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Brostallicin Hydrochloride

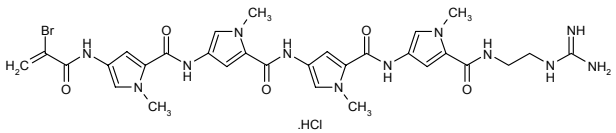
Minor Groove-Binding Agent
Oncolytic

Rec INNM

PNU-166196A

2-[4-[4-[4-[4-(2-Bromo-2-propenamido)-1-methylpyrrol-2-ylcarboxamido]-1-methylpyrrol-2-ylcarboxamido]-1-methylpyrrol-2-ylcarboxamido]-1-methylpyrrol-2-ylcarboxamido]ethylguanidine hydrochloride

InChI=1/C30H35BrN12O5.ClH/c1-16(31)25(44)36-17-9-22(41(3)12-17)27(46)38-19-11-24(43(5)14-19)29(48)39-20-10-23(42(4)15-20)28(47)37-18-8-21(40(2)13-18)26(45)34-6-7-35-30(32)33;/h8-15H,1,6-7H2,2-5H3,(H,34,45)(H,36,44)(H,37,47)(H,38,46)(H,39,48)(H4,32,33,35);1H



$C_{30}H_{36}BrClN_{12}O_5$
Mol wt: 760.0414

CAS: 203258-38-2

CAS: 203258-60-0 (free base)

EN: 262512

Abstract

Soft tissue sarcomas (STS) are a group of rare tumors, most of which are relatively resistant to standard chemotherapeutic agents. To date, favorable efficacy data exist for only a small number of agents in this disease. Minor groove binders (MGBs) are a new class of anticancer agents that theoretically provide a unique mechanism of antitumor activity. Brostallicin hydrochloride, a synthetic, second-generation MGB, has been shown to possess activity in STS. This article therefore aims to review the current evidence on the use of brostallicin in STS. Preclinical data have shown that brostallicin possesses certain features likely to be beneficial in the treatment of STS, such as activation in the presence of high levels of glutathione and/or glutathione *S*-transferase, as well as being unaffected by defects in DNA mismatch repair. Phase I trials established the maximum tolerated dose as 10 mg/m^2 and showed a partial response in a patient with a gastrointestinal stromal tumor. Phase II trials have shown encouraging progression-free survival figures in those with STS and further trials are ongoing to compare brostallicin with doxorubicin.

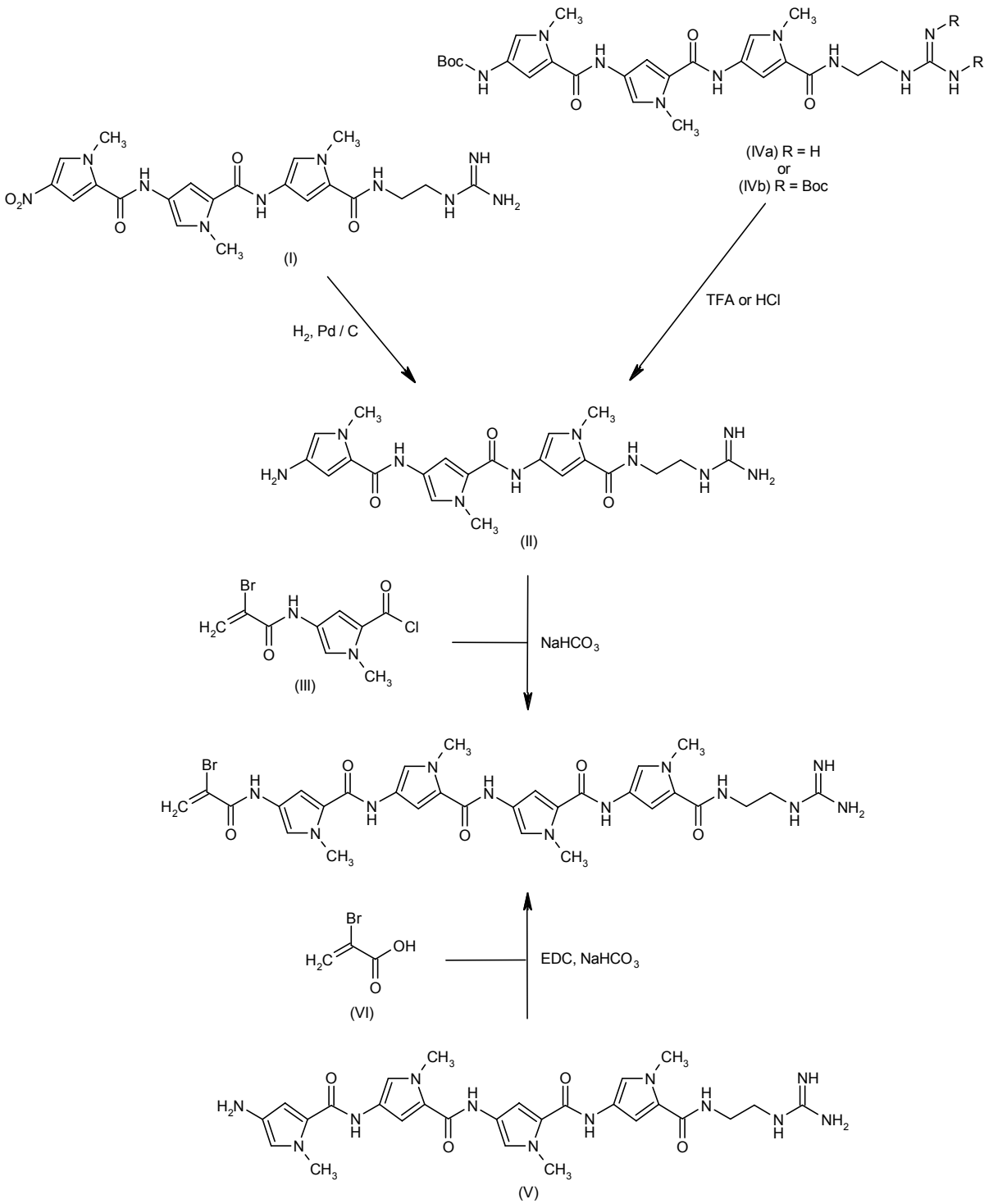
Synthesis[+]

