Published in final edited form as:

Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2014 ; 6(4): 349–368. doi:10.1002/wnan.1264.

Recent progress in the development of polysaccharide conjugates of docetaxel and paclitaxel

Aniruddha Roy¹, Mousumi Bhattacharyya¹, Mark J. Ernsting^{1,2}, Jonathan P May¹, and Shyh-Dar Li^{†,1,3,4}

¹ Drug Delivery and Formulation, Drug Discovery Program, Ontario Institute for Cancer Research, 101 College Street, Suite 800, Toronto, Ontario, Canada, M5G 0A3

² Faculty of Engineering and Architectural Science, Ryerson University, Toronto, Ontario, Canada, M5B 1Z2

³ Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada, M5S 3M2

⁴ The Techna Institute, University Health Network, Toronto, Ontario, Canada, M5G 1P5

Abstract

Taxanes are one of the most potent and broadest spectrum chemotherapeutics used clinically, but also induce significant side effects. Different strategies have been developed to produce a safer taxane formulation. Development of polysaccharide drug conjugates has increased in the recent years due to the demonstrated biocompatibility, biodegradability, safety and low cost of the biopolymers. This review focuses on polysaccharide taxane conjugates and provides an overview on various conjugation strategies and their effect on the efficacy. Detailed analyses on the designing factors of an effective polysaccharide drug conjugate are provided with a discussion on the future direction of this field.

Introduction

Cancer is a leading cause of death worldwide. It was estimated that in the United States, more than 1.6 million new cancer cases will be reported in 2013 and it will account for 0.6 million deaths in the same year. Due to this high prevalence and mortality rate, widespread research has been undertaken ranging from understanding the pathophysiology of the disease to developing innovative drugs and technologies for improved therapy. The established treatment strategies for cancer can be divided into four categories: surgery, radio-therapy, chemotherapy and immunotherapy. Amongst these, chemotherapy is regarded as the first line approach of treatment for advanced disease. Among those anticancer chemotherapeutic drugs that have emerged in the past decades, taxane diterpenoid anticancer agents such as docetaxel (Taxotere®, Sanofi-Aventis, Fig 1A) ¹ and paclitaxel (Taxol®, Bristol-Myers Squibb, Fig 1B) ² have shown significant potency against various cancers. Taxanes therapeutic effect is attributed to binding with microtubules, which are cytoskeletal elements with functions extending from cellular transport to cell motility and

[†] Corresponding author Shyh-Dar Li, Ph.D. 101 College St, MaRS Centre South Tower, Suite 800, Toronto, Ontario M5G 0A3, Canada sli@oicr.on.ca TEL: 647-260-7994.

mitosis³. Docetaxel and paclitaxel assist polymerization of microtubules to a hyper-stable and dysfunctional state, thus arresting the cell cycle at the G2/M phase, leading to cell death^{4, 5}. Taxanes are effective against a wide array of cancers including breast, ovarian, non-small cell lung and prostate cancers⁶. Although the antitumor spectrum of taxanes appears to be the broadest of any class of anticancer agents⁶, their use can be limited due to the toxicity associated with the drugs and the formulation excipients. Paclitaxel and docetaxel are insoluble in water and are currently formulated with Cremophor EL/ethanol/saline and Tween80/ethanol/saline, respectively. Both Cremophor EL and Tween 80 (especially Cremophor EL) cause severe hypersensitivity reactions, requiring premedication regimes^{7, 8}. The free drugs also cause severe dose limiting toxicity such as neutropenia and neuropathy due to the non-specific delivery^{9, 10}. Concerted attempts have been made to develop new delivery systems for taxanes with lower toxicity, and recent advances in nanomedicine have created an opportunity for not only development of a detergent free delivery system for taxanes, but also for a more potent and tumor-targeted dosage form.

Long before the term nanomedicine was first mentioned by Drexler, Peterson, and Pergamit in their popular book *Unbounding the Future* in 1991¹¹, interdisciplinary research was underway to utilize the advantages associated with drug–polymer conjugates in the treatment of cancer. The first practical exemplification of polymer conjugates as anticancer therapeutics was a polymer–protein conjugate: Maeda et al.¹² first demonstrated that the anticancer activity of a protein could be improved by conjugating with a polymer (SMANCS)¹². They demonstrated that the conjugated protein preferentially accumulated in the tumor tissue due to the increased molecular size, a characteristic which prolonged blood circulation and enhanced accumulation in the tumor through the leaky tumor vasculature. These conjugates also displayed reduced elimination from the tumor due to the impaired lymphatic drainage¹³. This phenomenon, which is commonly known as the enhanced permeability and retention (EPR) effect, resulted in improved efficacy and reduced toxicity of the drug. This discovery opened up significant potential for passive targeting of anticancer drugs to tumors, and has led to the development of numerous nanotherapeutic drug delivery systems, including biologically active polymeric drugs¹⁴, polymer–drug and polymer–protein conjugates¹⁵, nanoparticles and liposomes¹⁶, and non-viral vectors for gene/small interfering ribonucleic acid (siRNA) delivery^{17, 18}.

Polymer–drug conjugates have advantages over conventional polymeric nanoparticles that passively encapsulate drugs in terms of increased drug loading capacity, enhanced stability and prolonged plasma half-life in vivo¹⁹. Polymeric nanoparticles, where the active drug is physically encapsulated in the polymeric scaffold, often exhibit drug loading instability due to partitioning of the hydrophobic drug during systemic circulation, depleting the nanoparticles of drug content²⁰. Chemical conjugation of the active drug to the polymeric carrier provides increased control of the drug release rate if a site-selective, cleavable linker between the drug and the polymer is introduced, thereby reducing loss of the drug during circulation and improving tumor bioavailability. This controlled release of the drug can manifest enhanced pharmacokinetic and pharmacodynamic properties²¹.

A broad panel of polymers, both natural and synthetic, has been explored for their performance as anticancer drug–conjugates. Among these screened polymers,

polysaccharides have demonstrated advantages, including their low cost, high availability, stable physicochemical and biological characteristics, established safety profiles, permitted use as an excipient in pharmaceutical products and the presence of chemical functional groups suitable for bioconjugate modifications.

Polysaccharides are complex carbohydrate polymers consisting of two or more monosaccharides linked together covalently by α - or β -glycosidic linkages (Fig 1C). Depending on the composition, polysaccharides can be divided into two main types: homo-polysaccharides and hetero-polysaccharides. A homo-polysaccharide is defined to have only one type of monosaccharide repeating in the chain such as dextran and cellulose; whereas, a hetero-polysaccharide is composed of two or more types of monosaccharides such as heparin and hyaluronan. In both types of polysaccharide, the monosaccharide can link in a linear fashion or they can branch out into complex formations. As polysaccharides have a wide range of molecular weights and a large number of functional groups for chemical modification, they are an attractive choice as a polymeric backbone in polymer conjugates. The pharmacokinetics of polysaccharide conjugates are greatly influenced by their charge, MW, extent of chemical modifications, polydispersity and structure. Although polysaccharides have been extensively used for passive encapsulation of cytotoxic drugs ²², in this review we primarily focus on polysaccharide conjugates of taxanes that have been studied for anticancer therapy.

Hyaluronic acid

Hyaluronic acid (HA) is a biodegradable, biocompatible, and viscoelastic linear glycosaminoglycan composed of alternating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked together through alternating β -1,4 and β -1,3 glycosidic bonds (Fig 2A). HA is one of the main components of the extracellular matrix and is widely distributed throughout the connective, epithelial and neural tissues ²³. Due to its various biological functions, desirable physicochemical properties, biocompatibility, biodegradability and non-immunogenicity, HA and modified HA have been investigated and applied in different pharmaceutical and medical applications including arthritis treatment ²⁴, ophthalmic surgery ²⁵, drug development ²⁶ and tissue engineering ^{27, 28}. More recently, there has been growing research utilizing HA for tumor targeted drug delivery due to its potential for active tumor targeting (negating the need for additional targeting ligands). It has been reported that HA receptors such as cluster determinant 44 (CD44) and receptor for hyaluronate-mediated motility (RHAMM) are over expressed in certain types of cancers ^{29, 30}. Therefore, through conjugation with HA, cytotoxic drugs can be targeted to the cancer cells by receptor mediated endocytosis ³¹.

Different strategies have been developed for conjugating taxanes with HA. While hydroxyl and carboxylic acid sites are available, it is mainly the carboxylic group that is used as sites for drug conjugation (Fig 2). Linker molecules including succinic acid and amino acids for tuning the conjugation site chemistry have been investigated (Fig 2). Different solubilization techniques have been developed to dissolve polar HA and non-polar taxanes in a common solvent for the conjugation reaction. As an example, tetrabutylammonium interacts with the

carboxylates of HA via charge-charge interaction to increase the solubility in dimethyl formamide (DMF) for efficient chemical reaction with PTX.^{32, 33}

Adipic acid dihydrazide (ADH) is commonly used as a linker for conjugating taxanes to an HA backbone. Luo and Prestwich³⁴ first used the ADH modified HA for conjugation with PTX (Fig 2B). They reported three different levels of ADH modification (9%, 18%, 45%) in the HA backbone with varying PTX loading (from 1-15%). The degree of ADH substitution in the HA backbone influenced the CD44 targeting ability and cytotoxicity of these conjugates, with higher ADH substitution reducing the potency. Drug release was found to be relatively rapid: within 24h, 40%, 35% and 90% of PTX was released when the conjugate was incubated in human plasma, hyaluronidase and esterase respectively³⁵. Auzenne et al.³⁶ slightly modified the conjugation method and reported a HA-PTX conjugate with 10% ADH modification and 15-20% PTX loading. In general, potency of these conjugates was higher than free PTX against CD44 expressing cell lines (SKOV-3, NMP-1, HBL-100, and HCT-116), while no cytotoxicity was observed against CD44 negative cells (NIH 3-T-3) at concentrations up to 10 µg/mL PTX equivalent. In contrast, Galer et al.³⁷ reported in vitro potency of this conjugate to be ~2.5 times less than free PTX against human squamous cell carcinomas cell lines OSC-19 and HN5, both of which are CD44 positive. The CD44 targeting ability of the HA conjugate was demonstrated by selective uptake of FITC linked HA-PTX by CD44 expressing cells, which was blocked by anti-CD44 antibody and pre-incubation with free HA³⁶. The in vivo antitumor efficacy was improved compared to native PTX at the maximum tolerated doses (MTDs) in tumor xenograft (ovarian carcinoma SKOV-3ip, NMP-1)³⁶ and orthotopic models (squamous cell carcinoma OSC-19, HN-5)³⁷.

