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REVIEW ARTICLE

Streptomyces-Derived Nano-Doxorubicin: Clinical Status and Patents Granted

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Abstract

Streptomyces have been presented as a great source of antibiotics and anti-cancer drugs over the past century. Especially *Streptomyces* living in adverse conditions produce certain metabolites with cytolytic and anti-microbial activities, which have been utilised for manufacturing antimicrobial and anticancer drugs. Doxorubicin (DOX) is a potent anti-cancer drug derived from *Streptomyces*, that is widely used for various cancers, including cancers of the ovary, urinary bladder, GI tract, breast, thyroid gland, lung, bone, kidney (nephroblastoma) and blood (leukaemia). This anthracycline antibiotic is limited by its adverse effect profile, with the main adverse effects being nausea, vomiting, alopecia, infertility, cardiotoxicity, myelosuppression and nephrotoxicity. Nanoparticle delivery systems present a good solution to avoid adverse effects. Some nano-based formulations have reached the clinics, while many new ones in the pipeline show promising results. This review attempts to compile the existing literature on the clinical status of DOX highlight the need for the development of nanoparticles (NPs) that may serve as drug delivery agents, imaging probes and other multifunctional particulates. The integration of nanotechnology with *Streptomyces*-derived compounds can help shape the anti-cancer therapy of the future.

Key words: Doxorubicin; Nanostructures; *Streptomyces*; Nanoparticles; Microbial.

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Introduction

When it comes to producing antibiotics, *Streptomyces* is a very significant group of bacteria. These can be found in a variety of habitats ranging from the deep ocean to the highest mountains.[1, 2] *Actinobacteria* is a phylum of Gram-positive, filamentous, spore-forming bacteria. *Streptomyces* and *Kitasatospora* split around 382 million years ago during the late Devonian era, at the same time when terrestrial animals appeared.[3, 4] To spread, *Streptomyces* produces hyphae, which are threadlike filaments that burrow through surfaces in quest of nutrition. Strains such as *Streptomyces* are capable of surviving in adverse settings and dispersing quickly, making them ideal for places with limited resources.[5, 6]

These secondary metabolites are produced by *Streptomyces* during this development phase and may provide the organism with an edge over its competitors.[7] These metabolites benefit the vegetative bacteria cells by producing

antibiotics and siderophores and possessing the property to impart coloration (to protect them from UV radiation) and communication with other species. As a result of their relatively big genome, *Streptomyces* are capable of producing a wide range of molecular variations.

Bioactive chemicals produced by *Streptomyces* are well recognised.[8] Among naturally occurring antibiotics and medicinally important compounds, almost 75 % of these compounds were derived from *Streptomyces.*[9] All kinds of bioactive secondary metabolites, including those used in human health and agriculture, are reliable sources of microorganisms that attract the interests of scientists.[10] As a result, finding new strains of *Streptomyces* is an urgent and critical task.

Selective media formulations, such as those formulated to promote *Streptomyces* colonies while decreasing other bacteria, have been used to isolate this bacterium from soil. One *Streptomyces* selective media is Benedict's modified Lindenbein medium in which oil suspension is pretreated with extract of yeast and sodium dodecyl phosphate. After pretreatment, oil suspensions undergo heat shock when combined with humic acid vitamin agar, such as *Staphylococcus stifleri.*[11] Streptomyces numbers increase when soil solution is treated with 1.5 % phenol (30 °C for 30 min). This denatures proteins or destroys cell membranes of common actinomycetes such as bacteria and fungus.[12] This manuscript provides deep understanding about the mechanism of anthracyclines, with particular reference to doxorubicin (DOX) and its nano-formulations for the treatment of cancer.

Anthracyclines

Anticancer medications developed from *Streptomyces* bacteria are anthracyclines. Their antitumour efficacy was shown in the 1960s and they entered clinical usage in the 1970s. Anthracyclines are red aromatic polyketides that exist in many forms owing to structural variations in the aglycone base molecule and the various sugar residues attached. These medications are not specific to the cell cycle.

