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Current Perspective

Anthracycline-induced cardiotoxicity — are we about to clear this hurdle?

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Abstract Anthracyclines have contributed significantly to remarkable improvements in overall survival and are regarded as the most effective cytostatic drug for cancer treatment in various malignancies. However, anthracyclines are a significant cause of acute and chronic cardiotoxicity in cancer patients, and long-term cardiotoxicity can lead to death in about one-third of patients. Several molecular pathways have been implicated in the development of anthracycline-induced cardiotoxicity, although the underlying mechanisms of some molecular pathways are not fully elucidated. It is now generally believed that anthracycline-induced reactive oxygen species (resulting from intracellular metabolism of anthracyclines) and drug-induced inhibition of topoisomerase II beta are the key mechanisms responsible for the cardiotoxicity. To prevent cardiotoxicity, several strategies are being followed: (i) angiotensin-converting enzyme inhibitors, sartans, beta-blockers, aldosterone antagonists, and statins; (ii) iron chelators; and (iii) by development of new anthracycline derivatives with little or no cardiotoxicity. This review will discuss clinically evaluated doxorubicin analogues that were developed as potentially non-cardiotoxic anticancer agents and include recent development of a novel liposomal anthracycline (L-Annamycin) for the treatment of soft-tissue sarcoma metastatic to the lung and acute myelogenous leukaemia.

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1. Introduction

The anthracycline family of drugs (i.e. doxorubicin, daunorubicin, epirubicin, idarubicin) has significantly contributed to marked improvements of overall survival (OS) during the last few decades and represents the most potent cytostatic drugs for cancer treatment across various histologies (5-year OS of 80%) [1]. Based on these results, the US Food and Drug Administration has approved the anthracyclines as one of the most effective and commonly used antineoplastic drugs and they have also been named on the World Health Organisation list of essential drugs [2,3].

Several cellular targets have been identified following exposure of tumour cells to anthracyclines *in vitro* and *in vivo*. Anthracyclines were shown to bind DNA-forming adducts and crosslinks, which block DNA replication and transcription of genes [4]. Moreover, anthracyclines can impede the activity of DNA helicase and interfere with DNA strand separation. The main cellular target of all anthracyclines is topoisomerase II (TOPO-II), an enzyme that cleaves DNA strands and generates transient double-strand breaks. Anthracyclines bind and stabilise TOPO-II-DNA cleavable complexes, which leads to DNA double-strand breaks that are lethal if remain unrepaired. Two isoforms of TOPO-II have been identified: TOPO-II alpha (α) and TOPO-II beta (β). Several *in vitro* studies [4,5] have provided evidence that TOPO-IIα is highly active in proliferating cells and is overexpressed in malignant cells but not in quiescent tissues, and inhibition of TOPO-IIα is considered the main mechanism of anthracycline-induced cell death.

Anthracyclines can also contribute to DNA damage by generating reactive oxygen species (ROS), which occur during the intracellular breakdown of the drug. The generation of ROS can also result in protein modifications (lipid peroxidation) [6].

Following the discovery of anthracyclines, it became clear that the toxic effects of these drugs were not limited to tumour cells, and amongst all routinely administered antineoplastic agents the members of the anthracycline family were a significant cause of acute and chronic cardiotoxicity [7]. This observation has sparked considerable interest to further evaluate the long-term side-effects of anthracyclines (e.g. cardiotoxicity). In a recently published cohort study, 2000 cancer survivors were monitored over 7 years [8,9]. The authors found that approximately one-third of deaths could be attributed to long-term cardiotoxicity, whereas tumour-associated mortality was found to be 51% [8,9].

As the number of survivors following any cancer treatment is significantly increasing, the risk of developing severe long-term cardiac damage is also escalating and has led to the establishment of specialised cardio-oncology departments at several major cancer centres worldwide. This perspective focuses on clinically tested, novel, structurally altered anthracyclines and their potential reduced cardiotoxicity and anticancer activity.

2. Mechanisms of anthracycline-induced cardiotoxicity

Several molecular pathways have been implicated in the development of anthracycline-induced cardiotoxicity; however, to date, the exact underlying mechanisms are still not fully understood. It is now generally accepted that anthracycline-induced ROS (generated by the intracellular metabolism of anthracyclines) and drug-induced inhibition of TOPO-IIβ within cardiac myocytes are the key mechanisms for the observed cardiotoxicity (reviewed in [10]).