Rosato et al.³⁸ reported preparation of another HA-PTX conjugate using butyric acid as a linker by slightly modifying the method reported by Leonelli et al.³² (Fig 2C). PTX loading in this conjugate was about 20% wt/wt. The efficacy of the conjugate was found to be dependent on the tumor type in both the in vitro and in vivo assays. While in vitro potency of the conjugate against human bladder cancer cell lines (RT-4 and RT-112/84) was significantly higher than free PTX with a reduction of IC₅₀ by 120- to 800-fold³⁸, the conjugate exhibited much less potency against ovarian cancer cell lines (IGROV-1, OVCAR-3 and SKOV-3) with 5 – 50 times higher IC₅₀ values compared to native PTX^{39, 40}.

The in vivo antitumor efficacy of this conjugate was also dependent on the tumor type: while the conjugate exhibited slightly lower antitumor efficacy than native PTX against bladder carcinoma, it showed better efficacy against ovarian cancer. Tumor growth was significantly higher in the conjugate treated animals compared to native PTX against bladder carcinoma cell line RT-112/84. After treating the tumor bearing mice with the conjugate (100 mg/kg PTX equivalent i.p.) and free PTX (20 mg/kg i.v.) every 7 days for a total of 3 treatments, mean tumor volume of the conjugate and PTX treated animals were 711 and 574 mm³ respectively compared to 1335 mm³ of control mice³⁸. In contrast to this lower efficacy seen against bladder carcinoma, against ovarian cancer it exhibited a highly significant increase in the survival compared to native PTX treatment. The tumor was inoculated by i.p. injection of 5×10⁶ IGROV-1 and OVCAR-3 cells and treated with PTX (20 mg/kg i.v.) or conjugate (100 mg/kg PTX equivalent i.p.) at days 7, 14, and 21. Median

survival of the conjugate treated animals increased by 2.5-fold and 2-fold compared to free PTX against IGROV-1 and OVCAR-3 respectively⁴⁰. Improved in vivo efficacy of this conjugate against ovarian cancer was also reported by Stefano et al.³⁹. Tumor (OVCAR-3) was inoculated by i.p. injection of the cells. The conjugate was injected i.p. at 40 mg PTX equivalent/kg dose and free PTX was injected as i.v. or i.p. at 20 mg/kg dose. Conjugate treatment resulted in a 2.5 fold increase in median survival compared to the control. Treatment with free PTX (20 mg/kg/injection, i.p.) also produced a significant therapeutic benefit with a 2-fold increase in survival over control. On the other hand, the conventional i.v. PTX (20 mg/kg/injection) did not significantly increase survival. Similar results were obtained with the SKOV-3 model.

A phase I clinical trial was conducted with the conjugate first reported by Rosato et al.³⁸ for intravesical therapy of bacillus Calmette-Guérin (BCG) refractory carcinoma (n = 16)⁴¹. The treatment was given by weekly intravesical instillation starting at the dose of 30 mg PTX equivalent per patient which was stepwise elevated to 150 mg PTX equivalent in different group of patients. No dose limiting toxicity was noted in any of the dose level studied. A total of 11 adverse events were reported by 7 patients (40%) and 9 (60%) showed complete treatment response.

Lee et al.⁴² showed that their PTX-HA conjugate (Fig 2D) self-assembled into micellar nanoparticulate structure in an aqueous solution. HA was first mixed with PEG and it was then dissolved in DMSO to make a HA/PEG nanocomplex with a hydrodynamic diameter of 120 nm. PTX was conjugated with these nanocomplexes by direct esterification. PTX loading was found to be 10% w/w. In an aqueous solution, the conjugate self-assembled into micelles with average diameter of 196 ± 9.6 nm. This nanoconjugated PTX micelle exhibited increased cytotoxicity against CD44 expressing cell lines (HCT-116, MCF-7) compared to CD44 negative cell line (NIH-3T3). Recently, Mittapalli et al.⁴³ used the same method to conjugate an ultra-low molecular weight HA (~4–5 kDa) with PTX to enhance brain delivery, and achieved PTX loading of 8 wt %. Two distinct size populations were observed a 2 to 3 nm and the other at approximately 80 nm. The authors argued that the smaller conjugates can self-assemble into larger particles similar in structure to a lipid membrane. The conjugate showed increased efficacy in a preclinical model of brain metastases of breast cancer cells. Luc-2 transfected MDA-MB-231Br cells were injected through the intracardiac route. Animals were treated with PTX or HA-PTX (6 mg/kg (PTX equivalent), once a week, i.v. for five doses). Brain lesions were significantly decreased in the HA-PTX group compared with the control and PTX groups. The median survival, however, was not significantly improved with the HA-PTX treatment compared to native PTX.

Another self-assembled nano formulation of HA-PTX conjugate was reported by Xin et al.³³ (Fig 2E) where different amino acids (valine, leucine and phenyl alanine) were used as the linker. Drug loading varied between 10 and 14 % w/w. All the conjugates form core-shell nanoparticles of about 280 nm in diameter with a nominal neutral surface charge. Slight variation in release rate of PTX was found with different amino acid linker used. While valine was most stable with a half life of 63 h in PBS (pH 7.4), phenyl alanine

exhibited the highest release rate (half life 47 h). Potency of all the conjugates was higher than free PTX against MCF-7 with a 2- to 3-fold reduced IC₅₀.

Thierry et al.⁴⁴ prepared a polyelectrolyte multilayer (PEM) dosage form of chitosan and PTX-HA conjugate (Fig 2F). The HA (500 kDa) was first modified with ethylenediamine with which PTX-succinate was conjugated. To preserve water solubility of the conjugate, PTX loading was kept at 3 mol %. The PEM was formed by successive deposition of negatively charged PTX-HA and positively charged chitosan. A quick burst release of PTX was measured from the multilayer of the HA-PTX - Chitosan complex when incubated in PBS with a half life of ~ 3 h. Although the cytotoxic activity of the conjugate was not tested against any cancer cell line, murine macrophages J774 was found to be sensitive to the conjugate with almost 90% death in 4 days of incubation at 1 and 10 µg PTX /mL.

Although HA is one of the most widely used polysaccharides for taxane delivery with one product being tested in phase I clinical trials, these studies resulted in contradictory reports on effects in different cell lines. While most studies showed targeted cytotoxicity against CD44 overexpressing cell lines and minimal toxicity against CD44 (–) cells^{33, 34, 42}, some studies reported significant toxicity against CD44 non-overexpressing cells⁴⁴. The in vitro cytotoxicity also varied significantly with some reports showing dramatic increase in potency compared to the native drug³⁸ while, with the same conjugate, others reported significantly reduced potency^{39, 40}. Variation in effect may be due to the heterogeneity in the cell line tested: although all the cell line tested are CD44 positive, there may be variation in the level of CD44 expression among them, which could influence potency of the conjugate. A systemic study comparing the CD44 expression level in the cells and respective potency of HA-PTX conjugate could help resolve this discrepancy.

The biodistribution of HA conjugates should also be studied in greater depth. Apart from CD44, HA also interacts with the hyaluronic acid receptor for endocytosis (HARE) which presents on the liver and spleen sinusoidal endothelial cells⁴⁵. Significantly high liver uptake was reported with a cisplatin-HA conjugate which plateaued at a relatively high level for at least 3 days producing dose limiting toxicity and reducing the MTD of cisplatin by 2-fold⁴⁶. Liver toxicity of HA conjugates should be carefully studied.

Carboxymethyl cellulose

Carboxymethyl cellulose (CMC) or cellulose gum is a cellulose derivative with carboxymethylene groups (–CH₂–COOH) modification to some of the hydroxyl groups of the glucopyranose monomers that make up the cellulose backbone (Fig 3A). It is often used as its sodium salt, as a pharmaceutical excipient (FDA Inactive Ingredients Database). CMC has been approved by FDA for parenteral use in products such as Vivitrol, Sandolog, and Sandostatin and is reported to undergo hydrolysis in vivo to its precursors for excretion⁴⁷. The presence of a high number of carboxylate groups makes this polymer an attractive candidate for conjugation with different drugs with relatively high drug loading. However, due to its high polarity and very low solubility in non-aqueous organic solvents, conjugation strategies are limited. To overcome this problem, Ernsting et al.⁴⁸ modified CMC by esterification of the hydroxyl groups with acetic anhydride. Quantitative acetylation of all

