Short Communications

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Emerging Research and Future Directions on Doxorubicin: A Snapshot

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Abstract

Doxorubicin, a widely used anthracycline antibiotic, has been a cornerstone in cancer chemotherapy since the 1960s. In addition to doxorubicin, anthracycline chemotherapy medications include daunorubicin, idarubicin, and epirubicin. For many years, doxorubicin has been the chemotherapy drug of choice for treating a broad variety of cancers. Despite its efficacy, doxorubicin therapy is hindered by serious side effects, primarily cardiotoxicity, and the challenges of drug resistance. Recent research has focused on optimizing doxorubicin's therapeutic index by developing cardioprotective strategies, such as dexrazoxane, and utilizing non-invasive monitoring techniques to reduce cardiac risk. To counteract drug resistance, innovative formulations like nanoparticle-based delivery systems, enhance targeted drug delivery and overcome cellular resistance mechanisms. Furthermore, using combination approaches involving immunotherapy, photodynamic therapy, and genetic modulation, offer promising synergies to maximize tumor eradication. Personalized approaches, supported by pharmacogenomics and predictive biomarkers, are enhancing individualized treatment regimens, aiming to increase effectiveness and minimize toxicity. Future research on doxorubicin focuses on developing advanced drug delivery systems, such as nanoparticle and liposomal formulations, to enhance targeted delivery, minimize systemic toxicity, and improve therapeutic precision. Efforts are also underway to design combination therapies that integrate doxorubicin with immunotherapies, photodynamic approaches, and gene-based treatments, aiming to overcome resistance and increase tumor-specific effects. These advancements signify a transition toward more personalized and effective doxorubicin-based cancer therapies, prioritizing reduced side effects and improved patient outcomes. This article focusses on the ongoing innovations aimed at maximizing the therapeutic potential of doxorubicin while addressing its limitations.

Keywords: Anthracycline- Doxorubicin- Cardiotoxicity- Drug Resistance- Cancers- Photodynamic therapy

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Introduction

Doxorubicin, a potent anthracycline antibiotic derived from Streptomyces peucetius, has been a cornerstone in oncology since its introduction in the 1960s. Alongside other anthracyclines such as daunorubicin, idarubicin, and epirubicin, it is widely utilized for the treatment of diverse malignancies, including breast cancer, lymphomas, and leukemias. Its mechanism of action primarily involves DNA intercalation and inhibition of topoisomerase II, leading to disruption of DNA replication and transcription in cancer cells. Apart from advantages, Doxorubicin (DOX) has limitations such as cardiotoxicity, drug resistance, limited efficacy in certain tumor types, and the risk of secondary malignancies. To address these limitations, researchers worldwide are exploring novel approaches to maximize the therapeutic potential of doxorubicin and minimize its toxicity. This review highlights current advancements in doxorubicin research, with an emphasis on novel approaches aimed at overcoming its inherent limitations and optimizing its therapeutic potential in cancer management.

An important area of research is the reduction of doxorubicin-induced cardiotoxicity. The use of cardioprotective agents and cardiotoxicity monitoring are the two approaches that are being probed to achieve this goal. Cardioprotective drugs, like dexrazoxane, can reduce cardiac injury without compromising the anticancer efficacy of doxorubicin. Enhanced imaging techniques and novel biomarkers can help in non-invasive monitoring of cardiac function, thereby enabling early detection and management of cardiotoxicity.

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Reversing drug resistance is another key research priority for doxorubicin. Combination regimens that target signaling pathways associated with drug resistance, such as PI3K/AKT and MAPK, to sensitize doxorubicin-resistant cancer cells are being explored. Specific inhibitors of drug efflux transporters like P-glycoprotein, which enhance the intracellular accumulation and cytotoxicity of doxorubicin, are also being studied.

Innovative formulations and prodrug strategies are being actively investigated to improve doxorubicin delivery, minimize toxicity, and enhance therapeutic efficacy. Nanotechnology-based drug delivery platforms offer significant potential to increase doxorubicin's precision and effectiveness by reducing off-target effects. Liposomal formulations of doxorubicin have demonstrated improved pharmacokinetics and reduced cardiotoxicity in clinical applications. Additionally, prodrug modifications involve the development of inactive doxorubicin analogs that are selectively activated within the tumor microenvironment, thereby minimizing systemic toxicity while maintaining therapeutic potency.

Further, combining doxorubicin with other pharmaceuticals, such as immunotherapies and targeted therapies, is being investigated to overcome resistance and enhance treatment responses. The combination of doxorubicin with immune checkpoint inhibitors, for example, may enhance immune response against cancer and improve outcomes. The tumor microenvironment plays a crucial role in cancer progression, therapeutic response, and resistance. Targeting the microenvironment can enhance doxorubicin action and sensitize cancer cells to its effects. Combination therapies that target both cancer cells and the microenvironment have the potential to overcome resistance and improve treatment outcomes.

Personalized medicine is driving the evolution of doxorubicin research. Gene expression profiles, genetic polymorphisms, and microRNAs are being analyzed to find novel predictive and prognostic biomarkers to anticipate individual doxorubicin response and adverse effects. Utilizing these biomarkers to personalize dosage and treatment methods could maximize efficacy and minimize side effects.

Enhancing Safety and Efficacy of Doxorubicin

The concern surrounding cardiotoxicity remains significant when using doxorubicin for treatment. Doxorubicin induces cardiomyopathy, leading to congestive heart failure in a variable proportion of patients, depending on duration of treatment and underlying morbidities. The effect is dose-dependent and cumulative. It limits the extent to which they can be used safely. The incidence of heart failure is around 5% at a cumulative dose of 400mg/m^2 and rises exponentially at higher aggregate doses [1]. With chronic administration, cancer survivors are exposed to the risk of increased cardiovascular morbidity and mortality even years after treatment and with lower cumulative doses [2]. The principle mechanisms underlying doxorubicin cardiotoxicity are reactive oxygen species (ROS) generation and myocardial cell apoptosis. Depletion of sarcoplasmic reticulum Ca2+, increased Nitric oxide synthase (eNOS), mitochondrial dysfunction, dysregulation of autophagy, doxorubicin-iron complexes, topoisomerase II beta inhibition are among the proposed mechanisms for apoptosis (Figure 1) [3].

Cardioprotective agents

Recent research has been focused on developing strategies to mitigate doxorubicin-induced cardiotoxicity while preserving its effectiveness against cancer. One approach involves the use of cardioprotective agents, which have shown promise in reducing cardiac toxicity without compromising doxorubicin's ability to fight cancer [2].

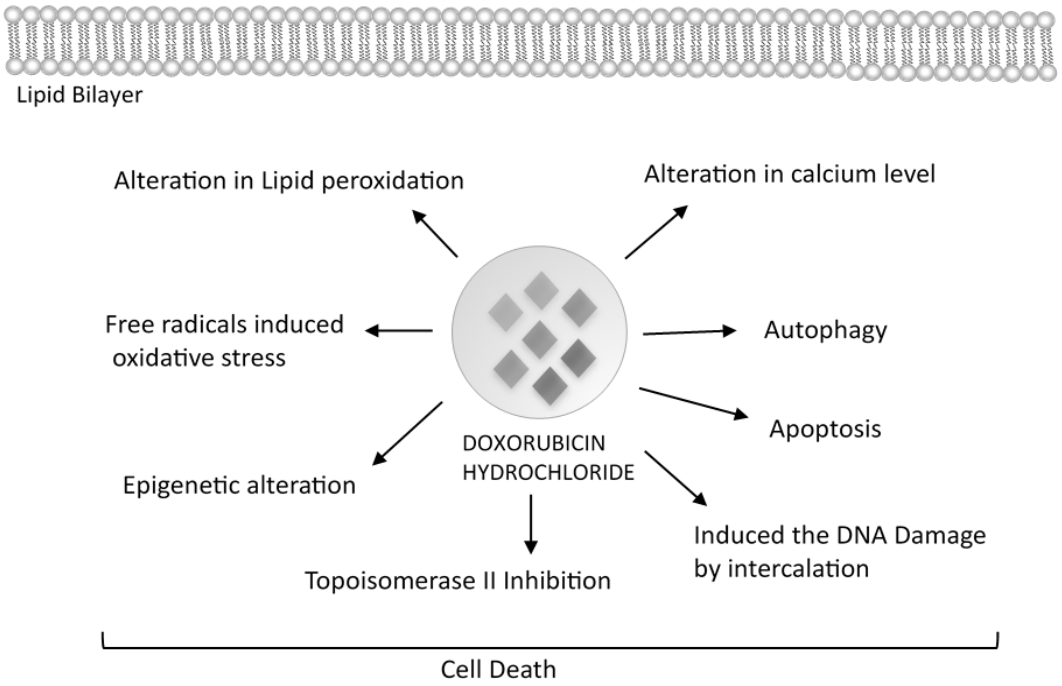

Figure 1. Different Mechanisms of Action of Doxorubicin-Induced Cell Death.

