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Review article

Developments and future clinical outlook of taxane nanomedicines

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ABSTRACT

Taxanes are highly valuable drugs for cancer treatment. Low water-solubility however puts a major challenge in obtaining formulations that are stable and easy-to-use in clinical practice. Initially, solubilization and lowering toxicity of taxanes has been the main research focus. However, emerging passive and active targeting strategies, especially in the field of nanomedicine, have been capital incentives to further broaden therapeutic index by improving efficacy. This review provides an up-to-date clinical track record of taxane nanomedicines in view of the current state-of-the-art in anti-cancer drug delivery. Additionally, the clinical status of taxane nanomedicines is discussed and considerations are provided for improving future clinical translation.

Contents

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1. Introduction

1.1. Chemotherapy

Cancer is a major cause of death worldwide, only exceeded by cardiovascular diseases [1,2]. Surgery and radiotherapy are effective and valuable treatment modalities for solid, well-localized tumors, but less suitable for treatment of metastatic cancer [1]. For the latter, small molecule chemotherapy is the current choice of treatment, as systemic circulation upon intravenous administration allows for drugs to distribute throughout the body and thus the likeliness of reaching metastatic sites is enhanced [3,4].

Several small molecule anti-neoplastic compounds targeting highly proliferative cells have already been identified half a century ago. Today however, the use of these compounds is often hampered by a low therapeutic index [5]. Table 1 summarizes the most common types of antineoplastic agents used in clinical setting. Although chemotherapeutic drugs exhibit substantial toxicity towards cancer cells as the latter exhibit high proliferation rates, healthy tissues featured by fast cell proliferation (e.g. hair follicles, bone marrow, gastrointestinal tract) are equally affected, thereby causing uncomfortable and sometimes life threatening side-effects [6]. These side-effects often have to be addressed by additional (pre)medication [7]. Due to the aspecific distribution of chemotherapeutic drugs throughout the body, relatively high doses need to be administered (e.g. 175 mg/m^2 Taxol in combination with carboplatin for treatment of advanced non-small cell lung cancer (NSCLC)) in order to achieve sufficient drug levels at the tumor site [8]. However, increasing dose is limited to a certain extent, as high systemic exposure further increases toxicity and severely reduces the quality of life (QoL) of patients [9].

Another issue arises from a pharmaceutical point of view. It is estimated that approximately one third of potent small molecule anti-cancer drugs are rather hydrophobic and thus have limited solubility in aqueous medium [14]. This in particular holds true for the taxanes paclitaxel (PTX) and docetaxel (DTX) (Fig. 1) and hence poses major challenges towards pharmaceutical formulation of these drugs [15,16]. This issue prompted the pharmaceutical industry to identify effective solubilizing excipients and concurrently triggered intensive research towards more advanced anti-cancer formulations capable of delivering drugs in a more effective, safer and patient-friendly fashion [17,18].

1.2. Nanomedicine

The knowledge of cancer biology and etiology has expanded substantially over the past few decades [19,20]. Not only has this led to novel classes of small molecule anti-cancer therapeutics (e.g. molecularly targeted drugs), but this improved understanding also provided a means to optimize the efficacy of conventional anti-cancer drugs. In view of increasing the therapeutic index of conventional, FDA-approved chemotherapeutics, great efforts have been put in the field of nanomedicine [21–23]. Initially investigated by merely liposomal formulations (e.g. Doxil, an FDA-approved liposomal formulation of doxorubicin) [24], similar endeavors have now also been developed using various other types of versatile, biocompatible polymeric nanomaterials such as dendrimers, polymerosomes, block copolymer micelles, polymer-drug conjugates and antibody-drug conjugates (Fig. 2) [25–31].

1.2.1. Assets

Several arguments are in favor of nanomedicines for enhancing the therapeutic efficacy of extremely hydrophobic chemotherapeutics such as taxanes. First of all, physical encapsulation or chemical conjugation of taxanes into amphiphilic nanostructures can significantly enhance drug solubility and the carrier vehicle can serve as a protective shield against chemical and biochemical degradation [32,33]. Second, hydrophobic compounds, solubilized by conventional surfactants (e.g. PTX is solubilized with Cremophor EL in Taxol and DTX is solubilized with Tween 80 in Taxotere), are susceptible to premature burst release into the bloodstream by supramolecular dissociation of the surfactant and/or by fast passive drug diffusion and subsequent interaction with plasma proteins [34]. Chemical conjugation or strong non-covalent interaction between drug and polymeric carrier can be crucial techniques for circumventing systemic drug release and hence side-effects [35–37]. Third, fast renal clearance of small molecule anti-cancer drugs can be avoided as drug-associated nanocarriers with size ≥ 5 nm do not easily pass the small fenestrae in renal vasculature [38–40]. The latter can drastically prolong drug half-life. Numerous in vivo studies indeed report on altered pharmacokinetic profiling of physically encapsulated and chemically conjugated nanomedicine drugs, compared to the corresponding free drugs [41–43]. Fourth, it is known that nanocarriers passively distribute throughout the body in a heterogeneous manner. Nanomaterials tend to accumulate in tissues with highly fenestrated vasculature [44–47]. This phenomenon can be exploited to provide a more selective delivery of drugs into tumors whether or not in combination with additional active targeting strategies. Passive and active targeting will be discussed into more detail in Section 1.2.2. Fifth, polymeric carriers can be chemically designed to trigger a response towards specific internal or external stimuli (e.g. change in pH, enzymes, redox, ultrasound, light). These bio- or stimuli-responsive properties can further enhance the selectivity and control in delivering anti-cancer agents and hence increase their therapeutic index [48–50]. Finally, nanomedicines can significantly alter the route of cellular drug uptake

Table 1
Classification of commonly used antineoplastic agents [10–13].

Drug class	Mechanism of action	Examples	Indications
Alkylating agents	Impair cell function by forming covalent bonds on important molecules (e.g. proteins, DNA, RNA)	Cisplatin, carboplatin, chlorambucil, cyclophosphamide	Ovarian, breast, testicular and bladder cancer, (non)-hodgkin lymphoma, leukemia
Anti-metabolites	Structural analogues of naturally occurring metabolites involved in DNA and RNA synthesis	5-Fluorouracil, methotrexate, gemcitabine, mercaptopurine	Gastric, colorectal, head, neck, lung, breast, ovarian and pancreatic cancer, osteosarcoma, leukemia, non-hodgkin lymphoma
Antitumor antibiotics	Intercalate DNA at specific sequences, creating free radicals which cause strand breakage	Bleomycin, anthracyclines (doxorubicin, epirubicin)	Breast, gastric, testicular, ovarian and thyroid cancer, (non)-hodgkin lymphoma, leukemia, neuroblastoma, squamous cell carcinoma
Topoisomerase inhibitors	Interfere with enzymes responsible for uncoiling of DNA during replication	Irinotecan, etoposide, camptothecin, SN-38	Colorectal and testicular cancer, small cell lung cancer
Tubulin-binding drugs	Taxanes block microtubule disassembly vinca alkaloids prevent microtubule formation	Taxanes (PTX, DTX) vinca alkaloids (vincristine, vinorelbine)	Breast, prostate, ovarian, and pancreatic cancer, NSCLC, leukemia, adenocarcinoma, AIDS-related Kaposi sarcoma
Mechanistic target of rapamycin (mTOR) inhibitors	Interfere with mTOR, a protein kinase involved in cellular metabolism, growth and proliferation	Everolimus, temsirolimus	Breast, pancreatic and lung cancer, renal cell carcinoma

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; SN-38, 7-ethyl-10-hydroxycamptothecin; PTX, paclitaxel; DTX, docetaxel; AIDS, acquired immune deficiency syndrome.

