

Novel insights into old anticancer drugs

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New insights into the activities and toxicities of the old anticancer drug doxorubicin







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ABSTRACT

The anthracycline drug doxorubicin is among the most used-and useful-chemotherapeutics. While doxorubicin is highly effective in the treatment of various hematopojetic malignancies and solid tumours, its application is limited by severe adverse effects, including irreversible cardiotoxicity, therapy-related malignancies and gonadotoxicity. This continues to motivate investigation into the mechanisms of anthracvcline activities and toxicities, with the aim to overcome the latter without sacrificing the former. It has long been appreciated that doxorubicin causes DNA double-strand breaks due to poisoning topoisomerase II. More recently, it became clear that doxorubicin also leads to chromatin damage achieved through eviction of histories from select sites in the genome. Evaluation of these activities in various anthracycline analogues has revealed that chromatin damage makes a major contribution to the efficacy of anthracycline drugs. Furthermore, the DNA-damaging effect conspires with chromatin damage to cause a number of adverse effects. Structure-activity relationships within the anthracycline family offer opportunities for chemical separation of these activities toward development of effective analogues with limited adverse effects. In this review, we elaborate on our current understanding of the different activities of doxorubicin and their contributions to drug efficacy and side effects. We then offer our perspective on how the activities of this old anticancer drug can be amended in new ways to benefit cancer patients, by providing effective treatment with improved quality of life.

INTRODUCTION

Doxorubicin, also known as adriamycin, is a member of the anthracycline anticancer drug family (Figure 1). The first anthracycline drug, daunorubicin, was isolated from



Figure 1. Structures of different anthracycline drugs and the structurally unrelated Topo II poison etoposide. *Aglycon rings are numbered in doxorubicin. Structural differences compared to doxorubicin are indicated in red.*

a soil sample found in Italy in 1960 [1, 2]. Daunorubicin is a pigmented antibiotic produced by the actinobacterium strain Streptomyces peucetius [2]. Soon, it was discovered that daunorubicin displayed anticancer activity in mice, which spurred its clinical use for the treatment of leukaemia. lymphoma and solid tumours in the late 1960s [3, 4]. In 1969, a daunorubicin homologue, doxorubicin, was isolated from a culture of chemically mutated Streptomyces peucetius [5]. Doxorubicin showed an even broader anticancer activity than daunorubicin, especially against solid tumours [6, 7]. However, quickly a major side effect of both otherwise highly potent anticancer drugs was noted — cardiotoxicity [8]. Cardiotoxicity incited by anthracyclines develops in a dose-dependent manner and can be lethal [9, 10]. As a result, treatment has to be stopped once the maximal tolerated cumulative dose is reached, while patients with poor heart function are excluded from chemo regimens containing anthracyclines. In addition to treatment-limiting cardiotoxicity, therapy-related malignancies and gonadotoxicity are also associated with anthracycline treatment [9, 11]. With latest improvements in cancer therapy, the emphasis in cancer management has changed from 'cure at any cost' to giving quality of life after treatment more consideration. In this light, quests to understand and alleviate the side effects incurred by anthracyclines have been revived. Here, we provide an overview of the mechanisms of action and toxicity of anthracycline drugs and discuss different attempts that have been made to improve them. This is followed by our perspective on how to detoxify doxorubicin for effective anticancer treatment with limited adverse effects.

MECHANISMS OF ACTION OF ANTHRACYCLINE DRUGS Topoisomerase II poison

The classical mechanism of action by which anthracyclines function is inhibition or poisoning of Topoisomerase II (Topo II) [12]. This enzyme plays a critical role in chromosome condensation, decatenation of intertwined DNA strands, and relaxation of tension in the DNA strand in front of the replication fork [13, 14]. Topo II acts by introducing a transient double-strand break (DSB) in one DNA strand (the G-segment). allowing another DNA strand (the T-segment) to pass through and subsequently closing the initial break by re-ligation of the two DNA ends (Figure 2) [13-18]. Most anthracyclines (e.g. doxorubicin, epirubicin, daunorubicin, idarubicin, and amrubicin) intercalate into DNA and poison Topo II in its catalytic step following initial break induction by forming Topo II-DNA complexes. These anthracyclines interfere at the interface of Topo II-DNA with their sugar moieties and the cyclohexane ring A [19]. In essence, the interfacial positioning makes these anthracvclines act as molecular doorstops and prevent Topo II from re-ligating the broken strand, which ultimately results in enzyme-mediated DNA damage in the form of DSB [12, 20, 21]. Although the protein structure of a Topo II-DNA-doxorubicin complex is not available (reason will be discussed in the latter part), the door-stopping act of doxorubicin can be deduced from the structure of a counterpart complex with the non-anthracycline Topo II poison, etoposide [22-24]. As a consequence of DSBs, DNA-damage response (DDR) and TP53 pathways are activated, which lead to cell cycle arrest and cell death [25]. Some anthracyclines interrupt Topo II at other steps of the catalytic cycle, such as preventing the enzyme binding to the DNA (e.g. aclarubicin) or inhibiting ATP binding [13]. Topo II is essential for the survival of rapidly dividing cells, such as cancer cells that are more sensitive to DNA breaks than normal quiescent cells; hence, anthracyclines create a chemotherapeutic window by hijacking the essential enzyme function in cells [26]. For the same reason, anthracyclines also cause side effects, such as hair loss, bone marrow suppression and gastrointestinal complications.



Figure 2. Schematic representation of the Topo II poisoning mechanism of anthracyclines. To entangle DNA or to remove DNA supercoils Topo II binds to DNA, introduce a transient double-strand break in one of the DNA strands (the G-segment), allowing the second DNA strand (the T-segment) to pass through. After re-ligation of the G-segment, the Topo II is released from DNA [15-18]. The majority of Topo II poisons, including most anthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin and amrubicin) and etoposide, stabilize the Topo II complex after it has introduced the DNA double-strand break and prevent the DNA break from being resealed [13, 26]. Anthracycline variants aclarubicin and diMe-doxorubicin inhibit the enzymatic activity by preventing Topo II from loading onto the DNA [13, 44]. Figure is inspired by [13].

DNA intercalation

Anthracyclines intercalate into the DNA helix with their anthraquinone moiety. While ring B and C of the tetracycline moiety overlap with adjacent DNA base pairs, and ring D passes through the intercalation site, the sugar moiety is pointed into the minor groove, which may compete for space with histones [19, 27]. In addition to stabilizing the Topo II-DNA complex, DNA intercalation of anthracyclines has additional effects, such as inhibiting DNA and RNA synthesis [28, 29].

Oxidative stress

The quinone moiety in ring C of anthracyclines can be transformed into a semiquinone by a number of oxidoreductases, including cytochrome P450 reductases, xanthine oxidase and NADH dehydrogenase (complex I) of the mitochondrial electron transport chain [30, 31]. Subsequently, this semiquinone quickly regenerates and thereby converts oxygen into reactive oxygen species (ROS), such as superoxide

anion (O_2) and hydrogen peroxide (H_2O_2) , or oxidize the bond between the sugar and the adjycon resulting in reductive deglycosylation. Eventually, O₂ and H₂O₂ are converted into more reactive hydroxyl radicals (OH) via the iron-catalysed Haber-Weiss reaction [32, 33]. In addition, anthracyclines can also mediate ROS production by directly interfering with iron metabolism. They can increase cellular levels of iron by interacting with iron regulatory proteins (IRP1 and/or IRP2) or accelerate the release of iron from ferritin, which then further amplifies iron-mediated oxidative stress [34-36]. The excessive ROS production can lead to lipid oxidation, genomic and mitochondrial DNA damage, which are toxic to cells. Nevertheless, the contribution of ROS formation to the anticancer activity of anthracyclines is still unclear and heavily discussed. It is worth noting that excessive ROS production is often observed when cells were exposed to anthracycline doses that are much higher than clinical relevant concentrations. Yet, at physiological concentrations, significant ROS formation was observed at late time points after drug removal, indicating this might be a secondary effect of anthracycline treatment rather than a direct mode of action [37]. Notwithstanding, it cannot be excluded that ROS formation may reinforce other mechanisms of anthracyclines.

Chromatin damage

To organize two meters of DNA in the nucleus of a single cell, DNA is compacted at several levels. One level of organization is the formation of nucleosomes, where a segment of 146 base pairs of DNA is wrapped around eight histone proteins [38]. As mentioned above, when an anthracycline intercalates into DNA, the sugar moiety emanates into the DNA minor groove and competes with histones for space, resulting in the collapse of nucleosomes. As a result, histories are evicted from chromatin (Figure 3) [27, 39]. In vitro experiments with reconstituted single nucleosomes showed that doxorubicin causes nucleosome dissociation in an ATP-, transcription-, and histone chaperone-independent manner, which may explain why the structure of Topo II-DNA-doxorubicin complex is not available [27]. Moreover, the doxorubicin metabolite doxorubicinone, which lacks the sugar molety of doxorubicin, was not able to dissociate nucleosomes under the same condition, suggesting a critical contribution of the sugar moiety to histone eviction [27, 40]. These data indicate that histone eviction induced by anthracyclines is a drug intrinsic process, which is cooperatively mediated by DNA intercalation of the anthraquinone group and nucleosome destabilization by the sugar moiety. This unique activity is not observed for other DNA intercalators (e.g. ethidium bromide [27]) or other chemotherapeutics (e.g. amsacrine or proflavin, data not published).