Brostallicin can be prepared by reduction of the synthetic nitro-tripyrrole (I) with H_2 and Pd/C, followed by acylation of the resulting amine (II) with 4-(2-bromoacryloylamino)-1-methylpyrrole-2-carbonyl chloride (III) under Schotten-Baumann conditions (1, 2). Alternatively, the intermediate tripyrrolyl-amine (II) can be obtained by acidic cleavage of the Boc-protected precursors (IVa) and (IVb), which are obtained by functional group manipulation of the natural product distamycin A (3-5). In a further strategy, brostallicin is prepared by acylation of the tetrapyrrolyl-amine (V) with 2-bromoacrylic acid (VI) in the presence of EDC (6). Scheme 1.

The tripyrrolyl precursor (I) can be synthesized as follows. Protection of ethylenediamine (VII) with Boc_2O in dioxane followed by treatment of the resulting mono-protected diamine (VIII) with either *S*-methylisothiourea (IXa) (1, 7) or *O*-methylisourea (IXb) (2, 6) gives guanidine (X), which by subsequent acidic cleavage of the Boc groups yields (2-aminoethyl)guanidine (XI) (1, 2, 6, 7). This compound is then acylated with 1-methyl-4-nitropyrrole-2-carbonyl chloride (XII) using $NaHCO_3$ in aqueous dioxane to afford the nitropyrrole-carboxamide (XIII), which is reduced to the aminopyrrole analogue (XIV) by catalytic hydrogenation over Pd/C. Aminopyrrole (XIV) is then subjected to a new coupling–hydrogenation cycle with acid chloride (XII) to provide the dipyrrole derivative (XV). A further coupling of (XV) with acid chloride (XII) furnishes the nitro-tripyrrolyl precursor (I) (2, 7, 8). An analogous sequence has been applied to the synthesis of isotopically labeled brostallicin, using either deuterim-labeled ethyl-

Eitan Amir, Richard W. Griffiths, Michael G. Leahy*. Department of Medical Oncology, Christie Hospital, NHS Foundation Trust, Manchester, UK. *Correspondence: michael.leahy@christie.nhs.uk. [+]Synthesis prepared by N. Serradell, E. Rosa, J. Bolós, R. Castañer. Prous Science, Provenza 388, 08025 Barcelona, Spain.