Zhang *et al.* [11] provided the first evidence that the deletion of TOPO-IIβ protects cardiomyocytes from doxorubicin-induced DNA double-strand breaks, suggesting that this interaction had a major impact on the development of cardiotoxicity. Since human cardiomyocytes do not express TOPO-IIα, but do express the TOPO-IIβ isoform, it was demonstrated that knockout of TOPO-IIβ in mice led to a significant reduction of doxorubicin-induced cardiomyocyte death. Furthermore, the TOPO-IIβ-doxorubicin complex was found to induce DNA double-stranded breaks in cardiomyocytes. As TOPO-IIβ is a regulator of the expression of various human genes (including those involved in mitochondrial biogenesis and antioxidant function), the interplay of anthracyclines and TOPO-IIβ is currently considered as a key element in the pathogenesis of anthracycline-induced cardiotoxicity [11].

Other mechanisms proposed to be responsible for the observed cardiotoxicity include mitochondrial iron accumulation, lipid peroxidation, protein nitrosylation, and calcium handling abnormalities (reviewed in [10,12]), suggesting that the observed anthracycline-induced cardiotoxicity has multifactorial underlayment.

Clinical research has provided evidence that anthracycline-induced cardiotoxicity (e.g. cardiac dysfunction, arrhythmias, and, rarely, acute myocarditis) can be diagnosed in up to 20% of all patients receiving anthracyclines, and 48% of all patients treated with high doses of anthracyclines [9].

It has also been shown that anthracycline-induced cardiotoxicity appears to be a continuous process that starts at the myocardial cell level and escalates progressively to heart failure [13]. Of note, cardiotoxicity can be rooted to the first anthracycline infusion as troponin levels increase shortly after administration [14]; however, clinical symptoms may not be observed until years after the initial damage [15].

Given the importance of anthracycline-based chemotherapy regimens for the treatment of cancer patients, prevention of their cardiotoxicity is a high unmet medical need and further research leading to the development of novel non-cardiotoxic therapeutic strategies is clearly warranted.

3. Cardiotoxicity-related clinical use limitations for anthracyclines and cardio-oncology guidelines

Despite the major impact of anthracyclines on OS in the treatment of a wide spectrum of solid tumours and haematologic malignancies (e.g. leukaemias, malignant lymphomas, small-cell lung cancer, breast cancer, multiple myeloma, sarcomas, etc.) the development of life-threatening cardiotoxicity still remains a huge challenge for physicians treating cancer patients.

Lotrionte and co-workers [16] have shown in a meta-analysis of 22,815 cancer patients (the majority of whom had breast cancers, with a follow-up nine years) that 17.9% of these patients developed early signs of cardiotoxicity, 6.3% developed clinically relevant cardiotoxicity, and 10.9% of patients exhibited cardiac events. Likewise, Cardinale et al. [13] evaluated a total of 2625 cancer patients on anthracycline therapy (median follow-up of 5.2 years) and found that cardiotoxicity developed in 9% of patients with the highest incidence in the first year after completion of therapy.

The other retrospective study of more than 4000 patients revealed clinically-apparent congestive heart failure in 2.2% of all anthracycline-treated patients [17]. Although the risk of developing heart failure following doxorubicin therapy is significantly increased at cumulative doses over 550 mg/m^2, there are several lines of evidence indicating that histologic alterations in endomyocardial biopsies can occur in patients receiving only 240 mg/m^2 doxorubicin [18], suggesting that other more predictive parameters are clearly needed.

Most recently, Larsen et al. [19] analysed the association of anthracycline treatments in breast cancer and lymphomas with heart failure in a retrospective study from 1985 to 2010 (812 patients with cancer, 1384 without cancer). They found no significant increase of cardiotoxicity up to a cumulative dose of 250 mg/m^2 (hazard ratio (HR) = 0.54). However, the increased risk of heart failure at higher doses persisted over time in cancer patients and was more than two-fold after 15 years that of the control group [19]. Furthermore, the authors identified age as an independent risk factor associated with heart failure.

Recommendations for the diagnosis and management of cancer drug-induced cardio-toxicities have most recently been published in updated guidelines [20]. In an agreement with the American Society of Echocardiography and the European Association of Cardiovascular Imaging, anthracycline-induced clinical cardiotoxicity has been divided into three groups (Table 1).

Furthermore, anthracycline-induced CTRCD (cancer therapy-related cardiovascular disease) is a dose-dependent and cumulative process of variable onset that may present with symptomatic or asymptomatic CTRCD [21,22]. The recommended monitoring protocol during anthracycline therapy according to baseline CTRCD risk should include repeated clinical assessment combined with cardiac biomarkers (i.e. cardiac troponin, natriuretic peptides) and (transthoracic echocardiography, including 3D-LVEF [Left Ventricular Ejection Fraction] and global systolic myocardial longitudinal strain, when available). This monitoring procedure has been shown to identify both symptomatic and asymptomatic CTRCD, with a reasonably high negative predictive value [23,24]. Patients with pre-existing cardiovascular disease should be treated according to guideline-based therapy [20] (see below).