The dynamic structure of chromatin is essential for many nuclear processes, including transcription and replication. Therefore, the assembly, spatial organizing, and compactization of chromatin is tightly regulated by various histone chaperones, ATP-dependent chromatin remodelling complexes and histone-modifying enzymes [41, 42]. Being the building blocks of chromatin, histones are directly involved in the regulation of these processes via different epigenetic modifications. Upon eviction, these modified histones are replaced by new/nascent ones with less or different epigenetic marks. This results in DDR delay, epigenetic and transcriptomic alterations, collectively termed as chromatin damage [43]. With the aid of next-generation sequencing, unbiased (epi)genomic analysis revealed that each anthracycline evicts histones at select (epi)genomic regions [27, 43]. More specifically, doxorubicin evicts histones at open genomic regions marked by H3K36me3; while aclarubicin, whose sugar moiety is different from doxorubicin, induces histone eviction in a wider range,



Figure 3. Schematic representation of chromatin damage induced by doxorubicin. *Besides DNA intercalation by its anthraquinone group, doxorubicin's sugar moiety destabilizes nucleosome by competing for space with histones. Histone eviction caused by doxorubicin is shown to be ATP-, transcription-, and histone chaperone-independent [27]. Histone eviction results in epigenetic and transcriptomic alterations and DSB repair attenuation, collectively referred to as chromatin damage. Part of the figure is reproduced from [27].*

including compacted chromatin regions decorated by H3K27me3. As a matter of fact, anthracyclines could therefore be considered as epigenetic modifiers with defined (epi)genomic selectivity.

How histone eviction exactly causes cell death remains unclear, but it is likely to play a major contribution to the anticancer activity of the anthracycline drugs. This is illustrated by the anthracycline drugs aclarubicin and *N*,*N*-dimethyl-doxorubicin (diMedoxorubicin), which induce histone eviction without generating DNA damage [44]. Aclarubicin is prescribed mainly for the treatment of acute myeloid leukaemia (AML), showing similar efficacy as doxorubicin [27, 39, 44]. While aclarubicin was once used worldwide, it is currently only used in Japan and China. The specific reason behind this is not clear, and there is no clinical data that can explain the halt of usage. On the other hand, the doxorubicin analogue diMe-doxorubicin was first reported in the 1980s [45]. It exhibited similar anticancer activity compared to doxorubicin in tissue culture experiments and in mice [27, 39, 44]. Further, its pharmacokinetics was tested in mice and rabbits [46], but no further follow-up was reported. Surprisingly, it was recently shown by our lab that diMe-doxorubicin only induces chromatin damage but no DSB, suggesting that chromatin damage rather that DNA breaks may be the dominant cytotoxic mechanism [44]. This is further substantiated by the anthracycline variant amrubicin, which only induces DSBs. Amrubicin is much less effective than doxorubicin, aclarubicin and diMe-doxorubicin in killing cancer cells, thus did not enter clinic. Taken together, this implies that chromatin damage rather than DSB formation constitutes the major anticancer activity of anthracyclines.

Immune modulation

Besides the direct effect on eliminating tumour cells, anthracyclines can also promote antitumour immunity. During cell death, cell contents can be released into the tumour microenvironment, including tumour antigens and danger signals (also known as damage-associated molecular patterns, DAMPs) [47]. These DAMPs can initiate inflammatory response, recruit immune cells and facilitate recognition of tumour cells. This process is known as immunogenic cell death (ICD) [48-50]. It has been shown that anthracyclines such as doxorubicin can induce ICD and thereby elicit a dendritic-cell-mediated tumour-specific CD8⁺ T cell response in a colon carcinoma mouse model [51]. Moreover, doxorubicin was reported to selectively deplete myeloid-derived suppressor cells from the tumour microenvironment, which relieved the immunosuppressive impact of these cells in a murine breast cancer model [52]. Recently, it is observed that the C-type lectin receptor Clec2d is activated by binding histones to induce inflammation and tissue damage responses [53]. So it would be interesting to test whether histories can be externalized by doxorubicin, detected by the Clec2d receptor and cause an inflammation response. The immune stimulatory activity of doxorubicin, in the context of immune checkpoint blockade, was confirmed in a multi-arm non-comparative phase II trial. Treatment of triple negative breast cancer patients with doxorubicin followed by PD1 blockade resulted in an overall response rate of 35%, compared to 17% for PD1 blockade alone [54]. Although this finding needs to be confirmed in larger cohorts, it suggests that the immune modulating function of anthracyclines may have a synergistic role in the overall anticancer activity in patients.

ANTHRACYCLINE-ASSOCIATED SEVERE SIDE EFFECTS AND PREVEN-TIVE SOLUTIONS

Although doxorubicin has been a cornerstone in cancer treatment for nearly five decades, its use is plagued with severe and treatment-limiting side effects. Next to common generally acute and reversible chemo-related adverse effects, such as nausea, vomiting, diarrhoea and bone marrow suppression, anthracycline treatment is associated with long-term side effects, namely cardiotoxicity, therapy-related malignancies and gonadotoxicity. These long-term adverse effects severely impact the quality of life of cancer survivors, which limit the further application of anthracyclines. Therefore, extensive research has been performed to understand and reduce the anthracycline-induced long-term side effects.

Cardiotoxicity

The most treatment-limiting and therefore probably the best studied side effect of anthracyclines is cardiotoxicity. Anthracycline-induced cardiotoxicity presents as cardiomyopathy, ventricular dysfunction, pericarditis-myocarditis syndrome or arrhythmias, and is dose-dependent and irreversible [10, 55, 56]. As a result, doxorubicin treatment is limited to a cumulative dose of $450 - 550 \text{ mg/m}^2$ [9, 10]. Besides cumulative dose, the risk of cardiotoxicity is also associated with treatment schedule, age extremes, and combinations with other drugs or radiotherapy in the heart region [57, 58]. Currently, there is no management or medication to relieve anthracycline-induce cardiotoxicity, and the only option for patients with severe symptoms is a heart transplantation. Therefore, doxorubicin is excluded from treating patients with a poor heart function, usually old patients. Thus, alleviating cardiotoxicity would greatly improve cancer treatment with anthracyclines.

Multiple mechanisms have been proposed, including mitochondrial dysfunction and/ or lipid peroxidation as a result of ROS formation, targeting topoisomerase II β (Topo II β) in cardiomyocytes, and effects on calcium homeostasis [59-62]. To reduce anthracycline-induced cardiotoxicity, several attempts to manipulate these pathways have been made. In the following sections we will discuss these in detail, and propose a possible solution based on recent data.

ROS alleviation

The most intensely studied mechanism of anthracycline-induced cardiotoxicity is ROS production through interference with redox cycling and mitochondrial function [63]. To meet the high demand of ATP supply, cardiomyocytes have a greater density of mitochondria compared to other tissues, which could explain why the heart is more affected by anthracycline-induced ROS production than other tissues [59, 64]. Green et al. showed that doxorubicin-induced mitochondrial dysfunction coincided with the production of ROS and cytochrome C release, which in turn activated caspase-3 and initiate apoptosis in H9C2 cardiac cells [65]. It was reported that pre-treatment with the free radical scavenger tocopherol reduced the cardiotoxicity of doxorubicin in a lymphoma mouse model, without affecting its antitumour efficacy [60]. Although similar results were observed in an AML animal model, the cardiac protective effects of radical quenchers in clinical trials were disappointing [66, 67].

Similar to ROS scavengers, most iron-chelating agents can reduce ROS formation and alleviate doxorubicin-induced cardiotoxicity in preclinical models. However, such benefits were not observed in patients [68, 69]. The iron chelator dexrazoxane is an exceptional case. It was reported to reduce anthracycline-induced cardiotoxicity in some clinical studies, albeit not in all [70, 71]. However, this reduced toxicity is likely mediated by mechanisms different from ROS quenching, since other iron chelators are not cardiac protective [72]. Several alternative mechanisms of dexrazoxane function have been proposed, including inhibition of both apoptosis and necroptosis of cardiomyocytes [73] and antagonizing doxorubicin-induced DNA damage by interfering with Topo II β [74].

Although it is convincingly shown that anthracyclines can induce ROS formation in *in vitro* studies, the discrepancy between the effectivity of ROS scavengers and iron chelators in preclinical studies and patients challenges the contribution of ROS production in anthracycline-induced heart damage. Using appropriate preclinical cardiotoxicity models and treatment with anthracyclines at clinical relevant concentrations and schedules may help clarifying this issue.

Precluding from targeting topoisomerase IIβ in cardiomyocytes

In human, Topo II enzymes are expressed in two isoforms, Topo II α and Topo II β [75]. Although these two isoforms are encoded by different genes, they share substantial amino acid sequence identity and exhibit almost identical enzymological properties

[76]. Notwithstanding their similarities, the expression patterns of Topo II α and Topo IIB are different. Topo IIa is mainly expressed in proliferating cells. and almost absent in guiescent and differentiated tissues. Topo IIg is associated with replication forks and stavs bound to chromosomes during mitosis, which makes its expression essential for proliferation. On the contrary, Topo IIB expression is independent of proliferation status and is high in most cell types [76]. In line with this notion, adult mammalian cardiomyocytes express Topo IIB, but no detectable Topo IIa. Zhang et. al. reported that targeting Topo IIB in cardiomyocytes by doxorubicin is important for the initiation of cardiotoxicity [61]. It was shown that mice with cardiomyocytes-selective conditional Topo II β knockout (Topo II $\beta^{+/\Delta}$ and Topo II $\beta^{\Delta/\Delta}$) were not susceptible to the cardiac impairment caused by doxorubicin as observed in Topo IIB^{+/+} mice. Further, Lyu et. al. reported that dexrazoxane reduced doxorubicin-induced DNA damage in cardiomyocytes in vitro by rapid proteasomal degradation of Topo IIß [74]. These studies indicate that the DSBs mediated by Topo IIB poisoning is a major cause of doxorubicin-induced cardiotoxicity. Nevertheless, DSB cannot be the only reason. since the structurally non-related Topo II poison etoposide does not cause cardiotoxicity. From a clinical point of view, it suggests that Topo IIα-specific anthracycline would prevent cardiotoxicity in patients and that Topo IIB expression could be used as a prognostic marker for cardiotoxicity. Unfortunately, no genuine Topo II α - or Topo IIβ-specific drugs are available in clinic at present.

