CLINICAL INVESTIGATION

Clinical evidence for the role of pixantrone in the treatment of relapsed or refractory aggressive non-Hodgkin's lymphoma

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Diffuse large B-cell lymphoma is the most common non-Hodgkin's lymphoma. Although over 50% of patients are cured with first-line therapy that includes an anthracycline, there are a number of patients who require systemic therapy for relapsed or refractory disease. Currently, no drug is approved for this indication. Pixantrone, a novel aza-anthracenedione, was developed to maintain clinical efficacy while minimizing the cardiac toxicity associated with anthracyclines. Initial results of clinical trials demonstrate that pixantrone induces responses in this difficult-to-treat patient population with tolerable side effects. Results of ongoing clinical trials will better define pixantrone's role in treating diffuse large B-cell lymphoma and help assess its long-term cardiac toxicity.

Keywords: anthracycline • aza-anthracenedione • BBR-2778 • diffuse large B-cell lymphoma • pixantrone

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma (NHL), accounting for one third of newly diagnosed cases [1]. This is an aggressive lymphoma that is curable with chemotherapy regimens that usually include an anthracycline [2–5]. However, 20–30% of patients will ultimately relapse and require additional treatment [5]. Although intensive chemotherapy followed by autologous stem cell transplant can be curative in patients with relapsed or refractory disease, there are a large number of patients who are not cured with currently available therapies [6–10]. For these heavily pretreated patients, new and effective options are needed.

Anthracyclines have an important role in the treatment of aggressive B-cell lymphomas and have been shown to improve overall survival in patients with DLBCL in the upfront setting [4]. Unfortunately, their use is limited by cardiac toxicity that increases with cumulative dose. In patients with lymphoma who have been treated with doxorubicin doses of 300–400 mg/m^2, episodes of clinical congestive heart failure (CHF) have been reported in 5% of patients [11–14]. As the dose increases, the risk of cardiac toxicity also increases, with an estimated incidence of cardiac events of 26% with a cumulative dose of 550 mg/m^2 [14]. It is clear that older patients and those with independent cardiac risk factors are at increased risk of development of CHF. There is no effective method to determine which patients, if any, can safely be exposed to additional anthracycline drug. Unfortunately, left ventricular ejection fraction (LVEF) alone is not predictive of subsequent cardiac toxicity since over half of patients who subsequently develop CHF have a drop in their LVEF of <30% during monitoring while receiving drug [14], but research is ongoing into the ability of serial troponin levels or measurement of diastolic function to predict cardiac outcomes [15–17]. For now, anthracyclines, which are known to have

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high efficacy in the treatment of DLBCL, are avoided in the relapsed/refractory setting. Pixantrone is an aza-anthracenedione that was designed to maintain the clinical benefit of anthracyclines while minimizing cardiac toxicity. It has demonstrated clinical activity in relapsed/refractory DLBCL and its role in the upfront setting is under active investigation.

Pixantrone

Anthracenediones are structurally related to the anthracyclines. However, structural changes have been made in an attempt to limit cardiac toxicity (Figure 1). The mechanism of action is similar to anthracyclines. The anthracenediones maintain the planar ring structure seen in the anthracyclines and it is this structure that enables them to intercalate between DNA base pairs and inhibit topoisomerase II leading to DNA double stand breaks [18]. Mitoxantrone is the first drug of the anthracenedione-class to be approved by the US FDA for use in hormone-refractory prostate cancer, acute nonlymphocytic leukemia and multiple sclerosis [19]. Pixantrone dimaleate (6,9-bis{[(2-amino) ethyl] amino} benzo[g]isoquinoline-5, 10-dione dimaleate; BBR 2778, Cell Therapeutics, Inc.) is a second-generation anthracenedione. The cardiac toxicity of anthracyclines and mitoxantrone is at least partially mediated through damage caused by oxygen free radicals [20]. In comparison to doxorubicin and mitoxantrone, pixantrone has side chains that are primary amino groups that do not bind iron, which may lead to decreased production of oxygen free radicals, thereby resulting in less cardiac toxicity [20,21].