Dexrazoxane, an iron chelator, has shown cardioprotective efficacy in patients undergoing doxorubicin-based chemotherapy regimens. By intracellular iron chelation, Dexrazoxane inhibits the formation of iron-mediated oxygen free radicals. It is also known to interfere with the topoisomerase II beta inhibiting activity of anthracyclines [4]. Carvedilol, a non-selective β-adrenergic antagonist with some α-antagonist and antioxidant effects, as monotherapy or in combination with ACE inhibitors (Enalapril) has reduced cardiac morbidity [5]. Studies on additional cardiotoxicity mechanisms, apart from oxidative stress, can help discover potential novel candidates for use as cardioprotective agents in patients on doxorubicin treatment. Several pre-clinical trials have shown that Doxorubicin (Dox)-induced cardiotoxicity can be effectively managed. Sadek et al. demonstrated the protective impact of proanthocyanidin on Dox-induced cardiotoxicity in rats by enhancing antioxidant levels and reducing inflammation. Proanthocyanidin was found to downregulate α-SMA and TGFβ1 expression while upregulating CDK4 and Rb protein expression, and it also reduced the activity of the NF-kB signaling pathway [6]. Additionally, Nie et al. reported that hydrogen sulfide treatment reduced endoplasmic reticulum stress and autophagy, leading to an increase in PI3K/AKT/mTOR protein expression, ultimately reducing myocardial fibrosis in Dox-treated rats [7]. The anti-allergy drug Tranilast improved Dox-induced myocardial hypertrophy and cardiac dysfunction by suppressing chymase expression, reducing angiotensin II levels, and modulating antioxidant levels. This intervention effectively prevented apoptosis and cardiomyocyte fibrosis [8]. Furthermore, Ahmed et al. demonstrated the beneficial effects of methyl gallate in mitigating oxidative stress, cardiac injury, ECG abnormalities, and the modulation of cardiac-related biochemical parameters in Dox-induced alterations in rats [9]. Finally, a bioactive peptide known as Xinmailong, extracted from American cockroaches, alleviated Dox-induced oxidative stress, enhanced antioxidant activity, upregulated *HO-1* expression, restored lysosomal function, and improved autophagy flux block in Dox-treated H9c2 cells [10].

Cardiotoxicity monitoring

Despite the introduction of cardioprotective agents, cardiotoxicity remains an important concern with doxorubicin therapy. Monitoring for early detection before the clinical onset of cardiomyopathy is of paramount importance in the management of patients receiving anthracycline-based chemotherapy. This allows early intervention at the subclinical stages for better outcomes. Traditionally, serial echocardiography (2D ECHO) based monitoring of cardiac function has been used for cardiotoxicity screening in patients receiving chemotherapy. In absence of clinical symptoms, cardiotoxicity is generally defined as a fall in left ventricular ejection fraction (LVEF) by more than 10%, or from baseline to less than 55% (or the lower limit of normal of the ECHO lab). Novel imaging methods like cardiac magnetic resonance imaging (CMR), as well as the utilization of biomarkers such as troponin and B-type natriuretic peptide (BNP), allow for early identification and intervention in individuals at risk of developing cardiotoxicity [2, 11]. These efforts aim to enhance doxorubicin's safety profile and improve long-term cardiovascular outcomes for cancer patients.

Advancements in Drug Resistance Reversal

The development of drug resistance presents a significant challenge to the effectiveness of doxorubicin in cancer treatment. Recent research has been dedicated to understanding the mechanisms underlying drug resistance and developing strategies to overcome it. Doxorubicin resistance is a multipronged phenomenon, and it results from reduced accumulation of the drug in the nucleus, reduced DNA damage and subsequent suppression of apoptotic pathways. Combination therapies that target these resistance mechanisms have shown promise in augmenting the anticancer effects of doxorubicin [3, 12].

Inhibition of Drug Efflux Pumps
P-glycoprotein

Multidrug resistance, a significant obstacle in cancer treatment, is often linked to the overexpression of drug efflux pumps like P-glycoprotein (P-gp), a member of the protein family of ATP-binding cassette (ABC) transporters, and responsible for actively transporting medications out of cancer cells [13, 14]. Inhibiting these efflux pumps presents an opportunity to enhance treatment outcomes by increasing intracellular drug concentrations [15,16]. Currently, various strategies are under investigation to inhibit P-gp and influence its role in doxorubicin resistance. Combining doxorubicin with a P-gp inhibitor, such as verapamil or cyclosporine, has shown promising results in animal models and early human studies [17]. Moreover, nanotechnology-based delivery systems, like nanoparticles or liposomes, can be developed to bypass efflux pumps and directly deliver doxorubicin into cancer cells, thereby circumventing drug resistance mechanisms [15, 18, 19, 20].

Targeting Signaling Pathways Associated with Drug Resistance

Drug resistance in cancer often arises due to disruptions in signaling pathways within cancer cells. Researchers are exploring innovative approaches to target these pathways and restore doxorubicin sensitivity. The rational design of these combination regimens involves identifying specific resistance pathways and selecting medications that can bypass these processes effectively. For instance, combining doxorubicin with DNA repair enzyme inhibitors like poly (ADP-ribose) polymerase (PARP) inhibitors has shown promise in both preclinical models and clinical trials [21]. Targeted treatments that disrupt specific signaling pathways associated with drug resistance, such as the PI3K/AKT/mTOR and JAK/STAT pathways, have demonstrated improved treatment outcomes when combined with doxorubicin [22, 23]. In preclinical studies, MAPK and STAT3 inhibitors, have shown promise when used in combination with doxorubicin [23, 24]. The investigation of epigenetic modulators, like histone deacetylase inhibitors, aims

to reverse drug resistance by altering gene expression patterns [25]. In cells exposed to DOX, the activation of Nrf2 signaling serves as a protective mechanism against cell death. Nrf2 is regulated by various upstream mediators in the context of DOX resistance. Given the pivotal role of Nrf2 signaling in potentially triggering resistance to DOX, significant attention has been focused on targeting this pathway to reverse chemoresistance. To address this objective, Singh and their colleagues have developed a small molecule inhibitor of Nrf2 signaling called ML385, which binds to the Neh1 domain of Nrf2 and impedes its DNA binding activity. This action results in an enhancement of DOX's anti-tumor efficacy against lung cancer cells [26]. In a separate study conducted by Mutlu et al., the analysis revealed differential expression of 186 genes in doxorubicin-sensitive and -resistant HeLa and K562 cell lines. Besides genes associated with the Wnt signaling pathway, genes responsible for regulating cell cycle, apoptosis, and drug metabolism (CDKN1A, CCND1, BAX, FAS), growth factors and their receptors (FGF2, IGF1R, IGF2R, MET), various transcription factors (HIF1A, NFKB1, NFKB2, RANK), as well as enzymes like CYP2E1 and CYP3A5, exhibited significant differences between the doxorubicin-resistant HeLa and K562 cell lines [27]. By addressing the dysregulated signaling pathways that contribute to drug resistance, researchers are striving to make significant advancements in cancer therapy, improving the effectiveness of doxorubicin and potentially leading to better patient outcomes. These innovative approaches hold the potential to enhance the efficacy of doxorubicin and overcome drug resistance in cancer patients, offering new possibilities in the fight against cancer [17].