Novel delivery strategies to reduce anthracycline-induced toxicity

Due to the unsatisfactory effects of ROS scavengers and iron chelators in the clinic, tumour-specific drug delivery systems were introduced in 1990s to reduce doxorubicin-induced toxicities. These delivery strategies included nanoparticle encapsulated liposomal doxorubicin (LD) and pegylated liposomal doxorubicin (PLD). LD and PLD both show prolonged serum half-life and a smaller volume of distribution compared to conventional doxorubicin [77]. LD and PLD can extravasate into the tumour via gaps in the micro vessels, whereas other tissues are much less permeable through tight junctions. Therefore, the long serum circulation of LD and PLD results in more specific tumour accumulation. Various animal models, as well as clinical trials, showed that these particles significantly decreased cardiotoxicity compared to conventional doxorubicin, without compromising antitumour efficacy [78-80]. Therefore, both LD and PLD are approved by the FDA for treating AIDS-related Kaposi's sarcoma, multiple myeloma, breast- and ovarian cancer, but their clinical application is limited by drug leakage and higher costs.

Separating chromatin damage from DNA damage

With the aim to identify more effective anthracyclines with fewer side-effects, thousands of doxorubicin analogues, either isolated from natural sources, produced by mutant enzymes or prepared by organic (semi)synthesis, have been evaluated in the past decades. However, only few variant drugs showed reduced cardiotoxicity without loss of anticancer activity. One such analogue which entered the clinic is epirubicin. In a meta-analysis, epirubicin treatment showed significantly less cardiotoxicity compared to doxorubicin (OR 0.39, 95% confidence interval: 0.20 – 0.78, p=0.008) and subclinical cardiotoxicity (OR 0.30, 0.16 – 0.57, p<0.001) without compromising antitumour efficacy [81]. Therefore, epirubicin can be used at higher cumulative dose (900 – 1000 mg/m²) compared to doxorubicin (450 – 550 mg/m²). Although epirubicin can be used at higher cumulative dose, its application is still limited by cardiotoxicity. The key question for the development of analogues with reduced toxicity is whether these toxic effects and anticancer activities are mediated by the same mechanism(s), which determines whether it is theoretically feasible to eliminate the cardiotoxicity of anthracycline without compromising its therapeutic efficacy. Recent work of our group provides some insight. We observed that aclarubicin, as well as the doxorubicin analogue diMe-doxorubicin, showed strongly reduced cardiotoxicity in various mouse models and human induced pluripotent stem cells-derived cardiomyocyte microtissues, without compromising anticancer activity [44]. N,N-dimethylation of the amino sugar eliminated the DNA-damaging capacity of these compounds, while retaining effective histone eviction activity (Figure 4). On the other hand, etoposide and amrubicin, with only DNA-damaging activity, are also not cardiotoxic in mouse models and patients, but display much lower anticancer activity. These observations indicate that the combination of DNA- with chromatin damage, as for doxorubicin and other clinically used anthracyclines, is responsible for the cardiotoxicity of these drugs [44]. Therefore, variants with only chromatin-damaging activity would be a promising direction for the development of next-generation anthracyclines. Furthermore, the identification of the structure-activity relationship of the sugar mojety and cardiotoxicity provides a new strategy for anthracycline development.

Therapy-related malignant neoplasms

Attributing to the increased survival of cancer patients which modern anticancer therapy has made possible, the long-term side effects, such as tumorigenicity, have become an issue. Currently, 17–19% of all new primary malignancies occur in cancer survivors [82, 83]. Among all the long-term adverse effects caused by chemotherapy, therapy-related malignant neoplasms (t-MNs) are one of the most deleterious, because of substantial morbidity and considerable mortality. Soon after discovery, anthracyclines (excluding aclarubicin hereafter in this section) has been found to cause transformation and mutagenesis in vitro and tumorigenic in vivo [84-90], and anthracvcline exposure is associated with increased risks of t-MNs in cancer survivors. The t-MNs most often ascribed to anthracyclines are AML [91-93], sarcoma [94-96] and female breast cancer [96, 97]. Thyroid cancer [98] and acute promyelocytic leukaemia (APL) [99, 100] have also been linked to antecedent anthracycline treatment. The anthracycline therapy-related AMLs (t-AMLs) frequently exhibit balanced chromosomal translocations at 11g23 (involving MLL1 gene) or 21g22 (involving AML1/ RUNX1/CBFA2 gene), however occurring at unique breakpoints than de novo AML with the same cytogenetics [101-104]. In contrast to alkylating agent-associated t-AMLs, these leukaemias are rarely preceded by a myelodysplastic phase [105]. They develop with a shorter latency, often within 1–3 years after the initial anthracycline-based chemotherapy and, in some cases, within 1 year [106]. Due to unfavourable, complex or monosomal karyotypes, these t-AMLs often present as aggressive diseases and are associated with poor prognosis compared to de novo AML [106, 107]. Anthracyclines are also involved in the development of therapy-related acute promyelocytic leukaemia (t-APL) featured with balanced translocation of t(15;17) [99], which results in a double dominant-negative fusion protein, PML-RAR α [100]. Anthracycline-associated t-APL also arises after a short latency period, usually without a preleukemic phase [99, 100]. After a peak at 2 years following primary anthracycline treatment, the incidence of t-APL quickly decreases with time. Although the chromosomal breakpoints induced by anthracyclines are distinct from those observed in de novo t(15;17) APL, the clinical outcomes of t-APL and de novo APL are similar after all-trans retinoic acid- and anthracycline-based treatments, for which the 5-year survival rate is about 80% [99, 100]. Anthracycline-associated solid tumours



Figure 4. Schematic overview of the activities and toxicities of the clinically used anthracyclines and their underlying mechanisms. *Most commonly used anthracyclines, including doxorubicin, epirubicin, daunorubicin and idarubicin, possess both DNA- and chromatin-damaging activities. As a consequence, these drugs are associated with cardiotoxicity, therapy-related malignancies and gonadotoxicity. N,N-dimethylation of the sugar moiety, as for aclarubicin (and diMe-doxorubicin), results in anthracycline variants with only chromatindamaging activity, which are effective anticancer drugs with limited toxicities.*

typically occur >10 years after exposure and in a dose-dependent manner [94-98]. There is not much known about the genetic alterations of anthracycline-related solid tumours, though a strong dose response correlation with doxorubicin was found in survivors of Li-Fraumeni syndrome-associated cancer types compared with other

childhood cancer survivors [96]. Furthermore, we recently reported that doxorubicin single drug treatment induced breast cancer development in Trp53^{+/-} female mice, indicating the direct contribution of doxorubicin treatment to tumour development [44]. Besides the tumoridenicity of anthracyclines, cancer survivors may be especially susceptible to developing t-MNs due to a variety of other risk factors. These include genetic predisposition (such as the abovementioned Li-Fraumeni syndrome), carcinogenic exposures in common (such as tobacco use or alcohol abuse), host effects (age, gender, immunodeficiency or obesity), and combination therapy with other mutagenic chemotherapeutics (alkylating agents, etoposide or radiotherapy) [82, 83. 95-971. Therefore, the exact mechanisms how anthracyclines contribute to t-MN development remains unclear. One option follows reports showing that leukaemiaassociated translocation t(8:21) can be detected in hematopoietic cells of healthy individuals with no overt leukaemia [108, 109], and anthracycline-related t(8;21) t-AMLs were found to be positive for JAK2 V617F mutation [110], which suggests that t-AML is the consequence of a series of genetic alterations. Anthracyclines may facilitate the complete transformation of preleukemic cells by introducing additional mutations. On the other hand, anthracyclines can cause chromosomal translocations through an indirect mechanism mediated by apoptotic nucleases [111-113]. Nevertheless, accumulating evidence suggests that anthracyclines play a direct role in causing t-MN associated genetic aberrations. Anthracyclines generate DSBs by hijacking Topo II, particularly at breakpoint hotspot regions of leukemic translocations [103]. Unfaithful repair by error-prone DNA repair pathways can then result in mutagenesis or chromosomal translocations [114]. Through a similar mechanism of action, the structurally unrelated Topo II poison etoposide was also found to be associated with t-MNs of similar karyotypes in a dose-dependent manner, albeit less potent than anthracyclines [95, 115, 116]. The inferior potency of etoposide in transformation is also observed in a Trp53^{+/-} mouse model treated with single agents of comparable dose and schedule, which excluded the influence of genetic predisposition of host and concurrent anticancer therapies [44]. This tumorigenic difference can be explained by the strongly delayed DNA repair of anthracycline due to eviction of histone variant H2AX [27].

H2AX is an important histone variant for DNA damage repair, which is phosphorylated at DNA damage sites and responsible for repair machinery recruitment. Eviction of H2AX by doxorubicin greatly attenuates DNA damage repair, consequently results in enhanced cell death and more transformation compared to etoposide [27, 43]. In line with this hypothesis, the same Trp53^{+/-} mouse experiment and *in vitro* data showed that aclarubicin and diMe-doxorubicin without DNA-damaging activity are not tumorigenic [44, 117, 118]. Collectively, DNA damage induced by Topo II poisons is a main cause of t-MNs.

As above mentioned, anthracyclines evict histones with different epigenomic selectivity. It is interesting to notice that t(11q23) AML with MLL1 translocation is also associated with epigenetic changes, since MLL1 is an H3K4 methyltransferase [119]. The C-terminal SET domain of MLL1, which is responsible for methylating H3K4, is missing in the fusion oncoprotein of 5'-MLL1–partner-3' rearrangement. Epigenetic profiling after MLL1 deletion or with MLL1 fusion proteins revealed reduced H3K4 methylation at promotor region of target genes [120, 121]. Considering the selectivity of doxorubicin for H3K4me3 at active promotors, this coincidence may provide another explanation for the development of t(11q23) AML and its resistance to doxorubicin-based regimens [122, 123]. As a result, anthracyclines with different histone eviction profiles, such as aclarubicin and diMe-doxorubicin, could provide alternative treatment options for doxorubicin-resistant AMLs, and vice versa [45, 124-128]. Due to limited understanding of the mechanisms of action, t-MN was previously considered as the original sin of anthracycline treatment because of resulted DNA damage. Hence, hope was laid on early detection of t-MNs by intense follow-up screening in susceptible cancer survivors or restraint of high cumulative dose of anthracyclines. However, the discovery of histone eviction activity of anthracyclines not only offers a new anticancer mechanism, but also provides a strategy to prevent t-MNs, which is experimentally illustrated by aclarubicin [44, 117]. The recent understanding on the structure-activity relationship of anthracyclines makes it possible to eliminate the DNA-damaging activity of anthracycline and related toxicities, while remaining their anticancer efficacy.

