

The Rediscovery of Bisantrene: A Review of the Literature

John Rothman*

Chief Scientific Officer Race Oncology Ltd Level
2, 50 Kings Park Rd, West Perth WA 6005 Australia

*Corresponding author

John Rothman, Chief Scientific Officer Race Oncology Ltd Level
2, 50 Kings Park Rd, West Perth WA 6005 Australia, Tel: +61296985414,
E-mail: John.rothman@raceoncology.com

Submitted: 01 July 2017; Accepted: 07 July 2017; Published: 14 July 2017

Abstract

Bisantrene is an anthracene with anthracycline-like antitumor activity that has been the subject of over 60 clinical trials but which was lost for over 30 years due to various merger and acquisition transactions. In over 2000 patients, bisantrene has been well tolerated and shown to lack the cardiac dose-limiting toxicity of the anthracycline class and perhaps to lack a propensity to induce multi-drug resistance. Aside from inhibition of topoisomerase II, macrophage-activating activity and telomerase inhibiting activity have been reported for this agent. Within an extensive body of publications comprising over 40 clinical trials, clinical activity has been documented in a number of indications, including lymphoma, refractory breast cancer, and ovarian cancer. In 7 phase 2 trials, therapeutic utility was seen in acute myeloid leukemia (AML) comparable or superior to drugs currently in development. Although never marketed, bisantrene was approved for the treatment of AML in France in 1991 under the name Zantrene. Originally developed in the 1970s and 1980s, bisantrene is a well-tolerated and useful drug that has resumed clinical development.

Keywords: Bisantrene, Acute myeloid leukemia (AML), Anthracycline, Breast cancer, Leukemia

Abbreviations

Acute Lymphoblastic Leukemia (ALL)
Acute Myeloid Leukemia (AML)
Acute Non-Lymphocytic Leukemia (ANLL)
National Cancer Institute (NCI)

Introduction

Anthracyclines have been the largest selling category of cancer chemotherapeutics for decades. They are first-line therapy for leukemia, lymphoma, breast, and other indications; however, they have significant toxicity. Lifetime limits of between approximately 0.5 to 1.0 mg/m², depending upon the specific drug, cannot be exceeded without inducing a potentially fatal congestive heart failure. Also, anthracyclines are associated with multi-drug resistance, which limits their administration.

Bisantrene was invented by Lederle Laboratories, a subsidiary of American Cyanamid Inc., in a program intended to develop less toxic anthracenes. From the early 1980s through the early 1990s, more than 40 clinical trials were conducted with this agent, including extensive study at the National Cancer Institute (NCI) under the name Orange Crush. In 1990, bisantrene was approved for human use in France under the trade name Zantrene®. However, the drug has never been marketed despite having been shown to possess a tolerable safety profile and therapeutic activity

in certain cancer indications, including leukemia, lymphoma, breast cancer, and others. While the reasons for this are unknown, it follows from a chain of events in the 1990s: at about the time of the French approval, American Cyanamid sold the entire Lederle drug portfolio to Wyeth, and Wyeth was sold shortly thereafter to Pfizer. A review of the investment literature of that time reveals that bisantrene was omitted from the list of assets in the Lederle portfolio.

Bisantrene is an unusual agent with direct cytotoxic actions as well as immunologic and other mechanisms of action, less cardiotoxicity than is typically associated with anthracyclines, and a documented ability to induce objective responses in heavily pretreated patients with progressive disease. After being lost to the clinical community for over 30 years, it is an agent that needs to be assessed in the light of contemporary science.

Chemistry

The chemical name for Bisantrene is 9,10-anthracenedicarboxaldehyde-bis [(4, 5-dihydro-1H-imidazole-2-yl) hydrazine] dihydrochloride (**Figure 1**), and it was originally classed as an anthracycline chemotherapeutic agent. These are drugs with planar configuration based around a resonant aromatic ring structure that intercalates within the helices of DNA and disrupts various functions, including replication, presumably due to a strong inhibitory effect on the enzyme topoisomerase II [1-4]. It was found that, like other anthracyclines, it could inhibit replication and kill tumor cells in clonogenic assays and intercalate with DNA, where it inhibits both DNA and RNA synthesis

[5-8]. In one study of tumor samples from 684 patients comprising 27 different histologic types of cancer, proliferation was inhibited by $\geq 70\%$ in cancers of the breast, ovary, kidney, lung (large and small cell) lymphoma, myeloid leukemia, melanoma, adenocarcinoma of unknown origin, adrenal gland, stomach, pancreas, and head and neck [5]. In another clonogenic study of 989 human tumor samples, doxorubicin reduced tumor colony-forming units by 50% in 14% of samples, mitoxantrone had a 21% response rate, and bisantrene was effective against 31% of the samples [9]. One of the major toxicities of anthracycline drugs is a cumulative and permanent cardiotoxicity. Early work with bisantrene revealed that it had the potential for fewer adverse events than other drugs in the class and a significantly lower cardiotoxic risk. Based upon these findings, clinical investigations for the use of bisantrene in the treatment of cancer were initiated.