Pharmacokinetics

Preclinical data in cell lines show that pixantrone is able to quickly form stable DNA adducts [18]. At low concentrations of drug (7.5 µm) complete stabilization of dsDNA occurred in cell lines within 7 h of exposure with a half-life of 2 h [18].

The highest concentration of drug was found in the skeletal muscle, liver and kidney with very low concentrations reaching the brain, suggesting that drug does not extensively cross the blood–brain barrier. Some accumulation of pixantrone has been seen after repeated dosing in rats but not in dog studies. Animal studies have shown that drug is primarily excreted

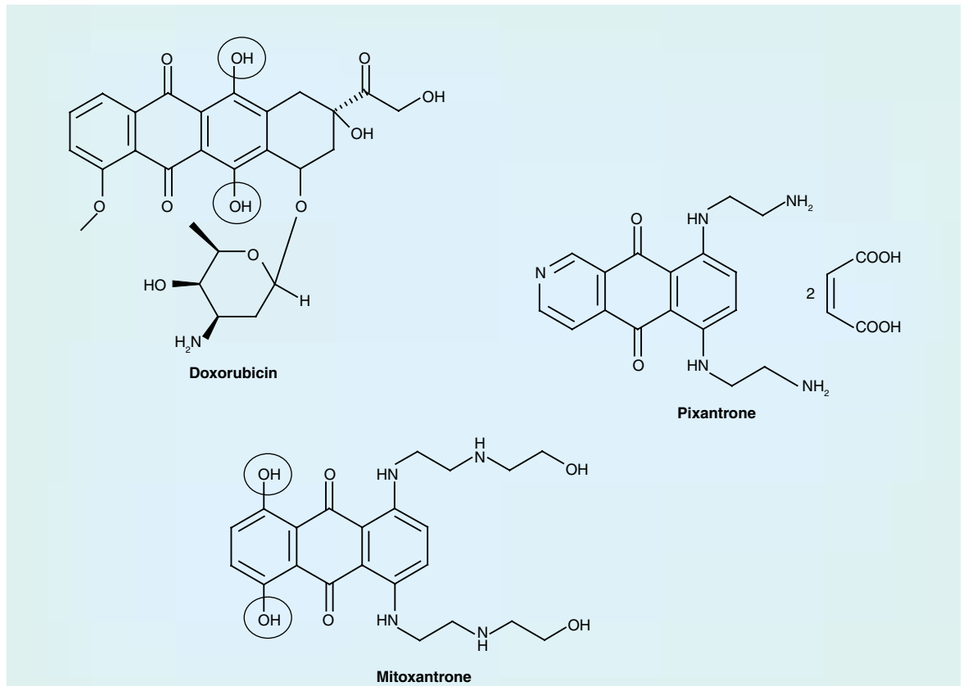

Figure 1. Doxorubicin, mitoxantrone and pixantrone.

in the feces [22]. In mice with disseminated YC-8 murine lymphoma, pixantrone was able to prolong the long-term survival compared with doxorubicin, mitoxantrone, cyclophosphamide and vincristine [22].

Phase I studies in humans showed linear pharmacokinetics (PK) with a terminal half-life of over 14 h (range: 14 to 20 h) [23–25]. The drug has a large volume of distribution and <10% unchanged drug is excreted in the urine [23–25]. PK testing has been performed after a first and second dose of pixantrone in one patient and no accumulation was seen, but the data are extremely limited [22].

Phase I clinical trials: determining the recommended dose of pixantrone

Neutropenia is the dose-limiting toxicity (DLT) found in Phase I trials of single agent pixantrone. The first published Phase I study of pixantrone was performed in patients with relapsed/refractory solid tumors with a median of two prior treatments (range: 1–6) [25]. Dose escalation was initially determined as per a standard Fibonacci schema and later, due to low plasma concentration of drug and few adverse events (AEs), escalation was decided using an accelerated schema based on toxicity and PK during the first cycle of drug. thirty patients were enrolled and received a median of two (range: 1–6) cycles of pixantrone. The maximum tolerated dose (MTD) was determined to be 112 mg/m^2 on days 1, 8 and 15 of a 28-day cycle. Six subjects were treated at the recommended dose for a total of 18 cycles. Grade III/IV neutropenia occurred during 50% of cycles with a nadir at day 14. As a result, the day 15 dose was held during 39% of the cycles due to hematologic toxicity. Pixantrone was otherwise well tolerated at this dose without any thrombocytopenia or nonhematologic side effects other than alopecia, grade I/II nausea and vomiting, and blue discoloration of the urine and skin. There were no symptomatic cardiac AEs reported.