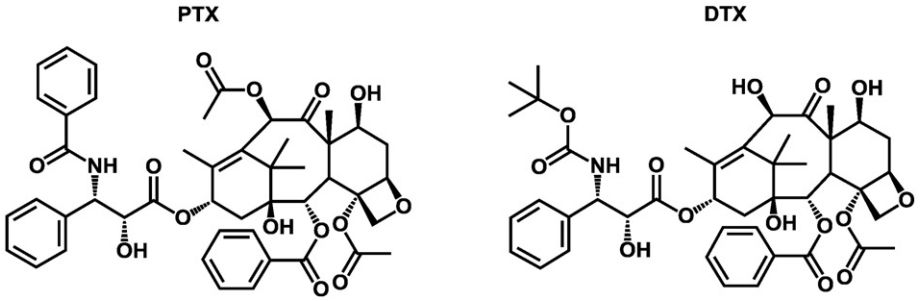

Fig. 1. Chemical structures of PTX and DTX.

and hence limit drug resistance. Hydrophobic small molecule drugs typically enter (tumor) cells by passive diffusion. Nanoparticles on the other hand are predominantly taken up by endocytosis, a mechanism that can be stimulated even more by active targeting strategies (Section 1.2.2) [51,52]. Altering the route of uptake can inherently change intracellular drug localization and concentration. Whilst gradual uptake by passive diffusion only leads to modest cellular drug concentrations, endocytosis of nanocarriers allows for delivering a high amount of drug cargo within a short period of time [53]. The resulting high intracellular drug concentrations can hence saturate efflux, mediated by cytosolic multiple drug resistance (MDR) proteins such as to P-glycoproteins (P-gp) [54]. This can be of great importance for improving the efficacy of chemotherapeutic agents for which drug resistance has been reported.

1.2.2. Altering pharmacokinetics and biodistribution

1.2.2.1. Passive targeting. On a cellular level, cancer can be characterized by several aberrations in normal processes (e.g. proliferation, metabolism, …) [19,20]. These malfunctions translate into tumors with physiological properties significantly different from healthy tissue. Nanomedicines enable the exploitation of these anomalies for tumor targeting by the so called enhanced permeability and retention effect (EPR) [46,55,56]. Blood vessels in solid tumors are often highly fenestrated due to aberrant angiogenesis. This results in endothelial gaps between 100 and 780 nm in size through which nanocarriers can easily extravasate into the interstitial fluid [4,57,58]. The lymphatic drainage of the latter is often impaired in tumors. This allows nanocarriers to reside longer in proximity of malignant tissues (Fig. 3). The FDA-approved formulation Doxil and the majority of the nanomedicines currently in clinical trials (e.g. Table 2 for taxane-based nanomedicines) rely on this passive targeting, mediated by the EPR effect [59].

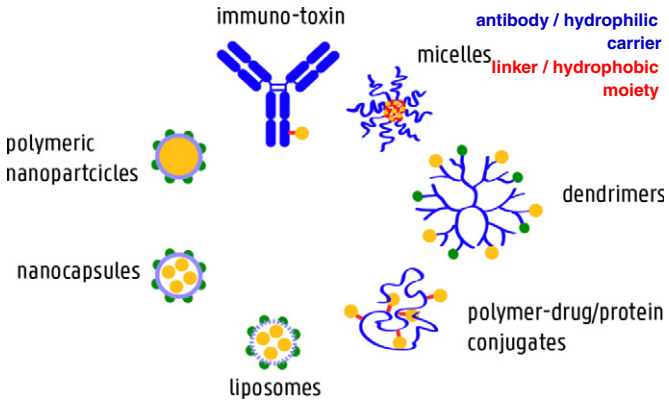

Fig. 2. Overview of prominent nanomedicines.

In order to achieve significant passive targeting, it is crucial for nanomaterials to possess long blood circulation half-lives [60]. This sets up challenges as the latter is a very complex biological matrix, containing several substances (e.g. enzymes, antibodies) that can induce systemic drug clearance. A key player in causing these phenomena is the reticulo-endothelial system (RES), also called the mononuclear phagocytic system (MPS) [61]. Certain plasma proteins, called opsonins, interact with non-endogenous materials. This renders them immunogenic and allows recognition by macrophages in liver (Kupffer cells), lymph nodes and spleen. This accelerated clearance can be avoided by decorating the surface of the nanocarriers with a hydrophilic corona (e.g. poly(ethylene glycol) (PEG)) [62]. It is also known that larger materials (over 200 nm) are more likely to be affected by MPS, irrespective of surface chemistry [63,64]. The optimal size to benefit from the EPR effect should be evaluated for each system, as it involves finding the right balance between prolonging circulation time (increased size) and tumor penetrating capacity (decreased size) [65,66].

1.2.2.2. Active targeting. Active targeting is a promising tool to further enhance the delivery of nanomedicines and hence broaden the therapeutic window. As mentioned before, MDR can be overcome as active targeted anti-cancer nanomedicines are known to be taken up by receptor-mediated endocytosis. The resulting high intracellular drug concentrations are less susceptible to P-gp-mediated cytosolic efflux [67]. Additionally, in many cases active targeting results in internalization and subcellular trafficking of drug loaded nanoparticles close to the target site. The latter can be exploited for drugs that cannot spontaneously pass cellular membranes (e.g. nucleic acid-based drugs) [68].

Active targeting involves decoration of the carrier vehicle with high-affinity ligands that can be recognized by receptors or antigens, specifically overexpressed in the targeted tissues (Fig. 3) [69]. A wide range of receptors have been evaluated, either for direct targeting of tumor cells (e.g. CD44 receptor [70–76], folate receptors (FRs) [77–79], transferrin receptors [80,81], prostate specific membrane antigen (PSMA) [82,83], epidermal growth factor receptors (EGFRs)) [84,85] and/or targeting of tumor-associated vasculature (e.g. αVβ3 integrins [86], PSMA [87], glycoprotein 60 (gp60)) [88]. Several types of targeting ligands have been used including proteins (e.g. transferrin and albumin for targeting transferrin receptors and gp60 respectively) [88,89], monoclonal antibodies and their fragments (e.g. for EGFRs targeting) [69,90], polysaccharides (e.g. hyaluronic acid (HA) for CD44 receptor targeting) [91–94], peptides (e.g. cyclic arginine-glycine-aspartate peptide (cRGD) for αVβ3 integrin targeting) [95–98], aptamers (e.g. A10 for PSMA targeting) [99,100] and small molecules (e.g. folic acid (FA) for FRs targeting) [77,101–103].