Novel Anthracycline Analogues and tumor targeted formulations

Strategies for improving the efficacy and safety of anthracyclines have moved along two domains: development of novel structural analogues and development of tumor-targeted formulations.

Designing Safer and More Effective Anthracycline Analogues

Much scientific work is focused on modifying the chemical structure of anthracyclines, to improve their therapeutic index and cardiotoxicity, while preserving their anticancer activity. These studies are guided by the biophysical principles of rational drug design and structure-function relationships.

One approach involves designing analogues with altered sugar moieties or amino sugar substitutions, which can influence drug stability, cellular absorption, and cardiotoxicity [28]. Modified analogues of doxorubicin are relatively less cardiotoxic than conventional doxorubicin. Epirubicin is a semisynthetic derivative of doxorubicin obtained by epimerization of a hydroxyl group in the amino sugar moiety (Figure 2). Due to better clearance and shorter half-life, it can be administered at higher cumulative doses and exhibits lower incidence of cardiotoxicity [29]. Recent research has been concentrated on altering the substituents on the anthracycline core structure to improve drug stability, lipophilicity, and cellular uptake. For instance, introducing lipophilic side chains or utilizing prodrug approaches can enhance intracellular accumulation of anthracyclines and overcome drug resistance mechanisms [30]. Additionally, researchers are studying structural modifications in the DNA-binding region of anthracyclines to increase their selectivity for cancer cells and reduce off-target effects [29, 30, 31].

Tumor targeted formulations

Targeted therapy utilizing novel drug delivery techniques to enhance the effectiveness and safety profile of doxorubicin has been a focus area in anthracycline research. The broad categories of research in this area are: i) carrier formulations like liposomal drug delivery systems that preferentially distribute the drug within tumors, and ii) conjugated carriers which specifically recognize targets/ligands on tumor cells for delivery (Figure 3).

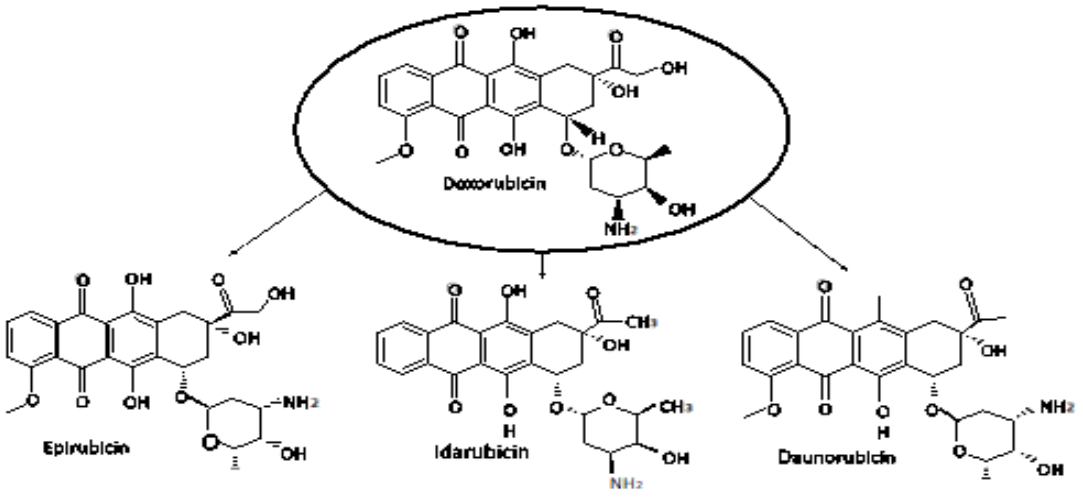

Figure 2. Doxorubicin and Its derivatives Epirubicin, Idarubicin and Daunorubicin is Derived from an Axial-to-Equatorial Epimerization of Hydroxyl Group (adapted from Minotti et al, 2004).

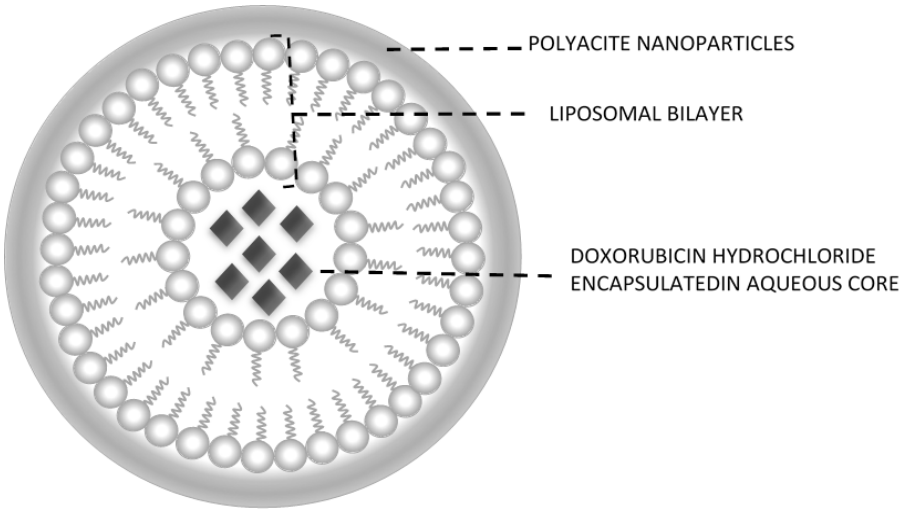

Figure 3. Schematic of PEGylated Liposomal Doxorubicin (PLD) Structure. It consists of a Doxorubicin loaded core within a liposomal formulation protected by methyoxypolyethylene glycol (Modified from SA Engelberth et al, Crit Rev Oncol, 2014)

Liposomal preparations of doxorubicin enhance drug delivery and bioavailability at the specific target organ, with reduced exposure to healthy tissues [31]. A polyethylene glycol (PEG) coating around the liposome bilayer further serves to protect the molecule from phagocytosis by mononuclear cells improving the plasma half-life. Pegylated liposomal doxorubicin formulations have demonstrated success in the therapy of ovarian and breast cancers with reduced cardiotoxicity [31,32].

Apart from liposomes, polymeric nanoparticles and mesoporous silica nanoparticles are being explored, to enable break down and release of drugs at controlled rates, enhancing drug accumulation and minimizing exposure to healthy tissues [33]. Surface modifications and ligand-based targeting is used to further optimize the stability and cancer specificity of nanoparticles. Conjugation strategies have been explored to attach various targeting ligands to anthracycline nanoparticles, to enable them to preferentially bind to cancer cells, improving selective delivery and minimizing off-target toxicity [28, 33]. They target tumors more effectively and are more amenable for combination therapy strategies [34]. Recently, Yang et al., demonstrated that a drug delivery system (LPS-RGD-Nb36-DOX) encompasses doxorubicin and anti-CTLA-4 Nb co-loaded and RGD specifically target tumor cells and enhance CD8+ T-cell activation. Moreover, in vivo experiments revealed the effectively directs treatment to tumor sites, stimulates T-cell proliferation, and elevates pro-inflammatory cytokine production. This combined therapeutic approach shows promise for synergistic tumor elimination, allowing for reduced chemotherapy doses and enhanced safety profiles [35]

Combination Strategies to Optimize Doxorubicin Treatment

Combination Therapies with Other Anti-Cancer Agents

To enhance the effectiveness of doxorubicin treatment and combat drug resistance, researchers have explored combining it with other anti-cancer medications. Recent studies have focused into various combination regimens that capitalize on the synergistic interactions between doxorubicin and other drugs. For instance, combining doxorubicin with taxanes like paclitaxel or docetaxel has demonstrated improved response rates and survival outcomes in breast cancer and other solid tumors [36, 37]. Park et al. and others employed pre-clinical models of breast and prostate cancer to illustrate that the combination of doxorubicin and the mitochondrio toxic Hsp90 inhibitor, gamitrinib, resulted in a remarkable reduction in the in vivo tumor growth in prostate and breast xenograft models, without an increase in cardiotoxicity [37, 38, 39]. In a study by Pondugula et al., it was demonstrated that a combination chemotherapy regimen of doxorubicin (DOX) and cyclophosphamide (CPS) did not induce significant hepatotoxicity in male C57BL/6J mice [40, 41]. More recently, Urla et al. used sildenafil, a potent and selective inhibitor of cGMP-specific PDE5, in human rhabdomyosarcoma (RMS) cell lines RH30 and RD. Their findings revealed that the combination of sildenafil with doxorubicin significantly increased the proportion of apoptotic cells and the production of reactive oxygen species (ROS) compared to treatment with Sildenafil alone. These results suggest that combining sildenafil with doxorubicin may hold promise as an approach in the treatment of pediatric RMS [42]. These combination approaches hold the potential to heighten doxorubicin's efficacy and expand its utility in various types of cancer.