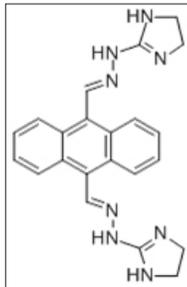


Figure 1: Bisantrene.

Mechanistically, Bisantrene preferentially binds to A-T rich regions of DNA [10], where it effects changes to supercoiling and initiates strand breaks in association with DNA-associated proteins [11,12]. This results from the inhibition of the enzyme topoisomerase II, which relaxes DNA coiling during replication and transcription [13].

A considerable amount of preclinical work was conducted at the NCI in the late 1970s and early 1980s. There it was found that while inactive orally, intravenous, intraperitoneal, or subcutaneous drug was effective in cancer models using colon 26, Lewis lung, Ridgway osteosarcoma, melanoma B16, Lieberman plasma cell, P388, or L1210 cancer cells [14]. In clonogenic assays from 684 patients diminished proliferation was seen in breast, small cell lung, large cell lung, squamous cell lung, ovarian, pancreatic, renal, adrenal, head and neck, sarcoma, gastric, lymphoma, and melanoma tumor cells, but not in colorectal [5,6,15]. Importantly, a lack of cross resistance with doxorubicin and mitoxantrone was found [9]. In stem cell assays, bisantrene demonstrated activity against a number of human cancers that included breast, ovarian, renal squamous cell, small cell lung, large cell lung, lymphoma, leukemia, melanoma adrenal, gastric, pancreatic, head and neck, and adeno carcinoma of unknown origin [5].

Toxicity studies in dogs and monkeys revealed that leukopenia, anorexia, diarrhea injection site necrosis, enterocolitis, muscle degeneration, and pulmonary edema were observed with high doses. Anthracyclines are known to be cardiotoxic; however, the cardiotoxicity of bisantrene was observed to be less than that of anthracyclines like doxorubicin [14,16-18].

Mechanisms of Action

Beyond the well-known cytotoxic mechanism of action of intercalation ascribed to anthracyclines, there is a growing body of data that indicates they have immunologic and other non-cytotoxic

effects as well. In 2009, Ferraro, et al. found that doxorubicin and daunorubicin induced apoptosis in hematopoietic cell lines in the G0-G1 stage of replication that appeared unrelated to the inhibition of topoisomerase II [19]. These agents also activated caspase-3 and induced production of ceramides. A seminal review of the immunologic role of chemotherapeutic agents in 2008 organized the data that showed these agents had effects on antigen uptake via the upregulation of cell surface calreticulin, antigen processing via the induction of HMGB-1 release, and T cell-dependent antitumor efficacy via T cell activation [20]. These authors reviewed studies that showed increased cytotoxicity of splenocytes from anthracycline-treated animals; enhanced potency of GM-CSF-treated tumor cell vaccines in the presence of anthracyclines; and anthracycline-enhanced survival of leukemic mice in the presence of IL-2 that appeared to be related to T cell and myeloid cell activation. An interesting study out of Hopkins in 2009 revealed that the anthracyclines doxorubicin and daunorubicin inhibited the binding of hypoxia inducible factor-1 (HIF-1) to DNA, an event associated with tumor proliferation under hypoxic conditions. These anthracyclines also inhibited the expression of various HIF-1 target genes that included CXCR4 and VEGF-R2, implying an anti-angiogenic role for anthracycline treatment [21]. Anthracyclines appear to have TLR-2- and TLR-9-mediated effects in that doxorubicin induced specific apoptosis of monocytes/macrophages associated with elevated IL-6 and MCP-1 and significantly diminished in the absence of MyD88, TLR-2, or TLR-9; this effect is reduced by TLR-9 antagonists [22]. The authors conclude that the specific action of the anthracycline to induce an immunogenic monocyte/macrophage apoptosis mediated by toll-like receptors via the generation of danger associated molecular patterns (DAMPs). Anthracyclines have been associated with tumor infiltrating lymphocytes (TILs) in breast cancer, and West, et al. found that in a population of estrogen receptor-negative breast cancer patients, TILs have a heightened sensitivity to anthracyclines that results in immediate immunologic responses and better long-term outcome [23]. These authors found this response to be predictive, such that patients who did not respond to anthracyclines with an immediate TIL response fared less well than those who did.