the hydroxyl groups was reported. This modification allowed this acetylated CMC to be dissolved in polar aprotic solvents like acetonitrile, increasing the coupling efficiency of hydrophobic drugs to this polymer. Acetylated CMC (CMC-Ac) was used for conjugation with DTX and poly-ethylene glycol (PEG) to prepare a polymeric conjugate of DTX (Cellax), composed of a PEGylated and acetylated CMC backbone with DTX attached via ester linkages (Fig 3B). A high drug loading of 37 wt% was achieved in this conjugate. As the polymeric conjugate contains both hydrophobic (DTX) and hydrophilic (PEG) components, the polymers condense into monodispersed ~120 nm particles in saline when the hydrophobic/hydrophilic ratio was balanced (20 – 37 wt% of DTX; 5 wt% of PEG). DTX release from this conjugate follows a near zero order kinetics (3.6% per day). Cellax exhibited significant cytotoxicity against a wide panel of cancer cell lines of both human and murine origin with 2- to 40-fold increased potency compared to native DTX. The increase in efficacy may be due to efficient cellular internalization of Cellax and the sustained release of DTX from Cellax: it was found that the fractionated dosing of native DTX mimicking the slow release exhibited enhanced cytotoxicity compared to a single bolus dose of DTX. In the in vivo biodistribution study, Cellax displayed significantly extended blood circulation time compared to the clinical formulation of DTX (Taxotere) with 5.2 times longer $t_{1/2}$ and 38.6 fold increased area under the curve (AUC)⁴⁹. Tumor accumulation of the drug was 5.5 fold higher with Cellax treatment compared to Taxotere. The maximum tolerated dose of DTX was significantly increased with Cellax compared to Taxotere: while Taxotere at 40 mg DTX/kg dose induced significant apoptosis in the kidney and lung of the treated mice, Cellax at 170 mg DTX/kg dose caused only minor weight loss (~5%). In vivo antitumor efficacy of Cellax was tested against both orthotopic and subcutaneous tumor model representing a wide array of tumors, including both human and murine origin. Compared to Taxotere, Cellax exhibited significantly higher antitumor efficacy against several cancer models in mice including breast cancer (EMT-6, MDA-MB-231), lung cancer (LL/2), prostate cancer (PC-3) and melanoma (B16F10): efficacy was found to be more against EMT-6, B16F10 and LL/2 tumors where Cellax exhibited more than 2 fold tumor growth inhibition at equivalent dose of Taxotere (40 mg DTX/kg). Against MDA-MB-231 and PC-3 models, Cellax showed similar efficacy at equitoxic (MTD) dose (170 mg DTX/kg for Cellax and 40 mg DTX /kg for Taxotere). Cellax also exhibited significantly better efficacy compared to the only clinically approved taxane nanoformulation, Abraxane® (an albumin stabilized nanoparticles of PTX). Systemic exposure (AUC) of the drug was found to be ~30 times higher with Cellax compared to Abraxane which translated into significantly higher tumor accumulation⁵⁰. Cellax uptake in the tumor at 3 h was 12 times higher than Abraxane, and declined gradually over 10 days, remaining above 5 µg/g. On the other hand, PTX levels in the Abraxane treated tumors declined rapidly to <1 µg/g within 6 h. In s.c. PC3 (prostate) efficacy model, Abraxane at the MTD dose (75 mg paclitaxel/kg) did not control tumor growth compared to the control (saline treated) group whereas Cellax completely inhibited tumor growth with undetectable tumor in 40% of animals. In an orthotopic 4T1 breast tumor model, Cellax reduced the incidence of lung metastasis to 40% with no metastatic incidence in other tissues. Mice treated with Abraxane displayed increased lung metastatic incidence (>85%) with metastases detected in the bone, liver, spleen and kidney.

It was also demonstrated that Cellax exerted an effect via a unique mode of action. Stromal cells, which are an important component of the tumor microenvironment and aid in tumor survival and metastasis, were found to be one of the main target of Cellax⁵¹. Studies of the 4T1 orthotopic breast tumor showed that more than 85% of the Cellax nanoparticles were delivered to the α -SMA+ stroma (mainly cancer associated fibroblasts), which resulted in ~80% reduction of stroma 4 days after treatment compared to the control tumor, whereas native DTX and Abraxane exerted no significant antistromal activity. As a result of significant reduction in stroma, tumor perfusion was increased by approximately 70-fold (FITC-lectin binding), tumor vascular permeability was enhanced by more than 30% (dynamic contrast-enhanced magnetic resonance imaging), tumor matrix was decreased by 2.5-fold (immunohistochemistry), and tumor interstitial fluid pressure was suppressed by approximately 3-fold after Cellax therapy compared with the control, native docetaxel, and Abraxane groups. The antistromal effect of Cellax treatment also resulted in a significantly enhanced antimetastatic effect: lung nodules were reduced by 7- to 24-fold by Cellax treatment, whereas native DTX and Abraxane treatments were ineffective. These data suggest that Cellax targets tumor stroma and performs more efficaciously than docetaxel and Abraxane.

Dextran

Dextran (Dex), another polysaccharide which is widely used for drug delivery, consists of repeating units of glucose connected by α -(1-6) glycosidic linkages between glucose molecules, while branches begin from α -(1-2), α -(1-3), and/or α -(1-4) linkages (Fig 4A). The solubility of dextrans depends upon the branch pattern. Dextrans with >43% branching through α -(1-3) linkages are water insoluble while the presence of >95% linear linkages makes it water-soluble, which is suitable for various applications⁵². Dextrans are mainly derived from bacterial sources (typically the *Lactobacillaceae* family). Clinical grade dextran is obtained by partial depolymerization of the primary product of microbiological synthesis, termed 'native-dextran'. Originally this poly-glucose biopolymer was approved as a plasma expander; but its desirable physicochemical characteristics along with its low cost and a history of clinical use make it an attractive system for drug delivery. Although the sulfate derivative of dextran, which is polyanionic in nature, has been used for improving encapsulation efficiency as well as for making electrostatic complexes with positively-charged cytotoxic drugs like doxorubicin, there are plenty of primary and secondary hydroxyl groups present on the dextran backbone which provide potential functional sites for drug conjugation through direct or indirect methods.

Carboxymethyl dextran (CM-Dex) has most often been used for preparing taxane bioconjugates, as this dextran contains a large number of carboxyl groups (degree of substitution: 0.2- 0.8) suitable for the drug attachment (Fig 4B). Sugahara et al.⁵³ demonstrated that the pharmacokinetic profile of the native polymer is dependent on the degree of carboxyl substitution (DS) as well as the molecular weight. While increased plasma AUC was seen with polymers exhibiting a DS value of 0.2 - 0.6, the AUC also increased marginally with increasing MW of the polymer and plateaued at 80 kDa. Tissue distribution of the native polymer was also affected by the MW and DS of the polymer and enhanced tumor accumulation was detected with increased DS and MW. Nevertheless, the

DS and MW of the native polymer did not significantly affect the in vivo antitumor efficacy when PTX was conjugated with these polysaccharides using a tetra peptide (Gly–Gly–Phe–Gly) as a linker. Tumors were inoculated by s.c injection of Colon26 carcinoma cells in BALB/c mice, and mice were dosed at 50 mg/kg (i.v.) of native PTX and 100 mg PTX/kg of CM-Dex-PTX (i.v.) on days 2, 9 and 16. At day 20, tumor growth inhibition varied between 82 – 97% with different DS and MW of CM-Dex compared to 30% for native PTX ⁵³.

The same author also reported conjugation of PTX with CM-Dex (MW 150 kDa; DS 0.5) using different amino acid (Gly, Ala, Leu, and Ile) ⁵⁴ as well as small peptides (Gly–Gly–Phe–Gly) as linkers ^{53, 55} (Fig 4C). Drug loading varied moderately among different conjugates and was slightly higher with single amino acid linkers (6.2 – 7.3 wt %) than the tetra-peptide (5.5 – 6.5 wt %). Drug release was dependent on the linker used and the tetra-peptide conferred the highest release rate (~ 75 % release at 48 h in PBS). Among the different amino acids, release was highest where Gly was used as the linker (more than 50% PTX release in PBS at 72 h) whereas Ile (< 10 % release at 72 h in PBS) was the most stable among them (stability in the order of Gly<Ala<Leu<Ile). All the different conjugates were well tolerated in mice with MTD exceeding 100 mg PTX equivalent but in vivo efficacy was dependent on the type of linker used. CM-Dex-**Gly**-PTX and CM-Dex-**Ala**-PTX exhibited significantly better tumor growth control compared to CM-Dex-**Leu**-PTX and CM-Dex-**Ile**-PTX against an s.c. Colon26 tumor model. The mean tumor volume of the CM-Dex-**Gly**-PTX and CM-Dex-**Ala**-PTX treated groups was ~1000 mm³ compared to ~2000 mm³ for CM-Dex-**Leu**-PTX, ~4000 mm³ for CM-Dex-**Ile**-PTX, and ~4000 mm³ for native PTX groups dosed at their MTDs. Further improved antitumor efficacy was noted when the amino acid was replaced with (Gly–Gly–Phe–Gly) tetra-peptide which exhibited almost 100% tumor growth inhibition against different tumor allograft (Colon26) and xenograft (HT-29, MX-1 and LX-1) models.

Nakamura et al. ⁵⁶ used unmodified Dex (70 kDa) to demonstrate the tumor targeting property of a folic acid (FA) labeled Dex-PTX conjugate (Fig 4D). Ethylenediamine was used as a linker between Dex and PTX. FA labeling was achieved by both ionic adsorption (positively charged –NH₂ group of FA with negatively charged –COOH group of Dex) and covalent conjugation. Degree of PTX conjugation varied from 1% to 5% degree of substitution depending on the reaction concentration. Cytotoxic activity of this conjugate was dependent on the degree of drug loading as well as FA labeling. In the non-FA labeled Dex-PTX conjugate, cytotoxicity was similar to the native PTX at PTX loading of 1 and 2 % degree of substitution, but introducing additional PTX reduced the activity. FA adsorption significantly increased the potency of the conjugate for FA receptor over-expressing cells (Caco2 and KB) compared to low FA receptor expressing cells (L929, MA104). Thus far, no in vivo data have been published.

Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine (Fig 5A). It is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans. Chitosan can also be produced by fungal fermentation ⁵⁷. Chitosan has a unique chemical

structure as a linear polyelectrolyte with a high charge density as well as reactive hydroxyl and amino groups. Protonated chitosan, which is water soluble, binds with negatively charged surfaces such as mucosal membranes. This property of chitosan leads to its exploitation as a haemostatic agent. Due to this mucoadhesive property of chitosan, it has been used for oral delivery of many different drugs. The presence of the reactive amino and hydroxyl groups in chitosan made it a potential candidate for conjugation with cytotoxic drugs.

Lee et al.^{58, 59} utilized the mucoadhesive property of chitosan to prepare an oral delivery dosage form of PTX and DTX (Fig 5B). Low molecular weight chitosan (MW <10 kDa) was used for conjugation and succinic acid was used as the linker. PTX loading was 12 % wt/wt whereas DTX loading was 8 %. Although the same polymeric backbone and linker were used for conjugation of both the drugs, a striking difference was found in the release kinetics of PTX and DTX from the respective conjugates. Release of PTX from the conjugate was highly resistant to acidic pH and only about 20% was released in simulated gastric fluid at pH 1.2 in 12 h whereas as much as 60% was released in cell culture medium within the same time. On the other hand, DTX release was highest in simulated gastric fluid (pH 1.2) and complete release of the drug was noted within 8 h of incubation. No explanation was provided on why these two taxane analogues displayed distinctive pH dependent release profiles, but as PTX is less stable than DTX⁶⁰, released PTX might be further degraded reducing the amount of detectable PTX in the media. Both the PTX and DTX conjugates exhibited similar cytotoxicity as that of the native drugs against a panel of cell lines including NCI-H358, SKOV-3, MDA-MB-231 and U87MG.