Combinatorial Approaches with Immunotherapy

The landscape of cancer treatment has been revolutionized by immunotherapy, which leverages the immune system's capability to identify and eliminate cancer cells [43, 44]. Doxorubicin has shown the ability to induce immunogenic cell death and stimulate anti-tumor immune responses, making it an attractive candidate for combination with immunotherapeutic interventions [20]. The integration of doxorubicin with targeted therapies, such as tyrosine kinase inhibitors or immune checkpoint inhibitors (ICI) viz. anti-PD-1 or anti-CTLA-4 antibodies, has shown promising results in preclinical models

and early-phase clinical studies, as it leverages their complementary modes of action, enhancing the immune response, and overcoming immunosuppression within the tumor microenvironment [45, 46, 47, 48, 49, 50]. Furthermore, the exploration of chimeric antigen receptor (CAR) T-cell therapy in combination with doxorubicin aims to target specific tumor antigens, leading to increased tumor cell destruction [49]. These combined approaches offer exciting prospects for augmenting doxorubicin's therapeutic impact in cancer treatment.

Concurrent Radiation Therapy and Doxorubicin

Recent research has shown that combining doxorubicin with radiation therapy can significantly improve patient outcomes, especially for locally advanced tumors or those that cannot be surgically removed. Scientists have investigated the optimal sequencing, timing, and dosage of doxorubicin and radiation therapy to achieve maximum therapeutic synergy. Preclinical studies indicate that doxorubicin can increase the sensitivity of tumor cells to radiation-induced DNA damage, leading in improved tumor control [51]. Clinical trials have been conducted to assess the effectiveness of concurrent or sequential administration of doxorubicin and radiation therapy in various cancer types, including sarcoma, breast cancer, and head and neck cancer [51, 52, 53]. These combined approaches hold the potential to enhance local tumor management and increase the chances of achieving curative outcomes for patients.

Photodynamic Therapy with Doxorubicin

Photodynamic therapy (PDT) is an emerging treatment approach that involves utilizing a photosensitizer and light to selectively kill cancer cells. Recent research has explored the potential benefits of combining doxorubicin with PDT to enhance its therapeutic effectiveness. Several studies have demonstrated increased cancer cell death and tumor regression with combination of doxorubicin and PDT [54]. One of the key advantages of PDT is its localized and focused nature, which allows for precise tumor ablation while minimizing damage to surrounding healthy tissues. To further improve the efficacy of doxorubicin and PDT, researchers are investigating various methods, including the development of new photosensitizers and advanced light delivery systems [18, 54]. These ongoing developments hold the potential to expand cancer treatment options, offering a promising avenue for enhancing the therapeutic outcomes of doxorubicin and PDT in the fight against cancer.

Gene Therapy Approaches

Gene therapy represents an innovative approach to enhance the efficacy of doxorubicin by targeting specific genetic abnormalities within cancer cells. Recent research has delved into using gene therapy to sensitize cancer cells to the lethal effects of doxorubicin. One strategy involves the overexpression of genes, such as *p53*, to restore tumor suppressor function and increase doxorubicin-induced apoptosis [55]. Chen et al. created a multifunctional carrier by utilizing a polycationic brush with "cyclodextrin-containing star polymers" as side chains to co-deliver DOX and a *p53* gene. These complexes effectively transported both DOX and the *p53* gene into the same cells and demonstrated high transfection efficiency in MCF-7 breast cancer cells. The authors observed an improved inhibition of cell growth with a reduced DOX dosage, which was attributed to the synergistic effect of co-delivering DOX and a *p53*-encoding gene. This discovery offers an efficient strategy for developing a co-delivery system for combination therapy [56]. In a separate study, Medrano et al. demonstrated that combining p19Arf/IFNβ with Dox (p19Arf/IFNβ + Dox) significantly increased cell death in vitro, enabling the use of a lower dose of adenovirus and Dox. This combination also boosted the immunogenicity of treated cells by enhancing ATP secretion and exposing HMGB1. In a therapeutic vaccine model, this approach resulted in superior antitumor protection compared to single therapy [57]. Moreover, gene-editing tools like CRISPR-Cas9 are being explored for their potential to modify genes involved in drug resistance pathways, thereby enhancing doxorubicin sensitivity [58]. These gene therapy techniques offer promising opportunities to overcome drug resistance and improve the therapeutic effects of doxorubicin in cancer treatment.

Photothermal Therapy in Combination with Doxorubicin: A Promising Cancer Treatment Strategy

Photothermal therapy (PTT) has emerged as a promising cancer treatment method, utilizing the unique properties of light-absorbing materials such as gold nanoparticles (AuNPs), carbon nanotubes (CNTs), and graphene oxide (GO) to elicit localized hyperthermia and tumor cell death [59]. PTT has the potential to improve treatment efficacy while minimizing systemic adverse effects when combined with conventional chemotherapeutic agents such as doxorubicin (DOX). Hyperthermia induced by PTT can enhance DOX uptake and intracellular release, thereby increasing its cytotoxicity. In addition, the local hyperthermia generated by PTT can elicit normalization of tumor vascularity, thereby enhancing DOX delivery to the tumor site. This synergistic strategy has the potential to decrease DOX concentrations, thereby reducing systemic toxicity [60, 61, 62]. L'Ecuyer et al, demonstrated in H9C2 cardiac myoblasts that the reduction of oxidative stress-induced activation of the DNA damage pathway and consequent cell death by mild hypothermia supports a possible protective role to reduce the clinical impact of DOX-induced cardiac toxicity [63]. Zhang et al., demonstrated that hollow polydopamine (HPDA)/Au@DOX accelerated DOX release and enhanced uptake by A549 cells, furthermore Both in vitro and in vivo experiments demonstrated that the photothermal-chemotherapy combination group (HPDA/Au@DOX+NIR) exhibited stronger anti-metastatic and anti-tumor activities compared to the monotherapy group (DOX) [64]. In support, Salvador et al, demonstrated that hyperthermia enhances DOX effect through cell cycle arrest, oxidative stress, and apoptotic cell death [65]. Several ongoing clinical trials are evaluating the safety and efficacy of PTT in combination with DOX for various forms of cancer. These clinical trials are essential for translating promising preclinical results into clinical

practice. However, issues such as optimizing treatment procedures, assuring precise targeting, and minimizing potential adverse effects must be solved to make this combination therapy more broadly available.

Targeting Specific Tumor Types
Doxorubicin in Breast Cancer

Breast cancer stands as one of the most prevalent cancers worldwide, and doxorubicin has been a fundamental component of breast cancer treatment for many years. Nevertheless, there is a growing necessity to optimize its usage to achieve maximum effectiveness while minimizing toxicity. Recent research has been dedicated to refining the administration schedules and dosage regimens of doxorubicin for breast cancer treatment. Al-Mahayri ZN et al. demonstrated that a dose-dense administration of doxorubicin in combination with other chemotherapeutic agents improved response rates and overall survival in early-stage breast cancer patients [36]. Swain et al. [4] demonstrated that dexrazoxane significantly lowered the incidence of cardiotoxicity in breast cancer patients undergoing doxorubicin-based chemotherapy while preserving its effectiveness. Other studies have sought to identify predictive biomarkers that can aid in selecting patients likely to benefit from doxorubicin-based therapies. For instance, the recognition of HER2/neu gene amplification and overexpression as predictive biomarkers has led to targeted therapies like trastuzumab in combination with doxorubicin, leading to improved outcomes for HER2-positive breast cancer patients [53]. Researchers are constantly exploring novel targeted approaches and combination therapies to enhance the efficacy of doxorubicin in breast cancer treatment.