Although bisantrene has not been marketed, and thus not studied as exhaustively as more commonly used and related class of anthracyclines, it is known that this agent also has immunologic properties. Wang et al observed that 2 days after mice were treated with bisantrene, and for 4 weeks there after, macrophages could be isolated from peritoneal exudate that had cytostatic antiproliferative functionality in cultures of P815 tumor cells (mastocytoma). The supernatants from bisantrene activated macrophages also had a protective cytostatic effect in the tumor cell cultures (**Figures 2 and 3**) [24,25]. Further work revealed that mice with EL-4 lymphomas in which macrophages activated with bisantrene were adoptively transferred more than doubled their median survival time, with 7 of 10 mice in the group being cured. Multiple administrations of activated macrophages were more effective than a single administration. The macrophage activity of bisantrene may be particularly useful in that these cells have important innate and adaptive immune functions. This immunogenic activation of macrophages may contribute to the efficacy seen with this agent, and may make it well suited for incorporation into combinatorial regimens that use cytotoxic and immunogenic agents.

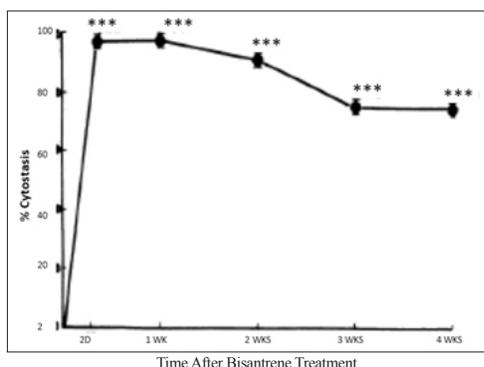


Figure 2: Persistence of activated macrophages following a single dose of bisantrene. Macrophages were prepared from mice at various time intervals after treatment with bisantrene (100 mg/kg). These macrophages were tested for tumor cytostasis at an effector:target cell ratio of 5:1. ***P< 0.001. Reprinted with permission from Wang 1984 [24].

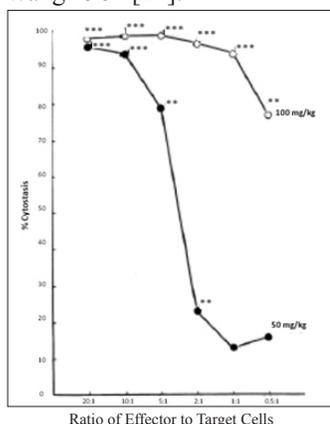


Figure 3: Activation of macrophages with bisantrene. Mice were treated intraperitoneally with bisantrene (50 or 100 mg/kg). Macrophages were prepared 4 days later and tested for tumor cytostasis at various effector:target cell ratios. **P< 0.01; ***P< 0.001. Reprinted with permission from Wang 1984 [24].

There is a brief mention in the literature a study that mentioned the possible effect of bisantrene on immunologic tumor target survivin. In this report, the survivin inhibitors NSC80467 and YM155 did so in a manner similar to the known mechanism of DNA expression inhibition of bisantrene[26]. This has not been explored further.

A non-immune, non-cytotoxic mechanism of action that appears to be unique to the anthracenebisantrene is its ability to interfere with the function of telomerase. The agent binds to DNA at a site called a G-quadruplex in which 4 guanines are associated by folding. Stabilization of the G-quadruplex can interfere with telomere-telomerase interactions and inhibit the activity of telomerase in various ways, including the displacement of telomerase binding proteins [27]. Analogs of bisantrene have been made in an attempt to improve upon the anti-telomerase activity [28]. Human melanoma (SK-Mel5) and colon cancer (LoVo) tumor cells were observed to lose their proliferative ability in the presence of these agents. Apoptosis was not observed; however, a loss of immortality was seen, with treated cells reacquiring the ability to become senescent, age, and die.

Bisantrene has been found to reduce platelet aggregation in response to collagen and to reduce the synthesis of prostaglandin E2 associated with collagen-induced platelet aggregation in a manner that can be effective in reducing the platelet-tumor cell aggregates that adhere to the vasculature and are found in microscopic tumor foci [29].

Pharmacokinetics

The clinical pharmacology and kinetics of bisantrene were well characterized in 6 clinical trials. In one trial of patients given a 90-min infusion at 260 mg/m², a biphasic elimination with an initial half-life of 65 ± 15 min, a terminal half-life of 1142 ± 226 min, and a steady state volume of distribution (V_{dss}) of 1845 L/m² was observed. Plasma clearance in this trial was 735 mL/min/m², with 11.3% of the administered dose excreted unchanged in the urine in 24 h [30]. At M.D. Anderson, doses of 80-250 mg/m² were assessed, and the initial and terminal half-lives were 0.6 h and 24.7 h, respectively, with a clearance of 1045.5 ± 51.0 mL/kg/h and a calculated volume of distribution of 42.1 ± 5.9 L/kg. In this work only 3.4% ± 1.1% of the administered dose was found in the urine over 96 h [31,32]. In 3 other single-dose studies, triphasic elimination was reported, one with t_{1/2} α, β, and γ of 3.44 min, 1.33 h and 26.13 h, respectively; another was 3 min, 1 h, and 8 h, respectively; and the last revealed clearances of 0.1 h, 1.9 h, and 43.9 h, respectively [6,33,34]. In one report, a large volume of distribution (687 L/m²) was interpreted as tissue sequestration of the drug with a subsequent depot effect [33]. In a 72-h infusion study at the Mayo Clinic, a plasma concentration of 12 ± 6 ng/mL was observed at a dose of 56 mg/m², while a dose of 260 mg/m² resulted in a plasma concentration of 55 ± 8 ng/mL. In this trial, plasma clearance was 1306 ± 179 mL/min/m² with urinary excretion of 4.6% of the dose in 24 h [35]. Finally, at St. Jude's, a 5-day schedule of 60-min infusions revealed a t_{1/2} α and β of 0.9 h and 9.7 h, respectively, with 7.1% of the dose excreted in the urine [36]. It is worth noting that Nicolau et al reported that, "The tumors contained relatively high drug concentrations as compared to most other tissues [37].