Gonadotoxicity

Owing to its mechanisms, doxorubicin also targets healthy tissues with high proliferating rates, such as myeloid and lymphoid tissues, gastrointestinal mucosa and gonads. Since the survival rates of cancer patients improved spectacularly in the last two decades, the number of cancer survivors suffering from doxorubicin-induced gonadotoxicity also strongly increased [129]. Gonadotoxicity not only causes psychosocial distress, but also increases the risk of subsequent complications, such as osteoporosis, infertility, and cardiovascular disease [130]. Gonadal damage caused by doxorubicin treatments happens to patients at all stages of life. Although many of the cancer survivors could regain gonadal functions in a few months or years after doxorubicin treatment [131], they may have a shortened reproductive lifespan or late effects on pregnancy than the age-matched normal population [132-134]. Currently, cryopreservation of gametes or embryos is the only option to preserver fertility in patients receiving doxorubicin-containing therapy. However, this approach is only applicable to patients in a reproductive age and can be problematic in adolescent patients. For patients who have not vet commenced puberty, there is no clinically approved method for fertility preservation at present [135], despite that previous doxorubicin treatment during prepubertal period can lead to severe injury of the adult fertility [136].

Several classes of compounds have been proposed to protect gonads from doxorubicin insult in mouse models, including hormone agonists [137], antioxidants [138, 139], proteasome inhibitors [140], tyrosine kinase- and DDR inhibitors [141]. Before validating these drugs in a patient cohort, it is more important to test whether these inhibitors alleviate the gonadotoxicity without compromising the anticancer activity of doxorubicin in vivo. Nevertheless, development of active anthracycline variants with limited gonadotoxicity would be a preferable strategy, if possible. The depletion of follicular reserve in females and depletion of spermatogenesis in males caused by doxorubicin treatment can be attributed to the DSBs generated by the drug and subsequent cell death of germ cells [134, 142-144]. Besides direct germ cell destruction, doxorubicin also causes DSBs in somatic cells, vasculature and apoptosis of the stromal compartments in gonads [136, 143, 145-147]. The latter then further impairs the development of fertile germ cells. Similar effects were also observed for the non-anthracycline Topo II poison etoposide, which also causes DSBs and destruction of gonads [148, 149]. These data suggest that the DNA-damaging activity of doxorubicin plays an important role in mediating gonadotoxicity. This observation is further strengthened by our recent study showing that aclarubicin and diMe-doxorubicin, both lacking DNA-damaging activity but with comparable antitumour capacity as doxorubicin, did not cause apoptosis of developing follicles in female mice [44].

However, diMe-doxorubicin, with different histone eviction profile than aclarubicin and doxorubicin (unpublished results) still induced depletion of spermatogenesis in male mice, albeit at a lower degree than doxorubicin.

Oxidative stress has also been proposed as a mechanism of doxorubicin-induced gonadotoxicity [150]. However, some work using spermatogonia and immature Sertoli cell lines has shown no increase of ROS formation before the onset of cytotoxicity [151]. In line with this observation, co-administration of antioxidants showed no protective effect on doxorubicin-induced testicular toxicity *in vivo* [139, 152]. Collectively, these data suggest that DNA-damaging activity of doxorubicin is a major cause for gonadotoxicity, especially in females, with perhaps some contribution of specific histone eviction in the case of diMe-doxorubicin in male gonadotoxicity.

PERSPECTIVES

Since the discovery of daunorubicin and doxorubicin in the 1960s, a search for less toxic vet effective alternatives to doxorubicin was initiated in the 1980s. Out of thousands of anthracycline variants tested, only a few entered the clinic, most notably epirubicin, idarubicin and aclarubicin. One reason for this limited number of successful compounds might be the lack of consensus on the mechanism of action of anthracyclines for their anticancer activity and toxicities. Furthermore, whether the severe toxicities of these drugs are intimately connected with their anticancer activity has been a lingering topic in the field. For a long time, DSB induction was considered as the main anticancer activity of anthracyclines. While only recently, a second activity -chromatin damage as a result of histone eviction- was proposed [27, 39]. Chromatin damage is not only a novel activity of anthracyclines, but also a new anticancer mechanism, which is not found in other types of chemotherapeutics. The ground-breaking discovery of chromatin damage is granted by modern molecular technologies, such as time-lapse confocal imaging, photoactivation and various next-generation sequencing techniques. Hence, it is still meaningful to re-investigate old drugs with modern technology. This may yield new mechanisms of action that can be explored to arrive at active and detoxified doxorubicin and other drug variants. Additionally, this resulted in the rediscovery of an anthracycline variant, aclarubicin, as a less toxic but very active drug in (relapsed) AML treatment.

While the potential cardiotoxicity-low/free anthracyclines need to be tested in clinic, some improvements of current anthracycline-containing chemotherapy regimen should be considered. For instance, it would be debatable to combine anthracyclines with etoposide in the same treatment regimen concerning the contribution of DNA-damaging activity to multiple toxicities, although this is frequently used in AML treatment. Likewise, specific anthracycline variant should be carefully selected for children cancer patients or patients with predisposal genetic disorder to avoid toxicities. The new mechanism, histone eviction with certain (epi)genomic selectivity, indicates that anthracyclines are in fact also epigenetic drugs. Preliminary data showed that diffuse large B-cell lymphoma cells with elevated levels of H3K27me3 were more susceptible to aclarubicin than daunorubicin [43], indicating anthracycline variant selection can be personalized for cancer treatment based on their histone eviction profiles.

The recent understanding on anthracycline anticancer activity and toxicities suggests that anthracycline development should focus on depleting DNA-damaging activity from chromatin-damaging activity. Such drugs should allow effective anthracycline-based therapies devoid of the major treatment-limiting adverse effects: cardiotoxicity, therapy-related malignancies and gonadotoxicity. This would especially benefit cancer patients with a poor heart function, which are currently excluded from anthracycline-based chemotherapy. In addition, drug variants lacking these side effects could be used in more intense and/or longer therapy, and could be used for relapsed patients with a history of anthracyclines-based therapies.

In conclusion, despite the long history of anthracyclines, the novel discovery of chromatin damage as the major antitumour activity and its collective contribution with DNA-damaging activity to toxicities, allows the development of potentially new treatment strategies to improve cancer therapy and the quality of life of cancer survivors.

AUTHOR CONTRIBUTIONS

SvdZ and XQ conceived the manuscript and constructed the text under supervision of JN.

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REFERENCES

1. Camerino B, P. G. (1960) Derivati della parazina II. Sulfonamdopir (in Italian). , Gazz Chim Ital 90: 1802–1815.

2. Di Marco, A., Cassinelli, G. & Arcamone, F. (1981) The discovery of daunorubicin, Cancer Treat Rep. 65 Suppl 4, 3-8.

 C. Tan, H. T., K.-P. Yu, M. L. Murphy and D. A. Karnofsky (1967) Daunomycin, an antitumor antibiotic, in the treatment of neoplastic disease, Cancer. 20, 333–353.
drugs.com, (2000). Drugs.com/daunorubicin. https://www.drugs.com/pro/daunorubicin.html. Accessed at april 8th 2020.

5. Arcamone, F., Cassinelli, G., Fantini, G., Grein, A., Orezzi, P., Pol, C. & Spalla, C. (1969) Adriamycin, 14-hydroxydaunomycin, a new antitumor antibiotic from S. peucetius var. caesius, Biotechnol Bioeng. 11, 1101-10.

6. A.D., H. (1996) New anthracyclines–a comparative analysis of efficacy and toxicity., In: Hiddemann, W, Büchner T, Wörmann B, Ritter J, Creutzig U, Plunkett W, Keating M, editors Acute Leukemias V Experimental approaches and management of refractory disease p. 591–4.

7. drugs.com, (2000). Drugs.com/doxorubicin. https://www.drugs.com/pro/doxorubicin.html. Accesed at april 8th 2020.

8. Tan, C., Tasaka, H., Yu, K. P., Murphy, M. L. & Karnofsky, D. A. (1967) Daunomycin an Antitumor Antibiotic in Treatmentt of Neoplastic Disease - Clinical Evaluation with Special Reference to Childhood Leukemia, Cancer. 20, 333-+.

9. Lotrionte, M., Biondi-Zoccai, G., Abbate, A., Lanzetta, G., D'Ascenzo, F., Malavasi, V., Peruzzi, M., Frati, G. & Palazzoni, G. (2013) Review and meta-analysis of incidence and clinical predictors of anthracycline cardiotoxicity, Am J Cardiol. 112, 1980-4.

10. Jones, R. L., Swanton, C. & Ewer, M. S. (2006) Anthracycline cardiotoxicity, Expert Opin Drug Saf. 5, 791-809.

11. Mistry, A. R., Felix, C. A., Whitmarsh, R. J., Mason, A., Reiter, A., Cassinat, B., Parry, A., Walz, C., Wiemels, J. L., Segal, M. R., Ades, L., Blair, I. A., Osheroff, N., Peniket, A. J., Lafage-Pochitaloff, M., Cross, N. C., Chomienne, C., Solomon, E.,

Fenaux, P. & Grimwade, D. (2005) DNA topoisomerase II in therapy-related acute promyelocytic leukemia, N Engl J Med. 352, 1529-38.

12. Tewey, K. M., Rowe, T. C., Yang, L., Halligan, B. D. & Liu, L. F. (1984) Adriamycin-Induced DNA Damage Mediated by Mammalian DNA Topoisomerase-li, Science. 226, 466-468.

13. Nitiss, J. L. (2009) Targeting DNA topoisomerase II in cancer chemotherapy, Nat Rev Cancer. 9, 338-50.

14. Nitiss, J. L. (2009) DNA topoisomerase II and its growing repertoire of biological functions, Nature Reviews Cancer. 9, 327-337.

15. Roca, J. & Wang, J. C. (1992) The Capture of a DNA Double Helix by an Atp-Dependent Protein Clamp - a Key Step in DNA Transport by Type-Ii DNA Topoisomerases, Cell. 71, 833-840.