The pharmacokinetic profiles of PTX and DTX conjugates were comparable: after oral administration, an extended plasma half-life was measured with the conjugates compared to the i.v. administered clinical formulations. The plasma $t_{1/2}$ for oral PTX-Chitosan and DTX-Chitosan was ~32 h and ~8 h respectively compared to ~1 h for PTX and ~½ h for DTX. A biodistribution study was done with only the PTX conjugate, and it was found that while liver accumulation of the orally administered conjugate was lower than the i.v. administered native PTX, the tumor accumulation was increased by 5-fold. Increased drug accumulation was also found in the intestine, colon and kidney with the oral delivery of the conjugate. In vivo antitumor efficacy of the PTX conjugate was evaluated against B16F10 murine melanoma and NCI-H358 human non-small cell lung cancer, and the DTX conjugate was tested against NCI-H358 human non-small cell lung carcinoma and U87MG human glioblastoma tumors. In both cases, oral administration of the conjugates exhibited similar antitumor efficacy compared to the i.v. administered clinical formulations. Importantly, i.v. administration of the native drugs lead to significantly higher body weight loss (~5-15%) compared to the orally delivered conjugates.

Heparin

Heparin (Hep) is a highly sulfated glycosaminoglycan and consists of a variably sulfated repeating disaccharide unit. The most common disaccharide unit is composed of a 2-O-sulfated iduronic acid and 6-O-sulfated, N-sulfated glucosamine (Fig 6A). The molecular weight of native heparin ranges from 3 kDa to 30 kDa. Heparin has long been used clinically

as anticoagulant, but it has also been shown that low molecular weight heparin inhibits cancer cell adhesion, deactivates heparanase, activates the attack by NK cells in the immune system and interferes with the activity of growth factors such as bFGF and VEGF while through this prevents tumor angiogenesis and metastasis^{61, 62}. It has been shown by Park et al.⁶³ that Hep-deoxycholic acid conjugate prevented cancer cell proliferation. They also demonstrated that increasing the deoxycholic acid ratio in the conjugate reduced the anticoagulant activity of Hep but the anti-angiogenic activity remained unaffected. This orally absorbable Hep derivative was also effective in controlling tumor growth in a mouse tumor model⁶⁴. Due to these reasons, it has received increasing attention as a drug carrier to improve efficacy of cytotoxic agents.

Wang et al.^{65, 66} tested both O-acetylated and succinylated Hep for delivery of PTX (Fig 6B, C). Different amino acids (Val, Leu and Phe) were also evaluated as linkers. It was found that although direct conjugation of PTX with the Hep backbone resulted in higher drug loading, use of the amino acid linkers facilitated release of PTX from the conjugate. In direct conjugation, about 25 wt% PTX was loaded whereas PTX loading varied from 16-18 wt% in the amino acid (AA) conjugate. However, only 5% of the drug was released from Hep-PTX conjugate, whereas about 60-70% PTX was released from different Hep-AA-PTX conjugates after 96 h incubation in PBS (pH 7.4). It was also noted that use of different amino acids as a linker influences drug release. A slight but statistically significant increase in release rate was measured with Phe (~70%) compared to Val and Leu (~55-60%) after 96 h incubation in PBS (pH 7.4). Interestingly, this trend reversed when the conjugate was incubated in human serum. About 90% release was noted with Val and Leu compared to about 75% release with Phe. The conjugates self-assembled into micellar structures with a diameter of 140-180 nm, exhibiting a zeta potential of -21 to -31 mV. These conjugates exhibited better or comparable cytotoxic potency compared to free PTX, although Hep-AA-PTX was found to be more potent than Hep-PTX. The *in vivo* antitumor activity of the conjugate was evaluated in a tumor xenograft model with a dose of 30 mg/kg dose of PTX equivalent, administered as *i.p.* injection. Both free PTX and the conjugate treatments resulted in similar tumor growth inhibition, but while treatment with free PTX caused body weight decrease, body weight of the conjugate treated animals remained constant, which implied that the conjugates were similarly bioactive but less toxic than free PTX.

Another Hep-PTX conjugate was reported where ethylenediamine was used as a linker⁶⁷ (Fig 6D). PTX loading varied between 0.8 to 4.1 mol %. Anticoagulant activity of these conjugates was found to be inversely proportional to the drug loading. The conjugate was shown to self-assemble into nanoparticles in aqueous solution with a mean diameter of 200 to 400 nm. These nanoparticles were effectively internalized by KB cells, which may be due to endocytic uptake. This higher and enhanced delivery of the conjugate resulted in increased cytotoxic activity of the conjugate compared to the free PTX.

Wang et al.⁶⁸ demonstrated that Hep-PTX-FA conjugates (Fig 6E, 15% PTX; 9% FA wt/wt) can form nanostructures via a self-assembly procedure when free PTX was incorporated into a DMSO solution of the conjugate and added dropwise to aqueous NaHCO₃. The size of the nanoparticles was 60 ± 10 nm with a zeta potential of -16.1 ± 1.1 mV. Cytotoxicity of this nanoparticle was dependent on the level of folate receptor (FR):

against a FR over-expressing cell line (KB-3-1), 90% reduction in colony formation was determined with the conjugate compared to 50% reduction with native PTX; however, when tested in a FR negative cell line (Tu212), similar inhibitory activity was measured with the conjugate and native PTX. This FR targeting was specific and could be blocked by free FR down-regulation. Tumor accumulation of this nanoparticle was not dependent on the presence of FA: comparable tumor accumulation was reported with Hep-PTX and Hep-PTX-FA nanoparticles but the in vivo antitumor efficacy against a tumor xenograft model was significantly increased with the presence of FA in the conjugate. The authors argued that nanoparticle extravasation into tumors via the EPR effect is a passive process, and presence of a ligand does not facilitate this process. However, a ligand can trigger receptor-mediated endocytosis after the conjugate/nanoparticle has extravasated and interacted directly with the tumor cells to increase the cellular bioavailability, leading to enhanced antitumor activity. Tumors were induced with s.c. injection of FR over-expressing KB-3-1 cells in nude mice and mice were dosed at 20 mg/kg PTX once per week for 5 weeks by i.v. route. The mean tumor volume of FA conjugated nanoparticles was $92.9 \pm 78.2 \text{ mm}^3$ compared to $1211.3 \pm 448.1 \text{ mm}^3$ for the non targeted nanoparticles and $1670.3 \pm 286.1 \text{ mm}^3$ for native PTX, suggesting an active targeting mechanism contributed to the enhanced efficacy. This nanoparticle system also exhibited better efficacy against a p-glycoprotein (P-gp) over-expressing PTX resistant (KB-8-5) cell line⁶⁹. After s.c. inoculation of the tumor cells, treatment was given with 40 mg/kg dose of PTX as free PTX or nanoparticle. Mean tumor volumes were found to be $1422.9 \pm 216.1 \text{ mm}^3$ for PTX, $1301.3 \pm 213.9 \text{ mm}^3$ for FA unconjugated nanoparticles, and $785.6 \pm 104.1 \text{ mm}^3$ for FA conjugated nanoparticle treated groups. These findings suggest that when the PTX was delivered as a targeted nanoparticle, the drug was more bioavailable to the P-gp-overexpressing cells than the free form of PTX.

Khatun et al.⁷⁰ demonstrated that when an absorption enhancer, tauricholic acid, was covalently attached to a Hep-DTX conjugate, the oral absorption of the conjugate was significantly enhanced (Fig 6F). The authors used diethyl amine as the linker to incorporate DTX and a high drug loading of 25-30 wt% was reported. The conjugate self-assembled to form nanoparticles in an aqueous medium with a diameter of ~120 nm with a PDI of 0.2. No drug release data was reported but the size of the nanoparticles remained unchanged in acidic to basic pH conditions as well as in plasma. Bioavailability of native Hep and the conjugate were compared by measuring the blood concentration of Hep. Native Hep showed no absorption whereas AUC of the conjugate varied between 250-350 $\mu\text{g/mL/min}$ with a T_{max} of 6 h. The in vitro cytotoxicity of the conjugate was significantly lower than native DTX: the conjugate showed measurable cell killing only at 0.5 $\mu\text{g/mL}$ against KB and MDA-MB-231 cells. In vivo efficacy of the conjugate was significantly better than native DTX at the dose of 10 mg DTX/kg administered orally against the KB tumor model.

One significant concern for Hep as a delivery vehicle is its anticoagulant activity. As most cancer patients undergo some forms of surgery, they are susceptible to bleeding related problems. Although in most of the studies discussed above, Hep was modified to reduce the anticoagulant activity; significant anticoagulant activity remained. A model system should be developed to evaluate the anticoagulant activity of these conjugates. Other than the traditional body weight loss monitoring and tissue histology, special attention should be given to measure internal hemorrhage, with and without a hemorrhagic stimulus.

Discussion

As described above, pilot research has been dedicated for the design, synthesis and characterization of polysaccharide–taxane conjugates, with some preliminary clinical evaluation. As the composition of these polymers varied significantly, the resulting conjugates also exhibited diverse pharmacokinetic and pharmacodynamic properties. It has been noted that the molecular weight of the polymer, extent of drug loading, type of linker used and solubility of the conjugate significantly influence the release of the conjugated drug, its cytotoxic potency and in vivo efficacy. The biological fate and biodistribution of the native polymer are dependent on the physicochemical nature of the polymer and different polymers exhibit different pharmacokinetic profiles. Sugahara et al.⁷¹ reported that different polysaccharides exhibit significantly different plasma AUC and tumor biodistribution. When the plasma AUC and tumor accumulation data for CM-Dex, Chitin, HA and Hep are compared, plasma AUC and tumor accumulation of CM-Dex was significantly higher compared to others across the panel of molecular weights tested (80–360 kDa). While plasma AUC of CM-Dex varied from 100–135% of dose h/mL (plasma AUC increased with increasing molecular weight), others varied between 0–25% of dose h/mL. This has also reflected in the tumor accumulation of the polymer. Tumor accumulation of CM-Dex varied between 1.5 – 4.1 % of dose /g (higher molecular weight exhibited increased accumulation) after 24 h of i.v. administration, whereas this was less than 0.5% of dose /g for other polymers in all the molecular weight tested. On the other hand, liver and bone marrow accumulation of HA and Hep was higher compared to CM-Dex. Chitin conjugates exhibited low plasma AUC as well as low accumulation in any other tissues tested. Depolymerization by lysozymes in the plasma has been identified as rationale for these low AUC observations, as it was demonstrated that Chitin was extensively hydrolyzed in the plasma and excreted through urine. CM-Dex, HA and Hep were found to be stable in plasma. To understand whether these polymers influence in vivo efficacy of a conjugated drug, a doxorubicin conjugate with these polymers was prepared using a Gly-Gly-Phe-Gly tetra-peptide as linker. CM-Dex conjugate exhibited significantly better antitumor efficacy and tumor distribution than the other polymers. Tumor accumulation of the drug was ~3% of the total dose per g tissue and a ~3 fold increase in efficacy was found with the CM-Dex conjugate whereas with other polymers (Chitin, HA and Hep), tumor accumulation varied from 0.04% - 0.35% of total dose / g tissue with minimal to no increase in antitumor efficacy.