In vivo and in vitro studies have shown that urinary excretion of 37% to 79% of 14C-labeled drug occurs in 24 h, and that in the presence of NADPH and O₂, bisantrene is oxidized to at least 2 polar metabolites with a 400% reduction in activity [38]. Because of its lack of aqueous solubility at physiologic pH, bisantrene precipitates in the body have been observed in studies of rabbits and calves. Deposition of drug into the tissues has been associated with phlebitis, and this finding has been explored as a mechanism for the delivery of high tissue concentrations of drug [31,32,39]. Buck and Kovach observed that UI-928 will precipitate when plasma concentrations exceed ~50 µg/mL and continues until the plasma concentration is no greater than 15 µg/mL, and that slower and more protracted infusion rates can lessen the occurrence of phlebitis and anaphylactoid adverse events [40]. Newer methods of synthesis have resulted in more polar formulations that are presumed to have better aqueous solubility and less toxicity, although they have yet to be tested [41-46].

Safety

The safety of bisantrene was clearly characterized in a number of Phase 1 and Phase 2 trials conducted at the NCI and other academic institutions. In Phase 1 trials, toxicity consisting of myelosuppression, phlebitis, erythema, and edema was observed in patients with melanoma, hypernephroma, renal cell, hepatoma, bladder, or lung

adenoma [36]. A Phase 1 pediatric study at doses between 10 and 120 mg/m²/d × 5 days every 3 weeks (q3w) observed leukopenia, neutropenia, minor liver function test elevations, transient blood pressure fluctuation during infusion, transient edema at injection site, and phlebitis [36]. A Phase 1 study at the Mayo Clinic found the maximum tolerated dose to be 300 mg/m² over 72 h with doses over 156 mg/m² requiring a central line due to phlebitis [36]. Allergic reactions, fever, dyspnea, and chest pain were observed. A UTX-San Antonio study found intra-arterial infusions to be of no benefit over intravenous administration [36]. The Children’s Cancer Study group used Phase 1 doses between 190 and 430 mg/m² q3w and found neutropenia to be the dose-limiting toxicity, and phlebitis was observed [36]. Another phase 1 trial in patients at UTX tested doses between 20 and 280 mg/m² and found leukopenia to be the dose-limiting toxicity, with hypotension, nausea, phlebitis, palpitations, and chest pain also observed. They concluded that bisantrene was “... an attractive candidate for clinical use” [47].

Thirty three Phase 2 trials of bisantrene at the NCI included patients with breast, colon, gastric, head and neck, hepatoma, non-small cell lung, small cell lung, melanoma, leukemia, Hodgkin’s and non-Hodgkin’s lymphoma, multiple myeloma, ovarian, pancreas, prostate, renal, bladder, sarcoma, and a variety of pediatric cancers. Most patients had been previously treated. Adverse events were similar to those observed in Phase 1 trials [36].

The allergic symptoms occasionally associated with bisantrene administration were type I (immediate), and believed to be associated with the release of vasoactive agents without the production of antibodies. A reduction in the rate of administration can eliminate the occurrence of these allergic symptoms [48,49].

As bisantrene is chemically similar to anthracyclines, and congestive heart failure has long been known to be a dose-limiting and persistent toxicity associated with anthracyclines, Phase 1 investigators assessed the ejection fraction in patients treated with bisantrene using radioangiography [50]. They found no loss of ejection fraction either acutely or in patients studied longitudinally. In large Phase 3 trials, the Southwest Oncology Group (SWOG) confirmed the preclinical observation that bisantrene was less cardiotoxic than other agents in the class, including mitoxantrone or doxorubicin [51-53].

It is worth noting that in the 7 published phase 1 safety studies, cardiac toxicity was not reported as an adverse event, and that in the clinical trials in which central venous administration was used, toxicity was noted to be considerably less than with peripheral venous administration.