A subsequent Phase I trial in 26 patients with relapsed/refractory NHL with a median of two (range: 1–7) prior treatment regimens demonstrated that the hematologic toxicities were more profound in this patient population [24]. The MTD of only 56 mg/m^2 on days 1, 8 and 15 of a 28-day cycle was much lower than the MTD of 112 mg/m^2 that was found in patients with solid tumors [25]. At the dose of 56 mg/m^2 no significant hematologic toxicities occurred. However, six patients received the next higher dose level of 84 mg/m^2, and three developed grade IV neutropenia while the other three patients had grade III neutropenia lasting more than a week. In addition, one patient had grade IV diarrhea that was positive for clostridium difficile and another patient had grade IV thrombocytopenia. Despite the frequent DLTs at the 84 mg/m^2, the authors recommended using this dose in future clinical trials of pixantrone in relapsed/refractory aggressive B-cell NHL. They made this decision because hematologic toxicities are not only accepted, but are expected in this patient population of relapsed/refractory DLBCL. In addition, the response rate seen at this dose was higher than at the 56 mg/m^2. Overall responses were seen in five of the 26 subjects enrolled at any dose level (19%) with three (11%) complete responses (CRs). All CRs occurred at the highest dose level (84 mg/m^2) with 50% (3/6) of subjects at this dose level achieving a CR despite prior doxorubicin and/or mitoxantrone in all subjects [24].

The clinical responses seen in this small Phase I trial were encouraging, particularly in such a heavily pretreated group. This higher dose of 84mg/m^2 is the dose that was used in the subsequent Phase II trial performed by the same group and, as reported below, it was relatively well tolerated [26].

Although most Phase I trials of pixantrone administer study drug on days 1, 8 and 15 of a 28-day cycle it can also be given less frequently at a higher dose. Phase I data of an every-3-week schedule in 24 patients with a malignancy for which there was no available effective therapy determined the MTD to be 180 mg/m^2 every 21 days [23]. As with the other Phase I trials of pixantrone given on a weekly basis, the DLT was neutropenia, which occurred in three out of five patients treated with 240 mg/m^2.

Phase II/III trials: clinical efficacy of single-agent pixantrone in aggressive NHL

Clinical trials of single-agent pixantrone have consistently produced responses in heavily pretreated patients with relapsed/refractory DLBCL, most of who have previously been exposed to an anthracycline.

The initial Phase II trial of 33 patients with relapsed/refractory DLBCL (n = 24), mantle cell lymphoma (n = 7) or other lymphoma (n = 2) administered pixantrone at 85 mg/m^2 weekly × 3 followed by 1 week off for up to six cycles [26]. Most patients had received a prior anthracycline and 78% had received two or more prior treatment regimens. The CR rate was 15% with an overall response rate (ORR) of 27%.

These encouraging results led to the EXTEND trial, a randomized Phase III trial of pixantrone versus investigator choice in patients with aggressive NHL that had relapsed after at least two prior chemotherapy regimens [27]. All patients had received prior anthracycline at least once, but were not refractory to anthracyclines as indicated by at least a partial response lasting ≥6 months to the last anthracycline received [28]. Patients with a LVEF <50% and those who had received

a cumulative dose of doxorubicin or equivalent that exceeded 450 mg/m^2 were excluded [28]. One hundred and forty patients were randomized to receive up to six cycles of pixantrone (85 mg/m^2) on days 1, 8 and 15 of a 28-day cycle versus investigator's choice. Oxaliplatin was the most frequently used comparator (45% of subjects) followed by ifosfamide (18%), vinorelbine (16%), etoposide (13%), mitoxantrone (6%) and gemcitabine (1%) [29]. Accrual to the study was slow so it was closed early after 140 patients were enrolled rather than the planned 320.