Early chemotherapeutic drugs in this family included daunorubicin and DOX. When physicians discovered that cancers gained resistance to those medications whose side effects, such as cardiotoxicity have restricted the dosage of patients that they could tolerate, the medicinal chemists attempted to create adaptations of these compounds - analogues with broader action and lower toxicity. Over the years, more than 2,000 analogues have been researched in an attempt to identify better anthracyclines.[13] However, only a few of anthracycline analogues, such as epirubicin and idarubicin,[14] have been authorised for clinical use. Cardiac toxicity continues to be a significant issue while taking anthracyclines. Anthracyclines are known to cause damage to DNA by several different processes, some of which include the creation of free radicals, the development of DNA-anthracycline adducts and the poisoning of topoisomerase-II. Anthracycline's semiquinone radical can intercalate between DNA base pairs, which can lead to DNA damage through the formation of reactive oxygen species (ROS).

Mechanism of action

Research on the exact method by which anthracycline functions in the body is still ongoing. However, it is recognised that the main target of popular anthracyclines like DOX is DNA. The basic mechanism involves planar tetracyclic chromophores intercalating between base pairs of DNA, which affects DNA transcription and translation. The cytotoxic activity of anthracyclines is not just dependent on the drug's affinity for binding DNA; other factors, including as the anthracycline's binding mechanism and location, also play a major role in cytotoxicity.

Each anthracycline has a different specificity, binding affinity and binding mechanism based on the DNA base sequence. Daunomycin binds more readily when two GC base pairs have AT between them, that is, GCAT-GC, according to structural, computational and solution-based analyses of the daunomycin-DNA complex.[15, 16] DNAase foot-printing

and equilibrium binding were used to examine daunorubicin's sequence and site specificity. These experiments show that daunomycin is specific to specific DNA sequences and that as the quantity of GC increases, so does its affinity for binding DNA.[17] Furthermore, to assess daunorubicin's sequence-specific binding, the effect of the restriction endonucleases EcoRI and PvuI on the cleavage of linear pBR322 DNA was examined.[17] EcoRI's and PvuI's recognition sequences are 5'-GAATTC-3' and 5'-CGATCG-3', respectively. PvuI inhibits pBR322 DNA digestion more quickly than EcoRI, as demonstrated by Chaires et al, suggesting that daunorubicin has preferred sequence specificity. Analyses of the crystal structure of daunomycin-DNA d (CpGpTpApCpG) complexes yielded comparable findings.[16] The selectivity for the GC base pair results from the formation of hydrogen bonds upon contact. The hydroxyl group on daunorubicin's C9 forms two hydrogen bonds with guanine's N2 and N3. This predilection for GC base pairs also explains why the drug's binding affinity increases as the GC content of DNA increases. Similar to DOX, epirubicin and idarubicin, the crystal structures of the anthracycline's DOX, epirubicin and idarubicin reveal a sequence-specific intercalative binding mechanism between DNA bases.

In addition, a number of studies were conducted to investigate the DNA intercalation mechanism of anthracyclines. Also created was a spectrofluorometric technique for estimating anthracycline intercalation in live cells and DNA solutions.[18] Using flow cytometry, Belloc et al[19] measured the intercalation of anthracyclines in the DNA of live cells. Using picogreen (a fluorescent dye binding DNA), Ashley et al[20] have proven the intercalative capability of anthracyclines in both nuclear and mitochondrial DNA. Mitochondrial toxicity is significantly affected by the intercalation of anthracycline into DNA of mitochondria. As a result of DOX's interaction with DNA, AFM investigations support the likely mechanism of intercalative binding mode.[20]

Mechanism of resistance

In addition to cardiotoxicity, therapy with anthracyclines induces anthracycline resistance even at the optimum cumulative dosage.[21] Natural and acquired medication resistance is both possible. Some cells exhibit natural resistance even before the medication is administered; while the acquired one arises with medication intake. Drug resistance is caused by a number of processes, such as changes in the drug's ATP-binding cassette-related efflux and accumulation, qualitative and quantitative changes in topoisomerase II, p53 activity, overexpression of enzymes that scavenge reactive oxygen species, etc. One of the main causes of anthracycline antibiotic resistance is thought to be ABC (ATP-binding cassette) transporter proteins [R]. One of the ABC proteins, P-glycoprotein (Pgp), is considered to cause anthracycline resistance through drug efflux, inflow suppression and drug accumulation within the cell.[22] The interaction between anthracycline and Pgp is linked to the active efflux of anthracycline through the cell's transmembrane domain. The *mdr1* gene, which codes for Pgp, is triggered by any chemical or environmental stimuli during cell differentiation. When the anthracycline comes into contact with the plasma membrane and exports it, Pgp recognises it. Therefore, the rising amount of Pgp produces an export-import imbalance.