Efficacy

The clinical models used for the assessment of efficacy in the bisantrene trials conducted in the 1980s and reported herein would not meet contemporary standards. Typically, the studies reported in the 1980s were only a few pages long and objective tumor response rates were reported (Table 1), but not survival or changes in performance status, and none of the immune-related criteria currently in use were in existence [54-59]. This is particularly important for an agent with immunologic effects such as bisantrene, as the current United States Food and Drug Administration guidelines on immune-related response criteria allow for increased survival independent of tumor responses, and that even minor responses of the type not

reported in the older studies can be attended by a favorable overall outcome in terms of performance status and survival. Moreover, the development models of the day were based exclusively upon a toxic chemotherapeutic mechanism of action in which the clinical dose was defined by tolerance, a model not appropriate for the development of agents with non-cytotoxic mechanisms of action. The maximum tolerated dose model for the development of bisantrene was attended by toxicities that prompted investigators to co-administer hydrocortisone to limit potential acute inflammatory and allergic reactions that may have negatively impacted the immunologic aspects of the drug that were not recognized at that time. Irrespective, the reported response rates for bisantrene in AML are comparable to, or better than, many of the drugs currently in development (Table 2) [60-64].

Table 1: Historical complete response rates in bisantrene trials in recurrent or refractory acute myelogenous leukemia.

Study	Phase	N	Complete Response
Marty et al 1985 [54]	I	17	23%
Marty et al 1987 [55]	II	40	50%
Leblanc et al 1994 [56]	II	22	46%
Tosi et al 1989 [57]	II	10	40%
Bezwoda and Seymour 1989 [58]	II	15	50%
Spadea et al 1993 [59]	II	7	72%

Table 2: Response rates for newer acute myelogenous leukemia therapies in development (bisantrene competitors).

Agent	Reference	% Complete Response
Vosaroxin	Roboz 2010 [60]	38%
LoDAC	Burnett et al 2006 [61]	18%
Clofarabine	ODAC Briefing Book 2009 [62]	46%
Laromustine	ODAC Briefing Book 2009 [62]	28%
Decitabine ^a	Cashen et al 2010 [63]	24%
Sapacitabine ^a	Garcia-Manero et al 2009 [64]	25%

a:Not approved.

Acute Myelogenous Leukemia (Acute Non-Lymphocytic Leukemia)

Bisantrene was approved in France for the treatment of acute myelogenous leukemia (AML). Gerad treated 12 patients with AML (acute non-lymphocytic leukemia [ANLL]) and one with acute lymphoblastic leukemia (ALL) [65]. Ten patients had received prior anthracyclines. Doses of 100, 200, 300, or 400 mg/m² were administered as peripheral intravenous infusions. Nine patients had 1 dose, 2 patients received 100 mg/m² followed 3 weeks later by 200 mg/m². Two patients received 200 and 250 mg/m² as 2 daily courses × 5 days. Four patients achieved hypocellular marrow and 8 did not. All patients required platelets. Significant toxicity was observed. Too few patients and too many dose levels and regimens did not allow for a conclusion. In 10 refractory relapsed AML patients treated by Tosi, et al. with 250 mg/m² × 7 days (repeated for 3 days as consolidation in complete response or as re-induction in partial response) 4 patients with complete response were observed, with no significant toxicity other than fever [57]. Bezwoda and Seymour reported in 1989 on 15 patients with

relapsed or refractory ANLL treated with 250 mg/m² bisantrene × 7 days who had received at least 2 prior cycles of Ara-C and daunorubicin. If a 50% reduction in blast infiltration was observed, then they received another cycle [58]. Six of 12 relapsed patients and 1 of 3 refractory patients achieved a complete response with durations of 4 to 8+ months. There was no cardiotoxicity observed.

A number of trials in AML were reported by a French group. In 1984, Marty, et al. presented data on 10 patients with acute promyelocytic leukemia (APL) of whom 9 had relapsed following a median of 8 different drugs (mean dose of doxorubicin of 540 mg/m²) [66]. Five patients received bisantrene at a dose of 200 mg/m², 4 received 250 mg/m², and 1 received 300 mg/m². Eight patients achieved a complete response. In 1985, Marty, et al. reported on 17 patients with relapsed AML who had all received a median of 8 anti-leukemic agents that included cytarabine and daunorubicin or zorubicin, or 6-mercaptopurine or 6-thioguanine [54]. Sixteen patients received a starting dose of 75 mg/m² as a 2-h infusion via a central line, which was increased by 25 mg/m² every 3 courses. Leukopenia, thrombocytopenia, and hepatic and renal toxicity were observed, as was alopecia. All toxicities reverted within 5-20 days. Four complete responses and 2 partial responses were observed for a response rate of 35%. An additional 4 minor responses were reported. In 1987, the same group twice reported in Phase 2 abstracts on the use of bisantrene in AML. In the first abstract they reported administering a dose of 250 mg/m² via a central line × 7 days with a second 3-day course beginning on Day 15 if an “empty marrow” was not observed [67]. They observed that in a population with a median number of 8 prior therapies that complete response was achieved in 1 of 7 patients with primary refractory disease, 16 of 30 patients in first relapse, and 8 of 11 patients with relapsing APL. The authors commented on reduced hematologic toxicity. In the second abstract, these authors reported on a group of 40 relapsed or refractory patients with AML and observed a 50% complete response rate with a 78% rate of complete response in the M3 subtype (cited in [57, 59]). In 1989, Mills et al [68] reported on a low-dose trial using 120 mg/m² in which a 15% complete response rate was observed [55].