In a separate study, Ernsting et al.⁴⁹ demonstrated that acetylated CMC (CMC-Ac) conjugated DTX (Cellax) exhibited increased plasma circulation time (53.8 h in $t_{1/2}$ compared to 10.3 h for DTX) and ~40-fold higher plasma AUC compared to the native drug. Tumor accumulation of CMC-Ac–DTX conjugate was 5% of total dose / g tissue compared to 1% of the injected dose / g tissue observed with native DTX.

Polymer molecular weight can also modulate drug release rate from the polymer-drug conjugates. Sugahara et al.⁵³ demonstrated reducing the molecular weight of CM-Dex increases the PTX release rate from the conjugate. While about 75% PTX release was found from 40 kDa polymer after 72 h incubation in plasma, about 60% PTX released from 250 kDa polymer. Conjugates prepared with low molecular weight polymers are less likely to

make secondary and tertiary structures when suspended or solubilized in an aqueous medium, assisting better exposure of the conjugate to the external medium for facilitated drug release. On the other hand, conjugates prepared with higher molecular weight polymer can make extensive secondary and tertiary structures in the solution which may prevent release of the cytotoxic drug.

Solubility of these conjugates was dependent on the percentage of drug loading. It was reported that with low drug loading (<5 wt%), the conjugates appeared to be water soluble but with increased loading, they tended to form nanoparticles. As the chemical structure of taxane is consisting of a tetracyclic 17-carbon (heptadecane) skeleton, which is sufficiently bulky and highly non-polar and hydrophobic in nature, it is difficult to make the parent drug water soluble without altering their skeletal structure. Conjugation with a water soluble polymer may improve hydration of the taxanes. In an aqueous medium, the polymer may form a shell around the conjugated drug by hydrophobic interaction and facilitate formation of a stable molecular suspension. If taxane loading in the polymer is sufficiently low, the size of these nanosuspensions would be small enough not to scatter any visible light and appear as soluble. With increase in the drug loading, the size of these nanoparticles increases to accommodate more drugs in the core of the particle. It was noted that in all the studies where drug loading was low (less than 5% wt/wt), the conjugate was reported to be “soluble” in water whereas high drug loading invariably lead to formation of self-assembled micellar nanoparticle in aqueous medium. The extent of drug loading of the conjugates influenced the release rate of the drug. It was generally observed that higher drug loading in the polymeric backbone resulted in slower drug release. Sugahara et al.⁵³ demonstrated that a change in drug loading from 6.3% to 13.2% reduced drug release from ~80% to ~60% in 72 h of incubation. As discussed previously, conjugates with low drug loading are better hydrated, which would expose the polymer-drug linker to the external medium, facilitating hydrolytic cleavage of the bond for increased drug release. With higher drug loading, the conjugate can self-assemble into a core-shell nanostructure with a highly hydrophobic taxane core, which is relatively less exposed to the external medium, reducing hydrolytic cleavage and release of the drug. It has been noticed that the in vitro potency of the conjugate is dependent on the rate of drug release and an increased release rate generally results in enhanced cytotoxicity⁵³.

Linker and linker chemistry also influence drug release and efficacy of the conjugate. It was demonstrated by Sugahara et al.⁵⁴, Xin et al.³³ and Wang et al.⁶⁶ that using an amino acid as a linker between the polymeric backbone and the cytotoxic agent significantly increases the drug release rate as well as the in vitro cytotoxic potency of the conjugate. It was noted that physicochemical nature of the amino acid used also influenced the drug release kinetics. Conjugates with a linker of amino acid with no side chain (Gly) or aromatic side chain (Phe) showed faster release compared with amino acids with aliphatic side chains (Leu, Val, Ala, Ile) when incubated in mouse plasma. The higher drug release also translated into increased in vitro cytotoxic potency. Further increased drug release and in vitro efficacy was seen when the amino acid was replaced with a tetra-peptide (Gly-Gly-Phe-Gly). It was also reported that the drug directly conjugated with the polymer has significantly less in vivo antitumor effect. Sugahara et al.⁷¹ postulated that a cleavable bond is necessary for efficient release of the drug for in vivo efficacy. As Gly-Gly-Phe-Gly tetra peptide can be cleaved

with protease, drug release is expected to be more efficient. Duncan et al.⁷² showed that a non-degradable Gly-Gly linker resulted in a significant decrease in efficacy compared to a degradable Gly-Phe-Leu-Gly linker in an animal model. It was hypothesized that due to the electron effect of the protonated amino group, amino acid linkers showed higher drug release rates³³.

These studies indicate the relationship between the polymer molecular weight, extent of drug loading and the physicochemical nature of the linker with the in vitro activity of the conjugate. Generally, conjugates with low molecular weights and low drug loading exhibit higher drug release and improved in vitro cytotoxic potency. Although conjugates with a high molecular weight polymer and a high drug loading display reduced drug release and decreased potency in vitro, they often show enhanced in vivo antitumor efficacy. As discussed previously, high molecular weight conjugates with high drug loading can form complex nano-structures which may prolong the pharmacokinetics of the conjugate by minimizing drug release during circulation and facilitate tumor accumulation by the EPR effect. This phenomenon may contribute to the better efficacy of the high molecular weight conjugates.

It was noted that among the advanced taxane-polymer conjugates, high molecular weight conjugates are favored: the HA-PTX conjugate currently in a phase I clinical trial incorporates HA of 200 kDa³⁸. Drug loading was also high in this conjugate with ~20 wt% PTX. This may emphasize the importance of utilizing high molecular weight polymer for enhanced drug delivery. As discussed above, high molecular weight polymer with high drug loading can render complex secondary and tertiary structures and even self-assemble into nanoparticulate structures. These nanoparticulate structures may facilitate better tumor targeted delivery due to the EPR effect compared to the solution dosage form. As they also display slower release kinetics, unwanted drug release during systemic circulation would be minimal, reducing side effects.

It was also noted that efficacy of these conjugates depend on the tumor type. The HA-PTX conjugate exhibited better efficacy against the ovarian cancer compared to bladder tumor. CMC-DTX conjugate (Cellax) was found to be more potent against syngeneic tumor model compared to xenograft tumor. The reason for this variability is still unknown. The biochemical and bio-physical interaction of nanoparticles with different tissues and cells is a relatively less researched area of nanoparticle drug delivery. As these interactions can significantly alter the bioactivity of the drug, a thorough understanding is warranted for proper design of nanotherapeutics.

It was also noted that most of the polymers used in these studies has more than one functional groups (–OH and –COOH in HA; –OH and –COOH in CM-Dex; –OH and –COOH in Hep; –NH₂ and –COOH in succinyl Chitosan etc) which can potentially react with one another to crosslink and self-polymerize at the reaction condition used for the drug conjugation. No study has been done to understand the cross linking of these polymers or the effect of crosslinking on the drug loading and overall function of the conjugate. Apart from Ernsting et al.⁴⁸, who acetylated the free –OH groups of CMC, no modification was done to the polymer in any other study to prevent polymer crosslinking, which can

significantly reduce the drug loading efficiency, and affect the physicochemical properties of the conjugate and drug release⁷³. It will be of interest to verify the effect of this on the efficacy of the conjugate.

Conclusion/Future direction/Expert opinion

Through numerous studies, it has been established that polymer conjugated delivery of taxanes is beneficial for cancer therapy with significant reduction in side-effects, increase in maximum tolerated dose and improved pharmacokinetics. Although many preclinical studies of polymer drug conjugates have been reported with promising results and a number of conjugates have entered clinical trials, the full potential of polysaccharide – drug conjugates is yet to be explored. A rational drug designing approach with focus on the optimal polymer molecular weight, drug loading, linker and drug release is needed to create a conjugate with prolonged pharmacokinetics, enhanced tumor delivery and increased tumor bioavailability. An important consideration should be placed on degree of PEGylation as this was shown to significantly modulate circulation time, plasma half life and tumor targeting of polymer-drug conjugates. PEGylation of the conjugate can reduce opsonization and reticulo-endothelial system (RES) clearance, but can also reduce the interaction between the conjugate and the target cell. PEGylation can also affect nanoparticle self-assembly: increased PEGylation often results in decreased particle size, leading to prolonged pharmacokinetics, improved vascular permeability and tissue penetration. However, over PEGylation can lead to insufficient condensation of the particles, affecting the stability. The degree of PEGylation should be carefully optimized in each case.

As nanomedicine is an entirely new class of drug delivery system, their pharmacokinetic and even pharmacodynamic properties differ significantly from the parent drug. Not only have they showed long circulation time and preferential accumulation in the tumor due to passive targeting, their mode of action and antitumor efficacy may substantially differ from the parent drug. It was recently demonstrated by Murakami et al.⁵¹ that treatment with Cellax, a nanoparticulate formulation of docetaxel, have a significant effect on the stroma cells in the tumor microenvironment whereas free DTX showed minimal to no effect. Stromal interaction significantly changed the in vivo efficacy of Cellax compared to native DTX. Interaction of the nanoparticles with biological systems including different tissues and cells is a complex phenomenon which is entirely different from that of the free drug. Hence a systematic and thorough study is necessary to understand the performance of the nano drug delivery formulation in the in vivo situation. Nanotherapeutics may also have different side effects compared to that of the free drug due to a major change in pharmacokinetics. For example, many of the nanoparticles accumulate in the liver and spleen due to interaction with RES⁷⁴. Hence particular importance should be given to determine the liver toxicity and effect on different immune cells which mature in the spleen.