Moreover, a change in topoisomerase II activity, either quantitatively, ie, a drop in the amount of enzyme, or qualitatively, ie, a change in the normal activity of enzyme owing to mutation or another cause, might confer resistance to the cell against additional doses of anthracyclines. The expression of p53 is required for anthracycline-mediated cell death. Consequently, inactivation or downregulation of p53 might result in drug resistance.[22] SOD, GSH and catalase are ROS scavengers and their overexpression during cytotoxicity may also confer anthracycline resistance.[39] Increased DNA repair also leads to anthracycline resistance in cells.

Doxorubicin

DOX (ATC code L01DB01) is an antibiotic having chemotherapeutic characteristics and is therefore used in cancer treatment.[23] It originated from the mutant strain of *S peucetius* spp *Caesius* ATCC 27952. *Farmitalia Carlo ErbaSpA* patented the medicine in 1971, with the European Medicines Agency (EMA) and the FDA approving various

products. DOX is one of the most effective anti-tumour anthracycline antibiotics frequently used to treat a variety of cancer types. Due to DOX's high toxicity, its application in medicine is limited [median fatal dosage - LD_{50} = 21,800 g/kg (rat, subcutaneous)]. DOX is strongly emetogenic, so anti-emetogenic medications must be prescribed; some patients also report delayed nausea.[24] It is also a vesicant, meaning that extravasation from the blood vessel produces significant tissue damage through necrosis, resulting in intense pain.

DOX is mutagenic and possibly carcinogenic in animals and humans. DOX is designated as FDA/pregnancy category D because it may cause gonadal suppression, resulting in amenorrhea or azoospermia, which may lead to infertility. Evidence of human foetal risk is associated with DOX based on adverse reactions, marketing experience, or human studies; however, the benefits of using DOX in pregnant women may often outweigh the risk, although breastfeeding should be avoided during chemotherapy. DOX also alters the male reproductive profile due to testicular injury and a considerable reduction in testicular weight. The damage to cell membranes, DNA and proteins is the result of two different mechanisms performed by DOX:

(i) Intercalation into DNA, leading to disruption of the DNA repair mechanism intervened by topoisomerase II and
(ii) Release of free radicals.

The unstable metabolite semiquinone is regenerated from DOX. The pathway of generating reactive oxygen species by DOX can result in oxidative stress, DNA damage, lipid peroxidation, membrane damage and cell death by persuading apoptotic pathways (Figure 1).

Cardiotoxicity has been found to be the most severe toxicity while using anthracyclines[25, 26] manifesting as either immediate or delayed consequences. Acute left ventricular failure is uncommon, although it may occur, especially in individuals who received more than the maximum recommended dosage of 550 mg/m^2. Due to the danger of irreparable heart damage, routine monitoring tests must be performed and in the event of suspected cardiotoxicity, the benefit must be considered. Concurrent administration of many medicines such as trastuzumab, paclitaxel – PTX, docetaxel and even radiation raises the menace of cardiotoxicity.[27]

Patients taking DOX-based chemotherapy often have myelosuppression,[28] notably of leukocytes, necessitating haematological function monitoring. DOX is a powerful immuno-suppressant; hence precautions must be taken to prevent secondary infections. Additionally, stomatitis and mucositis often emerge at the start of therapy.[29, 30] In extreme instances, it may manifest into mucous ulcers in a matter of days. DOX is mostly eliminated by the hepatobiliary system, hence