In a 1991 trial of amsacrine and rubidazole in APL (a subset of AML), the authors reported that 4 patients had resistant leukemia after initial salvage therapy and 3 of them were treated with bisantrene, resulting in complete response in 2 patients of 15 and 41+ months duration [69]. Overall, bisantrene as salvage therapy resulted in complete response in 3 of 4 patients who had failed 2 courses of rubidazole-AraC or Amsa-AraC. In another Phase 2 study of 27 evaluable relapsed and heavily pretreated AML patients receiving 120 mg/m²/d × 5 days, 3 complete responses and 1 partial response were observed; however, it should be noted that 61% of patients had ≥50% decrease in circulating blasts and 32% had ≥50% decrease in marrow blasts. The authors concluded that a low dose did not maximize the anti-leukemic effect of bisantrene. Leblanc et al treated 26 children whose leukemia had progressed subsequent to bone marrow transplant, 22 of whom were evaluable, including 13 AML comprising 4 with refractory disease and 9 in first relapse, and 10 ALL patients that included 2 with refractory disease and 8 in relapse, plus 4 undifferentiated patients (AUL) [49,56]. All were of poor prognosis. Patients received 250 mg/m²/d as a 1-h infusion via a central line × 5 days in combination with high dose Ara-C. The complete response rate was 46%, including complete response of 5 of 13 patients with AML, 5 of 9 patients

with ALL, and 2 of 4 AUL. Hepatic and renal toxicity occurred with infection being the predominant toxicity. Severe mucositis was occasionally observed and no cardiac failure was seen. Finally, Spadea, et al. treated 7 relapsed and heavily pretreated patients with 250 mg/m²/d × 7 days (followed by 3 days of consolidation in the case of complete response or 3 days of re-induction in the case of partial response), and observed 5 complete responses after a single induction course and 1 partial response for a response rate of 86% [59]. Responses were durable with complete response lasting 7, 11, and 12 months, and with 2 complete responses at 42+ and 46+ months. Toxicity was significant in this trial, with grade IV hepatic toxicity and grade III renal toxicity occurring, along with nausea, vomiting, and alopecia; however, all toxic symptoms resolved within 40 days of the last dose. It should be noted that despite heavy pretreatment with anthracyclines, no cardiotoxicity was observed.

Lymphoma

Encouraging results with bisantrene were also seen in lymphoma. In a trial of 50 patients with relapsed lymphoma and 2 patients with Hodgkin's lymphoma receiving bisantrene via central line at a dose of 350 mg/m² q3w or 300 mg/m² q3w in the event of insufficient marrow reserves, 14% of relapsed lymphoma patients experienced a complete response and 16% had a partial response, for an overall response rate of 60%, but neither of the 2 patients with Hodgkin's lymphoma responded. Patients with follicular lymphoma did particularly well, with a 31% complete response rate and a 25% partial response rate. The complete responses were durable, with durations of 16 to 24+ months. The drug was well tolerated [70]. Miller, et al. reported the effects of bisantrene in a very advanced and heavily pretreated population (92% stage IV) of 40 patients and reported 1 complete response and 2 partial response [71].

Breast Cancer

Clonogenic assays that compared bisantrene with other chemotherapeutic agents found it to be a particularly effective treatment in vitro (**Table 3**) [72]. The effect of bisantrene on refractory breast cancer was studied in Phase 2 and 3 studies (**Table w4**). When bisantrene was administered at a schedule of 250 to 300 mg/m² as a 1- to 2-h intravenous infusion in 40 evaluable patients who had received extensive prior therapy, including doxorubicin, there were 9 partial responses and 18 patients with stable disease, with responses seen in all major sites of organ involvement. At M.D. Anderson, a Phase 2 trial of bisantrene in refractory metastatic breast using 2 different regimens demonstrated a response rate of 14% in patients receiving a bolus of 300 mg/m² q3-4w, and 20% in patients given 80 mg/m²/d × 5 days q3-4w [35,73]. Four of these patients were refractory to doxorubicin. Another Phase 2 trial that used 260 mg/m² q3w in 30 women with metastatic breast cancer who had failed prior therapy resulted in 2 complete responses and 4 partial responses [74]. Although 4 patients died in the first 3 weeks of the trial, the response rate was 20%. A Phase 2 trial in Europe in which 49 evaluable patients, including women who were considered poor risks due to extensive radiotherapy and/or 4 or more chemotherapy regimens, were administered starting doses of 240-280 mg/m² with escalation in the absence of toxicity and observed a 6% response rate [75]. Seventeen women with metastatic breast cancer who had failed chemotherapy, but who had not received Doxorubicin, received 240 mg/m² as a 72-h infusion with 3 patients (18%)

were achieving a partial response [76]. SWOG conducted a Phase 3 trial of 412 patients using mitoxantrone, doxorubicin, or bisantrene in women with advanced breast carcinoma who had failed prior chemotherapy, with patient's crossing over from one drug to another upon progression. Response rates were 28% for doxorubicin, 18% for mitoxantrone, and 16% for bisantrene, with bisantrene being the best tolerated of these agents [51,53].