16. Roca, J. & Wang, J. C. (1994) DNA Transport by a Type-li DNA Topoisomerase - Evidence in Favor of a 2-Gate Mechanism, Cell. 77, 609-616.

17. Pommier, Y., Sung, Y. L., Huang, S. Y. N. & Nitiss, J. L. (2016) Roles of eukaryotic topoisomerases in transcription, replication and genomic stability, Nat Rev Mol Cell Bio. 17, 703-721.

18. Dong, K. C. & Berger, J. M. (2007) Structural basis for gate-DNA recognition and bending by type IIA topoisomerases, Nature. 450, 1201-U4.

19. Wang, A. H. J., Ughetto, G., Quigley, G. J. & Rich, A. (1987) Interactions between an Anthracycline Antibiotic and DNA - Molecular-Structure of Daunomycin Complexed to D(Cpgptpapcpg) at 1.2-a Resolution, Biochemistry-Us. 26, 1152-1163. 20. Pommier, Y., Schwartz, R. E., Zwelling, L. A. & Kohn, K. W. (1985) Effects of DNA Intercalating Agents on Topoisomerase-Ii Induced DNA Strand Cleavage in Isolated Mammalian-Cell Nuclei, Biochemistry-Us. 24, 6406-6410.

21. Tewey, K. M., Chen, G. L., Nelson, É. M. & Liu, L. F. (1984) Intercalative Antitumor Drugs Interfere with the Breakage-Reunion Reaction of Mammalian DNA Topoisomerase-Ii, Journal of Biological Chemistry. 259, 9182-9187.

22. Wu, C. C., Li, T. K., Farh, L., Lin, L. Y., Lin, T. S., Yu, Y. J., Yen, T. J., Chiang, C. W. & Chan, N. L. (2011) Structural Basis of Type II Topoisomerase Inhibition by the Anticancer Drug Etoposide, Science. 333, 459-462.

23. Wilstermann, A. M., Bender, R. P., Godfrey, M., Choi, S., Anklin, C., Berkowitz, D. B., Osheroff, N. & Graves, D. E. (2007) Topoisomerase II-drug interaction domains: Identification of substituents on etoposide that interact with the enzyme, Biochemistry-Us. 46, 8217-8225.

24. Bender, R. P., Jablonksy, M. J., Shadid, M., Romaine, I., Dunlap, N., Anklin, C., Graves, D. E. & Osheroff, N. (2008) Substituents on etoposide that interact with human topoisomerase II alpha in the binary enzyme-drug complex: Contributions to etoposide binding and activity, Biochemistry-Us. 47, 4501-4509.

25. Perego, P., Corna, E., De Cesare, M., Gatti, L., Polizzi, D., Pratesi, G., Supino, R. & Zunino, F. (2001) Role of apoptosis and apoptosis-related genes in cellular response and antitumor efficacy of anthracyclines, Curr Med Chem. 8, 31-7.

26. Liu, L. F. (1989) DNA Topoisomerase Poisons as Antitumor Drugs, Annu Rev Biochem. 58, 351-375.

27. Pang, B., Qiao, X., Janssen, L., Velds, A., Groothuis, T., Kerkhoven, R., Nieuwland, M., Ovaa, H., Rottenberg, S., van Tellingen, O., Janssen, J., Huijgens, P., Zwart, W. & Neefjes, J. (2013) Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin, Nature communications. 4, 1908.

28. Di Marco, A., Silvestrini, R., Di Marco, S. & Dasdia, T. (1965) Inhibiting effect of

the new cytotoxic antibiotic daunomycin on nucleic acids and mitotic activity of HeLa cells, J Cell Biol. 27, 545-50.

29. Munger, C., Ellis, A., Woods, K., Randolph, J., Yanovich, S. & Gewirtz, D. (1988) Evidence for inhibition of growth related to compromised DNA synthesis in the interaction of daunorubicin with H-35 rat hepatoma, Cancer Res. 48, 2404-11.

30. Davies, K. J. & Doroshow, J. H. (1986) Redox cycling of anthracyclines by cardiac mitochondria. I. Anthracycline radical formation by NADH dehydrogenase, J Biol Chem. 261, 3060-7.

31. Doroshow, J. H. & Davies, K. J. (1986) Redox cycling of anthracyclines by cardiac mitochondria. II. Formation of superoxide anion, hydrogen peroxide, and hydroxyl radical, J Biol Chem. 261, 3068-74.

32. Licata, S., Saponiero, A., Mordente, A. & Minotti, G. (2000) Doxorubicin metabolism and toxicity in human myocardium: role of cytoplasmic deglycosidation and carbonyl reduction, Chem Res Toxicol. 13, 414-20.

33. Minotti, G., Cairo, G. & Monti, E. (1999) Role of iron in anthracycline cardiotoxicity: new tunes for an old song?, FASEB J. 13, 199-212.

34. Gammella, E., Maccarinelli, F., Buratti, P., Recalcati, S. & Cairo, G. (2014) The role of iron in anthracycline cardiotoxicity, Front Pharmacol. 5, 25.

35. Demant, E. J. F. (1984) Transfer of Ferritin-Bound Iron to Adriamycin, Febs Letters. 176, 97-100.

36. Hasinoff, B. B. & Kala, S. V. (1993) The Removal of Metal-Ions from Transferrin, Ferritin and Ceruloplasmin by the Cardioprotective Agent Icrf-187 [(+)-1,2-Bis(3,5-DioxopiperazinyI-1-YI)Propane] and Its Hydrolysis Product Adr-925, Agents Actions. 39, 72-81.

37. Minotti, G., Menna, P., Salvatorelli, E., Cairo, G. & Gianni, L. (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity, Pharmacol Rev. 56, 185-229.

38. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. (1997) Crystal structure of the nucleosome core particle at 2.8 angstrom resolution, Nature. 389, 251-260.

39. Yang, F., Kemp, C. J. & Henikoff, S. (2013) Doxorubicin enhances nucleosome turnover around promoters, Curr Biol. 23, 782-7.

40. Jawad, B., Poudel, L., Podgornik, R., Steinmetz, N. F. & Ching, W. Y. (2019) Molecular mechanism and binding free energy of doxorubicin intercalation in DNA, Phys Chem Chem Phys. 21, 3877-3893.

41. Talbert, P. B. & Henikoff, S. (2017) Histone variants on the move: substrates for chromatin dynamics, Nat Rev Mol Cell Bio. 18, 115-126.

42. Alabert, C., Jasencakova, Z. & Groth, A. (2017) Chromatin Replication and Histone Dynamics, Adv Exp Med Biol. 1042, 311-333.

43. Pang, B., de Jong, J., Qiao, X., Wessels, L. F. & Neefjes, J. (2015) Chemical profiling of the genome with anti-cancer drugs defines target specificities, Nature chemical biology. 11, 472-80.

44. Qiao, X., van der Zanden, S. Y., Wander, D. P. A., Borras, D. M., Song, J. Y., Li, X., van Duikeren, S., van Gils, N., Rutten, A., van Herwaarden, T., van Tellingen, O., Giacomelli, E., Bellin, M., Orlova, V., Tertoolen, L. G. J., Gerhardt, S., Akkermans, J. J., Bakker, J. M., Zuur, C. L., Pang, B., Smits, A. M., Mummery, C. L., Smit, L., Arens, R., Li, J., Overkleeft, H. S. & Neefjes, J. (2020) Uncoupling DNA damage from chromatin damage to detoxify doxorubicin, Proc Natl Acad Sci U S A.

45. Schaefer, A., Westendorf, J., Lingelbach, K., Schmidt, C. A., Mihalache, D. L., Reymann, A. & Marquardt, H. (1993) Decreased resistance to N,N-dimethylated an-

thracyclines in multidrug-resistant Friend erythroleukemia cells, Cancer Chemother Pharmacol. 31, 301-7.

46. Egorin, M. J., Clawson, R. E., Ross, L. A., Chou, F. T. E., Andrews, P. A. & Bachur, N. R. (1980) Disposition and Metabolism of N,N-Dimethyldaunorubicin and N,N-Dimethyladriamycin in Rabbits and Mice, Drug Metab Dispos. 8, 353-362.

47. Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L. & Kroemer, G. (2015) Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents, Cancer Cell. 28, 690-714.

48. Obeid, M., Tesniere, A., Ghiringhelli, F., Fimia, G. M., Apetoh, L., Perfettini, J. L., Castedo, M., Mignot, G., Panaretakis, T., Casares, N., Metivier, D., Larochette, N., van Endert, P., Ciccosanti, F., Piacentini, M., Zitvogel, L. & Kroemer, G. (2007) Calreticulin exposure dictates the immunogenicity of cancer cell death, Nat Med. 13, 54-61.

49. Martins, I., Wang, Y., Michaud, M., Ma, Y., Sukkurwala, A. Q., Shen, S., Kepp, O., Metivier, D., Galluzzi, L., Perfettini, J. L., Zitvogel, L. & Kroemer, G. (2014) Molecular mechanisms of ATP secretion during immunogenic cell death, Cell Death Differ. 21, 79-91.

50. Yamazaki, T., Hannani, D., Poirier-Colame, V., Ladoire, S., Locher, C., Sistigu, A., Prada, N., Adjemian, S., Catani, J. P., Freudenberg, M., Galanos, C., Andre, F., Kroemer, G. & Zitvogel, L. (2014) Defective immunogenic cell death of HMGB1-deficient tumors: compensatory therapy with TLR4 agonists, Cell Death Differ. 21, 69-78.

51. Casares, N., Pequignot, M. O., Tesniere, A., Ghiringhelli, F., Roux, S., Chaput, N., Schmitt, E., Hamai, A., Hervas-Stubbs, S., Obeid, M., Coutant, F., Metivier, D., Pichard, E., Aucouturier, P., Pierron, G., Garrido, C., Zitvogel, L. & Kroemer, G. (2005) Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death, J Exp Med. 202, 1691-701.