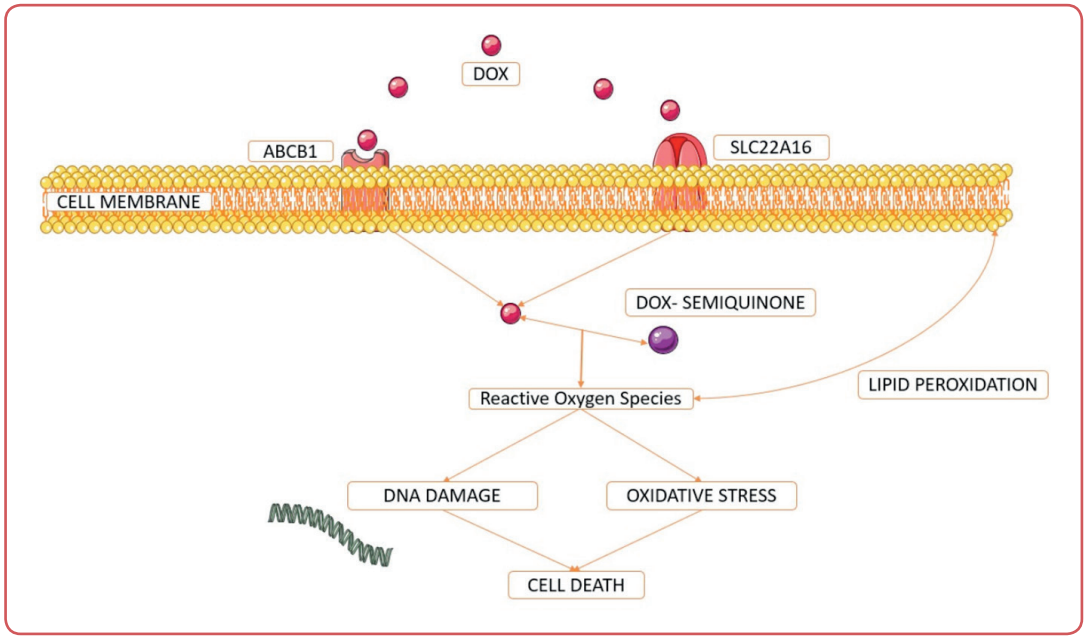

Figure 1: Mechanism of action of doxorubicin for its anticancer activity

should not be taken by the patients suffering with severe hepatic insufficiency.

DOX treatment is associated with an increase in plasma aspartate transaminase, alanine aminotransferase[31] and bilirubin levels. Focal hepatocyte destruction, vascular injury and steatosis may also occur. Some individuals who received DOX had lung complications such as pulmonary fibrosis, bronchospasm and local oedema, however, the underlying mechanisms are unknown. It also exhibits nephrotoxic action, as shown by severe congestion, intertubular haemorrhage with damaged epithelium and substantial degenerative alterations,[32] resulting in advanced renal damage which indicates that DOX induces chronic nephropathy that leads to end-stage renal failure.

In an effort to maintain DOX's broad-spectrum anticancer efficacy while minimising its toxicity, nanotechnology has been the primary focus. To put it simply, a nanomedicine is a system with nanocarriers and active moieties that may be utilised to treat or diagnose illness. No excipients are present and the nanomedicine exhibits improved bioavailability and drug distribution at the site of action with decreased toxicity.[33] Current chemotherapeutic methods have considerable drawbacks in comparison to nanomedicine. Drug release may be controlled in tumours using a well-designed nanoparticle technology. The increased permeability and retention (EPR) impact of nanoparticles (NPs) of suitable size (200 nm) and surface characteristics may allow them to passively target tumour tissues.[34] By shielding its payload medications from enzymes and the harsh physiological environment, NP formulations improve therapeutic effectiveness. There have been significant advances in the development of nanoparticles (NPs) that may serve as drug delivery agents, imaging probes and other multifunctional particulates. Several nano-delivery techniques, including polymeric, organic, inorganic, metallic and lipidic nano-formulations, have been used to lessen the adverse effects of medication delivery. Multiple imaging and targeted drug delivery modalities may be combined into a single particle for concurrent imaging and the treatment of tumour tissues. Using nontoxic, biocompatible and biodegradable polymeric nano-formulations for DOX is a potential strategy to deliver the drug at the desired location while also improving effectiveness and reducing adverse effects.[35] They have prolonged circulatory half-lives, increased permeability and EPR impact along with lower hepatic uptake.[36]

The mononuclear phagocyte system may identify traditional liposomes (Lip) after their systemic injection followed by prompt removal through reticuloendothelial system.[37] This is a key drawback of traditional liposomes. Proteins adsorption and formation of corona are often associated with the identification of Lip, along with other nanocarriers by the immune system followed by their removal from the systemic circulation. The technique that has been utilised most prevalently for minimising protein binding is the coating with inert biocompatible polymers, such as polyethylene glycol (PEG) on the surface of Lip to achieve the so-called 'stealth' surfaces.[38] Likewise, it has been proven that including Zwitter ionic lipids in Lip, alike PEGylation, permits decline in biding of proteins further enhancing the duration of plasma retention.[39] Moreover, a key issue confronting drug delivery method for treating cancer is the liposomal trigger at the tumour spot.[38] In this context, Lip may be built to limit or reduce the discharge of drug in the circulation and normal tissues and release their content only after receiving a precise trigger stimulus at the tumour location, yielding maximum anticancer effects.[37]