Doxorubicin in Hematological Malignancies

Doxorubicin plays a crucial role in treating hematological cancers e.g., leukemia, Hodgkin's/ Non-Hodgkin's lymphoma, multiple myeloma. However, its clinical application is limited by dose-dependent toxicity, particularly cardiotoxicity. Ongoing research aims to optimize the use of doxorubicin in hematological cancer treatment by reducing toxicity, boosting effectiveness, and identifying innovative targeted approaches [5]. One approach involves using cardioprotective agents, like dexrazoxane, to reduce doxorubicin-induced cardiotoxicity [4]. Additionally, researchers are exploring the potential of combining doxorubicin with targeted therapies, such as tyrosine kinase inhibitors (TKIs) or immunomodulatory compounds, to enhance its anticancer effects. An example is the combination of doxorubicin with rituximab, a monoclonal anti-CD20 antibody, which has shown improved outcomes for patients with diffuse large B-cell lymphoma [65]. These efforts are expected to lead to better treatment outcomes and improved quality of life for patients with hematological malignancies.

Doxorubicin in Other Solid Tumors

As a chemotherapeutic drug for solid tumors, doxorubicin has encountered challenges in treating certain tumor types due to inherent or acquired resistance. Various approaches to overcome chemoresistance and enhance therapeutic effectiveness are being explored. One strategy involves combining doxorubicin with other chemotherapeutic agents or targeted therapies. The combination of doxorubicin with ifosfamide has shown improved response rates and progression-free survival in treating advanced soft tissue sarcomas [66]. Novel drug delivery systems are also being developed to enhance doxorubicin's ability to target tumors more effectively. Nanoparticle-based formulations, like liposomes and polymeric nanoparticles, show promise in improving drug delivery while reducing systemic toxicity [67, 68]. Further, researchers are investigating the use of imaging techniques and biomarkers to predict doxorubicin response and monitor treatment efficacy in solid tumors. For example, positron emission tomography (PET) imaging with 18F-fluorodeoxyglucose (FDG) is being explored as a potential tool for assessing doxorubicin response in various solid tumors [29]. In colon cancer, doxorubicin resistance was reverted by targeting steroid receptor activator (SRC). Authors found that SRC act as common signaling node, leading to the overexpression of RTKs like EGFR and IGF-1R thereby elevating downstream AKT in doxorubicin resistant colon cancer cells, and combination of SRC inhibition with doxorubicin sensitizes these resistance cells by inducing apoptosis [69]. Bi et al. presented the initial findings showcasing the use of doxorubicin-loaded CalliSpheres® beads in transarterial chemoembolization (DEB-TACE) for patients with unresectable or recurrent esophageal carcinoma. The authors compellingly demonstrated that DEB-TACE is a secure and viable therapeutic approach, suggesting its inclusion among the treatment alternatives for such patients [69, 70]. In the recently completed Phase-3 ATLANTIS trial focusing on small cell lung cancer (SCLC), the researchers revealed that the combined treatment of lurbinectedin (a synthetic marine-derived anticancer agent) and doxorubicin did not result in an enhancement of overall survival compared to the control group in patients with relapsed SCLC. Nevertheless, the lurbinectedin plus doxorubicin combination exhibited a more favorable hematological safety profile when compared to the control group [71, 72]. In their investigations of prostate cancer through both in vitro and in vivo studies, Su et al. unequivocally illustrated the effectiveness of employing DOX nanomicelles tailored for prostate cancer targeting, coupled with photothermal therapy, as a successful treatment strategy for castration-resistant prostate cancer (CRPC) [73].These ongoing developments aim to enhance the therapeutic efficacy of doxorubicin in treating solid tumors and overcome resistance hurdles, ultimately improving patient outcomes.

Personalized Approaches in Doxorubicin Therapy

Currently, there is ongoing research in the field of identifying predictive biomarkers for doxorubicin response. Recent studies have been primarily focused on pinpointing molecular signals that could be utilized to predict how patients will respond to doxorubicin and aid in tailoring treatment approaches.

Genetic Variations and Pharmacogenomics

The response to doxorubicin treatment can be influenced by individual genetic variations. Pharmacogenomic research has been dedicated to identifying genetic differences associated with both doxorubicin's effectiveness and potential side effects. Researchers have explored drug transporter expression levels, such as ATP-binding cassette (ABC) transporters, which have shown potential as biomarkers for predicting doxorubicin resistance [74]. Additionally, there have been investigations into genetic changes in drug metabolism enzymes like carbonyl reductase 1 (CBR1) [75], UDP-glucuronosyltransferases (UGTs) and cytochrome P450 enzymes, to assess their impact on doxorubicin's effectiveness and toxicity. Through genome-wide association studies (GWAS), single nucleotide polymorphisms (SNPs) in genes related to drug metabolism, drug transport, and DNA repair pathways have been discovered that can modify doxorubicin response [76]. By integrating pharmacogenomic data into treatment decisions, it becomes possible to personalize doxorubicin therapy based on an individual's genetic profile, reducing undesirable effects, and improving treatment outcomes.

Individualized Dosing Strategies

To optimize the benefits of doxorubicin treatment and reduce its toxicity, personalized dosing regimens can be implemented. Recent research has focused on tailoring dosing techniques based on individual patient characteristics and pharmacokinetic data. Pharmacokinetic modeling and simulation methods have been employed to guide dose adjustments, considering factors such as age, body surface area, renal function, and concurrent administration of other medications [77]. Moreover, therapeutic drug monitoring (TDM) strategies, including monitoring doxorubicin concentrations in blood or saliva samples, have been investigated to assist in dose modifications and ensure adequate drug exposure [77]. These personalized dosing approaches hold great promise in maximizing doxorubicin's therapeutic benefits while minimizing the risk of adverse effects.

Future perspectives

The development and evaluation of doxorubicin and its formulations require accurate preclinical models and predictive tests. Three-dimensional cancer models, patient-derived xenografts (PDX), and organoids better simulate the tumor microenvironment and predict medication response. Organ-on-a-chip and microfluidic technologies can also allow evaluation of doxorubicin's effects on various organ systems, predicting toxicity and efficacy. To simulate clinical scenarios, long-term studies of anthracycline cardiotoxicity in animals must take precedence over short-term in vitro treatments of isolated cells.

There is need to study alternative mechanisms of anthracycline cardiotoxicity and identify noninvasive or systemic markers that predict or indicate early cardiac damage. With the advent of combination therapy, there is need to determine the interactions between the modalities, including toxicity. Applying doxorubicin research to clinical practice requires well-designed clinical trials of innovative treatments, such as combination therapies, tailored delivery systems, and precision medicine strategies. A recent study indicates that combining anti-programmed cell death receptor 1 (PD-1) immunotherapy with standard-of-care chemotherapy is a safe approach and may enhance therapeutic efficacy in the management of metastatic soft-tissue sarcoma [78]. In a phase I clinical trial combination of pembrolizumab and doxorubicin showed an encouraging response rate and robust T cell response dynamics in anthracycline-naïve patients with mTNBC [79]

Future doxorubicin research will continue to focus on addressing cardiotoxicity, drug resistance, limited efficacy, and secondary malignancies. Cardiovascular protection, drug resistance reversal, individualized medicine, nanotechnology, combination therapies, innovative formulations, microenvironment targeting, precise drug delivery, preclinical models, and clinical trials will shape the future of doxorubicin therapy. By integrating the latest scientific advancements and innovative therapeutic strategies, we are moving towards a more effective and targeted use of doxorubicin in cancer treatment, ultimately leading to improved patient outcomes and enhanced quality of life.