4. Prevention of anthracycline cardiotoxicity

In general, different strategies were considered to prevent or reduce anthracycline-induced cardiotoxicity and included use of (i) angiotensin-converting enzyme inhibitors, sartans, beta-blockers, aldosterone antagonists, and statins (reviewed in [15,21,22]); (ii) iron chelating agents (e.g. dexrazoxane) (reviewed in two recently published meta-analyses [7,17]); and (iii) development of novel anthracycline derivatives with little or no cardiotoxicity beyond pegylated liposomal doxorubicin (PLD) (Table 2).

5. Development of novel anthracyclines to limit cardiotoxicity in the treatment of soft-tissue sarcoma

To date, neither cytotoxic monotherapy nor combination regimens have been found to be superior when compared with doxorubicin monotherapy in terms of median overall survival (mOS) for the treatment of advanced or metastatic soft-tissue sarcomas (STSs). Van Glabbeke and co-workers [28] published a large meta-analysis with 2185 STS patients and reported an ORR (overall response rate) of 26% for the monotherapy with doxorubicin and a mOS of 12.7 months. Furthermore, many randomised clinical trials conducted over the last two decades failed to demonstrate a mOS benefit for the combination of doxorubicin and ifosfamide versus doxorubicin (reviewed in [29]), a finding that adds weight to the position that doxorubicin monotherapy still remains the gold standard for the first-line therapy for advanced or metastatic STSs.

5.1. *Aldoxorubicin*

Aldoxorubicin was designed to preferentially accumulate in tumours, thereby sparing the myocardial cells from its toxic effects [30]. It is a prodrug consisting of doxorubicin with a covalent linker and binds rapidly to cysteine-34 of serum albumin [30]. Following a preferential uptake into tumour cells, the conjugate is hydrolysed under acidic conditions with the release of doxorubicin [30]. In several preclinical models, aldoxorubicin demonstrated significantly reduced cardiotoxicity when compared to doxorubicin, although

Table 1
Definitions of cancer-related cardiovascular toxicity disorders (CTRCD) (modified from [20]).

CTRCD	Grade	Definition
Symptomatic CTRCD	very severe	HF requiring inotropic support, mechanical circulatory support, or consideration of transplantation
	severe	HF hospitalisation
	moderate	Need for outpatient intensification of diuretic and HF therapies
	mild	Mild HF symptoms, no intensification of therapy required
Asymptomatic CTRCD	severe	New LVEF reduction to < 40%
	moderate	New LVEF reduction by ≥10% to an LVEF of 40–49% *and* either new relative decline in GLS by > 15% from baseline *or* new rise in cardiac biomarkers
	mild	LVEF ≥50% *and* new relative decline in GLS by > 15% from baseline *and/or* new rise in cardiac biomarkers

HF, heart failure; GLS, global longitudinal strain; LVEF, left ventricular ejection fraction.

cardiotoxicity was not completely eliminated [31]. The maximum tolerated dose (MTD) was determined to be 350 mg/m^2 (equivalent to 260 mg/m^2 doxorubicin) in a phase I trial. In patients treated with high-dose aldoxorubicin, there was a dose-dependent upregulation of mitochondrial damage markers, suggesting that mechanisms underlying aldoxorubicin cardiotoxicity have not been completely eliminated [29].

In a phase II first-line study in advanced sarcoma patients (N = 126), an improved ORR and PFS (progression-free survival) over doxorubicin was demonstrated; however, there was no OS benefit [32]. Based on these findings, a subsequent randomised phase III trial (N = 443) including aldoxorubicin versus investigator's choice from a panel of chemotherapy regimens in the salvage setting in advanced sarcomas was conducted [27]. Two-thirds of the patients in each group had received prior doxorubicin treatment. The study failed to demonstrate benefits in medium PFS (mPFS) (4.1 versus 3.0 months, HR = 0.81) or mOS (12.9 versus 12.2 months, HR = 0.97) in the entire population, and no ORR differences were noted [27]. Although some evidence of subclinical cardiac effects was documented in a small portion of aldoxorubicin-treated patients, data from these two larger studies demonstrated a favourable cardiotoxicity profile of aldoxorubicin when compared with doxorubicin, based on LVEF measurements only.

Despite several limitations of the reported phase III trial with aldoxorubicin, the full potential of this compound has not been fully evaluated; thus, further randomised trials are clearly warranted to assess both efficacy as well as the degree of cardiotoxicity.

5.2. DTS-201 (CPI0004Na)

Protease-activated anthracycline conjugates are another promising approach to reduce cardiotoxicity. Using this technique, the payload (doxorubicin) is attached to an inactive, protease-cleavable linker capable of doxorubicin release in tumour microenvironment (TME). It was hypothesised that due to the lack of tumour-specific proteases in myocardial cells these drugs could be less cardiotoxic [32].