However, active targeting is not without risk. Additional functionalization can alter the physicochemical properties of the carrier vehicle [104]. This can in turn significantly influence the in vivo behavior. As these ligands are not always hydrophilic (e.g. FA), high densities onto the surface of the vehicle can result in colloidal instability and aggregation. Furthermore, stealth properties can be jeopardized when the

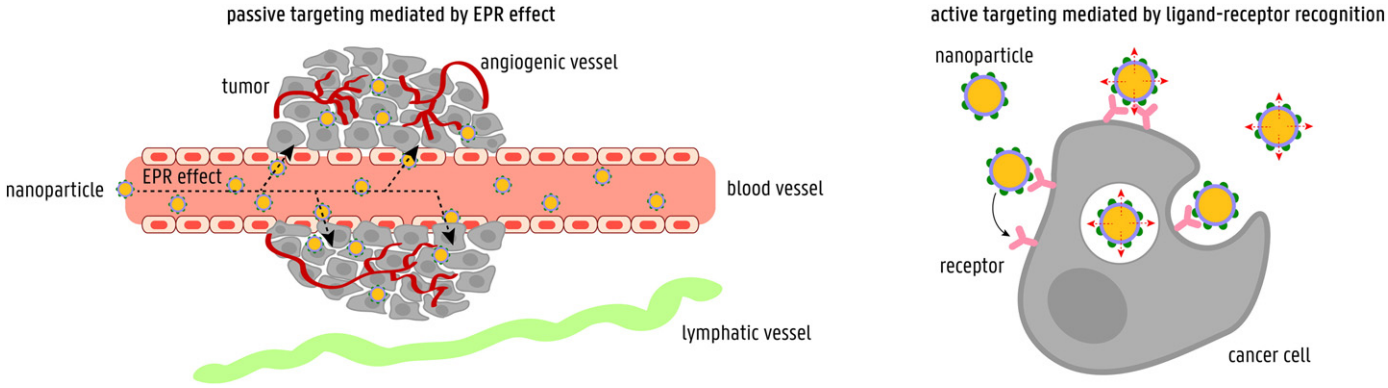

Fig. 3. Illustration of passive (EPR-mediated) and active targeting.

hydrophilic domains of the carrier are covered up to a high extent by more hydrophobic ligands, which can lead to higher RES-mediated clearance [105,106]. Indeed, preclinical evaluation of PSMA-targeted, DTX-loaded nanoparticles, developed by Langer and co-workers, demonstrated that an average 200 PSMA-ligands per nanoparticle resulted in the best in vivo outcome, even though up to 1000 molecules could be decorated per nanoparticle [82]. Finding this optimal degree of functionalization to obtain active targeting and to maintain in vivo stability has proven to be challenging and partly explains the lacking clinical translation of active targeted nanomedicines (Section 4) [69,107]. Recent literature also reports that active targeting can be both time- and dose-dependent. Effective active targeting has been demonstrated

Table 2
Summary of advanced taxane formulations in clinical trials [4,26,29,203,218,222,225,226,258,273,319–326].

Product	Composition	Ligand (target)	Indication	Clinical status
LEP-ETU	PTX liposome	/	Breast cancer, lung cancer, ovarian cancer	Phase II
EndoTAG-1	PTX liposome	/	Breast cancer, pancreatic cancer	Phase II
PNU-91934	PTX liposome	/	Oesophageal cancer	Phase II
LE-DT	DTX liposome	/	Advanced solid tumors	Phase II
ATI-1123	DTX liposome	/	Advanced solid tumors	Phase I
Abraxane (ABI-007)	PTX albumin-bound formulation	/	Breast cancer, pancreatic cancer, NSCLC	Approved (US, 2005)
ABI-008	DTX albumin-bound formulation	/	Metastatic breast cancer, hormone-refractory prostate cancer	Phase I/II
Genexol-PM (IG-001)	PTX PEG-PLA polymeric micelle	/	Breast cancer, lung cancer, ovarian cancer	Phase II/III, approved (South Korea, 2007)
Paxceed	PTX PEG-PLA polymeric micelle	/	Rheumatoid arthritis	Phase II
Paclical	PTX retinoid XR-17 polymeric micelle	/	Ovarian cancer	Phase III/orphan drug (US, 2009)
NK105	PTX PEG-p(Asp-Bz) polymeric micelle	/	Gastric cancer, breast cancer	Phase II/III
Nanoxel	PTX polymeric micelle	/	Advanced breast cancer	Phase I
DTX-PM (Nanoxel-PM)	DTX polymeric micelle	/		Phase I
Nanotax	PTX polymeric nanoparticle	/	Peritoneal neoplasms	Phase I
DTX-PNP	DTX polymeric nanoparticle	/	Advanced solid malignancies	Phase I
BIND-014	DTX PEG-PLGA/PEG-PLA nanoparticle	Small molecule ACUPA (PSMA)	Phase I: metastatic cancer phase II: metastatic castration-resistant prostate cancer, NSCLC	Phase I/II
Taxoprexin	DHA-PTX conjugate	/	Melanoma, liver cancer, kidney cancer, adenocarcinoma, NSCLC	Phase II/III
NKTR-105	4-armed PEG-DTX conjugate	/	Solid tumors, ovarian cancer	Phase I/II
CRLX301	Cyclodextrin-PEG-DTX conjugate	/	Refractory tumors	Phase I
DEP DTX (DTX-SPL8783)	Dendrimer-DTX conjugate	/	Advanced cancers	Phase I
PTX poliglumex (Opaxio, Xyotax, CT-2103)	Poly(L-glutamic acid)-PTX conjugate	/	Lung cancer, ovarian cancer	Phase III
Cripec DTX	DTX-conjugated PEG-p(HPMAm-Lac$_n$) core-crosslinked polymeric micelle	/	Solid tumors	Phase I
GRN1005 (ANG1005)	Angiopep 2-PTX conjugate	Low-density lipoprotein receptor-related protein 1 (LRP1)	Breast cancer with brain metastases, NSCLC with brain metastases	Phase II

shortly after intravenous injection, whilst over longer periods of time, passive targeting can predominate [108]. Additionally, active targeting has shown to be more efficient at lower doses. When nanomedicines are administered at high doses, passive targeting can prevail [109]. These findings should be taken into account for future evaluation of active targeted nanomedicines.