CR/complete response unconfirmed (CR/CRu) was significantly higher in the pixantrone arm with a CR/Cru rate of 24 versus 7% in the comparator arm (p = 0.009) [27]. The ORR was also significantly better at 40 versus 14% (p = 0.001). Although there was a significant improvement in progression-free survival for pixantrone, it was short in both arms at 5.3 versus 2.5 months (p = 0.005). The median overall survival for the pixantrone arm was 10.2 versus 7.6 months in the comparator arm, but this was not statistically significant (p = 0.251). Patients enrolled in this study were heavily pretreated and 57% of patients were considered refractory to most recent treatment regimens. Not surprisingly, the patients with refractory disease had a lower response rate in both arms, although responses were still seen with CR/CRu rates of 15% in refractory patients receiving pixantrone versus 29% of patients with chemosensitive disease [27].

The higher response rate and longer duration of response with pixantrone came at the expense of more toxicity. 76% of patients on the pixantrone arm experienced a grade III/IV toxicity versus 52% in the comparator arm. As a result, withdrawal due to AEs was more common in the pixantrone arm, with 21% of subjects stopping study drug early secondary to AEs versus only 13% in the comparator arm. AEs were predominantly hematologic with grade III/IV neutropenia in 41 versus 19% and febrile neutropenia in 7 versus 3% of patients in the pixantrone and comparator arms, respectively. In addition, as discussed below, there were more cardiac events (19 vs 10%) in the pixantrone arm [27]. Due to fewer responses to therapy, the rate of withdrawal due to progressive disease was higher in the comparator arm (56 vs 40%). As a result, despite more frequent AEs, patients on the pixantrone arm were able to stay on the study for a longer time, receiving a median of four treatment cycles versus three in the comparator arm [27].

Clinical efficacy of pixantrone drug combinations in relapsed/refractory aggressive NHL

Ultimately, pixantrone will likely be used as part of a multidrug regimen. There is a small Phase I/II trial of pixantrone, methylprednisolone, cisplatin and cytosine arabinoside (PSHAP) in which pixantrone replaces etoposide in an ESHAP-like regimen. Nineteen patients with relapsed/refractory NHL [30] were enrolled and the MTD of pixantrone was determined to be 80 mg/m^2 when combined with methylprednisolone 500 mg days 1–5, cisplatin 25mg/m^2 days 1–4, and cytarabine 2000 mg/m^2 on day 5 of a 21-day cycle. All patients had previously received an anthracycline. 63% had refractory disease to their most recent therapeutic regimen. The DLT in the Phase I portion of the trial was bone marrow suppression with grade III/IV anemia in 53% of subjects, grade III/IV neutropenia in 84%, grade III/IV thrombocytopenia in 95% and febrile neutropenia in 26%. These results were similar to those reported with standard ESHAP [31]. The ORR for this Phase I/II trial of PSHAP was 58% with a complete remission rate of 37%. 55% of the responders went on to stem cell transplant.

CPOP, a CHOP-like regimen that uses standard doses of cyclophosphamide (750 mg/m^2), vincristine (1.4 mg/m^2) and prednisone (100 mg days 1–5), but replaces doxorubicin with pixantrone, has been studied in patients with aggressive NHL who have relapsed after one or two prior chemotherapy regimens. Results from a Phase I study evaluating this combination determined the recommended Phase II dose of pixantrone to be 150 mg/m^2 on day 1 of a 21-day cycle [32]. As with prior Phase I studies, neutropenia was the DLT. A dose expansion Phase II study was performed using the 150 mg/m^2 dose in 30 patients to determine response rate. These patients had a mean age of 61 years (range: 26–76 years) and had DLBCL (67%), mantle cell lymphoma (27%) or grade III follicular lymphoma (7%). All subjects had previously received doxorubicin, 43% had received prior rituximab and 20% had prior stem cell transplant. In this group of patients, all of whom had relapsed after or were refractory to a prior anthracycline regimen, the ORR of 73% and CR/CRu rate of 47% is impressive and compares well with other salvage regimens (Table 1) [33–35]. The median duration of CR was 10.5 months, demonstrating some durability of response even in patients with relapsed/refractory NHL.

Table 1. Response rates of relapsed/refractory lymphoma to salvage regimens.