Author Contribution Statement

Conceptualization, writing, editing, review, finalization were performed by S.S., B.K, C.P.P., S.S.C., and M.K.

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None.

Conflict of Interest

The authors have declared that no conflict of interest exists.

References

1. Salvatorelli E, Menna P, Minotti G. Managing anthracycline-induced cardiotoxicity: Beginning with the end in mind. Future Cardiol. 2015;11(4):363-6. https://doi.org/10.2217/fca.15.35.
2. Ganatra S, Nohria A, Shah S, Groarke JD, Sharma A, Venesy D, et al. Upfront dexrazoxane for the reduction of anthracycline-induced cardiotoxicity in adults with preexisting cardiomyopathy and cancer: A consecutive case series. Cardiooncology. 2019;5(1):1. https://doi.org/10.1186/s40959-019-0036-7.
3. Jones IC, Dass CR. Doxorubicin-induced cardiotoxicity: Causative factors and possible interventions. J Pharm Pharmacol. 2022;74(12):1677-88. https://doi.org/10.1093/jpp/rgac063.
4. Swain SM, Whaley FS, Gerber MC, Weisberg S, York M, Spicer D, et al. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. J Clin Oncol. 1997;15(4):1318-32. https://doi.org/10.1200/jco.1997.15.4.1318.
5. Bosch X, Rovira M, Sitges M, Domènech A, Ortiz-Pérez JT, de Caralt TM, et al. Enalapril and carvedilol for preventing chemotherapy-induced left ventricular systolic dysfunction

in patients with malignant hemopathies: The overcome trial (prevention of left ventricular dysfunction with enalapril and carvedilol in patients submitted to intensive chemotherapy for the treatment of malignant hemopathies). J Am Coll Cardiol. 2013;61(23):2355-62. https://doi.org/10.1016/j.jacc.2013.02.072.
6. Sadek KM, Mahmoud SFE, Zeweil MF, Abouzed TK. Proanthocyanidin alleviates doxorubicin-induced cardiac injury by inhibiting nf-kb pathway and modulating oxidative stress, cell cycle, and fibrogenesis. J Biochem Mol Toxicol. 2021;35(4):e22716. https://doi.org/10.1002/jbt.22716.
7. Nie L, Liu M, Chen J, Wu Q, Li Y, Yi J, et al. Hydrogen sulfide ameliorates doxorubicin-induced myocardial fibrosis in rats via the pi3k/akt/mtor pathway. Mol Med Rep. 2021;23(4). https://doi.org/10.3892/mmr.2021.11938.
8. Zhan C, Bai N, Zheng M, Wang Y, Wang Y, Zhang L, et al. Tranilast prevents doxorubicin-induced myocardial hypertrophy and angiotensin ii synthesis in rats. Life Sci. 2021;267:118984. https://doi.org/10.1016/j.lfs.2020.118984.
9. Ahmed AZ, Satyam SM, Shetty P, D'Souza MR. Methyl gallate attenuates doxorubicin-induced cardiotoxicity in rats by suppressing oxidative stress. Scientifica (Cairo). 2021;2021:6694340. https://doi.org/10.1155/2021/6694340.
10. Jiang Y, Liu Y, Xiao W, Zhang D, Liu X, Xiao H, et al. Xinmailong attenuates doxorubicin-induced lysosomal dysfunction and oxidative stress in h9c2 cells via ho-1. Oxid Med Cell Longev. 2021;2021:5896931. https://doi.org/10.1155/2021/5896931.
11. Plana JC, Galderisi M, Barac A, Ewer MS, Ky B, Scherrer-Crosbie M, et al. Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: A report from the american society of echocardiography and the european association of cardiovascular imaging. Eur Heart J Cardiovasc Imaging. 2014;15(10):1063-93. https://doi.org/10.1093/ehjci/jeu192.
12. Cardinale D, Colombo A, Torrisi R, Sandri MT, Civelli M, Salvatici M, et al. Trastuzumab-induced cardiotoxicity: Clinical and prognostic implications of troponin i evaluation. J Clin Oncol. 2010;28(25):3910-6. https://doi.org/10.1200/jco.2009.27.3615.
13. Zhang H, Xu H, Ashby CR, Jr., Assaraf YG, Chen ZS, Liu HM. Chemical molecular-based approach to overcome multidrug resistance in cancer by targeting p-glycoprotein (p-gp). Med Res Rev. 2021;41(1):525-55. https://doi.org/10.1002/med.21739.
14. Szakács G, Annereau JP, Lababidi S, Shankavaram U, Arciello A, Bussey KJ, et al. Predicting drug sensitivity and resistance: Profiling abc transporter genes in cancer cells. Cancer Cell. 2004;6(2):129-37. https://doi.org/10.1016/j.ccr.2004.06.026.
15. Ween MP, Armstrong MA, Oehler MK, Ricciardelli C. The role of abc transporters in ovarian cancer progression and chemoresistance. Crit Rev Oncol Hematol. 2015;96(2):220-56. https://doi.org/10.1016/j.critrevonc.2015.05.012.
16. Ueda K. Abc proteins protect the human body and maintain optimal health. Biosci Biotechnol Biochem. 2011;75(3):401-9. https://doi.org/10.1271/bbb.100816.
17. Waghray D, Zhang Q. Inhibit or evade multidrug resistance p-glycoprotein in cancer treatment. J Med Chem. 2018;61(12):5108-21. https://doi.org/10.1021/acs.jmedchem.7b01457.
18. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of abc transporters in multidrug-resistant cancer. Nat Rev Cancer. 2018;18(7):452-64. https://doi.org/10.1038/s41568-018-0005-8.
19. Sarkadi B, Homolya L, Szakács G, Váradi A. Human multidrug resistance abcb and abcg transporters: Participation in a chemoimmunity defense system. Physiol Rev. 2006;86(4):1179-236. https://doi.org/10.1152/physrev.00037.2005.
20. Goebel J, Chmielewski J, Hrycyna CA. The roles of the human atp-binding cassette transporters p-glycoprotein and abcg2 in multidrug resistance in cancer and at endogenous sites: Future opportunities for structure-based drug design of inhibitors. Cancer Drug Resist. 2021;4(4):784-804. https://doi.org/10.20517/cdr.2021.19.
21. Joshi P, Vishwakarma RA, Bharate SB. Natural alkaloids as p-gp inhibitors for multidrug resistance reversal in cancer. Eur J Med Chem. 2017;138:273-92. https://doi.org/10.1016/j.ejmech.2017.06.047.
22. Hu X, Li J, Fu M, Zhao X, Wang W. The jak/stat signaling pathway: From bench to clinic. Signal Transduct Target Ther. 2021;6(1):402. https://doi.org/10.1038/s41392-021-00791-1.
23. Darici S, Alkhaldi H, Horne G, Jørgensen HG, Marmiroli S, Huang X. Targeting pi3k/akt/mtor in aml: Rationale and clinical evidence. J Clin Med. 2020;9(9). https://doi.org/10.3390/jcm9092934.
24. Germann UA, Furey BF, Markland W, Hoover RR, Aronov AM, Roix JJ, et al. Targeting the mapk signaling pathway in cancer: Promising preclinical activity with the novel selective erk1/2 inhibitor bvd-523 (ulixertinib). Mol Cancer Ther. 2017;16(11):2351-63. https://doi.org/10.1158/1535-7163.Mct-17-0456.
25. Kumar VE, Nambiar R, De Souza C, Nguyen A, Chien J, Lam KS. Targeting epigenetic modifiers of tumor plasticity and cancer stem cell behavior. Cells. 2022;11(9). https://doi.org/10.3390/cells11091403.
26. Singh A, Venkannagari S, Oh KH, Zhang YQ, Rohde JM, Liu L, et al. Small molecule inhibitor of nrf2 selectively intervenes therapeutic resistance in keap1-deficient nsclc tumors. ACS Chem Biol. 2016;11(11):3214-25. https://doi.org/10.1021/acschembio.6b00651.
27. Mutlu P, Yalçin Azarkan S, Taghavi Pourianazar N, Yücel M, Gündüz U. Determination of the relationship between doxorubicin resistance and wnt signaling pathway in hela and k562 cell lines. Excli J. 2018;17:386-98. https://doi.org/10.17179/excli2018-1129.
28. Teodori E, Dei S, Bartolucci G, Perrone MG, Manetti D, Romanelli MN, et al. Structure-activity relationship studies on 6,7-dimethoxy-2-phenethyl-1,2,3,4-tetrahydroisoquinoline derivatives as multidrug resistance reversers. ChemMedChem. 2017;12(16):1369-79. https://doi.org/10.1002/cmdc.201700239.
29. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev. 2004;56(2):185-229. https://doi.org/10.1124/pr.56.2.6.
30. Teodori E, Contino M, Riganti C, Bartolucci G, Braconi L, Manetti D, et al. Design, synthesis and biological evaluation of stereo- and regioisomers of amino aryl esters as multidrug resistance (mdr) reversers. Eur J Med Chem. 2019;182:111655. https://doi.org/10.1016/j.ejmech.2019.111655.
31. Rahman AM, Yusuf SW, Ewer MS. Anthracycline-induced cardiotoxicity and the cardiac-sparing effect of liposomal formulation. Int J Nanomedicine. 2007;2(4):567-83.
32. Engelberth SA, Hempel N, Bergkvist M. Development of nanoscale approaches for ovarian cancer therapeutics and diagnostics. Crit Rev Oncog. 2014;19(3-4):281-315. https://doi.org/10.1615/critrevoncog.2014011455.
33. Na K, Bae YH. Self-assembled hydrogel nanoparticles responsive to tumor extracellular ph from pullulan derivative/