52. Alizadeh, D., Trad, M., Hanke, N. T., Larmonier, C. B., Janikashvili, N., Bonnotte, B., Katsanis, E. & Larmonier, N. (2014) Doxorubicin Eliminates Myeloid-Derived Suppressor Cells and Enhances the Efficacy of Adoptive T-Cell Transfer in Breast Cancer, Cancer Research. 74, 104-118.

53. Lai, J. J., Cruz, F. M. & Rock, K. L. (2020) Immune Sensing of Cell Death through Recognition of Histone Sequences by C-Type Lectin-Receptor-2d Causes Inflammation and Tissue Injury, Immunity. 52, 123-+.

54. Voorwerk, L., Slagter, M., Horlings, H. M., Sikorska, K., van de Vijver, K. K., de Maaker, M., Nederlof, I., Kluin, R. J. C., Warren, S., Ong, S., Wiersma, T. G., Russell, N. S., Lalezari, F., Schouten, P. C., Bakker, N. A. M., Ketelaars, S. L. C., Peters, D., Lange, C. A. H., van Werkhoven, E., van Tinteren, H., Mandjes, I. A. M., Kemper, I., Onderwater, S., Chalabi, M., Wilgenhof, S., Haanen, J., Salgado, R., de Visser, K. E., Sonke, G. S., Wessels, L. F. A., Linn, S. C., Schumacher, T. N., Blank, C. U. & Kok, M. (2019) Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial, Nat Med. 25, 920-928.

55. Lefrak, E. A., Pitha, J., Rosenheim, S. & Gottlieb, J. A. (1973) A clinicopathologic analysis of adriamycin cardiotoxicity, Cancer. 32, 302-14.

56. Shan, K., Lincoff, A. M. & Young, J. B. (1996) Anthracycline-induced cardiotoxicity, Ann Intern Med. 125, 47-58.

57. Swain, S. M., Whaley, F. S. & Ewer, M. S. (2003) Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials, Cancer. 97, 2869-79.

58. Von Hoff, D. D., Layard, M. W., Basa, P., Davis, H. L., Jr., Von Hoff, A. L., Rozencweig, M. & Muggia, F. M. (1979) Risk factors for doxorubicin-induced congestive heart failure, Ann Intern Med. 91, 710-7.

59. Berthiaume, J. M. & Wallace, K. B. (2007) Adriamycin-induced oxidative mitochondrial cardiotoxicity, Cell Biol Toxicol. 23, 15-25.

60. Myers, C. E., McGuire, W. P., Liss, R. H., Ifrim, I., Grotzinger, K. & Young, R. C. (1977) Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response, Science. 197, 165-7.

61. Zhang, S., Liu, X. B., Bawa-Khalfe, T., Lu, L. S., Lyu, Y. L., Liu, L. F. & Yeh, E. T. H. (2012) Identification of the molecular basis of doxorubicin-induced cardiotoxicity, Nature Medicine. 18, 1639-+.

62. Wallace, K. B. (2007) Adriamycin-induced interference with cardiac mitochondrial calcium homeostasis, Cardiovasc Toxicol. 7, 101-7.

63. Mei, S. B., Hong, L., Cai, X. Y., Xiao, B., Zhang, P. & Shao, L. (2019) Oxidative stress injury in doxorubicin-induced cardiotoxicity, Toxicol Lett. 307, 41-48.

64. Barth, E., Stammler, G., Speiser, B. & Schaper, J. (1992) Ultrastructural quantitation of mitochondria and myofilaments in cardiac muscle from 10 different animal species including man, J Mol Cell Cardiol. 24, 669-81.

65. Green, P. S. & Leeuwenburgh, C. (2002) Mitochondrial dysfunction is an early indicator of doxorubicin-induced apoptosis, Biochim Biophys Acta. 1588, 94-101.

66. Sonneveld, P. (1978) Effect of alpha-tocopherol on the cardiotoxicity of adriamycin in the rat, Cancer Treat Rep. 62, 1033-6.

67. Legha, S. S., Wang, Y. M., Mackay, B., Ewer, M., Hortobagyi, G. N., Benjamin, R. S. & Ali, M. K. (1982) Clinical and Pharmacologic Investigation of the Effects of Alpha-Tocopherol on Adriamycin Cardiotoxicity, Ann Ny Acad Sci. 393, 411-418.

68. Dresdale, A. R., Barr, L. H., Bonow, R. O., Mathisen, D. J., Myers, C. E., Schwartz, D. E., Dangelo, T. & Rosenberg, S. A. (1982) Prospective Randomized Study of the Role of N-Acetyl Cysteine in Reversing Doxorubicin-Induced Cardiomyopathy, Am J Clin Oncol-Canc. 5, 657-663.

69. Ladas, E. J., Jacobson, J. S., Kennedy, D. D., Teel, K., Fleischauer, A. & Kelly, K. M. (2004) Antioxidants and cancer therapy: A systematic review, Journal of Clinical Oncology. 22, 517-528.

70. Speyer, J. L., Green, M. D., Sanger, J., Zeleniuchjacquotte, A., Kramer, E., Rey, M., Wernz, J. C., Blum, R. H., Hochster, H., Meyers, M. & Muggia, F. M. (1990) A Prospective Randomized Trial of Icrf-187 for Prevention of Cumulative Doxorubicin-Induced Cardiac Toxicity in Women with Breast-Cancer, Cancer Treat Rev. 17, 161-163.

 Lipshultz, S. E., Scully, R. E., Lipsitz, S. R., Sallan, S. E., Silverman, L. B., Miller, T. L., Borry, E. V., Asselin, B. L., Athale, U., Clavell, L. A., Larsen, E., Moghrabi, A., Samson, Y., Michon, B., Schorin, M. A., Cohen, H. J., Neuberg, D. S., Orav, E. J. & Colan, S. D. (2010) Assessment of dexrazoxane as a cardioprotectant in doxorubicin-treated children with high-risk acute lymphoblastic leukaemia: long-term follow-up of a prospective, randomised, multicentre trial, Lancet Oncol. 11, 950-961.
Hasinoff, B. B., Patel, D. & Wu, X. (2003) The oral iron chelator ICL670A (deferasirox) does not protect myocytes against doxorubicin, Free Radical Bio Med. 35, 1469-1479.

73. Yu, X. X., Ruan, Y., Huang, X. Q., Dou, L., Lan, M., Cui, J., Chen, B. D., Gong, H., Wang, Q., Yan, M. J., Sun, S. H., Qiu, Q., Zhang, X. Y., Man, Y., Tang, W. Q., Li, J. & Shen, T. (2020) Dexrazoxane ameliorates doxorubicin-induced cardiotoxicity by inhibiting both apoptosis and necroptosis in cardiomyocytes, Biochem Bioph Res

Co. 523, 140-146.

74. Lyu, Y. L., Kerrigan, J. E., Lin, C. P., Azarova, A. M., Tsai, Y. C., Ban, Y. & Liu, L. F. (2007) Topoisomerase II beta-Mediated DNA double-strand breaks: Implications in doxorubicin cardiotoxicity and prevention by dexrazoxane, Cancer Research. 67, 8839-8846.

75. Drake, F. H., Zimmerman, J. P., Mccabe, F. L., Bartus, H. F., Per, S. R., Sullivan, D. M., Ross, W. E., Mattern, M. R., Johnson, R. K., Crooke, S. T. & Mirabelli, C. K. (1987) Purification of Topoisomerase-Ii from Amsacrine-Resistant P388 Leukemia-Cells - Evidence for 2 Forms of the Enzyme, Journal of Biological Chemistry. 262, 16739-16747.

76. Austin, C. A. & Marsh, K. L. (1998) Eukaryotic DNA topoisomerase II beta, Bioessays. 20, 215-226.

77. Gabizon, A., Shmeeda, H. & Barenholz, Y. (2003) Pharmacokinetics of pegylated liposomal doxorubicin - Review of animal and human studies, Clin Pharmacokinet. 42, 419-436.

78. Vaage, J., Barberaguillem, E., Abra, R., Huang, A. & Working, P. (1994) Tissue Distribution and Therapeutic Effect of Intravenous Free or Encapsulated Liposomal Doxorubicin on Human Prostate Carcinoma Xenografts, Cancer. 73, 1478-1484.

79. Cabanes, A., Tzemach, D., Goren, D., Horowitz, A. T. & Gabizon, A. (1998) Comparative study of the antitumor activity of free doxorubicin and polyethylene glycol-coated liposomal doxorubicin in a mouse lymphoma model, Clin Cancer Res. 4, 499-505.

80. Harris, L., Batist, G., Belt, R., Rovira, D., Navari, R., Azarnia, N., Welles, L., Winer, E. & Group, T. D. S. (2002) Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma, Cancer. 94, 25-36.

81. Smith, L. A., Cornelius, V. R., Plummer, C. J., Levitt, G., Verrill, M., Canney, P. & Jones, A. (2010) Cardiotoxicity of anthracycline agents for the treatment of cancer: Systematic review and meta-analysis of randomised controlled trials, Bmc Cancer. 10.

82. Travis, L. B., Demark Wahnefried, W., Allan, J. M., Wood, M. E. & Ng, A. K. (2013) Aetiology, genetics and prevention of secondary neoplasms in adult cancer survivors, Nat Rev Clin Oncol. 10, 289-301.

83. Morton, L. M., Swerdlow, A. J., Schaapveld, M., Ramadan, S., Hodgson, D. C., Radford, J. & van Leeuwen, F. E. (2014) Current knowledge and future research directions in treatment-related second primary malignancies, EJC Suppl. 12, 5-17.

84. Bertazzoli, C., Chieli, T. & Solcia, E. (1971) Different incidence of breast carcinomas or fibroadenomas in daunomycin or adriamycin treated rats, Experientia. 27, 1209-10.

85. Sternberg, S. S., Philips, F. S. & Cronin, A. P. (1972) Renal tumors and other lesions in rats following a single intravenous injection of daunomycin, Cancer Res. 32, 1029-36.

86. Price, P. J., Suk, W. A., Skeen, P. C., Chirigos, M. A. & Huebner, R. J. (1975) Transforming potential of the anticancer drug adriamycin, Science. 187, 1200-1.