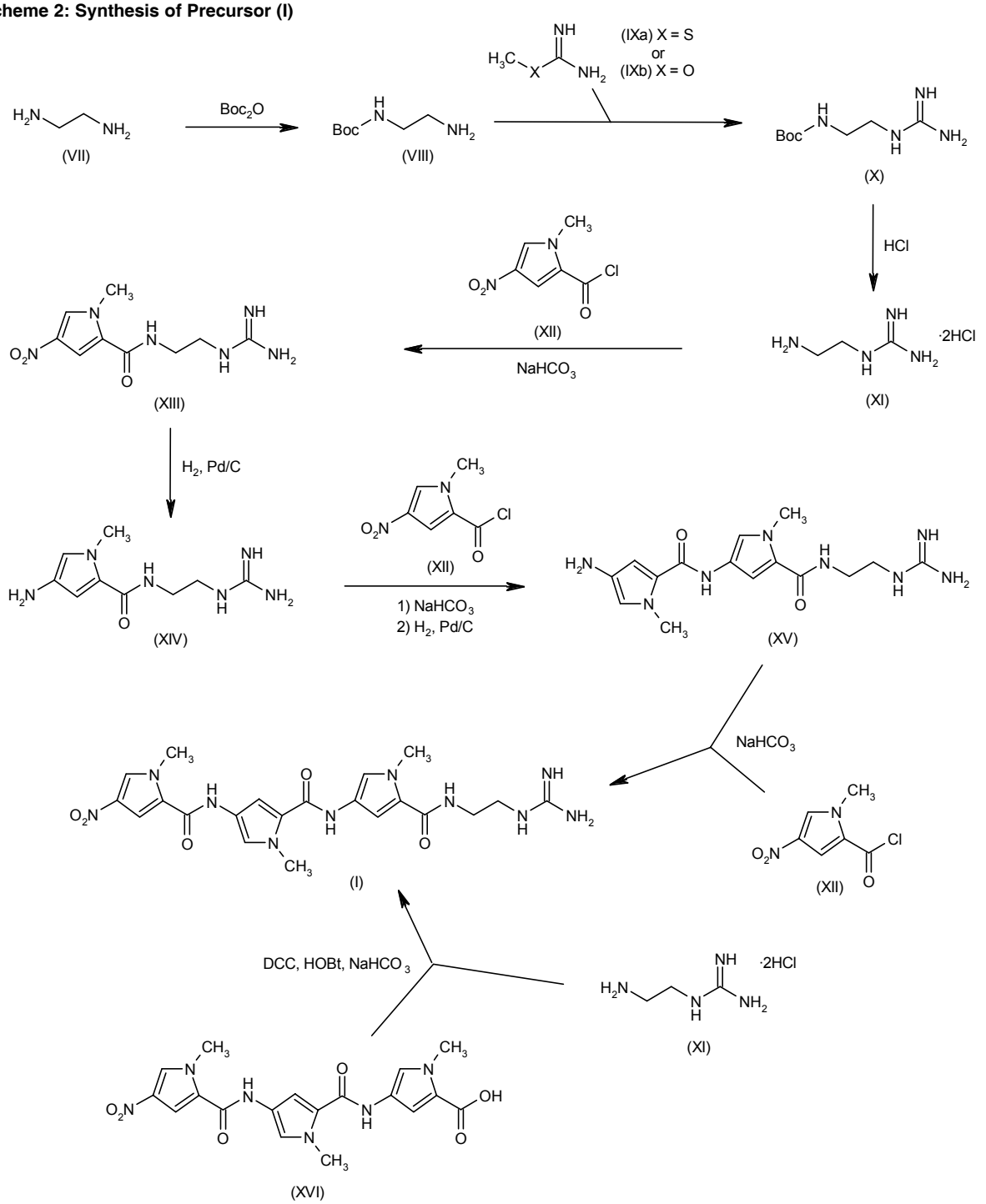
Scheme 1: Synthesis of Brostallicin



enediamine [^{2}H]-(VII) or 1-methyl-4-nitropyrrole-2-carbonyl chloride labeled with ^{14}C at the C-2 position [^{14}C]-(XII) (2). Alternatively, intermediate (I) can be prepared by coupling of aminoguanidine (XI) with the known tripyrrolyl carboxylic acid (XVI) in the presence of DCC and HOBt (1). Scheme 2.

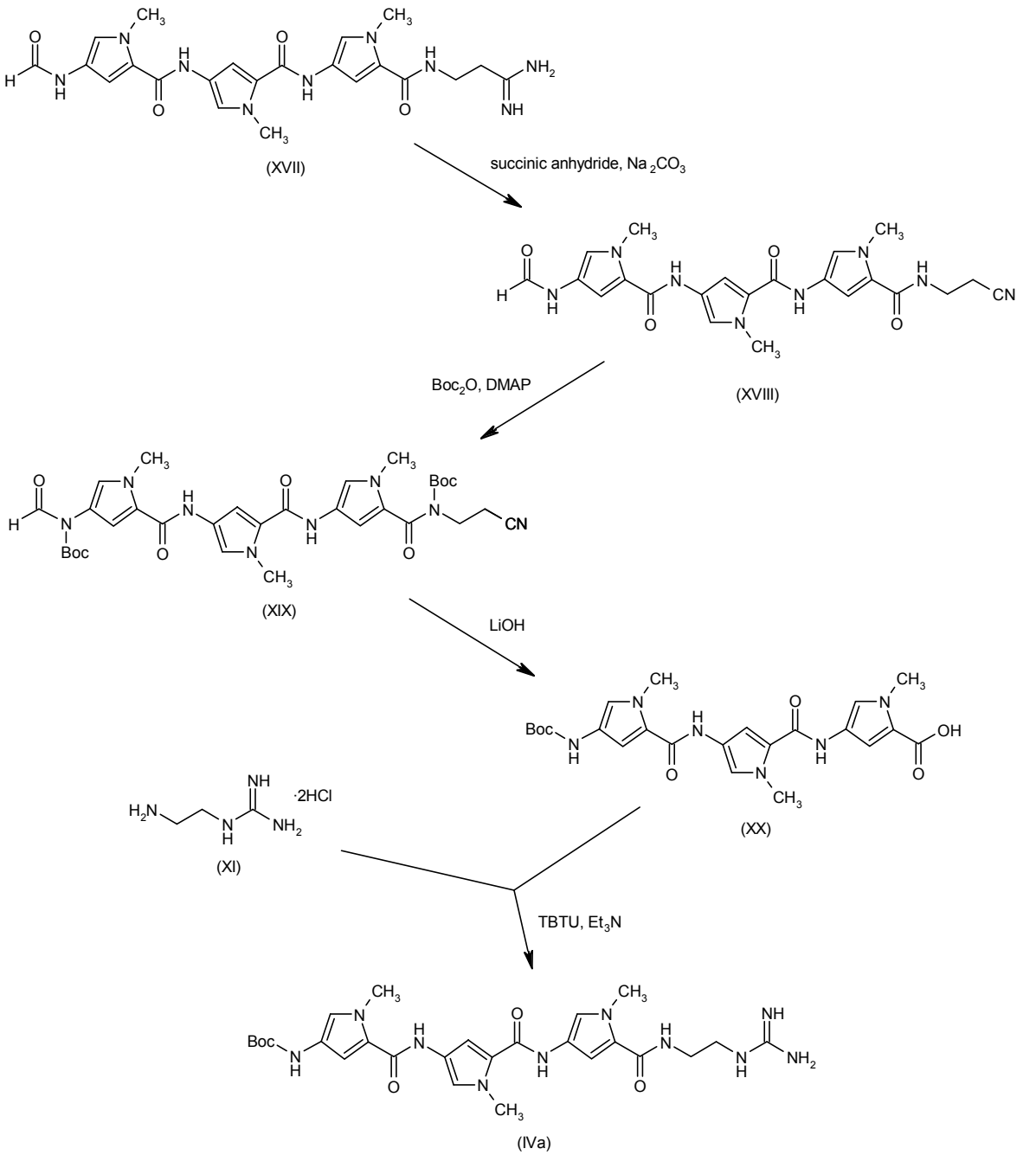
The Boc-protected precursor (IVa) can be prepared starting from the natural antibiotic distamycin A (XVII).