DTS-201 consists of the tetrapeptide N-succinyl-β-alanyl-L-leucyl-L-alanyl-L-leucine covalently bound to doxorubicin. The drug appears to be stable in blood but is cleavable by specific peptidases present in the TME [32]. Good efficacy and lower cardiotoxicity than doxorubicin was seen in preclinical models [33] and provided the rationale to evaluate DTS-201 in a phase I trial in patients with heavily pretreated solid tumours (N = 25) [34].

In a dose-finding study, DTS-201 was administered at levels ranging from 80 to 400 mg/m² every three weeks. Diarrhoea, nausea, and neutropenia were found to be dose-limiting toxicities (MTD = 400 mg/m^2; recommended dose for phase II testing). Of note, in line with preclinical data, DTS-201 administration was not related to any cardiotoxicity, even at higher doses. The drug showed clinical benefit in more than 50% of patients [PR (partial response), SD (stable disease)] with confirmed PR in one STS patient [35]. Details for monitoring drug-induced cardiotoxicity were not stated.

However, despite these encouraging results, the development of DTS-201 is currently on hold reputedly due to funding issues.

5.3. Camsirubicin (GPX-150)

Several lines of research have provided evidence that anthracycline-associated cardiotoxicity is, at least in part, a consequence of drug-induced inhibition of TOPO-IIβ [10]. In an attempt to eliminate cardiotoxic side-effects, a novel 13-deoxy-5-imino derivative of doxorubicin was synthesised (GPX-150, camsirubicin) and was found to selectively target TOPO-IIα, and spared TOPO-IIβ present in cardiomyocytes (IC_{50} for doxorubicin: TOPO-IIα 3.8 μmol/l versus TOPO-IIβ 40.1 μmol/l; IC_{50} for camsirubicin: TOPO-IIα 35.2 μmol/l versus TOPO-IIβ not detectable) [36].

In line with these topoisomerase activity assays, no cumulative dose-dependent cardiotoxicity was seen in animal models [36]. This prompted the investigators to conduct an initial phase I dose-escalation trial to determine the MTD for camsirubicin. Consistently with preclinical data, no evidence of cardiotoxicity was

Table 2
Summary of anthracycline derivatives without cardiotoxicity undergoing clinical trials.

Compound	Manufacturer	Molecular Features	Development Phase	Target Tumours	Comments
Doxorubicin (PLD) (Doxil®, Caelyx®)	Baxter, Janssen	pegylated liposomal doxorubicin	approved	Kaposi sarcoma, MBC, ovarian carcinoma, multiple myeloma	Overall ORR 26%; PLD did not show significant superiority to other approved conventional chemotherapies [25] . Cumulative PLD dose remains a clinical concern [26].
Aldoxorubicin	ImmunityBio	Hydrazone derivative of doxorubicin	III	r/r STS	A phase III study of aldoxorubicin versus investigator's choice from a panel of chemotherapy regimens in the salvage setting did not demonstrate a benefit in mPFS or mOS in the entire population [27].
DTS-201	Diatos	tetrapeptide pro-drug	I	Solid tumours	Development on hold due to funding issues
Camsirubicin	Monopar	13-deoxy-5-imino analogue of doxorubicin	II	STS	Significant myelosuppression (→ co-treatment with PEG-G-CSF required)
Annamycin	Moleculin	Iodine sugar derivative *and* liposomal formulation	I/II	STS, AML (*de novo*, r/r); pancreatic carcinoma	mdr-1 independent; 30-fold enrichment in lungs targeting; no cardiotoxicity (FDA-certified)

STS, soft-tissue sarcoma; mdr, multidrug resistance; AML, acute myelogenous leukaemia, r/r, relapsed/refractory; G-CSF, granulocyte colony-stimulating factor; MBC, metastatic breast cancer; mOS, median overall survival; mPFS, median progression-free survival; FDA, US Food and Drug Administration; ORR, overall response rate; PLD, pegylated liposomal doxorubicin.

observed in 24 patients (including four patients who had been previously treated with anthracyclines). The dose-limiting toxicity was found to be neutropenia, and SD was demonstrated in 5/7 patients, including three patients with STS [36].

Based on these results, a phase II study in STS was conducted [37]. Eligible patients could not have received prior anthracycline chemotherapy for their current sarcoma, except for gemcitabine and/or docetaxel as adjuvant therapy completed at least six months prior to the first planned dose of camsirubicin. The drug was administered every three weeks (265 mg/m^2) for up to 16 doses (with prophylactic PEG-G-CSF) until progression or death. The mPFS in this cohort (21 patients were eligible for assessment of efficacy) was found to be 38% and 12% at 6 and 12 months, respectively, and the mOS was reported to be 74% and 45% at 6 and 12 months, respectively. The DCR (disease control rate, including ORR and SD) was 45.5%. Again, no cardiotoxicity was recorded and neutropenia was the most prominent adverse event.