87. Philips, F. S., Gilladoga, A., Marquardt, H., Stemberg, S. S. & Vidal, P. R. (1975) Some Observations on the Toxicity of Adriamycin.

88. Marquardt, H., Philips, F. S. & Sternberg, S. S. (1976) Tumorigenicity in vivo and induction of malignant transformation and mutagenesis in cell cultures by adriamycin and daunomycin, Cancer Res. 36, 2065-9.

89. Solcia, E., Ballerini, L., Bellini, O., Sala, L. & Bertazzoli, C. (1978) Mammary

tumors induced in rats by adriamycin and daunomycin, Cancer Res. 38, 1444-6. 90. (1987) Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42, IARC Monogr Eval Carcinog Risks Hum Suppl. 7, 1-440.

91. Diamandidou, E., Buzdar, A. U., Smith, T. L., Frye, D., Witjaksono, M. & Hortobagyi, G. N. (1996) Treatment-related leukemia in breast cancer patients treated with fluorouracil-doxorubicin-cyclophosphamide combination adjuvant chemotherapy: the University of Texas M.D. Anderson Cancer Center experience, J Clin Oncol. 14, 2722-30.

92. Praga, C., Bergh, J., Bliss, J., Bonneterre, J., Cesana, B., Coombes, R. C., Fargeot, P., Folin, A., Fumoleau, P., Giuliani, R., Kerbrat, P., Hery, M., Nilsson, J., Onida, F., Piccart, M., Shepherd, L., Therasse, P., Wils, J. & Rogers, D. (2005) Risk of acute myeloid leukemia and myelodysplastic syndrome in trials of adjuvant epirubicin for early breast cancer: correlation with doses of epirubicin and cyclophosphamide, J Clin Oncol. 23, 4179-91.

93. Le Deley, M. C., Suzan, F., Cutuli, B., Delaloge, S., Shamsaldin, A., Linassier, C., Clisant, S., de Vathaire, F., Fenaux, P. & Hill, C. (2007) Anthracyclines, mitoxantrone, radiotherapy, and granulocyte colony-stimulating factor: risk factors for leukemia and myelodysplastic syndrome after breast cancer, J Clin Oncol. 25, 292-300.

94. Henderson, T. O., Whitton, J., Stovall, M., Mertens, A. C., Mitby, P., Friedman, D., Strong, L. C., Hammond, S., Neglia, J. P., Meadows, A. T., Robison, L. & Diller, L. (2007) Secondary sarcomas in childhood cancer survivors: a report from the Childhood Cancer Survivor Study, J Natl Cancer Inst. 99, 300-8.

95. Henderson, T. O., Rajaraman, P., Stovall, M., Constine, L. S., Olive, A., Smith, S. A., Mertens, A., Meadows, A., Neglia, J. P., Hammond, S., Whitton, J., Inskip, P. D., Robison, L. L. & Diller, L. (2012) Risk factors associated with secondary sarcomas in childhood cancer survivors: a report from the childhood cancer survivor study, Int J Radiat Oncol Biol Phys. 84, 224-30.

96. Teepen, J. C., van Leeuwen, F. E., Tissing, W. J., van Dulmen-den Broeder, E., van den Heuvel-Eibrink, M. M., van der Pal, H. J., Loonen, J. J., Bresters, D., Versluys, B., Neggers, S., Jaspers, M. W. M., Hauptmann, M., van der Heiden-van der Loo, M., Visser, O., Kremer, L. C. M., Ronckers, C. M. & Group, D. L. S. (2017) Long-Term Risk of Subsequent Malignant Neoplasms After Treatment of Childhood Cancer in the DCOG LATER Study Cohort: Role of Chemotherapy, J Clin Oncol. 35, 2288-2298.

97. van Leeuwen, F. E. & Ronckers, C. M. (2016) Anthracyclines and Alkylating Agents: New Risk Factors for Breast Cancer in Childhood Cancer Survivors?, J Clin Oncol. 34, 891-4.

98. Veiga, L. H., Bhatti, P., Ronckers, C. M., Sigurdson, A. J., Stovall, M., Smith, S. A., Weathers, R., Leisenring, W., Mertens, A. C., Hammond, S., Neglia, J. P., Meadows, A. T., Donaldson, S. S., Sklar, C. A., Friedman, D. L., Robison, L. L. & Inskip, P. D. (2012) Chemotherapy and thyroid cancer risk: a report from the childhood cancer survivor study, Cancer Epidemiol Biomarkers Prev. 21, 92-101.

99. Beaumont, M., Sanz, M., Carli, P. M., Maloisel, F., Thomas, X., Detourmignies, L., Guerci, A., Gratecos, N., Rayon, C., San Miguel, J., Odriozola, J., Cahn, J. Y., Huguet, F., Vekhof, A., Stamatoulas, A., Dombret, H., Capote, F., Esteve, J., Stoppa, A. M. & Fenaux, P. (2003) Therapy-related acute promyelocytic leukemia, J Clin Oncol. 21, 2123-37.

100. Rashidi, A. & Fisher, S. I. (2013) Therapy-related acute promyelocytic leukemia: a systematic review, Med Oncol. 30, 625.

101. Pedersen-Bjergaard, J. & Philip, P. (1991) Balanced translocations involving

chromosome bands 11q23 and 21q22 are highly characteristic of myelodysplasia and leukemia following therapy with cytostatic agents targeting at DNA-topoisomerase II, Blood. 78, 1147-8.

102. Larson, R. A., Le Beau, M. M., Ratain, M. J. & Rowley, J. D. (1992) Balanced translocations involving chromosome bands 11q23 and 21q22 in therapy-related leukemia, Blood. 79, 1892-3.

103. Felix, C. A., Kolaris, C. P. & Osheroff, N. (2006) Topoisomerase II and the etiology of chromosomal translocations, DNA Repair (Amst). 5, 1093-108.

104. Cowell, I. G. & Austin, C. A. (2012) Mechanism of generation of therapy related leukemia in response to anti-topoisomerase II agents, Int J Environ Res Public Health. 9, 2075-91.

105. Pendleton, M., Lindsey, R. H., Jr., Felix, C. A., Grimwade, D. & Osheroff, N. (2014) Topoisomerase II and leukemia, Ann N Y Acad Sci. 1310, 98-110.

106. Park, S. H., Chi, H. S., Cho, Y. U., Jang, S. & Park, C. J. (2013) Evaluation of prognostic factors in patients with therapy-related acute myeloid leukemia, Blood Res. 48, 185-92.

107. Kayser, S., Dohner, K., Krauter, J., Kohne, C. H., Horst, H. A., Held, G., von Lilienfeld-Toal, M., Wilhelm, S., Kundgen, A., Gotze, K., Rummel, M., Nachbaur, D., Schlegelberger, B., Gohring, G., Spath, D., Morlok, C., Zucknick, M., Ganser, A., Dohner, H., Schlenk, R. F. & German-Austrian, A. (2011) The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML, Blood. 117, 2137-45.

108. Basecke, J., Cepek, L., Mannhalter, C., Krauter, J., Hildenhagen, S., Brittinger, G., Trumper, L. & Griesinger, F. (2002) Transcription of AML1/ETO in bone marrow and cord blood of individuals without acute myelogenous leukemia, Blood. 100, 2267-8.

109. Mori, H., Colman, S. M., Xiao, Z., Ford, A. M., Healy, L. E., Donaldson, C., Hows, J. M., Navarrete, C. & Greaves, M. (2002) Chromosome translocations and covert leukemic clones are generated during normal fetal development, Proc Natl Acad Sci U S A. 99, 8242-7.

110. Schnittger, S., Bacher, U., Kern, W., Haferlach, C. & Haferlach, T. (2007) JAK2 seems to be a typical cooperating mutation in therapy-related t(8;21)/ AML1-ETO-positive AML, Leukemia. 21, 183-4.

111. Stanulla, M., Wang, J., Chervinsky, D. S., Thandla, S. & Aplan, P. D. (1997) DNA cleavage within the MLL breakpoint cluster region is a specific event which occurs as part of higher-order chromatin fragmentation during the initial stages of apoptosis, Mol Cell Biol. 17, 4070-9.

112. Betti, C. J., Villalobos, M. J., Diaz, M. O. & Vaughan, A. T. (2001) Apoptotic triggers initiate translocations within the MLL gene involving the nonhomologous end joining repair system, Cancer Res. 61, 4550-5.

113. Betti, C. J., Villalobos, M. J., Diaz, M. O. & Vaughan, A. T. (2003) Apoptotic stimuli initiate MLL-AF9 translocations that are transcribed in cells capable of division, Cancer Res. 63, 1377-81.

114. Mays, A. N., Osheroff, N., Xiao, Y., Wiemels, J. L., Felix, C. A., Byl, J. A., Saravanamuttu, K., Peniket, A., Corser, R., Chang, C., Hoyle, C., Parker, A. N., Hasan, S. K., Lo-Coco, F., Solomon, E. & Grimwade, D. (2010) Evidence for direct involvement of epirubicin in the formation of chromosomal translocations in t(15;17) therapy-related acute promyelocytic leukemia, Blood. 115, 326-30.

115. Kudo, K., Yoshida, H., Kiyoi, H., Numata, S., Horibe, K. & Naoe, T. (1998) Etoposide-related acute promyelocytic leukemia, Leukemia. 12, 1171-5. 116. Le Deley, M. C., Leblanc, T., Shamsaldin, A., Raquin, M. A., Lacour, B., Sommelet, D., Chompret, A., Cayuela, J. M., Bayle, C., Bernheim, A., de Vathaire, F., Vassal, G., Hill, C. & Societe Francaise d'Oncologie, P. (2003) Risk of secondary leukemia after a solid tumor in childhood according to the dose of epipodophyllotoxins and anthracyclines: a case-control study by the Societe Francaise d'Oncologie Pediatrique, J Clin Oncol. 21, 1074-81.

117. Westendorf, J., Marquardt, H., Ketkar, M. B., Mohr, U. & Marquardt, H. (1983) Tumorigenicity in vivo and induction of mutagenesis and DNA repair in vitro by aclacinomycin A and marcellomycin: structure-activity relationship and predictive value of short-term tests, Cancer Res. 43, 5248-51.