Scheme 2: Synthesis of Precursor (I)



Elimination of ammonia from distamycin A (XVII) by heating with succinic anhydride and Na_2CO_3 in DMF leads to nitrile (XVIII), which by stepwise protection of its formamide NH group with Boc_2O and DMAP in CH_2Cl_2 and then the amide NH with further addition of Boc_2O in DMF affords the di-Boc compound (XIX). Subsequent hydrolysis of the formyl and cyanoethyl amide groups of (XIX) using LiOH in THF/H_2O provides the tripyrrolyl carboxylic acid (XX), which is finally coupled with (2-aminoethyl)guanidine (XI) by means of TBTU in DMF (3, 5). Scheme 3.

Scheme 3: Synthesis of Precursor (IVa)

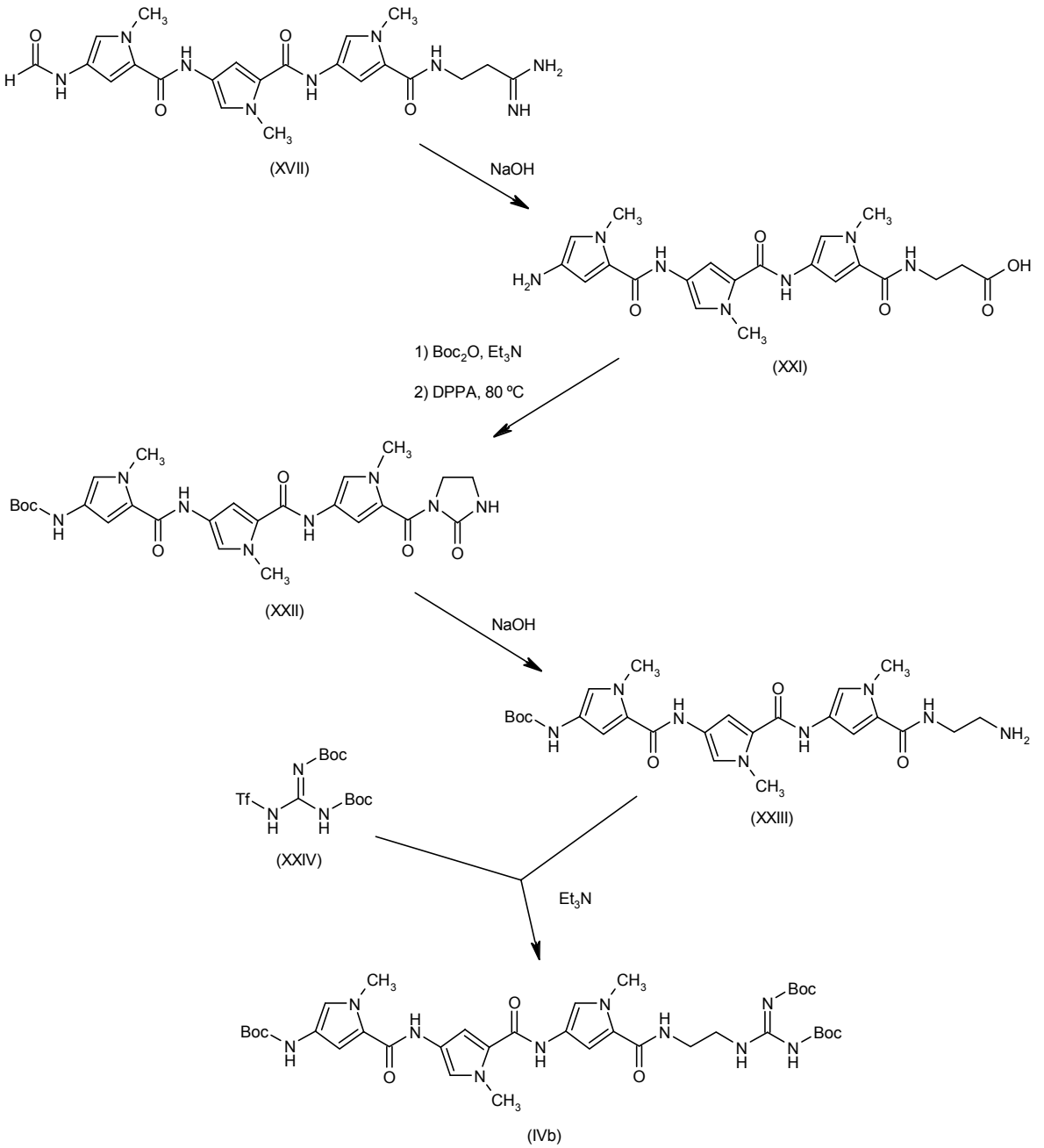


(XVII) (XVIII) (XIX) (XX) (XI) (IVa)