The prior phase I trial [36] determined a MTD of 265 mg/m^2 due to concerns of acute neutropenia. In an attempt to overcome this issue, a subsequent phase I trial was initiated with concomitant PEG-GCSF administration (to date N = 11 enroled; and the trial is ongoing to evaluate the MTD: NCT05043649). The current dose level is 520 mg/m^2 with a reported clinical outcome of SD at 50%. Again, no cardiotoxicity was seen [38]. Although the trial is still ongoing, the current results should be interpreted with caution for several reasons. First, only patients with ECOG 1 were recruited, which does not reflect the clinical situation for heavily pretreated STS patients. Second, the concomitant administration of PEG-GCSF can hardly be justified in this palliative situation. Additionally, cardiotoxicity was only monitored by measuring LVEF and since no troponin levels were reported, the early onset of camsirubicin-induced cardiotoxicity cannot be ruled out (see Table 1).

Overall, camsirubicin appears to have a better safety profile with regard to cardiotoxicity and seems to be better tolerated than doxorubicin [39]. The finding that this innovative doxorubicin analogue that selectively targets TOPO-II alpha but not the cardiomyocyte-specific TOPO-IIβ isoform is a novel observation that, if confirmed in larger randomised clinical trials, may have major clinical implications.

5.4. *Pegylated liposomal doxorubicin (Doxil®, Caelyx®)*

PLD (attachment of polyethylene glycol polymer chains) has been shown to have reduced cardiotoxicity when compared with doxorubicin [40]; however, its cumulative dose for cardiotoxicity still remains a clinical concern. PLD is probably the most commonly used

anthracycline to date in patients with reduced myocardial function.

Some studies have provided evidence that PLD accumulates in the tumour, but not in the myocardial tissue (38.1%,41). Moreover, the release rate of doxorubicin in the myocardial tissue was found to be much slower when compared with other tissues. PLD has also been proven to overcome P-170-associated multidrug resistance (mdr) which makes it an ideal candidate for relapsed and/or refractory cancers [42]. In general, it is recommended that the PLD dose should not exceed 550 mg/m^2 [41]. However, a randomised trial reported by Pendleburg et al. [43] has demonstrated that when the cumulative dose reached 1061 mg/m^2, less than 2% of patients developed cardiotoxicity. This is in line with other studies which showed that even cumulative doses of over 2800 mg/m^2 were not associated with any cardiotoxicity [44,45]. Finally, it has been confirmed that patients with PLD at cumulative doses over 550 mg/m^2 or PLD combined with previous doxorubicin had no PLD-related heart failure after 10 years of follow-up [46].

A number of clinical trials have explored this drug in the sarcoma setting (reviewed in [23]). However, although no cardiotoxicity was reported at lower doses, the clinical activity of PLD appeared to be modest. For example, in a small randomised phase II EORTC study in STS (N = 94), the ORR was found to be only 10% for liposomal doxorubicin and 9% for conventional doxorubicin [47] suggesting that doxorubicin and PLD are equally effective in STS patients. Despite this low response rate in STSs, it should be noted that PLD is a very effective formulation for the treatment of aggressive fibromatosis [48] and may provide long-term clinical benefit in several patients.

5.5. Dexrazoxane

Several lines of experimental studies have provided evidence that the cardio-protective mechanism of dexrazoxane is associated with iron chelation which prevents the binding of anthracyclines to iron and, in turn, the formation of ROS. Dexrazoxane has been found to bind to TOPO-II and thereby cardio-protection is conferred by TOPO-IIβ [49].

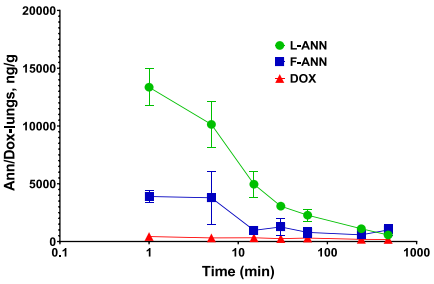
The role of dexrazoxane has been extensively evaluated in patients with advanced breast cancer, STS and childhood leukaemias which resulted in approval for extended anthracycline dosing. A published meta-analysis has demonstrated that dexrazoxane together with anthracyclines (doxorubicin or epirubicin) reduced the rates of subclinical and clinical cardiotoxicity when compared to doxorubicin or epirubicin alone [50]. In addition, Asselin et al. [51] reported the cardioprotective effects of dexrazoxane versus doxorubicin alone in 537 patients with haematological malignancies. After 3 years, LV function assessed by fraction shortening was significantly lower in the doxorubicin-only group than the dexrazoxane group (p = 0.05) and the 5-year event-free survival and the rate of secondary malignancies were not statistically different between dexrazoxane and doxorubicin alone.