Regimen	No. of prior treatments	ORR (%)	CR/Cru (%)	Ref.
CPOP	1 (53%); ≥2 (47%)	73	47	[32]
RICE	1	52	27	[35]
RICE vs R-DHAP	1 vs 1	64 vs 64		[41]
R-EPOCH	Median of 4	68	28	[33]

CPOP: Cyclophosphamide, vincristine, prednisone and pixantrone; CR: Complete response; Cru: complete response unconfirmed; ORR: Overall response rate; R-DHAP: Dexamethasone, cytarabine and cisplatin plus rituximab; R-EPOCH: Etoposide, vincristine, doxorubicin, cyclophosphamide and prednisone plus rituximab; RICE: Ifosfamide, carboplatin and etoposide plus rituximab.

Pixantrone in the front-line setting

Pixantrone has the potential to be equally effective and less toxic than doxorubicin; however, before it can become a part of first-line therapy in patients with DLBCL this theoretical benefit needs to be confirmed. An ongoing randomized Phase II study of R-CHOP versus R-CPOP as first-line therapy for DLBCL will help to answer these questions [36]. This study is closed to enrollment and we await final results; however, an interim analysis was presented in abstract form at the American Society of Hematology annual meeting in 2007. This was a preplanned interim analysis after 40 subjects were enrolled, so the numbers are small and must be interpreted with care.

Patients with stage II–IV DLBCL without prior history of indolent lymphoma and with a LVEF ≥50% were eligible. Subjects were randomized to standard R-CHOP (rituximab 375 mg/m^2 on day 1, cyclophosphamide 750 mg/m^2 on day 1, doxorubicin 50 mg/m^2 on day 1, vincristine 1.4 mg/m^2 on day 1, prednisone 100 mg on days 1–5) or R-CPOP in which pixantrone 150 mg/m^2 replaced doxorubicin, but all other drugs and doses remained the same. Cycles were given every 21 days with staging studies repeated after four cycles. Subjects with a CR received two more cycles for a total of six while subjects in a partial response received four additional cycles for a total of eight and subjects with stable or progressive disease after four cycles were removed from the study [36].

Response rates after four cycles of chemotherapy were similar between the two arms with an overall response and CR rate of 86 and 33%, respectively, in the R-CPOP arm and 84 and 32% in the R-CHOP arm. Both regimens were equally well tolerated although there was a trend towards decreased AEs in the R-CPOP arm (Table 2). Four patients (21%) in the R-CHOP arm withdrew from the trial due to AEs (one neutropenia, one febrile neutropenia, one neutropenia and paresthesias and one fatigue) versus none in the R-CPOP arm. However, there were no deaths within 30 days of the last dose of study drug in the R-CHOP arm versus three deaths (14%) in the R-CPOP arm. Two of the deaths were considered treatment-related due to neutropenia and pneumonia in one patient and noncardiogenic pulmonary edema secondary to infection in a non-neutropenic patient. No episodes of symptomatic heart failure occurred in either arm although decreases in LVEF occurred in both arms with a mean decrease of 14% in the R-CPOP arm and 17% in the R-CHOP arm. This study is closed to accrual and we await longer follow-up in more patients to better assess the efficacy and tolerability of R-CPOP compared with R-CHOP [36].

Table 2. Adverse events and response rates with R-CHOP versus R-CPOP.

	R-CHOP (n = 39)†	R-CPOP (n = 39)†
Any AE attributed to treatment	24 (62%)	21 (54%)
Grade III/IV AE attributed to treatment	15 (38%)	12 (31%)
Any serious AE	11 (26%)	10 (24%)
Grade III/IV neutropenia	10 (26%)	9 (23%)
Grade III/IV infection	7 (18%)	2 (5%)
Decline in LVEF:		
■ 10–15%	1	6
■ 16–20%	5	2
■ ≥21%	2	0
Death within 30 days of last dose of study drug	0	3
Withdrawal secondary to AE	4	0
ORR (%)	84	86
CR (%)	32	34

†At the time of this interim analysis, 40 patients were evaluable for response but 78 patients were evaluable for safety.
AE: Adverse event; CR: Complete response rate; LVEF: Left ventricular ejection fraction; ORR: Overall response rate; R-CHOP: Cyclophosphamide, vincristine, prednisone and doxorubicin plus rituximab; R-CPOP: Cyclophosphamide, vincristine, prednisone and pixantrone plus rituximab.
Data taken from [36].