The tri-Boc derivative (IVb) can also be prepared from distamycin A (XVII) by the following method. Hydrolysis of (XVII) with NaOH in refluxing methanol gives the amino acid (XXI), which by subsequent protection with Boc_2O and Et_3N in DMF followed by Curtius rearrangement of the carboxy group by treatment with diphenylphosphoryl azide affords imidazolidinone (XXII). Hydrolysis of compound (XXII) with NaOH in DMF provides the aminoethyl amide (XXIII), which is converted to the Boc-protected guanidine (IVb) by reaction with *N,N*'-di-Boc-*N*''-trifluoromethylsulfonylguanidine (XXIV) in the presence of Et_3N in DMF (4). Scheme 4.

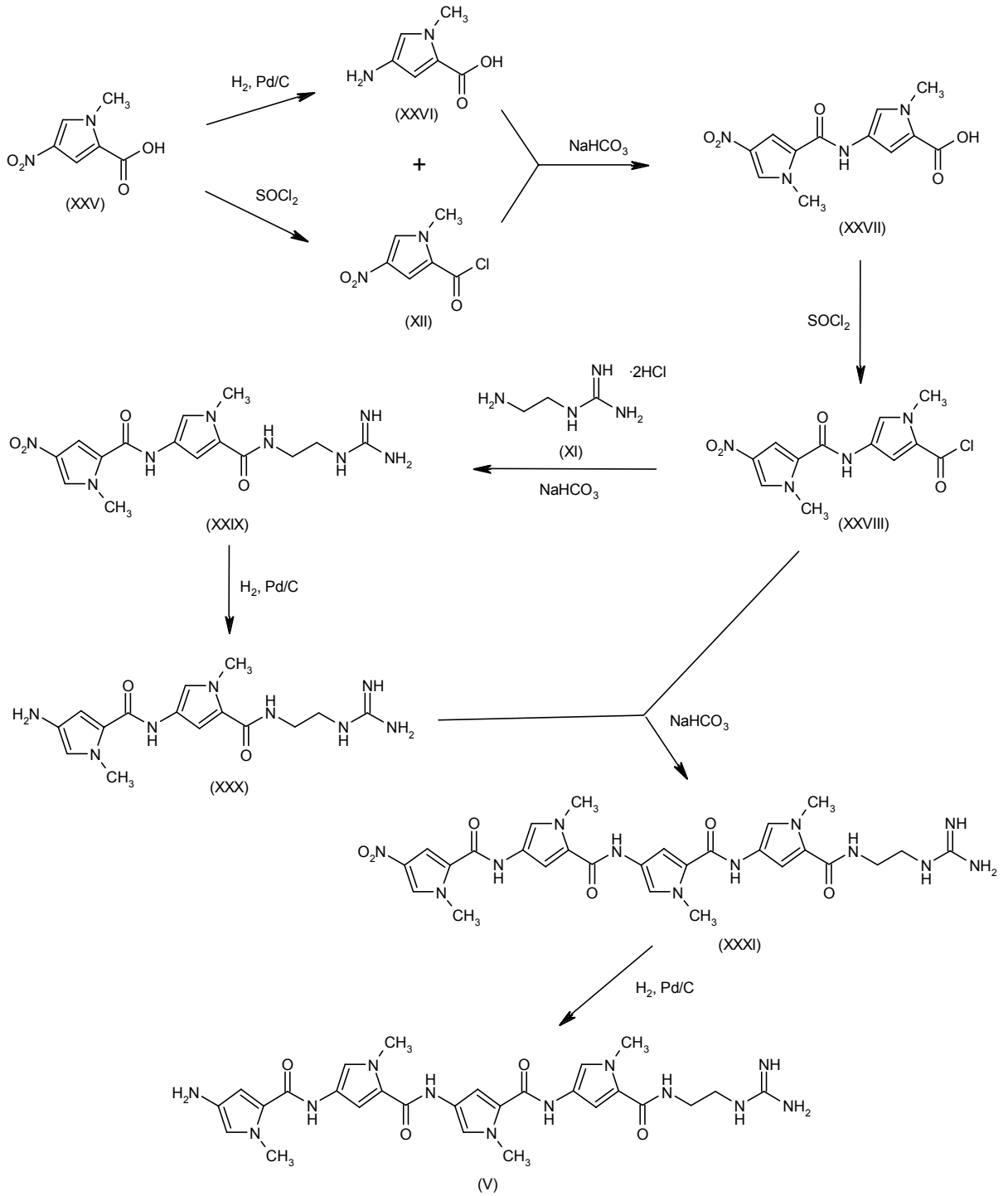
The tetrapyrrolyl-amine precursor (V) is obtained by the following procedure. 1-Methyl-4-nitropyrrole-2-car-