1.2.2.3. Stimuli-responsive release. Systemic drug exposure and side-effects can be further restricted by triggering a release under specific conditions. These triggers can be either external or internal [110]. Examples of external stimuli include ultraviolet or near-infrared light which are often used within the field of photodynamic therapy (PDT) [111]. Other examples are ultrasound and magnetic forces which will not be further discussed. This review focuses on the use of internal triggers. For instance, various nanocarrier systems reported in literature rely on change in pH for inducing drug release [112–117]. Due to the high metabolic rate of tumors, the tumor microenvironment is slightly more acidic (i.e. pH 6.5) than the physiological level (i.e. pH 7.4). Additionally, *endo*- and lysosomal vesicles are characterized by higher acidity (i.e. pH 5.5–5) [118,119]. Furthermore, the expression of certain enzymes is often upregulated in cancerous tissue (e.g. matrix metalloproteinases (MMPs), cathepsin B) [120,121]. Also, certain tumors that are low in oxygen and nutrient levels are often rich in reductive agents [122]. All these features can be exploited for bio-responsive release of anti-cancer drugs by designing acid-sensitive, enzyme-sensitive and redox-sensitive carrier systems respectively.

A variety of acid-sensitive (e.g. ketals, acetals, hydrazones, oximes, orthoesters), enzyme-sensitive (e.g. peptide sequences, esters), base (esters, carbonate esters) and redox-sensitive (e.g. disulfides) functionalities have been exploited to design responsive nanomedicines (Fig. 4) [123–132]. With regard to stimuli-responsive polymeric micelles, these functionalities can be introduced inside the hydrophobic core either by using functionalized monomers or by post-modification [133–135]. The stimulus will trigger degradation of the hydrophobic core into hydrophilic moieties, resulting in complete disassembly and release of encapsulated drug [136]. Additionally, for cross-linked systems (e.g. cross-linked block copolymer micelles, nanogels) stimuli-responsive cross-linkers can be used, allowing full degradation of the nanovehicle into hydrophilic degradation products that can be cleared from the body by renal filtration [137,138]. For polymer-drug conjugates, the goal is to covalently bind drug and polymer through a responsive linker, allowing for tumor-specific, oxidative/reductive, enzyme- or pH-triggered cleavage and avoiding systemic premature burst release [139]. Importantly, as several stimuli are not highly tumor-specific (e.g. low pH in *endo*- and lysosomes, enzymes such as esterases, …), combinations with active targeting should be considered to maximize the therapeutic benefit [140].

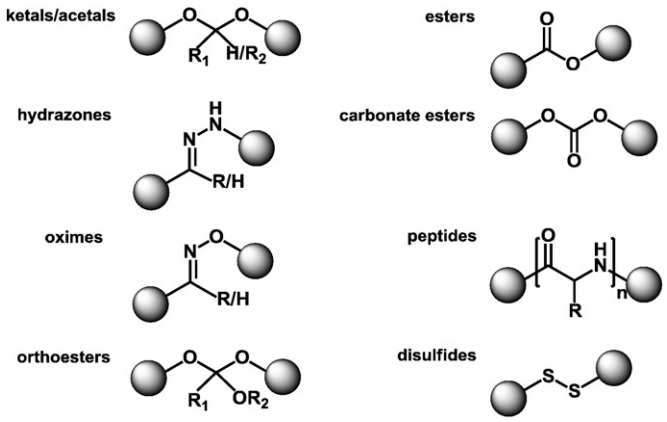

Fig. 4. Overview of bio-responsive linker chemistries.

2. Taxanes

2.1. History

In 1962, a plant screening operation was organized by the US National Cancer Institute. It was found that an extract of the bark of *Taxus brevifolia* (or Pacific yew) exerted cytotoxicity in vitro against the human KB cancer cell line. Initially, only modest anti-cancer activity was detected in vivo, therefore the discovery initially did not receive overall positive response. In the 1970s, the complete structure elucidation of the active compound (i.e. paclitaxel (PTX)) was performed along with more extensive in vivo studies. Contrarily, the latter showed very promising results. The subsequent discovery of the mechanism of action (Section 2.3) fueled the scientific interest even further which eventually led to clinical translation of PTX [141]. Phase I and II trials started in 1984 and 1985, respectively. The first report on clinical anti-cancer activity against ovarian cancer was published in 1989. Few years later, similar positive results were obtained for breast cancer treatment with PTX and in 1992, Bristol-Myers Squibb got FDA-approval for Taxol (formulation details are described in Section 3.1) for treatment of ovarian, breast and NSCLC [142,143].

The scarcity of PTX triggered the exploration towards alternative, renewable sources (Section 2.2). In 1981, a collaboration started between the pharmaceutical company Rhône-Poulenc Rorer Inc. and the Institut de Chemie des Substances Naturelles. In 1986, this collaboration resulted in the discovery of the semisynthetic analogue docetaxel (DTX) [144]. DTX exhibited widespread in vitro and in vivo activity with at least similar potency compared to PTX [145]. Due to the regenerative capacity of the source (i.e. needles), DTX experienced swift clinical development [146]. In 1992, Rhône-Poulenc Rorer Inc. and the National Cancer Institute (NCI) signed a cooperative research and development agreement (CRADA) to seek approval for Taxotere (formulation details are described in Section 3.1) [147]. Phase I and II trials started in 1992 and 1993, respectively [148,149]. Substantial single agent activity was observed in treatment of breast, ovarian and NSCLC [150]. In 1996, DTX was granted accelerated approval for second-line treatment of breast cancer [151].

2.2. Production

PTX can be extracted from the bark of Pacific yew trees, but only in very low yields (i.e. 0.01%). Approximately 2500 trees need to be harvested to obtain 1 kg of PTX [152]. Additionally, these trees are characterized by a very slow growth rate, impeding large scale cultivation. This led to a supply crisis for clinical phase III trials of Taxol in the 1990s and urged the pharmaceutical industry to search for alternative production methods [153]. Extensive research was conducted in finding protocols for the total synthesis of PTX [154–158]. Even though methods have been developed and described in literature, none of these, however, appeared to be viable on an industrial scale. The most successful strategy that eventually resolved the PTX supply crisis involved a semisynthetic approach out of 10-deacetyl baccatin III [142]. The latter is a precursor that can be extracted out of renewable sources (i.e. needles) of a broader spectrum of yew tree varieties with faster growth rates (e.g. European yew (*Taxus baccata*)) [159,160]. This approach also led to the discovery and development of DTX (Section 2.1). Novel biotechnological approaches including production from fungal endophytes and plant cell cultures are currently gaining interest as well. Semisynthesis of DTX and PTX from 10-deacetyl baccatin III, obtained from either yew trees or plant cell cultures, are the most commonly applied strategies for the commercial supply of taxanes. Extensive efforts are currently being devoted to achieving higher yields, lowering the production costs and improving the environmental sustainability [161].

2.3. Mechanism of action

Taxanes are known for their interaction with microtubules [162]. These hollow cylindrical macromolecular structures are built out of 13 longitudinal protofilaments composed of tubulin, a dimeric protein containing an α- and a β-subunit (Fig. 5a) [163]. Microtubules are important building blocks of the cytoskeleton and play pivotal roles in various dynamic processes including cell migration, organelle movement and spindle formation during mitosis. The latter renders microtubules an attractive drugable target, as the proportion of cells in active cell cycle phase is substantially higher in tumor tissue than in normal tissues. Taxanes bind to the inner surface of microtubules, specifically through interaction with the β-tubulin subunit and thereby promote both their formation and their stabilization (Fig. 5b) [164]. DTX possesses a 2-fold higher microtubule binding affinity compared to PTX [165]. The latter partly explains the lower dose of DTX required for obtaining similar in vitro and in vivo anti-cancer effects (Section 3.1). This stabilization thus prevents depolymerization of microtubules from occurring as during normal mitosis, but instead arrests cells in the late G2/M-phase which eventually leads to apoptotic cell death.

