malignancies. Two patients with melanoma had received a licensed tumor vaccine (Melacine). Cell culture *ex vivo* was achievable over 13–29 days in culture with an average 118-fold expansion. This was less than expected from previous studies,[83] probably due to technical difficulties experienced in scaling up production consistent with a clinical trial. No responses were documented, but no toxicities over and above those attributable to the IL-2 infusion were noted except for sterile abscess formation at the site of vaccine administration in one patient. Whilst the feasibility and safety of this approach is now documented it may be that its future clinical utility will lie as an adjuvant approach to surgery in patients at high risk of relapse rather than in patients with suppressed immunity secondary to advanced malignancy.

Conclusions

Unfortunately, the promising preclinical activity reported for the bryostatins has not yet been translated into the clinic. Phase II trials of bryostatin-1 as a single agent have yielded disappointing response rates in a wide variety of solid and hematological malignancies. Further optimization of treatment schedules is hampered by the lack of pharmacokinetic data from human studies. The wide variety of MTDs (25–60 μg/m^2/week) derived from phase I single-agent and combination studies is also of concern as the reasons for this are unclear.

Preliminary reports from phase I studies of combination regimens including bryostatin-1 are promising, with treatments being well tolerated with no unexpected toxicities. Clearly, further investigation in randomized phase III trials against standard regimens is necessary to determine the true efficacy of bryostatin-1 combinations. Pertinent to this, useful information could be obtained more quickly by trials in which bryostatin-1 is added to conventional combination treatment in patients whose disease has progressed on that regimen.

The use of bryostatin-1 as a T cell activator also holds promise but clinical experience of adoptive immunotherapy is still preliminary.

If bryostatin-1 is proven in future to have a significant role in the pharmacological management of cancer, its availability to patients may still be hindered by the lack of an effective chemical synthesis pathway. In summary, the remarkable journey from marine bryozoan to pharmacy shelf still has several obstacles to overcome en route.

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