Scheme 4: Synthesis of Precursor (IVb)



boxylic acid (XXV) is reduced to the aminopyrrole (XXVI) by catalytic hydrogenation in the presence of Pd/C. Alternatively, chlorination of acid (XXV) with $SOCl_2$ provides the pyrrolecarbonyl chloride (XII). Then, condensation between aminopyrrole (XXVI) and acid chloride (XII) using $NaHCO_3$ in aqueous dioxane gives the dipyrrolyl amide (XXVII), which is converted to the acid chloride (XXVIII) by treatment with $SOCl_2$ and a catalytic amount of DMF. Condensation of acid chloride (XXVIII) with (2-aminoethyl)guanidine (XI) yields compound (XXIX), which by reduction of the nitro group with H_2 and Pd/C leads to the aminopyrrole (XXX). This compound is then coupled with a second moiety of the dipyrrolyl-acid chloride (XXVIII) to provide the tetrapyrrolyl compound (XXXI), which is finally reduced at the nitro group by catalytic hydrogenation over Pd/C (6). Scheme 5.

Scheme 5: Synthesis of Precursor (V)



Background

Sarcomas are rare tumors with an estimated annual incidence of approximately 2-3 per 100,000 persons, and therefore account for < 1% of all solid malignancies (9, 10). These tumors usually, but not exclusively, arise from fat, muscle, nerve, nerve sheath, blood vessels, bone and other connective tissues. Although often previously

lumped together, they are a heterogeneous group of malignancies of mesenchymal cell origin with diverse clinical and pathological features. Within this group, a number of distinct clinical entities are recognized which have specific treatment protocols, including systemic therapy: osteosarcomas, the Ewing's family of tumors (including peripheral primitive neuroectodermal tumors [PNETs]), rhabdomyosarcoma and gastrointestinal stromal tumors (GISTs) (11, 12). For the remainder of histotypes (which together form the majority of cases), surgery remains the mainstay of treatment, with systemic therapy reserved for patients with advanced disease.

Soft tissue sarcomas (STSs) can occur anywhere in the body, but most originate in an extremity (59%), the trunk (19%), the retroperitoneum (15%), or the head and neck (9%) (13). There are more than 50 histological subtypes, with the most common being fibrosarcoma (18%), leiomyosarcoma (16%), liposarcoma (6%) and synovial sarcoma (1%) (10). In many tumors, it may be difficult or impossible to identify specific features of morphological differentiation, and these have been given different names over the last 50 years, causing some confusion (14).

With the exception of those tumor types mentioned above, current treatment protocols for metastatic STS are doxorubicin-based chemotherapy regimens with or without ifosfamide as front-line therapy. These drugs have been shown to have single-agent activity, with data showing response rates in the range of 10-30% (15-18). Data on dose-intense chemotherapy supported by hematopoietic growth factors or peripheral blood stem cell transplantation (PBSCT) have shown trends towards increased response rates of up to 50-60%. Unfortunately, there has been no substantial improvement in overall survival (19-23). Furthermore, dose escalation of anthracyclines is limited by nonhematological side effects, especially cardiotoxicity.

Second-line chemotherapeutic options in advanced STS after progression during or after doxorubicin are limited. Established drugs in this setting are ifosfamide (if not previously coadministered with doxorubicin as first-line therapy) and dacarbazine (24, 25). Progress in the development of other agents for use in STS has sadly been slow. Many different cytotoxic agents have been tested in phase II trials accepting all-comers with different STS subtypes, and most of these have been classed as negative trials. More recently, a number of new distinct tumor types have emerged from the pack which may respond differently to systemic agents and deserve individual treatment protocols. These include, most dramatically, the treatment of GISTs with imatinib (26), and also the treatment of Kaposi's sarcoma with liposomal doxorubicin, cutaneous angiosarcoma with paclitaxel (27) and perhaps some leiomyosarcomas with gemcitabine and docetaxel (28).

More recently, a new group of cytotoxic agents with a novel mechanism of action has attracted attention in the treatment of sarcoma. Minor groove binders (MGBs) represent a class of anticancer agents whose DNA sequence specificity leads to a highly selective mechanism of action (29-31). Unlike most cytotoxic agents, which bind the DNA major groove, MGBs fit into the space formed between the two phosphate–sugar backbones in the double helix. MGBs tend to have high selectivity for sequences rich in thymine–adenine and this theoretically provides their unique mechanism of action (32, 33). First-generation MGBs, such as agents derived from either CC-1065 (34-36) or the nitrogen mustard derivative of distamycin A tallimustine (37, 38), were found to be very active against experimental tumors unresponsive to other antineoplastic agents. Unfortunately, they did not proceed in clinical studies because of their severe dose-limiting myelotoxicity (39, 40).