Two other studies using dexrazoxane in doxorubicin-treated STS patients added weight to the hypothesis that the compound has the potential to significantly reduce doxorubicin-induced cardiotoxicity. In a retrospective trial, Schuler and co-worker [52] analysed 32 sarcoma patients and found a cardio-protective effect of dexrazoxane to prevent doxorubicin-induced cardiotoxicity (measured by transthoracal echocardiography). Patients were found to safely receive a median cumulative doxorubicin dose of 750 mg/m^2 until disease progression [52]. Similar results were details by Tap et al. [53] who reported the results of the phase III ANNOUNCE trial (doxorubicin plus olaratumab versus doxorubicin plus placebo, first-line STS, N = 509). Dexrazoxane was allowed prior to doxorubicin administration starting at cycle 1 and was recommended with cycle 5. The cardiac monitoring was performed using echo-cardiogrammes or multigated acquisition scans and was performed after 4, 6, and 8 cycles, then every 3 months for the first year, every 6 months for the second year, and annually thereafter [53]. After assessment of cardiotoxcity, cardiac dysfunction (≥ grade 3) was found to be similar in both groups [54]. Interestingly, treatment up to 8 cycles of doxorubicin (cumulative dose: 600 mg/m^2) was allowed and administered to a total of 90 patients (17.9%) with no increase of cardiac adverse events [53].

Based on these findings, the ASCO guidelines recommend the use of dexrazoxane in anthracycline-treated patients, who have received more than 300 mg/m^2 doxorubicin [55].

5.6. Doxorubicin treatment beyond the recommended cumulative dose

A higher cumulative dose of doxorubicin for advanced or metastatic STSs is known to be associated with a significantly better efficacy. Although the rechallenging (or continuation) of anthracyclines beyond the maximal cumulative dose might be a promising approach, an increase of cardiotoxicity cannot be ruled out. Further support for this proposal came from a retrospective study published by Tian et al. [56]. They compared 146 patients with STS in the standard-dose group (defined as first-line) with 24 patients in the overdose group (defined as more than one treatment line). The average cumulative anthracycline dose in the standard-dose group was 364.04 ± 63.81 mg/m^2 and 714.38 ± 210.09 mg/m^2 in the overdose group [56]. The authors reported an ORR of 15.07% (standard dose) versus 16.67% (overdose) and a mPFS of 6 months (standard dose) versus 4 months (overdose). Heart failure occurred in 5 patients (standard-dose) and one patient in the overdose group.



Agent	C_{max} ng/ml	Ratio of ANN C_{max} to Dox C_{max}
DOX	3,625	1
F-ANN	1495	0.4
L-ANN	6110	1.7

Agent	C_{max} ng/g	Ratio of ANN C_{max} to DOX C_{max}
DOX	430	1
F-ANN	3899	9
L-ANN	13352	31

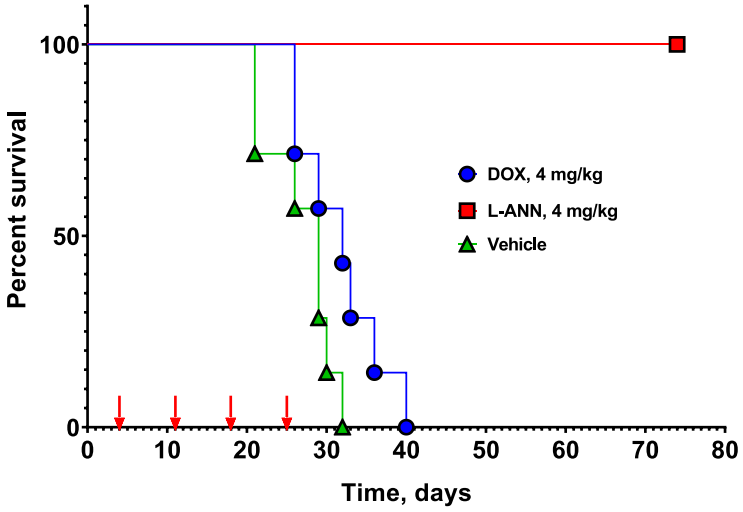


Fig. 1. Experimental design: Rats CD®IGS [Crl:CD(SD)], with body weights at dosing ranging from 261 to 355 g for males and 195–292 g for females, were administered with a single i.v. dose of liposomal Annamycin (L-ANN), free Annamycin (F-ANN) and free doxorubicin (DOX). The animals were sacrificed at the indicated timepoints followed by extraction of plasma and selected organs (left). The concentration of ANN and DOX in plasma (middle) and lungs (right) was measured using LC/MS. In addition, female C57NL/6J mice were injected with 1 x 10^5 cells through tail vein. Mice received 4 weekly doses of L-ANN or DOX at 4 mg/kg. Median survival of vehicle-receiving mice was 29 days, and was very similar to DOX-treated mice (32 days, not statistically different). There was no single death in L-ANN-treated group up to day 74 (ongoing study) (bottom). Data provided by Professor Waldemar Priebe and co-workers [57]. LC, liquid chromatography; MS, mass spectroscopy.