sulfonamide conjugate: Characterization, aggregation, and adriamycin release in vitro. Pharm Res. 2002;19(5):681-8. https://doi.org/10.1023/a:1015370532543.
34. Wu H, Xu XF, Zhu JQ, Wang MD, Li C, Liang L, et al. Mesoporous silica nanoparticles for potential immunotherapy of hepatocellular carcinoma. Front Bioeng Biotechnol. 2021;9:695635. https://doi.org/10.3389/fbioe.2021.695635.
35. Yang W, Sun Q, Zhang X, Zheng L, Yang X, He N, et al. A novel doxorubicin/ctla-4 blocker co-loaded drug delivery system improves efficacy and safety in antitumor therapy. Cell Death Dis. 2024;15(6):386. https://doi.org/10.1038/s41419-024-06776-6.
36. Al-Mahayri ZN, Patrinos GP, Ali BR. Toxicity and pharmacogenomic biomarkers in breast cancer chemotherapy. Front Pharmacol. 2020;11:445. https://doi.org/10.3389/fphar.2020.00445.
37. Park HK, Lee JE, Lim J, Jo DE, Park SA, Suh PG, et al. Combination treatment with doxorubicin and gamitrinib synergistically augments anticancer activity through enhanced activation of bim. BMC Cancer. 2014;14:431. https://doi.org/10.1186/1471-2407-14-431.
38. Cui W, Aouidate A, Wang S, Yu Q, Li Y, Yuan S. Discovering anti-cancer drugs via computational methods. Front Pharmacol. 2020;11:733. https://doi.org/10.3389/fphar.2020.00733.
39. McDonald PC, Swayampakula M, Dedhar S. Coordinated regulation of metabolic transporters and migration/invasion by carbonic anhydrase ix. Metabolites. 2018;8(1):20. https://doi.org/10.3390/metabo8010020.
40. Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of anthracyclines. Front Cardiovasc Med. 2020;7:26. https://doi.org/10.3389/fcvm.2020.00026.
41. Pondugula SR, Salamat JM, Abbott KL, Flannery PC, Majrashi M, Almaghrabi M, et al. A clinically relevant combination treatment with doxorubicin and cyclophosphamide does not induce hepatotoxicity in c57bl/6j mice. Liver Res. 2021;5(4):239-42. https://doi.org/10.1016/j.livres.2021.04.002.
42. Urla C, Stagno MJ, Fuchs J, Warmann SW, Schmid E. Combination therapy of doxorubicin and sildenafil inhibits the growth of pediatric rhabdomyosarcoma. J Cancer Res Clin Oncol. 2023;149(6):2513-22. https://doi.org/10.1007/s00432-022-04092-0.
43. Kopecka J, Salaroglio IC, Perez-Ruiz E, Sarmento-Ribeiro AB, Saponara S, De Las Rivas J, et al. Hypoxia as a driver of resistance to immunotherapy. Drug Resist Updat. 2021;59:100787. https://doi.org/10.1016/j.drup.2021.100787.
44. Rafalko A, Iliopoulos O, Fusaro VA, Hancock W, Hincapie M. Immunoaffinity enrichment and liquid chromatography-selected reaction monitoring mass spectrometry for quantitation of carbonic anhydrase 12 in cultured renal carcinoma cells. Anal Chem. 2010;82(21):8998-9005. https://doi.org/10.1021/ac101981t.
45. Medina MA, Oza G, Sharma A, Arriaga LG, Hernández Hernández JM, Rotello VM, et al. Triple-negative breast cancer: A review of conventional and advanced therapeutic strategies. Int J Environ Res Public Health. 2020;17(6). https://doi.org/10.3390/ijerph17062078.
46. Zitvogel L, Apetoh L, Ghiringhelli F, André F, Tesniere A, Kroemer G. The anticancer immune response: Indispensable for therapeutic success? J Clin Invest. 2008;118(6):1991-2001. https://doi.org/10.1172/jci35180.
47. Li D, Li X, Zhou WL, Huang Y, Liang X, Jiang L, et al. Genetically engineered t cells for cancer immunotherapy. Signal Transduct Target Ther. 2019;4:35. https://doi.org/10.1038/s41392-019-0070-9.
48. Groen HJ, Socinski MA, Grossi F, Juhasz E, Gridelli C, Baas P, et al. A randomized, double-blind, phase ii study of erlotinib with or without sunitinib for the second-line treatment of metastatic non-small-cell lung cancer (nsclc). Ann Oncol. 2013;24(9):2382-9. https://doi.org/10.1093/annonc/mdt212.
49. Titov A, Valiullina A, Zmievskaya E, Zaikova E, Petukhov A, Miftakhova R, et al. Advancing car t-cell therapy for solid tumors: Lessons learned from lymphoma treatment. Cancers (Basel). 2020;12(1). https://doi.org/10.3390/cancers12010125.
50. Dirix LY, Takacs I, Jerusalem G, Nikolinakos P, Arkenau HT, Forero-Torres A, et al. Avelumab, an anti-pd-l1 antibody, in patients with locally advanced or metastatic breast cancer: A phase 1b javelin solid tumor study. Breast Cancer Res Treat. 2018;167(3):671-86. https://doi.org/10.1007/s10549-017-4537-5.
51. Brackstone M, Palma D, Tuck AB, Scott L, Potvin K, Vandenberg T, et al. Concurrent neoadjuvant chemotherapy and radiation therapy in locally advanced breast cancer. Int J Radiat Oncol Biol Phys. 2017;99(4):769-76. https://doi.org/10.1016/j.ijrobp.2017.06.005.
52. Haddad R, Concha-Benavente F, Blumenschein G, Jr., Fayette J, Guigay J, Colevas AD, et al. Nivolumab treatment beyond recist-defined progression in recurrent or metastatic squamous cell carcinoma of the head and neck in checkmate 141: A subgroup analysis of a randomized phase 3 clinical trial. Cancer. 2019;125(18):3208-18. https://doi.org/10.1002/cncr.32190.
53. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against her2 for metastatic breast cancer that overexpresses her2. N Engl J Med. 2001;344(11):783-92. https://doi.org/10.1056/nejm200103153441101.
54. Korbelik M, Sun J. Photodynamic therapy-generated vaccine for cancer therapy. Cancer Immunol Immunother. 2006;55(8):900-9. https://doi.org/10.1007/s00262-005-0088-4.
55. Hu J, Cao J, Topatana W, Juengpanich S, Li S, Zhang B, et al. Targeting mutant *p53* for cancer therapy: Direct and indirect strategies. J Hematol Oncol. 2021;14(1):157. https://doi.org/10.1186/s13045-021-01169-0.
56. Chen W, Zhang M, Shen W, Du B, Yang J, Zhang Q. A polycationic brush mediated co-delivery of doxorubicin and gene for combination therapy. Polymers (Basel). 2019;11(1). https://doi.org/10.3390/polym11010060.
57. Medrano RFV, Salles TA, Dariolli R, Antunes F, Feitosa VA, Hunger A, et al. Potentiation of combined p19arf and interferon-beta cancer gene therapy through its association with doxorubicin chemotherapy. Sci Rep. 2022;12(1):13636. https://doi.org/10.1038/s41598-022-17775-y.
58. Tian X, Gu T, Patel S, Bode AM, Lee MH, Dong Z. Crispr/cas9 - an evolving biological tool kit for cancer biology and oncology. NPJ Precis Oncol. 2019;3:8. https://doi.org/10.1038/s41698-019-0080-7.
59. Taylor ML, Wilson RE, Jr., Amrhein KD, Huang X. Gold nanorod-assisted photothermal therapy and improvement strategies. Bioengineering (Basel). 2022;9(5). https://doi.org/10.3390/bioengineering9050200.
60. Huang X, Tang S, Mu X, Dai Y, Chen G, Zhou Z, et al. Freestanding palladium nanosheets with plasmonic and catalytic properties. Nat Nanotechnol. 2011;6(1):28-32. https://doi.org/10.1038/nnano.2010.235.
61. Chandra S, Michael Nguyen H, Wiltz K, Hall N, Chaudhry S, Olverson G, et al. Aptamer-functionalized hybrid nanoparticles to enhance the delivery of doxorubicin into breast cancer cells by silencing p-glycoprotein. J