The first MGB with a favorable efficacy and toxicity profile was ecteinascidin-743 (ET-743, trabectedin, Yondelis®; PharmaMar), a natural marine product derived from the tunicate *Ecteinascidia turbinata*. Phase II trials in Europe and in the U.S. have revealed that, although it was associated with low objective response rates of only 5-10% (41, 42), it had particularly high antitumor activity in liposarcoma, especially the myxoid liposarcoma subtype (43), and treatment led to disease stabilization (progression arrest) in a high proportion of patients. Retrospective analysis of the European Organisation for the Research and Treatment of Cancer (EORTC) database of clinical trials in STS has identified that progression-free survival (PFS) may be a good discriminant of active compounds and more sensitive than the traditional clinical trial endpoint of overall objective response rate (44). When the PFS of patients with STS treated with trabectedin was analyzed, it was found to segregate with the PFS seen in trials with doxorubicin and ifosfamide and was significantly different from the PFS seen in trials of agents considered inactive.

More recently, favorable data have been reported for the use of the MGB brostallicin. Brostallicin hydrochloride (PNU-166196A; Pfizer) is a synthetic, second-generation DNA MGB comprising a distamycin A backbone covalently linked to an α-bromoacryloyl group. As with trabectedin, brostallicin was selected for clinical development because of its positive antitumor activity, again based partly on significant prolongation of expected PFS, as well as its favorable toxicity profile (30, 45-47). This article will therefore review the development of brostallicin and its establishment in the treatment of STSs.

Preclinical Pharmacology

Initial laboratory work showed that brostallicin could exert antitumor activity against several murine and human tumor xenografts (45). The mechanism of action of brostallicin appears to be unique among MGB agents in that it has no DNA-alkylating activity *per se*, but is activated in the presence of high levels of glutathione (GSH) and/or glutathione *S*-transferase (GST) (48-50). Some chemotherapeutic drugs and other MGBs rely on their direct DNA-alkylating effect for cytotoxicity, and as such display little activity against tumor cell lines deficient in DNA mismatch repair enzymes (51-53). Notably, the

cytotoxicity of brostallicin is unaffected by defects in mismatch repair or other DNA repair enzymes, such as the products of the ataxia telangiectasia mutated (*ATM*) gene and DNA-dependent protein kinase (DNA-PK) (50). This is of relevance to STS, where DNA mismatch repair enzyme deficiencies have been identified and correlated with inferior survival (54).

In vitro evaluations of brostallicin have also shown potential for efficacy in refractory tumors, particularly those expressing high levels of GSH/GST, which has been associated with primary or acquired resistance to a number of anticancer drugs, including nitrogen mustards, platinum agents and anthracyclines (49, 55-59). Both *in vitro* and *in vivo* studies using isogenic cell systems differing only in the expression of the GST-π isozyme have shown that sensitivity to brostallicin occurred not only in cultured cell lines but also in tumors transplanted into nude mice (40, 49).

In vitro data from these experiments have shown that brostallicin possessed 3-fold higher activity in melphalan-resistant murine leukemia cells than in the parental cell line. These melphalan-resistant cell lines have increased levels of GSH in comparison with the parental cells. Conversely, depletion of GSH using buthionine sulfoximine in a human ovarian carcinoma cell line significantly decreased both the cytotoxic and the proapoptotic effects of brostallicin. It was postulated that GSH, as an intracellular reactive nucleophilic species, could react with the α-bromoacrylamide moiety of brostallicin, leading to the formation of a highly reactive GSH complex representing the real effective agent of brostallicin activity. Further *in vitro* work has confirmed that the α-bromoacryloyl moiety of brostallicin reacts with GSH, allowing it to covalently bind with DNA, inducing cell cycle arrest and activating proapoptotic signaling pathways via as yet uncertain mediators. The reaction between brostallicin and GSH is thought to be catalyzed by GST, with the π and μ isozymes being more effective than the α isozyme (49).

In vivo data derived from human ovarian carcinoma clones implanted into nude mice have also confirmed that the antitumor activity of brostallicin was higher in the GST-π-overexpressing tumors. In this experiment, ovarian cancer clones with different GST-π content were implanted into nude mice and the antitumor activity of brostallicin was evaluated. Results showed that brostallicin exhibited greater activity in the GST-π-overexpressing tumors than in the tumors expressing normal levels of the enzyme, without increased toxicity (49).

In an attempt to extrapolate brostallicin's enhanced activity in the presence of high GSH/GST levels, further work has evaluated the possible augmentation of the efficacy of brostallicin using thiol antioxidants. In this study, human nasopharyngeal squamous carcinoma and human hepatic carcinoma cell lines were treated with *N*-acetylcysteine and silibinin and brostallicin, either alone or in combination. Paradoxically, results showed that thiol antioxidants in fact reduced brostallicin's cytotoxicity. It was proposed that the mechanism underlying this interaction involved the inhibition of apoptosis, as an increase in Bcl-2 protein levels and a decrease in caspase-3 activity were detected with the silibinin–brostallicin combination (60). There are little further data in the literature relating to the combination of brostallicin with other agents and this is an area of research that requires further work.