Table 3: In vitro response rates to various anticancer agents tested against tumor colony-forming units of human breast cancer. Reprinted with permission from Jones et al [72].

Drug	Number Tested	% Sensitive ^a
Dasorubicin	36	14
Bisantrene	26	54
Vinblastine	24	33
Mitomycin	22	36
Interferon (clone A)	22	23
5-Fluoruracil	20	20
Methotrexate	18	17
Interferon (leukocyte)	15	33
Mitoxantrone	12	42
Cyclophosphamide (bioactivated)	12	25
m-AMSA	12	16
Melphalon	10	10

a: Survival of tumor colony-forming units \leq 50% of control.

Table 4: Bisantrene response rates in heavily pretreated breast cancer treatment failures.

Reference	N	RR	Notes
Yap, et al. 1983 [73]	40	23%	71% objective + minor responses
Holmes, et al. 1986 [35]	35	34%	Responses in patients refractory to other drugs
Cavalli, et al. 1985 [75]	40	6%	Heavily pretreated refractory salvage patients
Ingle, et al. 1986 [76]	17	18%	Heavily pretreated refractory salvage patients
Osborne, et al. 1984 [74]	30	20%	Heavily pretreated refractory salvage patients
Cowan, et al. 1991 [51]	146/411	13%	Pretreated estrogen receptor + bisantrene safest therapy (Phase 3)

RR = response rate.

Ovarian Cancer

Cowan, et al. studied 36 patients who had failed treatment for ovarian cancer at a dose of 260 mg/m² q3w, and of whom 30 were evaluable for efficacy. There were 3 CR, including one of 30+ month duration in a patient who had failed 7 prior agents [77]. Leukopenia was the major toxicity with little thrombocytopenia, no anaphylaxis cardiac renal or hepatic toxicity and phlebitis was seen only in patients who received peripheral, but not central, drug administration. Less activity was reported by Kavanagh, et al. who observed only 1 PR in 23 heavily pretreated women with

progressive ovarian malignancies [78].

Other Tumor Types

Phase 2 trials at the NCI revealed marginal clinical benefit in a number of different tumor types, with only leukemia, non-Hodgkin's lymphoma, and breast cancer having response rates of > 10% [35]. In other trials at academic institutions; 4 trials in renal cell did not indicate clinical utility, 5 trials in lung cancer did not support the clinical use of bisantrene, 2 trials in colon cancer, in metastatic melanoma, and in liver cancer, reported negative results, as did 1 trial each in sarcoma, head and neck cancer, and gastric cancer [79-96]. Minor activity was reported in a trial of poor prognosis patients with advanced hormone-resistant prostate cancer, with 1 of 3 patients with measurable disease responding and 3 of the remaining 11 patients having stable disease, decline in serum acid phosphatase, no increase in bone disease, or decrease in performance status or body weight [97].

Drug Resistance

Anthracycline-induced multidrug resistance (MDR) has been well documented, but poorly understood with highly variable occurrence. It is a complex phenomenon that has been associated with the overexpression of drug efflux pumps, overexpression of P-glycoprotein (P-gp) and the MDR/TAP family of genes, alterations in topoisomerase II activity, and changes in glutathione metabolism [50]. It is believed that anthracycline therapy induces the expression of P-gp, which results in resistance to all anthracyclines and other drugs (vinblastine, etoposide, etc.). Specific investigations into bisantrene resistance has shown that it is a substrate for P-gp, and that this protein is induced in the presence of bisantrene. Topoisomerase II activity is reduced, as would be expected from a topoisomerase-II poison; however, there is no reduction in the levels of this protein [98]. As the authors note, though, the bisantrene literature contains many examples of patients who responded to bisantrene after failing prior anthracycline therapies (in some cases many different anthracyclines), which are known to induce P-gp. These findings reviewed herein would indicate that MDR to bisantrene is, at best, partial [9, 35, 50, 51, 53-57, 59, 69-71, 74-77].

It should be noted that work published after most of the trials reviewed in this document were published has shown that MDR could be reduced by various agents, and that erapamil, as well as many other reversal agents for MDR, are thought to bind and inhibit P-gp [98,99]. This effect blocks the efflux of antitumor agents and thereby kills resistant cells. It was observed that, "At concentrations that were nontoxic alone, verapamil slightly (but consistently) reversed resistance to bisantrene in S1 and markedly resensitized S1-B1-20 cells [99]. In addition, verapamil resensitized KB 8-5, KB V1, and MDR1 transfected cells to bisantrene." These authors concluded that overexpression of MDR protein does not induce resistance to bisantrene, and that therapeutic results with bisantrene may be improved with the concomitant use of effective reversal agents. They speculate that bisantrene may have application in gene therapy paradigms where P-gp is transfected into stem cells."