118. Westendorf, J., Marquardt, H. & Marquardt, H. (1984) Structure-activity relationship of anthracycline-induced genotoxicity in vitro, Cancer Res. 44, 5599-604.

119. Del Rizzo, P. A. & Trievel, R. C. (2011) Substrate and product specificities of SET domain methyltransferases, Epigenetics. 6, 1059-67.

120. Jakovcevski, M., Ruan, H., Shen, E. Y., Dincer, A., Javidfar, B., Ma, Q., Peter, C. J., Cheung, I., Mitchell, A. C., Jiang, Y., Lin, C. L., Pothula, V., Stewart, A. F., Ernst, P., Yao, W. D. & Akbarian, S. (2015) Neuronal Kmt2a/Mll1 histone methyltransferase is essential for prefrontal synaptic plasticity and working memory, J Neurosci. 35, 5097-108.

121. Xu, J., Li, L., Xiong, J., denDekker, A., Ye, A., Karatas, H., Liu, L., Wang, H., Qin, Z. S., Wang, S. & Dou, Y. (2016) MLL1 and MLL1 fusion proteins have distinct functions in regulating leukemic transcription program, Cell Discov. 2, 16008.

122. Zuber, J., Radtke, I., Pardee, T. S., Zhao, Z., Rappaport, A. R., Luo, W., Mc-Currach, M. E., Yang, M. M., Dolan, M. E., Kogan, S. C., Downing, J. R. & Lowe, S. W. (2009) Mouse models of human AML accurately predict chemotherapy response, Genes Dev. 23, 877-89.

123. Estey, E. & Dohner, H. (2006) Acute myeloid leukaemia, Lancet. 368, 1894-907.

124. Erttmann, R., Munchmeyer, M., Looft, G. & Winkler, K. (1991) Conserved cytostatic activity of aclarubicin in a doxorubicin selected Friend leukaemia cell line with multifactorial multidrug resistance, Eur J Cancer. 27, 1064.

125. Natale, R. B., Cody, R. L., Simon, M. S. & Wheeler, R. H. (1993) An in vivo and in vitro trial of aclarubicin in metastatic breast cancer: a novel approach to the study of analogs, Cancer Chemother Pharmacol. 31, 485-8.

126. Lehne, G., De Angelis, P., Clausen, O. P. & Rugstad, H. E. (1996) Human hepatoma cells rich in P-glycoprotein are sensitive to aclarubicin and resistant to three other anthracyclines, Br J Cancer. 74, 1719-29.

127. Liu, L., Qu, Q., Jiao, W., Zhang, Y., Li, X., Ding, C. & Wu, D. (2015) Increasing aclarubicin dose in low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor (CAG regimen) is efficacious as salvage chemotherapy for relapsed/refractory mixed-phenotype acute leukemia, Leukemia research. 39, 805-11.

128. Qu, Q., Liu, L., Zhang, Y., Li, X. & Wu, D. (2015) Increasing aclarubicin dosage of the conventional CAG (low-dose cytarabine and aclarubicin in combination with granulocyte colony-stimulating factor) regimen is more efficacious as a salvage therapy than CAG for relapsed/refractory acute myeloid leukemia, Leukemia research. 39, 1353-9.

129. Delessard, M., Saulnier, J., Rives, A., Dumont, L., Rondanino, C. & Rives, N. (2020) Exposure to Chemotherapy During Childhood or Adulthood and Consequences on Spermatogenesis and Male Fertility, International journal of molecular

sciences. 21.

130. European Society for Human, R., Embryology Guideline Group on, P. O. I., Webber, L., Davies, M., Anderson, R., Bartlett, J., Braat, D., Cartwright, B., Cifkova, R., de Muinck Keizer-Schrama, S., Hogervorst, E., Janse, F., Liao, L., Vlaisavljevic, V., Zillikens, C. & Vermeulen, N. (2016) ESHRE Guideline: management of women with premature ovarian insufficiency, Hum Reprod. 31, 926-37.

131. Viviani, S., Santoro, A., Ragni, G., Bonfante, V., Bestetti, O. & Bonadonna, G. (1985) Gonadal toxicity after combination chemotherapy for Hodgkin's disease. Comparative results of MOPP vs ABVD, Eur J Cancer Clin Oncol. 21, 601-5.

132. Byrne, J., Fears, T. R., Gail, M. H., Pee, D., Connelly, R. R., Austin, D. F., Holmes, G. F., Holmes, F. F., Latourette, H. B., Meigs, J. W. & et al. (1992) Early menopause in long-term survivors of cancer during adolescence, Am J Obstet Gynecol. 166, 788-93.

133. Green, D. M., Whitton, J. A., Stovall, M., Mertens, A. C., Donaldson, S. S., Ruymann, F. B., Pendergrass, T. W. & Robison, L. L. (2002) Pregnancy outcome of female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study, Am J Obstet Gynecol. 187, 1070-80.

134. Ben-Aharon, I., Bar-Joseph, H., Tzarfaty, G., Kuchinsky, L., Rizel, S., Stemmer, S. M. & Shalgi, R. (2010) Doxorubicin-induced ovarian toxicity, Reprod Biol Endocrinol. 8, 20.

135. Anderson, R. A., Mitchell, R. T., Kelsey, T. W., Spears, N., Telfer, E. E. & Wallace, W. H. (2015) Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults, Lancet Diabetes Endocrinol. 3, 556-67.

136. Brilhante, O., Stumpp, T. & Miraglia, S. J. I. J. M. S. (2011) Long-term testicular toxicity caused by doxorubicin treatment during pre-pubertal phase. 3, 52-60.

137. Manabe, F., Takeshima, H. & Akaza, H. (1997) Protecting spermatogenesis from damage induced by doxorubicin using the luteinizing hormone-releasing hormone agonist leuprorelin: an image analysis study of a rat experimental model, Cancer. 79, 1014-21.

138. Kropp, J., Roti Roti, E. C., Ringelstetter, A., Khatib, H., Abbott, D. H. & Salih, S. M. (2015) Dexrazoxane Diminishes Doxorubicin-Induced Acute Ovarian Damage and Preserves Ovarian Function and Fecundity in Mice, PloS one. 10, e0142588.

139. Levi, M., Tzabari, M., Savion, N., Stemmer, S. M., Shalgi, R. & Ben-Aharon, I. (2015) Dexrazoxane exacerbates doxorubicin-induced testicular toxicity, Reproduction. 150, 357-66.

140. Roti Roti, E. C., Ringelstetter, A. K., Kropp, J., Abbott, D. H. & Salih, S. M. (2014) Bortezomib prevents acute doxorubicin ovarian insult and follicle demise, improving the fertility window and pup birth weight in mice, PloS one. 9, e108174.

141. Tuppi, M., Kehrloesser, S., Coutandin, D. W., Rossi, V., Luh, L. M., Strubel, A., Hotte, K., Hoffmeister, M., Schafer, B., De Oliveira, T., Greten, F., Stelzer, E. H. K., Knapp, S., De Felici, M., Behrends, C., Klinger, F. G. & Dotsch, V. (2018) Oocyte DNA damage quality control requires consecutive interplay of CHK2 and CK1 to activate p63, Nat Struct Mol Biol. 25, 261-269.

142. Perez, G. I., Knudson, C. M., Leykin, L., Korsmeyer, S. J. & Tilly, J. L. (1997) Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction, Nature medicine. 3, 1228-32.

143. Roti Roti, E. C., Leisman, S. K., Abbott, D. H. & Salih, S. M. (2012) Acute doxorubicin insult in the mouse ovary is cell- and follicle-type dependent, PloS one. 7, e42293.

144. Beaud, H., van Pelt, A. & Delbes, G. (2017) Doxorubicin and vincristine affect undifferentiated rat spermatogonia, Reproduction. 153, 725-735.

145. Marcello, M. F., Nuciforo, G., Romeo, R., Di Dino, G., Russo, I., Russo, A., Palumbo, G. & Schiliro, G. (1990) Structural and ultrastructural study of the ovary in childhood leukemia after successful treatment, Cancer. 66, 2099-104.

146. Kobayashi, H., Urashima, M., Hoshi, Y., Uchiyama, H., Fujisawa, K., Akatsuka, J., Maekawa, K. & Hurusato, M. (1996) Testicular morphological changes in children with acute lymphoblastic leukemia following chemotherapy, Acta Paediatr Jpn. 38, 640-3.

147. Brilhante, O., Okada, F. K., Sasso-Cerri, E., Stumpp, T. & Miraglia, S. M. (2012) Late morfofunctional alterations of the Sertoli cell caused by doxorubicin administered to prepubertal rats, Reprod Biol Endocrinol. 10, 79.

148. Stumpp, T., Sasso-Cerri, E., Freymuller, E. & Miraglia, S. M. (2004) Apoptosis and testicular alterations in albino rats treated with etoposide during the prepubertal phase, Anat Rec A Discov Mol Cell Evol Biol. 279, 611-22.

149. Ortiz, R. J., Lizama, C., Codelia, V. A. & Moreno, R. D. (2009) A molecular evaluation of germ cell death induced by etoposide in pubertal rat testes, Mol Hum Reprod. 15, 363-71.

150. Bar-Joseph, H., Ben-Aharon, I., Rizel, S., Stemmer, S. M., Tzabari, M. & Shalgi, R. (2010) Doxorubicin-induced apoptosis in germinal vesicle (GV) oocytes, Reprod Toxicol. 30, 566-72.

151. Tremblay, A. R. & Delbes, G. (2018) In vitro study of doxorubicin-induced oxidative stress in spermatogonia and immature Sertoli cells, Toxicol Appl Pharmacol. 348, 32-42.

152. Hou, M., Chrysis, D., Nurmio, M., Parvinen, M., Eksborg, S., Soder, O. & Jahnukainen, K. (2005) Doxorubicin induces apoptosis in germ line stem cells in the immature rat testis and amifostine cannot protect against this cytotoxicity, Cancer research. 65, 9999-10005.