Toxicity of pixantrone

Pixantrone has demonstrated clinical efficacy even in heavily pretreated patients with relapsed/refractory DLBCL. However, this beneficial effect needs to be carefully weighed against the potential toxicities. This was very clearly shown in preliminary data from the EXTEND trial of pixantrone versus investigator's choice [27]. In this study the dropout rate between the two arms was very similar (71 and 77%) but the reason for dropout differed with more dropouts due to AEs in the pixantrone arm (21 vs 13%) and more dropouts

Table 3. Response and grade III/IV hematologic toxicity with single-agent pixantrone in aggressive non-Hodgkin's lymphoma.

Clinical trial	Drug dose	Grade III/IV neutropenia (n [%])	Febrile neutropenia (n [%])	ORR (%)	CR/Cru (%)	Ref.
Phase I	Pixantrone 5–84 mg/m^2 days 1, 8 and 15 of 21-day cycle	6/6 (100) 84 mg/m^2 dose	0/6 (0) 84 mg/m^2 dose	19 (all dose levels)	11 (all dose levels)	[24]
Phase II	Pixantrone 85mg/m^2 days 1, 8 and 15 of 21-day cycle	19/33 (57.6)	n/a	27	15	[26]
EXTEND	Pixantrone 85mg/m^2 days 1, 8 and 15 of 21-day cycle	28/68 (41.2)	5/68 (7.4)	25.7	15.7	[27]
	Comparator	13/67 (19.4)	2/67 (3.0)	8.6	4.3	

CR: Complete response; Cru: complete response unconfirmed; ORR: Overall response rate.

due to progressive disease in the comparator arm (40 vs 56%).

Neutropenia is the most frequent grade III/IV AE that occurs with single-agent pixantrone (Table 3) [24,26,27,29,32]. Fortunately, febrile neutropenia is rare, occurring in only 7.4% of subjects on the pixantrone arm of the Phase III extend trial [27]. The role of granulocytic growth factor has not been explored with the weekly pixantrone doses, but with the low incidence of febrile neutropenia primary prophylaxis is not recommended by the American Society of Clinical Oncology growth factor guidelines [37]. Given that most patients receiving single-agent pixantrone are likely relapsed or refractory to multiple prior therapies, managing neutropenia by dose delays or reductions is a reasonable approach.

The most unusual AE that occurs with pixantrone is a reversible blue discoloration of skin and urine that occurs secondary to the dark blue color of pixantrone itself. Autopsies of animals receiving pixantrone reveal a blue pigmentation of all organs secondary to its wide volume of distribution [24]. In the Phase I trial in patients with NHL, all patients had a blue/green tinge to their urine and one out of 26 patients developed a blue discoloration of the skin that resolved after 3 days [24]. In subjects with non-lymphoid solid tumors, weekly doses of 75 mg/m^2 or higher led to transient blue coloration of the skin [25].

Cardiac toxicity of pixantrone

The main goal for the development of the anthracenediones was to create a clinically active drug with minimal cardiac toxicity. Mitoxantrone was the first drug of this class and it is approved by the US FDA for use in hormone refractory prostate cancer, acute nonlymphocytic leukemia and multiple sclerosis [19]. Unfortunately, research and long-term follow-up have failed to demonstrate an improved side-effect profile. In fact, there is one study in Hodgkin's lymphoma that found increased delayed cardiotoxicity in the mitoxantrone arm compared with the doxorubicin or epirubicin arms [38]. The cardiotoxic effects of mitoxantrone may be at least partially due to the 5,8-dihydroxyphenyl ring, which was removed and replaced with a pyridine ring in the second-generation anthracenedione, pixantrone [8]. Long-term cardiac data on pixantrone are lacking, but early results suggest that it is well tolerated even in patients with previous exposure to anthracyclines.