As many of these carbohydrate polymers have multiple functional groups, they can be a suitable backbone for the development of multi-therapeutic dosage form by conjugating more than one drug. If drugs with complimentary mode of action and non-overlapping toxicity can be incorporated, the therapeutic activity may be enhanced. The molar ratio of drugs in combination has been demonstrated as an important factor for determining the

therapeutic effect by Mayer et al.⁷⁵. When different classes of anticancer drugs with distinct molecular mechanisms are given as a combination, they can produce synergistic, additive or antagonistic effect depending on the ratio. As these drugs have different pharmacokinetic properties, it is difficult to control their plasma concentration once injected as the solution dosage form through i.v. route. Nanoparticles, on the other hand, may deliver the exact ratio of the drugs to the tumor interstitium for synergistic activity. For example, camptothecin and doxorubicin produced synergistic activity at a molar ratio of 1.5:1 but a strong antagonistic activity was observed at 5:1⁷⁶. However, even if these two drugs are given at 1.5:1 molar ratio, the drug ratio in the plasma changes over time due to the different pharmacokinetic profiles of these two drugs, leading to compromised activity. Mayer et al.⁷⁷ demonstrated that a fixed ratio of irinotecan and floxuridine delivered in a liposome successfully maintained the optimal drug ratio in the plasma over time, inducing enhanced efficacy. A similar strategy may be applied to polymeric conjugates. Additionally, multiple components other than the active pharmaceutical ingredient can be incorporated to one molecule of polysaccharide, including PEG to shield the conjugate from RES recognition and clearance, an imaging agent to report drug delivery or therapeutic response in a real time fashion, a targeting ligand to increase the cellular bioavailability of the conjugate. Again, the ratio of the multiple components in the conjugate has to be carefully optimized for maximized effect.

References

1. Bissery MC, Guenard D, Gueritte-Voegelein F, Lavelle F. Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. *Cancer Res.* 1991; 51:4845–4852. [PubMed: 1680023]
2. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc.* 1971; 93:2325–2327. [PubMed: 5553076]
3. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer.* 2004; 4:253–265. [PubMed: 15057285]
4. Wang TH, Wang HS, Soong YK. Paclitaxel-induced cell death: where the cell cycle and apoptosis come together. *Cancer.* 2000; 88:2619–2628. [PubMed: 10861441]
5. Hsiao JR, Leu SF, Huang BM. Apoptotic mechanism of paclitaxel-induced cell death in human head and neck tumor cell lines. *J Oral Pathol Med.* 2009; 38:188–197. [PubMed: 19200178]
6. Rowinsky EK. The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. *Annu Rev Med.* 1997; 48:353–374. [PubMed: 9046968]
7. Gelderblom H, Verweij J, Nooter K, Sparreboom A, Cremophor EL. the drawbacks and advantages of vehicle selection for drug formulation. *Eur J Cancer.* 2001; 37:1590–1598. [PubMed: 11527683]
8. Shelley WB, Talanin N, Shelley ED. Polysorbate 80 hypersensitivity. *Lancet.* 1995; 345:1312–1313. [PubMed: 7746084]
9. Baker J, Ajani J, Scotte F, Winther D, Martin M, Aapro MS, von Minckwitz G. Docetaxel-related side effects and their management. *Eur J Oncol Nurs.* 2009; 13:49–59. [PubMed: 19201649]
10. Singla AK, Garg A, Aggarwal D. Paclitaxel and its formulations. *Int J Pharm.* 2002; 235:179–192. [PubMed: 11879753]
11. Eric Drexler, CP. *Unbounding the Future: The Nanotechnology Revolution.* William Morrow and Company, Inc.; New York: 1991. Gayle Pergamit..
12. Maeda H, Ueda M, Morinaga T, Matsumoto T. Conjugation of poly(styrene-co-maleic acid) derivatives to the antitumor protein neocarzinostatin: pronounced improvements in pharmacological properties. *J Med Chem.* 1985; 28:455–461. [PubMed: 3156994]

13. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* 1986; 46:6387–6392. [PubMed: 2946403]
14. Dhal PK, Polomoscank SC, Avila LZ, Holmes-Farley SR, Miller RJ. Functional polymers as therapeutic agents: concept to market place. *Adv Drug Deliv Rev.* 2009; 61:1121–1130. [PubMed: 19682515]
15. Duncan R, Vicent MJ. Polymer therapeutics-prospects for 21st century: the end of the beginning. *Adv Drug Deliv Rev.* 2013; 65:60–70. [PubMed: 22981753]
16. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. *Trends Pharmacol Sci.* 2009; 30:592–599. [PubMed: 19837467]
17. Li SD, Chen YC, Hackett MJ, Huang L. Tumor-targeted delivery of siRNA by self-assembled nanoparticles. *Mol Ther.* 2008; 16:163–169. [PubMed: 17923843]
18. Li SD, Huang L. Targeted delivery of siRNA by nonviral vectors: lessons learned from recent advances. *Curr Opin Investig Drugs.* 2008; 9:1317–1323.
19. Maeda H, Seymour LW, Miyamoto Y. Conjugates of anticancer agents and polymers: advantages of macromolecular therapeutics in vivo. *Bioconjug Chem.* 1992; 3:351–362. [PubMed: 1420435]
20. Letchford K, Liggins R, Wasan KM, Burt H. In vitro human plasma distribution of nanoparticulate paclitaxel is dependent on the physicochemical properties of poly(ethylene glycol)-block-poly(caprolactone) nanoparticles. *Eur J Pharm Biopharm.* 2009; 71:196–206. [PubMed: 18762253]
21. Duncan R. Polymer conjugates as anticancer nanomedicines. *Nat Rev Cancer.* 2006; 6:688–701. [PubMed: 16900224]
22. Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z. Polysaccharides-based nanoparticles as drug delivery systems. *Adv Drug Deliv Rev.* 2008; 60:1650–1662. [PubMed: 18848591]
23. Jackson DG. Immunological functions of hyaluronan and its receptors in the lymphatics. *Immunol Rev.* 2009; 230:216–231. [PubMed: 19594639]
24. Balazs EA, Denlinger JL. Viscosupplementation: a new concept in the treatment of osteoarthritis. *J Rheumatol Suppl.* 1993; 39:3–9. [PubMed: 8410881]
25. Takeuchi K, Nakazawa M, Yamazaki H, Miyagawa Y, Ito T, Ishikawa F, Metoki T. Solid hyaluronic acid film and the prevention of postoperative fibrous scar formation in experimental animal eyes. *Arch Ophthalmol.* 2009; 127:460–464. [PubMed: 19365025]
26. Oh EJ, Park K, Kim KS, Kim J, Yang JA, Kong JH, Lee MY, Hoffman AS, Hahn SK. Target specific and long-acting delivery of protein, peptide, and nucleotide therapeutics using hyaluronic acid derivatives. *J Control Release.* 2010; 141:2–12. [PubMed: 19758573]
27. Lataillade JJ, Albanese P, Uzan G. [Implication of hyaluronic acid in normal and pathological angiogenesis. Application for cellular engineering]. *Ann Dermatol Venereol.* 2010; 137(Suppl 1):S15–22. [PubMed: 20435250]
28. Kim IL, Mauck RL, Burdick JA. Hydrogel design for cartilage tissue engineering: a case study with hyaluronic acid. *Biomaterials.* 2011; 32:8771–8782. [PubMed: 21903262]
29. Wang C, Thor AD, Moore DH 2nd, Zhao Y, Kerschmann R, Stern R, Watson PH, Turley EA. The overexpression of RHAMM, a hyaluronan-binding protein that regulates ras signaling, correlates with overexpression of mitogen-activated protein kinase and is a significant parameter in breast cancer progression. *Clin Cancer Res.* 1998; 4:567–576. [PubMed: 9533523]
30. Kokko LL, Hurme S, Maula SM, Alanen K, Grenman R, Kinnunen I, Ventela S. Significance of site-specific prognosis of cancer stem cell marker CD44 in head and neck squamous-cell carcinoma. *Oral Oncol.* 2011; 47:510–516. [PubMed: 21514878]
31. Qhattal HS, Liu X. Characterization of CD44-mediated cancer cell uptake and intracellular distribution of hyaluronan-grafted liposomes. *Mol Pharm.* 2011; 8:1233–1246. [PubMed: 21696190]
32. Leonelli F, La Bella A, Francescangeli A, Joudioux R, Capodilupo A-L, Quagliariello M, Migneco LM, Bettolo RM, Crescenzi V, De Luca G, et al. A New and Simply Available Class of Hydrosoluble Bioconjugates by Coupling Paclitaxel to Hyaluronic Acid through a 4-Hydroxybutanoic Acid Derived Linker. *Helvetica Chimica Acta.* 2005; 88:154–159.