A normal pH value for biological fluids is between 7.4 and 7.6. Tumours have an external pH of 6.5–7.2, while their internal endosomes (5.5–5.0) and lysosomes (4.0–4.5) have even lower pH values.[40, 41] For this reason, pH-sensitive Lips have been created as delivery systems for anticancer drugs as they have good endosomal membrane fusing capacity, thereby releasing the medication into the cell's cells.[37, 38] When the Lip components have acid- base ionisable groups or acid-cleavable bonds, the pH sensitivity may be achieved. To add insult to injury, tumours are known to be hyperthermic because of their fast metabolic rate. Cancer cells are more sensitive than healthy ones to temperature fluctuations, which has inspired the creation of Lip nanocarriers that are thermo- responsive and can deliver anticancer therapies.[37, 42] It has been shown that phospholipids with a gel-to-liquid crystal phase transition at a few degrees above physiological temperature can be used to create a lip that is responsive to temperature changes,[43] as demonstrated by 1,2- dipalmitoyl- sn- glycero-3- phosphocholine (DPPC).

When treating breast cancer, fat layers in the mammary glands assist to accumulate medication into the breast tissue by oral delivery or direct injection of drug into the fat layers of the breast tissue. Researchers decided to use anionic SAIL [Ch][Ol] because of this consideration.[44] Oleic acid, a component of [Ch][Ol], has been shown to improve penetrability and absorption in breast fat layers.[45,46] Literature describes some SAILs including the studies about their aggregation behaviour.[47,48] Cholinium fatty acid-based ILs were investigated by Tanner et al for transdermal medication delivery.[49] For the non-invasive administration of insulin, the same IL was tested.[50] L-glutamic acid and L-alanine's solubility was improved by using [Ch][Ol].[51] Among all choline carboxylate ILs, [Ch][Ol] is the least hazardous, with greater permeability through the fat layers of the breast and improved aggregation behaviour.[52] The [Ch][Ol] micelles and surface-active characteristics have already been studied.[53] Because of the hydrophobic interactions between the oleate ions' carbon chains, [Ch][Ol] may self-aggregate to form nano-sized micelles. Zeta potential measurements from the infrared show the self-assembled structure as anionic one. An external additive, like GA, reduces electrostatic repulsions among head groups, increasing packing and transforming nano-sized micelles into vesicles (SANS data shown in the infra).[54] Intermolecular H-bonds with choline oleate result in the formation of densely packed aggregated structures at neutral pH when GA stays in its Zwitterionic state, which is composed of both COO^- and NH_3^+ ions. Because of its anticancer action,[55,56] pH sensitivity and propensity of improving the system's permeability, GA was selected above all other amino acids. Biocompatible and pH-responsive, the system might be the greatest drug delivery technology for delivering medications without impacting their effectiveness *via* localised and targeted drug administration. Since both [Ch][Ol] and GA have been shown to increase drug permeation to target cells in the mammary fat layer or by topical application, it is reasonable to assume that this two-scaffold system would work well for administering chemotherapy drugs to target cells in the mammary fat layer or to cells on the skin. Vesicles loaded with DOX were also analysed at acidic and neutral pH. The system displayed the release of 94 % of drug during 16 h at neutral pH compared to 95 % of drug release within 1 h at acidic pH, which indicates a sustained release of drug at neutral pH. Additionally, the DOX-loaded vesicle system is more cytotoxic to MCF-7 cell lines as compared to free DOX.