Although this study has several limitations (e.g. retrospective trial, low patient number in the overdose group, methods to detect and monitor cardiotoxicity not known), it adds weight to the proposal that even patients with later treatment lines respond to rechallenging with doxorubicin, a finding that, however, needs to be further evaluated in prospective clinical trials with well-defined cardiotoxicity criteria.

5.7. *Annamycin (L-ANN)*

The innovative idea of combining both liposomal formulations and novel chemical structure modifications that could lead to reduced cardiotoxicity and increased anti-tumour activity against mdr cancers has led to the development of L-Annamycin (L-ANN). An initial key modification leading to the design of Annamycin was the replacement of a basic amine at the C-3' position with a hydroxy group, which was shown to significantly reduce cardiotoxicity when compared with doxorubicin [57]. Removal of the basic amine from doxorubicin not only decreased cardiotoxicity, but also led to increased activity against mdr-1 tumours. In addition to the C-3' hydroxylation, Annamycin incorporates several important structural modifications, including demethoxylation at C-4, epimerization at C-4', and for the first time in this class of agents, an iodine atom was introduced at C-2' position. L-ANN has been shown to be a consistently more potent inducer of apoptosis than doxorubicin and more efficacious *in vivo* against mdr-1 tumours [58]. Separated studies documented L-ANN as a potent TOPO-II poison [57]. Another critical property differentiating L-ANN from doxorubicin is its organotropism. This indicates that there is a high uptake of L-ANN in several organs, including the lungs, which significantly exceeds that of doxorubicin. An incremental increase was noted for L-ANN and in two studies the C_{max} of L-ANN in lungs was > 30-fold greater than that of doxorubicin [59] (Fig. 1). With weekly intravenous doses of 5.2 mg/kg L-ANN for 6 weeks or 3.1 mg/kg and 4.2 mg/kg L-ANN for 10 weeks, the cardiotoxicity of L-ANN was less than equitoxic doses of doxorubicin [60].

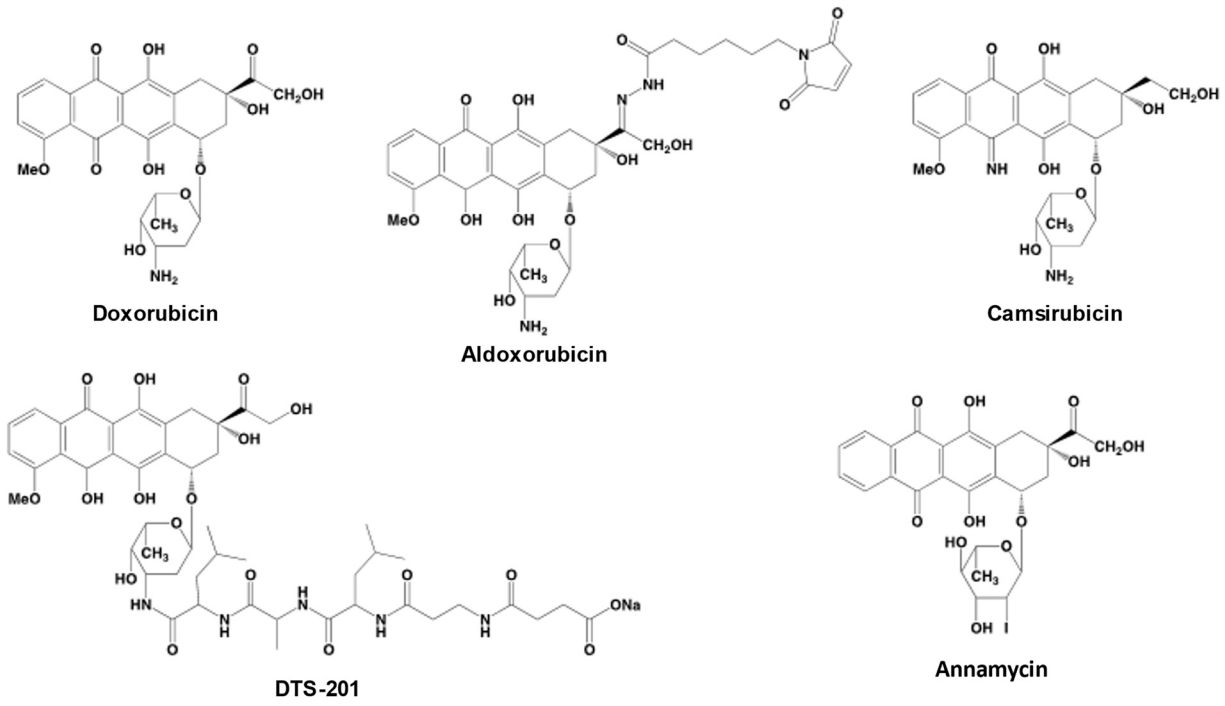


Fig. 2. Chemical structures of doxorubicin and analogues described in this paper.