3. Taxane solubilization

3.1. Taxol and taxotere

Despite their high potency in cancer treatment, taxanes are used only to a modest extent in clinic today. The hydrophobic structure of PTX, discovered in the 1970s, already highlighted that taxanes possess extremely low water-solubility. For example, it was found that the solubility of PTX in water is below 0.3 μg/mL. Thus, finding a suitable pharmaceutical formulation was challenging for Bristol-Myers Squibb. The company developed a formulation of PTX composed of a 50:50 ethanol:Cremophor EL mixture and commercialized it under the brand name Taxol [166]. In clinic, often 175 mg/m^2 of PTX is administered by intravenous infusion every 3 weeks [167]. This formulation is diluted into an iso-osmotic solution (e.g. 5% dextrose), which is subsequently administered by intravenous infusion over a time course of several hours [168]. Taxol has been FDA-approved for treatment of several cancers including NSCLC, AIDS-related Kaposi sarcoma, ovarian and breast cancer [169].

Even though slightly more hydrophilic, DTX also exhibits very limited water-solubility. For this reason, Rhône-Poulenc Rorer Inc. (now Sanofi), formulated DTX in a 50:50 ethanol:polysorbate 80 mixture and commercialized it under the brand name Taxotere [145]. A DTX dose of 100 mg/m^2 is often administered upon dilution, every 3 weeks within 1–2 h of intravenous infusion [167]. In phase III trials, Taxotere has shown both similar and superior efficacy compared to Taxol (e.g. in platinum combination therapy for treatment of ovarian cancer and as single agent for treatment of anthracycline-resistant metastatic breast cancer respectively) [170–173]. Neutropenia was often more abundant in DTX treatment but generally, less neuropathy was observed than with PTX [170]. Taxotere is currently FDA-approved for treatment of adenocarcinoma, squamous cell carcinoma of head and neck, NSCLC, breast and prostate cancer [174].

However, the surfactants of both Taxol (i.e. Cremophor EL, a pegylated derivative of castor oil) and Taxotere (i.e. polysorbate 80, an ester of pegylated sorbitan with oleic acid), cause serious hypersensitivity reactions in patients, even within minutes during infusion [175–181]. Therefore, patients undergoing therapy with Taxol or Taxotere have to be pre-treated with antihistamines and/or corticosteroids to temper severe, possibly fatal allergic reaction. Polysorbate 80 in particular can also induce fluid retention and often requires additional treatment with diuretics [177]. This has somewhat hampered extensive use of Taxol and Taxotere in clinic, but also instigated scientific interest for developing alternative taxane formulations using more biocompatible excipients in order to administer taxanes in a safer fashion.

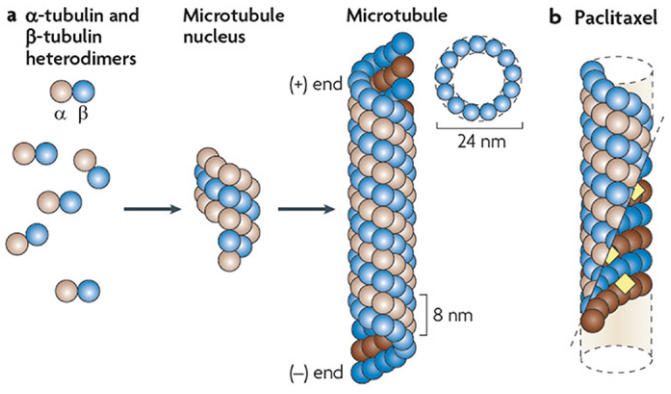

Fig. 5. (A) composition, size and structural organization of microtubules, (B) microtubule binding site of PTX (DTX shares the same binding site). Reproduced with permission from [162].

3.2. Abraxane

In 2005, the first and so far only alternative PTX formulation was FDA-approved for treatment of metastatic breast cancer. It is devoid of ethanol and toxic Cremophor EL and contains human serum albumin (HSA) instead. PTX exhibits high systemic protein binding. Albumin is the most abundant plasma protein and known to function as carrier molecule for lipophilic drugs based on physical, hydrophobic interaction [88,182–184]. The formulation can be prepared by dissolving 2–3% of HSA in water, adding 2 to 4% (v/v) of chloroform and finally adding PTX in a quantity between 5 and 20% by weight relative to the weight of the albumin present in solution. The latter mixture is subjected to high pressure homogenization (i.e. between 9000 and 40,000 psi), to give a nanoemulsion which is frozen and lyophilized. The obtained powder can be reconstituted in saline (i.e. 0.9% NaCl), subsequently diluted in physiological solution and administered through intravenous infusion [185,186]. Reconstitution generates 130 nm particles. Upon dilution into the bloodstream, the only modest hydrophobic interaction causes the particles to rapidly disassemble into single PTX-albumin complexes [187].

Phase I studies showed absence of cremophor-related toxicities which resulted in a relatively higher maximum tolerated dose (MTD; i.e. 300 mg/m^2 for Abraxane versus 200–250 mg/m^2 for Taxol) [188] and greater efficacy in phase II and III trials [88,189–192]. After FDA-approval, additional retrospective in vitro and in vivo studies showed higher tumor accumulation of Abraxane compared to Taxol at equal dose. The latter could be correlated to binding of the PTX-albumin complexes to endothelial gp60-receptors and subsequent transcytosis [193]. Once entered in the tumor interstitium, the complex can bind the secreted protein acidic and rich in cysteine (SPARC), which could in turn facilitate the delivery of PTX into tumor cells. Even though SPARC is not a tumor specific protein, high expression has been associated with malignant transformation [180,194–196]. Despite the preclinical evidence, further clinical research should be conducted to confirm whether these mechanisms also hold true in humans. It has been suggested that elevated expression of SPARC could be a positive biomarker for Abraxane efficacy, although this might apply only for specific tumor types [197–201]. This advanced formulation is generally not considered a nanomedicine. Abraxane exhibits similar short PTX half-lives as compared to Taxol, hence EPR-mediated delivery is unlikely to occur [202]. The success of the platform most probably lies in its ability to significantly lower the toxicity of PTX by avoiding Cremophor EL its formulation, allowing higher dosing and hence improving therapeutic effect. Recently, 2 more indications have been added for treatment with Abraxane: advanced NSCLC and late-stage pancreatic cancer [203]. Based on the same technology, the company (i.e. Celgene) has

developed an albumin-based formulation of DTX (ABI-008), which currently undergoes phase I/II trials.