Preclinical data have nicely demonstrated a lack of cardiotoxicity in mouse models (Box 1) [39]. In order to more closely mimic its use in a human population of patients with relapsed/refractory DL BCL, all the mice received three weekly doses of doxorubicin prior to being exposed to pixantrone. 6 weeks after receiving doxorubicin, the mice received normal saline, more doxorubicin, pixantrone or mitoxantrone for one to two cycles. Mice were sacrificed after the first or second cycle in order to perform histopathological evaluation of the heart. There was no difference in cardiomyopathy between the mice that received normal saline and those that received pixantrone, but significantly more cardiomyopathy was seen in the mitoxantrone and doxorubicin arms.

Equally encouraging outcomes with little cardiotoxicity have been seen in human trials of pixantrone. Echocardiograms were performed pretreatment and at the end of treatment in 17 out of 24 subjects with any malignancy enrolled in a Phase I dose escalation study of pixantrone [23]. The mean cardiac function was unchanged with a LVEF of 60% pretreatment (range: 41–77%) and 58% post-treatment (range: 46 to 74%). One patient had a 20% decrease in LVEF (67 to 46%) on echocardiogram after the fourth cycle of pixantrone, but was asymptomatic.

Preliminary results from the EXTEND trial of pixantrone versus investigator's choice suggest that the cardiac toxicity seen with anthracyclines and other anthracenediones has not been completely eradicated with the structural changes made when developing pixantrone. The subjects enrolled in the trial had previously been exposed to anthracyclines/anthracenediones such that after receiving six cycles of pixantrone in this trial they had been exposed to a median of 700 mg/m^2 of doxorubicin equivalents [29]. Thus they were at high risk of cardiac toxicity, even at the start of the trial. 9% of patients on the pixantrone arm had a serious adverse cardiac event versus 4.5% in the comparator arm [27]. However, although 19% of patients in the pixantrone arm had a decrease in the LVEF ≥10%, a grade III decrease in LVEF only occurred in two patients (3%). No grade III/IV events occurred in the comparator arm. Overall, changes in the LVEF were minor in both arms with a median decrease in the LVEF of only 5% in the pixantrone arm versus a 1% increase in the comparator arm [29]. These results certainly suggest some increase in cardiac toxicity with the pixantrone, but much of it was asymptomatic changes in LVEF and these results are based on preliminary data.

Other studies of pixantrone in patients with relapsed/refractory NHL have also shown that there is some decrease in LVEF after exposure to pixantrone, but again, these changes were mostly asymptomatic. The Phase I/II trial of CPOP in patients with NHL previously exposed to an anthracycline enrolled 65 patients with a mean prior doxorubicin-equivalence exposure of approximately 300 mg/m^2 [32]. A cardiac event occurred in 32% of patients, but these were predominantly decreases in LVEF of ≤10% although two patients had a grade III decrease in LVEF (20–39%). The multigated acquisition scan performed at the end of the study showed a mean decrease in LVEF of 6% in the Phase 1 portion and only 1.8% in the Phase II portion. Similarly, when combined with fludarabine, dexamethasone and rituximab in low-grade lymphomas, grade I/II decreases in LVEF occurred in 27% of patients, but were symptomatic in only 7% of patients [40].

The randomized Phase II trial of R-CHOP versus R-CPOP in the first-line setting will provide important data about the cardiac toxicity of the aza-anthracenedione pixantrone, compared with the anthracycline doxorubicin [36]. Data are not finalized, but an interim analysis after 78 subjects had received at least one dose of study drug reported that no subjects in either arm had symptomatic CHF. However, some cardiotoxicity was seen with asymptomatic decreases in LVEF occurring in eight subjects in each arm with a mean decrease of 14% in the R-CPOP arm and 17% in the R-CHOP arm.

Longer follow-up from clinical trials, in particular the R-CHOP versus R-CPOP trial, will further help to define the actual incidence and severity of pixantrone-induced cardiac damage. No in-depth examination has been performed yet to assess which patients are at highest risk of developing cardiac toxicity, but it is likely that factors such as increasing age, male gender, co-morbidities such as hypertension and diabetes, or prior exposure to an anthracycline will all increase the risk of cardiac toxicity from the pixantrone. Therefore, the pros and cons of pixantrone use should be carefully explored prior to beginning therapy in any high-risk patients.