33. Xin D, Wang Y, Xiang J. The use of amino acid linkers in the conjugation of paclitaxel with hyaluronic acid as drug delivery system: synthesis, self-assembled property, drug release, and in vitro efficiency. *Pharm Res.* 2010; 27:380–389. [PubMed: 19876721]
34. Luo Y, Prestwich GD. Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bioconjugate. *Bioconjug Chem.* 1999; 10:755–763. [PubMed: 10502340]
35. Luo Y, Ziebell MR, Prestwich GD. A hyaluronic acid-taxol antitumor bioconjugate targeted to cancer cells. *Biomacromolecules.* 2000; 1:208–218. [PubMed: 11710102]
36. Auzenne E, Ghosh SC, Khodadadian M, Rivera B, Farquhar D, Price RE, Ravoori M, Kundra V, Freedman RS, Klostergaard J. Hyaluronic acid-paclitaxel: antitumor efficacy against CD44(+) human ovarian carcinoma xenografts. *Neoplasia.* 2007; 9:479–486. [PubMed: 17603630]
37. Galer CE, Sano D, Ghosh SC, Hah JH, Auzenne E, Hamir AN, Myers JN, Klostergaard J. Hyaluronic acid-paclitaxel conjugate inhibits growth of human squamous cell carcinomas of the head and neck via a hyaluronic acid-mediated mechanism. *Oral Oncol.* 2011; 47:1039–1047. [PubMed: 21903450]
38. Rosato A, Banzato A, De Luca G, Renier D, Bettella F, Pagano C, Esposito G, Zanovello P, Bassi P. HYTAD1-p20: a new paclitaxel-hyaluronic acid hydrosoluble bioconjugate for treatment of superficial bladder cancer. *Urol Oncol.* 2006; 24:207–215. [PubMed: 16678050]
39. De Stefano I, Battaglia A, Zannoni GF, Prisco MG, Fattorossi A, Travaglia D, Baroni S, Renier D, Scambia G, Ferlini C, et al. Hyaluronic acid-paclitaxel: effects of intraperitoneal administration against CD44(+) human ovarian cancer xenografts. *Cancer Chemother Pharmacol.* 2011; 68:107–116. [PubMed: 20848284]
40. Banzato A, Bobisse S, Rondina M, Renier D, Bettella F, Esposito G, Quintieri L, Melendez-Alafort L, Mazzi U, Zanovello P, et al. A paclitaxel-hyaluronan bioconjugate targeting ovarian cancer affords a potent in vivo therapeutic activity. *Clin Cancer Res.* 2008; 14:3598–3606. [PubMed: 18519794]
41. Bassi PF, Volpe A, D'Agostino D, Palermo G, Renier D, Franchini S, Rosato A, Racioppi M. Paclitaxel-hyaluronic acid for intravesical therapy of bacillus Calmette-Guerin refractory carcinoma in situ of the bladder: results of a phase I study. *J Urol.* 2011; 185:445–449. [PubMed: 21167517]
42. Lee H, Lee K, Park TG. Hyaluronic acid-paclitaxel conjugate micelles: synthesis, characterization, and antitumor activity. *Bioconjug Chem.* 2008; 19:1319–1325. [PubMed: 18481885]
43. Mittapalli RK, Liu X, Adkins CE, Nounou MI, Bohn KA, Terrell TB, Qhattal HS, Geldenhuys WJ, Palmieri D, Steeg PS, et al. Paclitaxel-Hyaluronic NanoConjugates Prolong Overall Survival in a Preclinical Brain Metastases of Breast Cancer Model. *Mol Cancer Ther.* 2013; 12:2389–2399. [PubMed: 24002934]
44. Thierry B, Kujawa P, Tkaczyk C, Winnik FM, Bilodeau L, Tabrizian M. Delivery platform for hydrophobic drugs: prodrug approach combined with self-assembled multilayers. *J Am Chem Soc.* 2005; 127:1626–1627. [PubMed: 15700982]
45. Zhou B, Weigel JA, Fauss L, Weigel PH. Identification of the hyaluronan receptor for endocytosis (HARE). *J Biol Chem.* 2000; 275:37733–37741. [PubMed: 10952975]
46. Li SD, Howell SB. CD44-targeted microparticles for delivery of cisplatin to peritoneal metastases. *Mol Pharm.* 2010; 7:280–290. [PubMed: 19994852]
47. Turaev AS. Dependence of the biodegradability of carboxymethylcellulose on its supermolecular structure and molecular parameters. *Chemistry of Natural Compounds.* 1995; 31:254–259.
48. Ernsting MJ, Tang WL, MacCallum N, Li SD. Synthetic modification of carboxymethylcellulose and use thereof to prepare a nanoparticle forming conjugate of docetaxel for enhanced cytotoxicity against cancer cells. *Bioconjug Chem.* 2011; 22:2474–2486. [PubMed: 22014112]
49. Ernsting MJ, Tang WL, MacCallum NW, Li SD. Preclinical pharmacokinetic, biodistribution, and anti-cancer efficacy studies of a docetaxel-carboxymethylcellulose nanoparticle in mouse models. *Biomaterials.* 2012; 33:1445–1454. [PubMed: 22079003]
50. Ernsting MJ, Murakami M, Undzys E, Aman A, Press B, Li SD. A docetaxel-carboxymethylcellulose nanoparticle outperforms the approved taxane nanoformulation, Abraxane, in mouse tumor models with significant control of metastases. *J Control Release.* 2012; 162:575–581. [PubMed: 22967490]

51. Murakami M, Ernsting MJ, Undzys E, Holwell N, Foltz WD, Li SD. Docetaxel conjugate nanoparticles that target alpha-smooth muscle actin-expressing stromal cells suppress breast cancer metastasis. *Cancer Res.* 2013; 73:4862–4871. [PubMed: 23907638]
52. Purama RK, Goswami P, Khan AT, Goyal A. Structural analysis and properties of dextran produced by *Leuconostoc mesenteroides* NRRL B-640. *Carbohydrate Polymers.* 2009; 76:30–35.
53. Sugahara S, Kajiki M, Kuriyama H, Kobayashi TR. Carrier effects on antitumor activity and neurotoxicity of AZ10992, a paclitaxel-carboxymethyl dextran conjugate, in a mouse model. *Biol Pharm Bull.* 2008; 31:223–230. [PubMed: 18239277]
54. Sugahara S, Kajiki M, Kuriyama H, Kobayashi TR. Paclitaxel delivery systems: the use of amino acid linkers in the conjugation of paclitaxel with carboxymethyldextran to create prodrugs. *Biol Pharm Bull.* 2002; 25:632–641. [PubMed: 12033505]
55. Sugahara S, Kajiki M, Kuriyama H, Kobayashi TR. Complete regression of xenografted human carcinomas by a paclitaxel-carboxymethyl dextran conjugate (AZ10992). *J Control Release.* 2007; 117:40–50. [PubMed: 17126446]
56. Nakamura J, Nakajima N, Matsumura K, Hyon SH. Water-soluble taxol conjugates with dextran and targets tumor cells by folic acid immobilization. *Anticancer Res.* 2010; 30:903–909. [PubMed: 20393013]
57. Zamani A, Edebo L, Sjöström B, Taherzadeh MJ. Extraction and precipitation of chitosan from cell wall of zygomycetes fungi by dilute sulfuric acid. *Biomacromolecules.* 2007; 8:3786–3790. [PubMed: 18039008]
58. Lee E, Kim H, Lee IH, Jon S. In vivo antitumor effects of chitosan-conjugated docetaxel after oral administration. *J Control Release.* 2009; 140:79–85. [PubMed: 19712714]
59. Lee E, Lee J, Lee IH, Yu M, Kim H, Chae SY, Jon S. Conjugated chitosan as a novel platform for oral delivery of paclitaxel. *J Med Chem.* 2008; 51:6442–6449. [PubMed: 18826299]
60. Pazdur R, Kudelka AP, Kavanagh JJ, Cohen PR, Raber MN. The taxoids: paclitaxel (Taxol®) and docetaxel (Taxotere®). *Cancer Treatment Reviews.* 1993; 19:351–386. [PubMed: 8106152]
61. Mousa SA, Petersen LJ. Anti-cancer properties of low-molecular-weight heparin: preclinical evidence. *Thromb Haemost.* 2009; 102:258–267. [PubMed: 19652876]
62. Niers TM, Klerk CP, DiNisio M, Van Noorden CJ, Buller HR, Reitsma PH, Richel DJ. Mechanisms of heparin induced anti-cancer activity in experimental cancer models. *Crit Rev Oncol Hematol.* 2007; 61:195–207. [PubMed: 17074500]
63. Park K, Lee GY, Kim YS, Yu M, Park RW, Kim IS, Kim SY, Byun Y. Heparin-deoxycholic acid chemical conjugate as an anticancer drug carrier and its antitumor activity. *J Control Release.* 2006; 114:300–306. [PubMed: 16884806]
64. Park JW, Jeon OC, Kim SK, Al-Hilal TA, Jin SJ, Moon HT, Yang VC, Kim SY, Byun Y. High antiangiogenic and low anticoagulant efficacy of orally active low molecular weight heparin derivatives. *J Control Release.* 2010; 148:317–326. [PubMed: 20869408]
65. Wang Y, Xin D, Liu K, Zhu M, Xiang J. Heparin-paclitaxel conjugates as drug delivery system: synthesis, self-assembly property, drug release, and antitumor activity. *Bioconjug Chem.* 2009; 20:2214–2221. [PubMed: 19950889]
66. Wang Y, Xin D, Liu K, Xiang J. Heparin-paclitaxel conjugates using mixed anhydride as intermediate: synthesis, influence of polymer structure on drug release, anticoagulant activity and in vitro efficiency. *Pharm Res.* 2009; 26:785–793. [PubMed: 19015960]
67. Park I-K, Kim YJ, Tran TH, Huh KM, Lee Y-k. Water-soluble heparin–PTX conjugates for cancer targeting. *Polymer.* 2010; 51:3387–3393.
68. Wang X, Li J, Wang Y, Cho KJ, Kim G, Gjyzezi A, Koenig L, Giannakakou P, Shin HJ, Tighiouart M, et al. HFT-T, a targeting nanoparticle, enhances specific delivery of paclitaxel to folate receptor-positive tumors. *ACS Nano.* 2009; 3:3165–3174. [PubMed: 19761191]
69. Wang X, Li J, Wang Y, Koenig L, Gjyzezi A, Giannakakou P, Shin EH, Tighiouart M, Chen ZG, Nie S, et al. A folate receptor-targeting nanoparticle minimizes drug resistance in a human cancer model. *ACS Nano.* 2011; 5:6184–6194. [PubMed: 21728341]
70. Khatun Z, Nurunnabi M, Reeck GR, Cho KJ, Lee YK. Oral delivery of taurocholic acid linked heparin-docetaxel conjugates for cancer therapy. *J Control Release.* 2013; 170:74–82. [PubMed: 23665255]

71. Sugahara S, Okuno S, Yano T, Hamana H, Inoue K. Characteristics of tissue distribution of various polysaccharides as drug carriers: influences of molecular weight and anionic charge on tumor targeting. *Biol Pharm Bull.* 2001; 24:535–543. [PubMed: 11379776]
72. Duncan R, Hume IC, Kopecková P, Ulbrich K, Strohalm J, Kopeček J. Anticancer agents coupled to N-(2-hydroxypropyl)methacrylamide copolymers. 3. Evaluation of adriamycin conjugates against mouse leukaemia L1210 in vivo. *Journal of Controlled Release.* 1989; 10:51–63.
73. Gander B, Gurny R, Doelker E, Peppas NA. Effect of polymeric network structure on drug release from cross-linked poly(vinyl alcohol) micromatrices. *Pharm Res.* 1989; 6:578–584. [PubMed: 2508077]
74. Li SD, Huang L. Nanoparticles evading the reticuloendothelial system: role of the supported bilayer. *Biochim Biophys Acta.* 2009; 1788:2259–2266. [PubMed: 19595666]
75. Mayer LD, Janoff AS. Optimizing combination chemotherapy by controlling drug ratios. *Mol Interv.* 2007; 7:216–223. [PubMed: 17827442]
76. Pavillard V, Kherfellah D, Richard S, Robert J, Montaudon D. Effects of the combination of camptothecin and doxorubicin or etoposide on rat glioma cells and camptothecin-resistant variants. *Br J Cancer.* 2001; 85:1077–1083. [PubMed: 11592782]
77. Mayer LD, Harasym TO, Tardi PG, Harasym NL, Shew CR, Johnstone SA, Ramsay EC, Bally MB, Janoff AS. Ratiometric dosing of anticancer drug combinations: controlling drug ratios after systemic administration regulates therapeutic activity in tumor-bearing mice. *Mol Cancer Ther.* 2006; 5:1854–1863. [PubMed: 16891472]

Figure 1.