3.3. Genexol-PM

Genexol-PM was approved in South Korea in 2007 for treatment of metastatic breast cancer. The solubilizing agent in this formulation is an amphiphilic block copolymer, comprised of monomethoxy poly(ethylene-glycol)-*b*-poly(lactic acid) (PEG-PLA; Fig. 6A), which is obtained by ring-opening polymerization (ROP) [204,205]. The formulation can be prepared by solvent diffusion. Briefly, PTX and block copolymer are dissolved in acetonitrile and stirred. After solvent evaporation, the obtained gel matrix is dispersed in water to acquire a clear micellar solution with physically entrapped PTX. The latter is filtered (0.22 μm) and subsequently lyophilized [206]. The obtained powder is reconstituted in saline solution and diluted in 5% dextrose before administration by intravenous infusion [207]. Similar to Abraxane, Genexol-PM showed much lower toxicity in phase I studies compared to Taxol (MTD was more than doubled), which allowed high dose administration [208]. However, no differences in plasma AUC were observed at equal PTX dose [207]. This suggests the micelles rapidly disassemble after administration and/or the drug diffuses out of the particles and strongly interacts with abundant plasma proteins such as albumin. Again, the main asset of this formulation is the ability to solubilize PTX without Cremophor EL, not necessarily through acting as a nanomedicine. Genexol-PM has now also been approved in South Korea for treatment of NSCLC and ovarian cancer. No FDA-approval has yet been granted for the formulation in the US, but several clinical trials are currently ongoing.

4. Nanomedicines for improved taxane delivery

The clinical success of the aforementioned Cremophor EL-free formulations re-highlighted the great potential of taxanes. However, the rationale of these formulations was merely to solubilize these hydrophobic compounds. Even though Cremophor EL-related toxicity was avoided, high systemic drug exposure evoked by these formulations still leads to intrinsic taxane side-effects including myalgia, neutropenia and neuropathy [188,207]. Based on the assets mentioned before, there is a great opportunity in the field of nanomedicine for further broadening the therapeutic index of taxanes. A summary of all taxane formulations in clinical trials is presented in Table 2. In the following paragraphs, a selection of novel, pioneering technologies will be discussed to scope the current trends in advanced taxane delivery.

4.1. Physical encapsulation

As previously mentioned, PTX-encapsulated in albumin nanoparticles (i.e. Abraxane) and polymeric micelles (i.e. Genexol-PM) are likely to dissociate and/or swiftly release PTX once injected in the bloodstream [202]. To truly be defined as nanomedicines, drug-encapsulated formulations have to ensure strong mutual hydrophobic interactions between amphiphiles intrinsically, as well as between amphiphile and drug, in order to avoid particle dissociation and premature drug release, respectively [37].

Kataoka and co-workers designed a micellar PTX formulation based on PEG-poly(aspartate) (i.e. NK105), in which half of the aspartate moieties are functionalized with 4-phenyl-1-butanol (PEG-p(Asp-Bz); Fig. 6B), to yield an amphiphilic block copolymer that forms micellar structures in aqueous environment and exerts strong interaction with PTX [209]. The block copolymer is synthesized by ring-opening polymerization of β-benzyl L-aspartate *N*-carboxy anhydride, initiated by the terminal primary amine group of a α-methoxy-ω-aminopoly(ethylene glycol) macroinitiator [210]. After polymerization, the polymer can be dialyzed, filtered and lyophilized. Micelles are formed upon reconstitution in water. Next, PTX is dissolved in organic solvent (e.g. ethanol) and subsequently added to the micellar dispersion, allowing physical entrapment of the drug into the hydrophobic core of the micelles. The obtained formulation can be lyophilized, reconstituted in suitable iso-osmotic medium and administered through intravenous infusion [211]. Preclinical studies showed 90-fold higher plasma AUC and 25-fold higher tumor AUC of PTX compared to the Taxol formulation [212,213]. Phase I studies showed a significant drop in systemic neurotoxicity and clinical efficacy was proven in a phase II study [214]. These results indicate that NK105 truly acts as a nanocarrier, avoiding premature systemic burst release of PTX and facilitating drug targeting via the EPR effect. NK105 has recently finalized a phase III trial in the US for the treatment of metastatic breast cancer. Unfortunately, the primary endpoint of the study (i.e. in terms of progression free survival (PFS)), did not meet the prespecified statistical criteria [215]. This can put in question whether merely passively targeted nanomedicines could be used as a standard chemotherapeutic treatment modality. The latter will be further discussed in Section 5.

A related block copolymer system has recently been reported by Hennink and co-workers, in which the hydrophobic block consists of

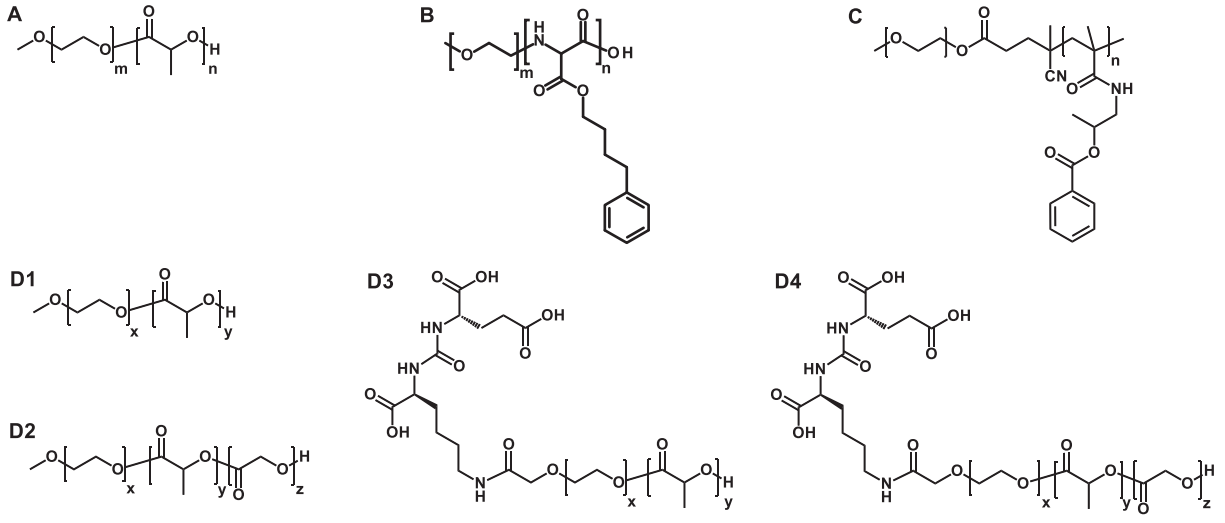

Fig. 6. Molecular structures of polymeric systems used for physical encapsulation of PTX and DTX. (A) PEG-PLA, (B) PEG-p(Asp-Bz), (C) PEG-p(HPMAm-Bz), (D) BIND-014, which is composed of both PEG-PLA (D1) and PEG-PLGA (D2), and PEG-PLA (D3)/PEG-PLGA (D4), functionalized with the ACUPA targeting moiety at the hydrophilic PEG chain end.