It is widely accepted that carbon-based nano-materials, such as graphene oxide (GO), are among the most sophisticated carriers for the effective delivery of medicines and biomolecules. Biocompatibility, large surface area, cheap cost and high drug loading capacity are only some of the advantages of using GO as a drug carrier.[57] Functional groups such as hydroxyl, epoxy and carboxyl may be found in GO.[58] It is known that GO nanosheets may be loaded with pharmaceuticals more efficiently *via* van de Waals forces and by using the p-conjugated structure of GO. Even yet, the existence of sharp edges in GO makes it unsuitable for drug delivery since it may disrupt normal cells and aggregate on cell membranes, resulting in toxicity.[59] Nanoparticles that are functionalised with biocompatible polymers, such as GO nanoparticles, to minimise cytotoxicity, boost solution solubility and maintain their stability under physiological circumstances are often used to address these constraints. Their biodegradability, low toxicity and biocompatibility make polysaccharide nanoparticles ideal drug delivery carriers. Polymers functionalised onto GO sheets include gum Arabic, gelatine, chitosan, starch, glycogen polyethylene glycol, cellulose, poly-caprolactone, polyvinylpyrrolidone and polylactic acid. GL-GA-GO tri-nanocomposite for DOX loading was developed in work as a unique green biocompatible nontoxic material.[60] Good biocompatibilities, nano size stability and high DOX loading were all shown in the newly developed tri-nanocomposite. The release of DOX from the loaded composite was pH-dependent, in a way of higher release rate at malignant pH as compared to physiological pH. Both the WI-38 and the A549 cells were unaffected by the prepared unloaded tri-nanocomposite. Nanocomposite loaded with DOX was shown to be more effective against A549 cells than conventional DOX, with IC_{50} values of 51.9 and 86.6 g/mL, respectively. For WI-38 cells, the DOX-loaded composite was less hazardous than conventional DOX, bearing IC_{50} values of 1.08 and 0.46 µg of DOX/mL, respectively.

Hernandes et al studied the effects of iron-oxide magnetic nanoparticles coupled with the anticancer medication DOX on human breast cancer cells *in vitro*. The findings of study demonstrat-

ed that magnetic nanoparticles containing DOX (NpMag+Dox) contribute to the development of a cellular redox imbalance in MCF-7 cells. Additionally, researchers demonstrate that iron-oxide nanoparticles that have been functionalised with DOX generate oxidative stress, which may be observed in the form of DNA damage, lipid peroxidation, disruption of the cell membrane and loss of mitochondrial potential values. Because of this, NpMag+Dox causes MCF-7 cells to pause the cell cycle and reduce the amount of cell migration that occurs. A better delivery of DOX to MCF cells was produced by the association of NpMg+Dox, primarily in the presence of a magnetic field. This resulted in an increase in the death of MCF cells, which ultimately led to a reduction in the toxicity of the therapy for healthy cells, resulting in a more effective treatment.[61]

An innovative radio-bioconjugate has been developed for targeted therapy of cancers that overexpress HER2 receptors.[62] This multimodal treatment combines a potent chemotherapeutic agent (DOX), a β-emitter (^{198}Au) and a guiding vector (trastuzumab, Tmab). The integration of these components allows for a more precise and effective approach to treating cancer. In order to accomplish this objective, 30 nm radioactive gold nanoparticles (^{198}AuNPs) were synthesised and then coated with a poly-ethylene glycol (PEG) linker. This linker was conjugated to DOX and monoclonal antibody (Tmab) through peptide bond formation. Through *in vitro* experiments, it was observed that the radio-bioconjugate showed a strong binding to HER2 receptors and was efficiently taken up by the cells. Using the MTS assay, researchers observed a notable decrease in the viability of SKOV-3 cells in cytotoxicity experiments. After 48 h of treatment with 2.5 MBq/mL, a synergistic cytotoxic effect was observed as a result of the simultaneous presence of DOX and ^{198}Au. Flow cytometry analysis revealed that DOX-^{198}AuNPs-Tmab predominantly caused cell cycle arrest in the G2/M phase and late apoptosis. In spheroid models, the radio-bioconjugate demonstrated dose-dependent additive and synergistic effects. *Ex vivo* biodistribution experiments were conducted in mice with SKOV-3 tumours to study the distribution of ^{198}AuNPs-DOX and DOX-^{198}AuNPs-Tmab after intravenous and intertumoral administration. Finally, the *in vivo* therapeutic efficacy studies on the same animal model revealed highly encouraging results. They demonstrated a remarkable halt in tumour growth for up to 28 days after a single intertumoral injection of 10 MBq. Hence, the suggested multimodal radio-bioconjugate exhibits immense promise for the targeted therapy of HER2+ cancers.[62]

Patents related to nano-doxorubicin in cancer treatment

The patents related to Nano-Dox has been tabulated in Table 1.