Resistance also occurs in hypoxic environments in which bisantrene uptake by tumor cells is not affected, but DNA strand breaks are reduced [100].

Cardiotoxicity

It is worth noting that research with the anthracenebisantrone demonstrated this drug had a substantially reduced risk for congestive heart failure when compared to anthracyclines, which was one of the reasons for moving this agent in to clinical development. As discussed above, preclinical studies in beagle dogs, radioangiographic assessment of ejection fractions in Phase I patients [101], or extensive observations in numerous clinical trials (reviewed herein) found evidence of the reduced ejection fraction or congestive heart failure that typifies anthracycline use. McLaughlin reported that, "There was minimal cardiotoxicity with cumulative doses of up to 7080 mg/m² of bisantrene [18,70,101]. However, while numerous studies report no cardiotoxic adverse events associated with bisantrene administration, Artman, et al. did observe congestive consequences in 2 children who received bisantrene after failing prior therapy [102].

Discussion

Bisantrene was developed as an anthracene with structural similarities to the well-known class of anthracycline drugs derived originally from doxorubicin, but without the anthracene nucleus. These drugs revolutionized cancer therapy in their day by reducing tumor burdens, extending survival, and occasionally curing patients. However, they are associated with toxicities of the marrow, liver, kidney, and heart, amongst others, and these toxicities could be grade III and IV. Most of the anthracycline-related toxicities resolved upon discontinuation of treatment; however, persistent, cumulative cardiotoxicity did not resolve with the cessation of drug administration. Bisantrene was found to have less associated cardiotoxicity than other drugs in the class. Coupled with good activity in clonogenic assays, a strong rationale was made to bring bisantrene forward into clinical development.

The clinical models and reporting structures used at the time of bisantrene development were not sufficient to fully appreciate the potential mechanisms of action of the drug or to capture all of the important clinical outcomes that might have occurred during clinical testing. Cancer drug development in the 1980s, especially anthracycline development, was predicated upon a maximum tolerated dose model. That is, since the drug is a toxin and the objective is to kill cells (hopefully more cancer cells than healthy normal cells), the way to determine the most effective dose was to see how much drug could be given before doing irreparable damage to the patient. In this model, deaths due to drug overdose were an unfortunate but accepted aspect of treatment. Immunotherapy was in its infancy, and immunologic efficacy was not a consideration in the development of anticancer agents of the day. Today we know that a maximum tolerated dose is not necessarily the ideal dose for drugs with mechanisms of action other than cytotoxic activity, such as immunotherapies. Further, many of the bisantrene studies reported in the 1980s used a classical Response Evaluation Criteria In Solid Tumors (RECIST)-reporting schema that quantified objective responses, but did not quantify changes in performance status or look at overall survival. We now know that agents with immune and other mechanisms of action aside from cytotoxicity (eg, kinase inhibitors) can increase survival significantly, even in the absence of objective responses, and have salutary effects on performance status independent of tumor burden, which are responses that would have been missed by the prevailing clinical models of the day. This is particularly germane for an objective assessment of bisantrene if, in fact, it has the ability to inhibit

telomerase in a manner that causes tumor cells to revert to mortal phenotypes that become senescent and subsequently die.

The existing data for bisantrene clearly demonstrated activity in AML (it was already approved for this use), and in other indications including lymphoma, refractory breast cancer, and ovarian cancer. Moreover, the response rate associated with bisantrene in AML accompanied by its putative non-cytotoxic mechanisms of action, which have never been assessed in the clinic, and the remitting/relapsing nature of the indication, allow for the possibility that a combinatorial regimen that uses bisantrene in an induction regimen, followed by an immunotherapeutic consolidation regimen, might improve the outcomes in this disease.

The ability of bisantrene to manifest cytotoxic and macrophage-activating properties as well as the ability to interfere with the actions of telomerase provides a unique set of antitumor mechanisms. In the past 2 decades since bisantrene was tested, the landscape in cancer research has grown to include numerous classes of drugs that did not exist when the original work with this compound was conducted. Small molecules, antibodies, and tumor vaccines, amongst others, are currently in development to treat cancer by attacking many different pathways, and possible ways to integrate the use of these agents with the pre-existing armamentarium of cytotoxic drugs is a timely topic. This kind of combinatorial research is a very active area of investigation, as simultaneous or sequential attacks on disparate pathways is a methodology that has shown therapeutic synergy in the past. The rediscovery of bisantrene may provide a useful tool to develop methods for integrating the well-known cytotoxic actions of DNA-intercalating agents with immune and non-immune actions of newer agents, resulting in the development of novel, effective, combinatorial regimens for various cancers.

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