Conclusion & future perspective

Pixantrone has demonstrated efficacy and good tolerability in the treatment of aggressive NHL. Cell Therapeutics, Inc. (CTI) is seeking approval of pixantrone for use in patients with relapsed/refractory DLBCL after at least two prior treatment regimens. If approved this would be the first drug approved specifically for use in multiply relapsed DLBCL.

The FDA reviewed pixantrone in 2010 and declined approval for its use in the USA, although they did encourage CTI to conduct more trials for possible resubmission. The FDA's decision was due to concerns about the results and AE profile seen in the EXTEND trial of pixantrone versus investigator's choice [101]. In addition, the trial closed early due to slow enrollment, which changed the preplanned statistical analysis parameters, and since only eight patients were enrolled in the USA there were concerns that results were not representative of the US population. Furthermore, the FDA was concerned about the high rate of AEs seen in the pixantrone arm with 21% of subjects dropping out due to AEs versus only 13% in the control arm. As the data from the EXTEND trial has matured and

Box 1. Single-agent pixantrone cardiac toxicity.

- Phase I trial in NHL [24]
  - 26 subjects
  - No cardiac toxicity
- Phase I trial in solid tumors [25]
  - 17 subjects with pre- and post-pixantrone echocardiogram
  - Mean LVEF 60% pre- and 58% post-pixantrone
  - 1 subject with >20% decrease in LVEF
- Phase II trial in NHL [26]
  - 33 subjects enrolled
  - 3 subjects with >10% decrease in LVEF
- Phase III trial of pixantrone versus comparator in NHL [27]
  - Serious cardiac event: pixantrone (8.8%), comparator (4.5%)
  - >10% decrease in LVEF: pixantrone (19%), comparator (10%)
  - Grade I/II decrease in LVEF: pixantrone (16%), comparator (10%)
  - Grade III decrease in LVEF: pixantrone (3%), comparator (0%)

LVEF: Left ventricular ejection fraction; NHL: Non-Hodgkin's lymphoma.

Executive summary

Introduction
- Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma (NHL), accounting for one third of newly diagnosed cases.
- Although a curable disease, patients with primary refractory disease or those relapsing, especially after prior stem cell transplant, have few effective therapies and there is no drug approved for use in relapsed/refractory DLBCL.

Phase II/III trials: clinical efficacy of single-agent pixantrone in aggressive NHL
- Pixantrone has demonstrated increased response rates and progression-free survival when compared with investigator's choice in a Phase III randomized clinical trial.

Pixantrone in the front-line setting
- Results from a clinical trial of cyclophosphamide, vincristine, prednisone and doxorubicin plus rituximab versus cyclophosphamide, vincristine, prednisone and pixantrone plus rituximab are pending and will help answer two important questions on the efficacy and cardiac toxicity of pixantrone in comparison with the anthracycline doxorubicin.

Toxicity of pixantrone
- Neutropenia is the most frequent toxicity that occurs with pixantrone, but febrile neutropenia is uncommon.

Cardiac toxicity of pixantrone
- More long-term data are required to fully assess whether pixantrone is less cardiotoxic than anthracyclines.

Conclusion & future perspective
- Cell Therapeutics, Inc. will likely resubmit pixantrone to the US FDA for evaluation for new drug approval in late 2011. If approved, pixantrone would be the first drug approved specifically for use in relapsed/refractory DLBCL.

new data are becoming available from the PIX203 trial of R-CPOP versus R-CHOP, the FDA has agreed to allow CTI to resubmit [102]. It is expected that CTI will likely resubmit the new drug application for pixantrone in late 2011/early 2012.

Pixantrone was developed to provide the clinical efficacy of an anthracycline while minimizing the cardiac toxicity. The PIX 203 trial of R-CPOP versus R-CHOP should bring us closer to answering the question of pixantrone's clinical benefit and assessing its cardiac toxicity in comparison with an anthracycline in the front-line setting. For now, the data suggest that this will be a good drug that will induce clinical responses in subjects with relapsed/refractory DLBCL. However, cardiac toxicity can often be delayed so long-term follow-up is required before pixantrone becomes a standard agent in the treatment of aggressive B-cell NHL, particularly in the front-line setting.

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