Clinical Studies

The seminal phase I trial of brostallicin was reported by Ten Tije and colleagues in their study in 27 patients with a variety of solid tumors. This trial concluded that brostallicin was well tolerated, neutropenia being the principal toxicity. The maximum tolerated dose from this study was 10 mg/m^2 on a 21-day cycle. Common Terminology Criteria for Adverse Events v3.0 (CTCAE) (61) grade 4 neutropenia was the only dose-limiting toxicity at 12.5 mg/m^2, whereas grade 4 thrombocytopenia and grade 4 neutropenia were the dose-limiting toxicities at 15 mg/m^2. Of note, 1 partial response was observed in a patient with a GIST (62).

In view of the favorable response in the patient with GIST and due to the high level of GSH and GSH-related enzymes in STSs, confirmatory phase II trials were carried out to investigate the potential role of brostallicin in STS.

To date, there have been two phase II trials designed to evaluate the use of brostallicin in STS. The first of these trials was undertaken by the Soft Tissue and Bone Sarcoma Group (STBSG) of the EORTC. In this study (EORTC 62011) (63), the antitumor activity of brostallicin in patients with locally advanced or metastatic STS who had failed one prior chemotherapy treatment was explored. The patient population was stratified into two groups according to histological diagnosis, with one group comprising GIST tumors only and the other group including patients with any other type of STS (non-GIST). The primary endpoint was overall response rate. Predefined secondary endpoints included time to tumor progression, duration of objective response and the safety profile of brostallicin. Twenty-one patients with GIST and 43 patients with non-GIST were recruited. In the GIST group, there were no patients with a confirmed response and hence recruitment into this arm was discontinued subsequent to the first interim analysis. In the non-GIST group, there were 2 confirmed partial responses among the 40 patients with evaluable disease (5% of cohort). Twenty patients (50%) had stable disease. The 3- and 6-month PFS was 46% and 22%, respectively, in the non-GIST group and 33% and 21%, respectively, in the GIST group. Median survival in the non-GIST group was 231 days (range 159-421 days), while in the GIST group a figure of 298 days (range 188-?) was recorded. In general, the drug was well tolerated. Neutropenia was the most common toxicity, with CTCAE grade 3 or grade 4 toxicities observed in 22% and 48% of patients, respectively. Pyrexia associated with such neutropenic toxicity was seen in 14% of patients, while there was a single confirmed toxic death due to neutropenic septicemia. Fatigue was the most common nonhematological toxicity.

CTCAE grade 3 or grade 4 fatigue was recorded in 25% of patients. Finally, 3 patients had clinically significant allergic reactions to the 249 cycles of treatment delivered.

Evidence shows that a favorable PFS after administration of second-line agents in STS may suggest antitumor activity in the first-line setting. In a retrospective analysis of 11 agents investigated by the EORTC STBSG (64), it was shown that patients treated with either of the two agents with known clinical activity in first-line treatment (doxorubicin or ifosfamide) had improved outcome in second-line treatment. In this setting, the PFS at 3 and 6 months was significantly higher than in patients treated with agents classed as inactive. It was therefore suggested that 3-month PFS > 40% or 6-month PFS > 20% after second-line therapy might be a signal for clinically significant benefit in first-line therapy.

In view of the encouraging PFS seen in the above phase II study, the authors concluded that further investigation of brostallicin in the first-line treatment of non-GIST STS appeared to be warranted. Consequently, the EORTC has commenced a trial to evaluate the efficacy of brostallicin in the first-line setting (EORTC 62061, BRTA-0100-015) (65). In this ongoing phase II trial, patients with advanced or metastatic STS are being randomized to receive either brostallicin or doxorubicin. The predefined primary endpoint is 6-month PFS, with secondary endpoints including overall PFS, objective tumor response, toxicity, duration of response and overall survival. This study will include different strata for the main subtypes of STS. It is planned for 108 patients to be recruited into this study. Interim results are expected in the next 12 months.

Summary

Brostallicin is a novel cytotoxic agent with an interesting mechanism of action, suggesting that it may be able to overcome some chemoresistant mechanisms identified in human cancers. It is a generally well-tolerated antineoplastic agent with mild to moderate myelosuppression as the principle toxicity. In studies performed thus far, this has been a manageable and reversible side effect. Early trials have shown that, although objective tumor responses are infrequent, the drug is associated with a favorable 3-month PFS of about 40% in a group of patients with a range of STS histological subtypes. There are, however, major weaknesses in the data published on the use of brostallicin in STS. It is becoming well recognized that different histological subtypes of STS demonstrate different sensitivities to different agents and should perhaps be treated differently (44, 66). It is therefore imperative that future trials be sufficiently powered to present robust data on the efficacy of brostallicin in different subtypes of STS. The rarity of STS makes this a considerable challenge for oncologists, but it is hoped that the EORTC 62061 study will go some way to providing such information.

Source

Cell Therapeutics, Inc. (US).

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