Chemical structures of Docetaxel (A) and Paclitaxel (B). Both drugs have been evaluated for polysaccharide conjugated delivery. Conjugation of the drug to polymers principally occurs at the reactive 2' -OH group, which is labeled in blue. C. Chemical structure of a monosaccharide unit which is the building block of polysaccharides. Monosaccharides linked together covalently by glycosidic linkage to form a polysaccharide. When a single monosaccharide unit is repeated, the resultant polysaccharide is called the homo-polysaccharide, whereas, a hetero-polysaccharide is composed of two or more types of

monosaccharides. There are two anomeric form of monosaccharide: α and β . They are defined by the position of the $-OH$ group at the C-1 position: in α anomer the $-OH$ group point downward axially and in β anomer the $-OH$ group would point upward equatorially. The numbering system in the monosaccharide is depicted in blue in the figure.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2.

Chemical structures of hyaluronic acid (A) and different HA-taxane conjugates (B-F). Hyaluronic acid is composed of glucuronic acid and N-acetyl glucosamine (A). Mainly carboxylate groups of the glucuronic acid component is used for drug conjugation. In the conjugates, chemical structure of paclitaxel is depicted as PTX for simplification. Linkers are shown in blue colour and PTX is conjugated via the 2' -OH group. Structures of different conjugates prepared by different groups are shown (B-F).

Figure 3.
Chemical structures of carboxy-methyl cellulose (A) and CMC-DTX conjugate (B).

Figure 4.
 Chemical structures of Dextran (A), CM-Dextran (B) and different Dextran-PTX conjugates (C-D).

Figure 5.
Chemical structures of Chitosan (A) and Chitosan-taxane conjugate (B).

Figure 6.
 Chemical structures of Heparin (A) and Heparin-taxane conjugates (B-F).

Figure 7.

Schematic representation of low MW polymer with low drug loading (1), high MW polymer with low drug loading (2) and high MW polymer with high drug loading (3). With higher drug loading, high MW polymers tend to form complex secondary and tertiary structures in an aqueous medium and can self assemble into core-shell nanostructures with a hydrophobic drug core which can reduce the hydrolytic release of the drug.

Table 1

Different polysaccharide-taxane conjugates.

Hyaluronic Acid						
Drug	Linker	Type of bond	% of drug loading	Drug release	In vitro studies	In vivo studies
PTX	ADH-SA (Fig 2B)	PTX-(ester)-SA-(amide)-ADH-(amide)-HA	5-20 wt%	~40% in 24h(plasma)	Selective uptake and cytotoxicity against SK-OV-3, HBL-100, and HCT-116 cell lines, while no cytotoxicity against NIH 3T3	Increased antitumor efficacy and decreased toxicity against CD44(+) human ovarian carcinoma xenografts (NMP-1 and SKOV-3ip) compared to free PTX
PTX	BA (Fig 2C)	PTX-(ester)-BA - (ester)-HA	20 wt%	In 6h, no PTX release (incubated in urine)	Significant cytotoxicity against RT-4 and RT-112/84 bladder tumor cells, decreased cytotoxicity against OVCAR-3 and SKOV-3 human ovarian cancer cell lines	Decreased antitumor activity against bladder tumor RT-112/84; increased antitumor activity against xenotransplanted OVCAR-3 and SKOV-3 human ovarian cancer cell lines
PTX	Direct conjugation (Fig 2D)	PTX-(ester)-HA	1-10 wt%	In 3h, no release at pH 7, increased release as pH decreases	More cytotoxicity for HCT-116 and MCF-7 cells, but reduced cytotoxicity for NIH-3T3 cells	With low MW HA, increased efficacy against brain metastases of MDA-MB-231Br cells
PTX	AA (Val, Leu, or Phe) (Fig 2E)	PTX-(ester)-AA-(amide)- HA	10-14 wt%	Variable depending on the linker, release in the order of Phe>Leu>Val	Better cytotoxicity against MCF-7 than native PTX.	No data
PTX	ED-SA (Fig 2F)	PTX-(ester)-SA-(amide)-ED-(amide)-HA	3 mol %	Burst release (PBS)	Significant cytotoxicity against J774 macrophages	No data
Carboxymethyl cellulose						
Drug	Linker	Type of bond	% of drug loading	Drug release	In vitro studies	In vivo studies
DTX	Direct conjugation (Fig 3B)	DTX-(ester)-CMC	~37 wt%	Linear release at 3.6%/day in plasma.	Enhanced cytotoxicity against a panel of cancer cell lines including LL/2, PAN02, EMT-6, MDA-MB-231 and PC3. Significant cellular uptake after 24h of incubation	Enhanced antitumor efficacy against breast cancer (EMT-6, MDA-MB-231), lung cancer (LL/2), prostate cancer (PC-3) and melanoma (B16F10). Prolonged pharmacokinetic and improved tumor bioavailability compared to the clinically used taxanes.

Dextran						
Drug	Linker	Type of bond	% of drug loading	Drug release	In vitro studies	In vivo studies
PTX	Gly-Gly-Phe-Gly (Fig 4C)	PTX-(ester)-Gly-Gly-Phe-Gly-(amide)-Dextran	5-6 wt%	More than 80% after 24-48 h incubation in plasma or serum	No data	Increased antitumor efficacy against murine tumor models (MX-1, LX-1, HT-29, colon26)
PTX	AAs (Ile, Leu, Ala, Gly) (Fig 4C)	PTX-(ester)-AA-(amide)-Dextran	6-7 wt%	Variable depending on the linker, release in the order of Gly>Ala>Leu>Ile.	No data	Efficacy is dependent on the linker: Gly and Ala exhibited increased activity compared to Leu and Ile against s.c. Colon26 tumor model.
PTX (and FA as targeting ligand)	ED (Fig 4D)	PTX/FA-(amide)-ED-(amide)-Dextran	1-5 wt%	No data	Increased activity with FA labeling for FA receptor over-expressing cells (Caco2 and KB)	No data
Ref. 53, 55						
Ref. 54						
Ref. 56						

Chitosan						
Drug	Linker	Type of bond	% of drug loading	Drug release	In vitro studies	In vivo studies
PTX	SA (Fig 5B)	PTX-(ester)-SA-(amide)-Chitosan	12 wt%	~8% in PBS, 30% in plasma, 60% in cell culture medium after 12 h incubation	Similar cytotoxicity against NCI-H358, SK-OV-3, and MDA-MB-231 cells compared to free PTX.	Significant bioavailability after oral delivery and comparable antitumor activity as i.v. PTX against B16F10 and NCI-H358 tumor models.
DTX	SA (Fig 5B)	DTX-(ester)-SA-(amide)-Chitosan	8 wt%	~90% release in plasma after 72h incubation	Similar cytotoxicity against NCI-H358 and U87MG cell lines compared to free DTX.	Significant plasma level after oral delivery and comparable antitumor activity to i.v. DTX against NCI-H358 and U87MG tumor xenograft models.
Ref. 58						
Ref. 59						

Heparin						
Drug	Linker	Type of bond	% of drug loading	Drug release	In vitro studies	In vivo studies
PTX	Direct conjugation or amino acid spacer (Val, Leu, or Phe) (Fig 6B, C)	PTX-(ester)-Heparin Or PTX-(ester)-AA-(amide)-Heparin	16- 25 wt%	Variable depending on the linker, release in the order of Phe>Leu>Val>> no linker	Increased cytotoxicity against MCF7 cells and Hep-AA-PTX was more potent than Hep-PTX	Decreased toxicity and comparable antitumor activity against SKOV3 tumor xenograft model relative to PTX
PTX	ED (Fig 6D)	PTX-(ester)-ED-(amide)-Heparin	4% (mole)	No data	Slightly higher cytotoxicity against KB cells	No data
PTX (FA as	SA (Fig 6E)	PTX/FA-(ester)-SA-(ester)-Heparin	15-26 wt%	No data	Increased cytotoxicity against FA expressing cell line (KB-3-1) than	Increased efficacy against FA over expressing tumors in
Ref. 65, 66						
Ref. 67						
Ref. 68, 69						

Heparin						
Drug	Linker	Type of bond	% of drug loading	Drug release	In vitro studies	In vivo studies
targeting ligand)					FA negative cell line (Tu212).	both taxane sensitive and resistant models
DTX	ED (Fig 6F)	DTX-(amide)-ED-(amide)-Heparin	~20-30 wt%	No data	Cytotoxicity against KB and MDA-MB-231 cells at µg/mL concentration	Better control on tumor volume after oral delivery compared to oral DTX against KB tumor model
Ref.						
						70

Abbreviations used:

- AA- Amino acid
- ADH- Adipic acid dihydrazide
- BA- Butyric acid
- CE- Ceramide
- CMC- Carboxymethyl cellulose
- DTX- Docetaxel
- ED- Ethylenediamine
- FA- Folic acid
- HA- Hyaluronic acid
- P85- Pluronic P85
- PTX- Paclitaxel
- SA- Succinic acid