N-(2-hydroxypropyl)methacrylamide (HPMA), bearing aromatic benzoyl groups (PEG-p(HPMAm-Bz); Fig. 6C) [36]. The formulation was evaluated in a subcutaneous xenograft mouse model and showed prolonged blood circulation kinetics, effective retention of PTX within the micellar core, low toxicity, high EPR-mediated tumor accumulation and efficient tumor regression [37].

Systems based on physical encapsulation are also being explored for active targeting by surface decoration with high-affinity ligands. So far, BIND-014 is the first and only active targeted polymeric taxane nanoformulation that has reached clinical trials [216]. This system, developed by the Langer and co-workers, comprises encapsulation of DTX into nanoparticles, composed of PEG-PLA and poly(ethylene glycol)-*b*-poly(lactic-*co*-glycolic acid) (PEG-PLGA) block copolymers, of which a fraction is functionalized with a small molecule high-affinity ligand for prostate specific membrane antigen (PSMA) (i.e. S,S-2-(3-(5-amino-1-carboxypentyl)-ureido)-pentanedioic acid (ACUPA); Fig. 6D) [82]. Preclinical development showed superior efficacy of the PSMA-targeted DTX nanoformulation compared to the corresponding non-targeted DTX-loaded nanoparticles. BIND-014 was in clinical phase I for treatment of advanced cancer and phase II for metastatic prostate cancer and NSCLC. However, disappointing results have recently put clinical translation of BIND-014 on hold [217]. This case study highlights the necessity for future nanomedicine development to use preclinical models providing a better predictive value towards clinical outcome, and to exploit concurrent screening to enable careful patient selection for targeted nanomedicine therapy. This will be further discussed in Section 5.

4.2. Chemical conjugation

At present, the nanomedicines with the highest approval rate are drug conjugates. These therapeutics comprise active agents, covalently attached to targeted antibodies, peptides or water-soluble polymers. Conjugating PTX to a hydrophilic polymeric carrier encompasses multiple advantages. First of all, it allows for the preparation of PTX derivatives with substantially higher aqueous solubility [182]. Second, uncontrolled, systemic passive diffusion of drug out of the nanocarrier vehicle is limited as chemical bonds have to be cleaved first. This is a highly attractive asset for polymer-drug conjugates to compete with the numerous liposomal and polymeric micelle and nanoparticle formulations in clinical trials (Table 2). Third, when the polymeric carrier is of high molecular weight (HMW) and/or the polymer-drug conjugate self-assembles into a nanoparticle, renal excretion is limited and thus passive targeting is feasible. Finally, conform to the Ringsdorf model, additional ligands can be attached to the carrier vehicle for active targeting [218].

The approval of Oncaspar (pegylated asparaginase) in 1994 demonstrated that PEG is a highly attractive carrier molecule for bioconjugation [219]. Not surprisingly, only a few years after Taxol was marketed, the first linear PEG-PTX conjugates were reported by Greenwald and colleagues [220]. A series of conjugates (PEG MW of 5 or 40 kDa) were synthesized by conjugating PEG derivatives with a terminal carboxylic acid group to PTX using carbodiimide chemistry (Fig. 7A). Even though both low molecular weight (LMW) and HMW PTX-conjugates showed promising in vitro efficacy, the best in vivo activity was obtained with HMW PTX-conjugates [221]. The latter showed an increased PTX half-life due to slow renal filtration. Thus, the observed in vivo activity most probably resulted from EPR-mediated tumor targeting, although reports have not been very clear. In 2001, Enzon Pharmaceuticals tested these PTX-conjugates in a phase I clinical trial, but decided to discontinue further development in 2003 [222]. A possible reason could be the high molecular weight and limited possibility to functionalize linear PEG, which does not allow high drug loading and impedes administration to patients. Nektar Therapeutics therefore developed a 4-armed PEG structure to which 4 DTX molecules were conjugated [223]. Improved activity and pharmacokinetics were observed in rats and dogs. This conjugate (i.e. NKTR-105) is undergoing phase I/II for treatment of several solid tumors. Other hydrophilic hyperbranched structures are now also being explored for chemical conjugation of DTX. Two of them have now entered phase I clinical trials. One is based on cyclodextrins (i.e. CRLX301) the other involves dendrimer technology (i.e. DEP DTX/DTX-SPL8783) [224–226].

Amongst the PTX-polymer conjugates that are currently being investigated, most progress has been made by PTX poliglumex (also known as Opaxio, Xyotax, CT-2103). This is a biodegradable, HMW (i.e. 48 kDa) conjugate of poly(L-glutamic acid) to which PTX is conjugated by esterification of its C2′ hydroxyl group with the carboxylic acid side groups of the polymer (Fig. 7B) [227]. As the C2′ hydroxyl group is crucial for binding tubulin [228], this water-soluble nanoformulation is in fact a PTX prodrug. Preclinical evaluation showed considerably prolonged plasma half-life, higher MTD, tumor exposure and anti-tumor efficacy compared to Taxol [229]. It has also been proposed that release of PTX predominantly occurs after cellular uptake and is mediated by lysosomal enzymes (e.g. cathepsin B) [230,231]. These features demonstrate the prodrug and passive targeting capabilities of the formulation. Although a phase III trial for first line chemotherapy of advanced NSCLC was somewhat disappointing, the formulation (in combination with platinum drugs) shows promising results in a clinical phase I/II trial for treatment of ovarian cancer and as radiosensitizers for treatment of several cancers [232–234].

Another conjugated system with high profile preclinical results is the CriPec platform. This core-crosslinked polymeric micelle technology is based on methoxy poly(ethylene glycol)-*b*-poly(*N*-(2-hydroxypropyl) methacrylamide-lactate) (PEG-p(HPMAm-Lac_n); Fig. 7C) block copolymers, developed by Hennink and co-workers [235]. These polymers can self-assemble upon heating due to their lower critical solution temperature (LCST) behavior. In the CriPec platform, a fraction of the lactate side chains is reacted with methacrylic anhydride. Next, using a fast heating solvent displacement method, a methacrylated DTX-derivative is loaded into the block copolymer micelles [236]. The micellar dispersion is then transferred to a buffer containing potassium persulfate, in which the polymeric micelles are crosslinked (i.e. by mutual reaction between the pending block copolymer methacrylate moieties) and covalently loaded with DTX (i.e. by reaction between the methacrylate moieties of both the DTX-derivative and those of the block polymer) [237]. Loading capacities up to around 10% can be achieved. The robustness of this polymer synthesis and drug formulation strategy enables to tailor the micelles in terms of size, nanocarrier degradation and drug release kinetics [238]. This can offer opportunities in customizing DTX-formulations in function of patient-specific tumor characteristics (e.g. smaller particle sizes for tumors with more narrowly fenestrated endothelium, faster release kinetics for tumors exerting higher clearance rates [225]), which could be assessed by imaging nanodiagnostics (e.g. iron oxide nanoparticles; Section 5). A phase I study is currently recruiting patients with solid tumors for determining the highest safe dose [239].