Most recently, initial data from a clinical trial (MB-105) in patients with relapsed or refractory (r/r) acute myelogenous leukaemia were reported [61]. In this dose-escalation phase I/II trial, 20 patients were treated with L-ANN (NCT03388749). The study met its primary endpoint at the MTD as a single agent in which there was an ORR of 80% and no cardiotoxicity was detected using the guidelines cited above. Another study of L-ANN for STSs is ongoing (NCT04887298). L-ANN appears to be a highly promising non-cardiotoxic, potent TOPO-II poison able to overcome mdr-1 that, due to its unique organotropism, is also able to target distant metastasis. These properties differentiate L-ANN from all other anthracyclines that have shown limited or no cardiotoxicity to date and highlight it as a very promising anticancer agent. L-ANN is currently undergoing several early-phase clinical trials in different indications (Table 1).

The chemical structures of doxorubicin and its analogues described in this paper are shown in Fig. 2.

6. Conclusion

Several lines of preclinical and clinical research have provided evidence that the development of non-cardiotoxic anthracyclines is a feasible and elegant approach to eliminate this potentially life-threatening adverse event in patients with cancer. Using STSs as an initial tumour indication, the early results look promising and will likely spark further clinical research. In this context, STSs trials should be regarded as a proof-of-concept model for these novel drugs since the early assessment of heavily pretreated STS patients can be guided based on published guidelines for anthracycline-induced cardiotoxicity, albeit due to a limited remaining lifespan may not be evaluable in the chronic clinical setting.

Of great interest, however, is the development of these non-cardiotoxic compounds for the therapy of children and younger adults with tumours that are treated with a curative intent (e.g. chondrosarcomas, osteosarcomas, Ewing sarcomas, Hodgkin's disease, malignant lymphomas, acute leukaemias, adjuvant breast cancer, etc.) in which the elimination of long-term anthracycline-induced cardiotoxicity is still a high unmet medical need. Particularly for the adjuvant and palliative therapy of HER-2/neu-positive breast cancer, the combination of trastuzumab, which has been shown to be cardiotoxic, and non-cardiotoxic anthracyclines could offer a new treatment strategy if the initial preclinical data are confirmed in clinical trials.

Finally, to make some of these novel drugs 'comparable' in terms of their cardiotoxicity profiles, the standardisation of future trials is urgently needed and recommended guidelines for the monitoring of cardiotoxicity should be followed throughout (e.g. LVEF, troponin, brain natriuretic peptides, and others). Ideally, this could lead to the identification of novel biomarkers for the early onset of anthracycline-induced cardiotoxicity.

In summary, the design and development of novel, potentially non-cardiotoxic anthracyclines that are already under clinical evaluation is sufficient proof that the approach based on structural modification leading to elimination of drug interactions with cardiotoxic pathways/targets is a valid and highly promising

approach. These promising results suggest that the identification of non-cardiotoxic, clinically effective anthracycline-based anticancer agent is a real possibility.

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CRediT authorship contribution statement

Wolfram C.M. Dempke: Conceptualisation, Methodology, Validation, Formal analysis, Investigation, Data curation. **Rafal Zielinski:** Validation, Formal analysis, Investigation, Data curation. **Christina Winkler:** Validation, Formal analysis, Investigation, Data curation. **Sandra Silberman:** Validation, Formal analysis, Investigation, Data curation. **Susanne Reuther:** Validation, Formal analysis, Investigation, Data curation. **Waldemar Priebe:** Methodology, Supervision, Validation, Formal analysis, Investigation, Data curation.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Drs. Dempke and Silberman are employees of Moleculin Inc. Dr. Priebe is an inventor of patents covering Annamycin and its formulation. He is the Chair of the Scientific Advisory Board (SAB) of Moleculin and a shareholder of Moleculin, CNS Pharmaceuticals, and WPD Pharmaceuticals. His research is supported in part, by a sponsor research grant from Moleculin. Dr. Zielinski is a shareholder and serves as a consultant at Moleculin. Drs. Winkler and Reuther have no conflicts of interest to declare.

Disclosures

Drs. Dempke and Silberman are employees of Moleculin Inc. Dr. Priebe is an inventor of patents covering Annamycin and its formulation. He is the Chair of the Scientific Advisory Board (SAB) of Moleculin and a shareholder of Moleculin, CNS Pharmaceuticals, and WPD Pharmaceuticals. His research is supported in part, by a sponsor research grant from Moleculin. Dr. Zielinski is a shareholder and serves as a consultant at Moleculin. Drs. Winkler and Reuther have no conflicts of interest to declare.

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