Table 1: Patents related to nano-doxorubicin in cancer treatment

Patent No	Details of patent	Ref
CN-117085147-B	A biological medicine-related carbon-based drug delivery system loaded with doxorubicin and its manufacture and use were disclosed in the invention. First, carbonising poloxamer, 3-hydroxytyrosol, ferrous ammonium sulphate hexahydrate, 1,3, 5-trimethylbenzene and ammonia water to obtain black nano particles CDF; then, sequentially reacting the black nano particles CDF with ammonia water, tetraethyl silicate, 3-methacryloxypropyl trimethoxy silane, mercaptopropionic acid, dimethyl maleic anhydride, hydrazine hydrate, dialdehyde polyethylene glycol and ace. Dispersing the carbon-based nanomaterial and doxorubicin in water and stirring at room temperature in a dark environment to load the nanomaterial with the medication. The acid-responsive drug delivery mechanism protected against cardiotoxicity caused by doxorubicin compared to tumour-released doxorubicin.	63

RU-2808909-C1	Invention described a method of treating connective tissue sarcoma M-1 in rats using a conjugate of diprop-oxybacteriopurpurin with doxorubicin, which included photodynamic therapy and chemotherapy, adminis-tered intravenously and laser-exposed to the tumour node during a drug-light interval.	64
CN-115444810-B	Poly(lactic-co-glycolic) acid (PLGA) and lecithin are weighed and dissolved in methylene dichloride to be used as an oil phase, an internal water phase was added and probe ultrasound was performed under the ice water bath to obtain colostrum; transferring the colostrum into Tween-80 solution, ultrasonic treatment to obtain compound emulsion and reduced pressure distillation to remove dichloromethane to obtain DOX-NPs suspension and centrifugation. The doxorubicin-loaded nanoparticle composite temperature-sensitive gel is easy to prepare and has biodegradability, slow-release performance and higher bioavailability.	65
RU-2794798-C1	Patent included doxorubicin in phospholipid nanoparticles with AS_{1411} aptamer conjugated with DSPE-PEG_{2000}-Mal for targeted tumour administration of a cytostatic drug and a method for generating the compo-sition. In one embodiment, the pharmaceutical composition comprised phospholipids, doxorubicin and AS_{1411} aptamer coupled with DSPE-PEG_{2000}.	66, 67
CN-114209824-B	The invention described doxorubicin prodrugs that were designed to be used in combination with photo-thermal tumour penetration enhancement and chemotherapy. The improved intertumoral permeation and combination photothermal and chemotherapeutic properties of the nano-medicament made according to the invention make it more effective against tumour cells. The drug accomplished the goal of combining photo-thermal and chemotherapy by using an intelligent size regulation strategy, having a large-size enhanced long circulation in blood, disintegrating into small-size DOX-photothermal conversion nano-particles in a tumour to improve penetration and then generating heat under near infrared light to release DOX once the nano-par-ticles have penetrated the deepest part of the tumour.	68

Conclusion and future directions

Nature already has a vast variety of natural molecules that have a large variety of biological activities,[69-74] but due to their poor physical properties, they are not able to permeate or perform their activity to optimum, as a result, nanotechnology-based approaches are used. However, nanotechnology uses various approaches for synthesis or preparation and these are somewhat complex.[75-79] Organic and inorganic nanoparticles of varying formulation and fabrication procedures that achieve controllable size, shape and high versatility, have been the subject of numerous recent papers evaluating their relative merits. They contain multiple chemotherapeutic and active molecules and are functionalised for targeted therapy. Despite promising outcomes in preclinical research, only a small fraction of those findings is being put into practice in hospitals. Some nanomedicines are already in clinical use. First, using smart strategies in cancer nanomedicines for the stratification of patients, various protocols to look for the microenvironment of the tumour and identification of suitable individuals to be included in clinical trials by imaging-based tumour accumulation, was proposed by Van der Meel and colleagues.[80] Secondly, designing vigilant rational pharmacological combined regimens would amplify the pharmacodynamic and/or pharmacokinetic benefits. They proposed to use tactful approaches for designing modular (pro)drug and drug-delivery systems and library screening to increase the likelihood of formulations developed and experimented in a preclinical setting. The application, translation and use of nanomedicine will all benefit from these astute methods.

Ethics

This study was a secondary analysis based on the currently existing data and did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

Author contributions

Conceptualisation: HC
Methodology: HC, DDB
Data curation: DDB, PK
Writing - original draft: HC, DDB
Writing - review and editing: PK

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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