Cancer Treatment Diagn. 2020;4(1):1-13. https://doi.org/10.29245/2578-2967/2020/1.1176.
62. Hosseini NF, Amini R, Ramezani M, Saidijam M, Hashemi SM, Najafi R. As1411 aptamer-functionalized exosomes in the targeted delivery of doxorubicin in fighting colorectal cancer. Biomed Pharmacother. 2022;155:113690. https://doi.org/10.1016/j.biopha.2022.113690.
63. L'Ecuyer TJ, Aggarwal S, Zhang JP, Van der Heide RS. Effect of hypothermia on doxorubicin-induced cardiac myoblast signaling and cell death. Cardiovasc Pathol. 2012;21(2):96-104. https://doi.org/10.1016/j.carpath.2011.02.001.
64. Zhang X, Xu B, Ni J, Xiang Y, He Z. Combined chemo- and photothermal therapies of non-small cell lung cancer using polydopamine/au hollow nanospheres loaded with doxorubicin. Int J Nanomedicine. 2024;19:9597-612. https://doi.org/10.2147/ijn.S473137.
65. Salvador D, Bastos V, Oliveira H. Hyperthermia enhances doxorubicin therapeutic efficacy against a375 and mnt-1 melanoma cells. Int J Mol Sci. 2021;23(1). https://doi.org/10.3390/ijms23010035.
66. Pfreundschuh M, Trümper L, Osterborg A, Pettengell R, Trneny M, Imrie K, et al. Chop-like chemotherapy plus rituximab versus chop-like chemotherapy alone in young patients with good-prognosis diffuse large-b-cell lymphoma: A randomised controlled trial by the mabthera international trial (mint) group. Lancet Oncol. 2006;7(5):379-91. https://doi.org/10.1016/s1470-2045(06)70664-7.
67. Judson I, Verweij J, Gelderblom H, Hartmann JT, Schöffski P, Blay JY, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: A randomised controlled phase 3 trial. Lancet Oncol. 2014;15(4):415-23. https://doi.org/10.1016/s1470-2045(14)70063-4.
68. Kenchegowda M, Rahamathulla M, Hani U, Begum MY, Guruswamy S, Osmani RAM, et al. Smart nanocarriers as an emerging platform for cancer therapy: A review. Molecules. 2021;27(1). https://doi.org/10.3390/molecules27010146.
69. Xiong S, Xiao GW. Reverting doxorubicin resistance in colon cancer by targeting a key signaling protein, steroid receptor coactivator. Exp Ther Med. 2018;15(4):3751-8. https://doi.org/10.3892/etm.2018.5912.
70. Farokhzad OC, Jon S, Khademhosseini A, Tran TN, Lavan DA, Langer R. Nanoparticle-aptamer bioconjugates: A new approach for targeting prostate cancer cells. Cancer Res. 2004;64(21):7668-72. https://doi.org/10.1158/0008-5472.Can-04-2550.
71. Bi Y, Shi X, Ren J, Yi M, Han X, Song M. Clinical outcomes of doxorubicin-eluting callispheres® beads-transarterial chemoembolization for unresectable or recurrent esophageal carcinoma. BMC Gastroenterol. 2021;21(1):231. https://doi.org/10.1186/s12876-021-01816-3.
72. Aix SP, Ciuleanu TE, Navarro A, Cousin S, Bonanno L, Smit EF, et al. Combination lurbinectedin and doxorubicin versus physician's choice of chemotherapy in patients with relapsed small-cell lung cancer (atlantis): A multicentre, randomised, open-label, phase 3 trial. Lancet Respir Med. 2023;11(1):74-86. https://doi.org/10.1016/s2213-2600(22)00309-5.
73. Su X, Li C, Xu K, Su W, Mao X, Zou Y, et al. The effect of prostate cancer-targeting doxorubicin nanomicelles combined with photothermal therapy on castration-resistant prostate cancer. J Biomed Nanotechnol. 2022;18:1276-88. https://doi.org/10.1166/jbn.2022.3335.
74. de Gooijer MC, Kemper EM, Buil LCM, Çitirikkaya CH, Buckle T, Beijnen JH, et al. Atp-binding cassette transporters restrict drug delivery and efficacy against brain tumors even when blood-brain barrier integrity is lost. Cell Rep Med. 2021;2(1):100184. https://doi.org/10.1016/j.xcrm.2020.100184.
75. Kassner N, Huse K, Martin HJ, Gödtel-Armbrust U, Metzger A, Meineke I, et al. Carbonyl reductase 1 is a predominant doxorubicin reductase in the human liver. Drug Metab Dispos. 2008;36(10):2113-20. https://doi.org/10.1124/dmd.108.022251.
76. Aminkeng F, Bhavsar AP, Visscher H, Rassekh SR, Li Y, Lee JW, et al. A coding variant in rarg confers susceptibility to anthracycline-induced cardiotoxicity in childhood cancer. Nat Genet. 2015;47(9):1079-84. https://doi.org/10.1038/ng.3374.
77. van der Zanden SY, Qiao X, Neefjes J. New insights into the activities and toxicities of the old anticancer drug doxorubicin. Febs J. 2021;288(21):6095-111. https://doi.org/10.1111/febs.15583.
78. Livingston MB, Jagosky MH, Robinson MM, Ahrens WA, Benbow JH, Farhangfar CJ, et al. Phase ii study of pembrolizumab in combination with doxorubicin in metastatic and unresectable soft-tissue sarcoma. Clin Cancer Res. 2021;27(23):6424-31. https://doi.org/10.1158/1078-0432.Ccr-21-2001.
79. Egelston CA, Guo W, Yost SE, Ge X, Lee JS, Frankel PH, et al. Immunogenicity and efficacy of pembrolizumab and doxorubicin in a phase i trial for patients with metastatic triple-negative breast cancer. Cancer Immunol Immunother. 2023;72(9):3013-27. https://doi.org/10.1007/s00262-023-03470-y.