No active targeted taxane-polymer conjugates are currently in clinical trials. However, the first active targeted polymeric nanoformulation ever reported was a polymer-drug conjugate, moreover a p(HPMA)-doxorubicin conjugate (PK2, Pfizer Inc.). This platform involves attachment of doxorubicin to the pending hydroxyl groups of pHPMA, using a tetrapeptide Gly-Phe-Leu-Gly spacer [240]. The latter was designed by Kopeček and co-workers and can be cleaved specifically by lysosomal enzymes (i.e. cathepsin B) [241,242]. Additionally, the backbone was functionalized with galactosamine. The latter is a ligand with affinity towards lectins, present on the surface of mammalian liver cells. A phase II trial showed modest improved hepatic targeting for PK2, compared to the conjugate lacking the galactose residues (PK1) [243]. No further clinical results have been published for this system. Table 2 gives a summary of all the aforementioned innovative taxane formulations that are currently undergoing clinical evaluation.

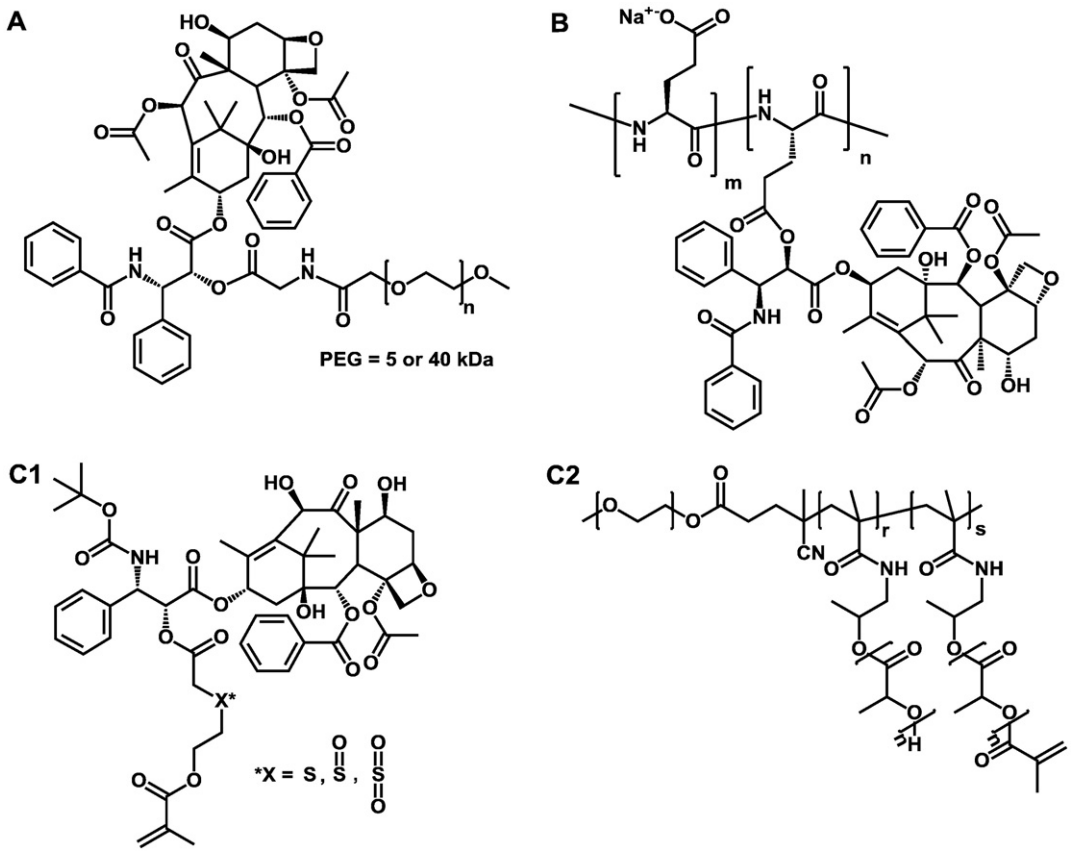

Fig. 7. Molecular structures of polymeric systems used for chemical conjugation of PTX and DTX. (A) PEG-PTX, (B) poly(L-glutamic acid)-PTX, (C) Cripec, where DTX is modified with a methacrylate moiety (C1) through a hydrolysable spacer and encapsulated in core-crosslinked amphiphilic block copolymer micelles through copolymerization with methacrylate moieties on the side chain of the hydrophobic block of PEG-p(HPMAm-Lac$_n$) (C2).

4.3. Antibody-drug conjugates

Although not always considered as nanomedicines, antibody-drug conjugates (ADCs) comprise the current state-of-the-art in active targeted drug delivery and are therefore mentioned in this review. ADCs could also set the benchmark for novel active targeted taxane nanomedicines as the latter can compete with ADCs with regard to drug loading. Monoclonal antibodies (mAbs) bind antigens with meticulous specificity [244]. Therefore, they have been extensively investigated over the past 20 years as carrier vehicles for targeted delivery of small molecule cytotoxic agents. To obtain an ADC, a cytotoxin is coupled to a mAb via a linker molecule. Even though stable linkers have proven to be successful, the latest trends in linker design aim at maintaining stability in the bloodstream whilst releasing drug payload at the target site. This release can be triggered either by pH-, redox- or enzyme-mediated stimuli [245].

The high potential of ADC-technology is reflected by its clinical track record. Over 40 ADCs are currently undergoing clinical evaluation and 2 have been FDA-approved [246]. Brentuximab vedotin comprises the chimeric mAb cAC10, directed against CD30 and conjugated to monomethyl auristatin E (MMAE), through an cathepsin-cleavable, self-immolative dipeptide (i.e. valine-citrulline) linker (Fig. 8A). Like taxanes, MMAE is a very potent antimitotic agent that interferes with microtubule dynamics, but not through promotion of formation and stabilization of microtubules, but by blocking tubulin polymerization [247]. Based on positive results obtained in single-arm multicenter clinical trials, an accelerated FDA-approval was granted in 2011 for treatment of relapsed or refractory hodgkin lymphoma and anaplastic large-cell lymphoma, which are often featured by an overexpression of CD30 [248]. Trastuzumab emtansine was the first approved ADC for treatment of solid tumors. The drug consists of an anti-HER2 mAb, conjugated by its lysine residues to the cytotoxic maytansinoid emtansine through a non-cleavable thioether linker (Fig. 8B). Emtansine (DM1) also inhibits the formation of microtubules, predominantly by binding tubulin [249]. Release occurs after receptor-mediated uptake and lysosomal degradation. Proved safety and effectiveness in a phase III trial involving 991 patients led to a full market approval in 2013 for treatment of HER2-positive metastatic breast cancer patients who previously received trastuzumab and a taxane. Another ADC called gemtuzumab ozogamicin was approved in 2000 but has been withdrawn in 2010 after showing lack of benefit over conventional therapy and concomitant hepatotoxicity [250].

The latter example along with the fact that all the approved ADCs still induce systemic toxicity (e.g. neutropenia, nausea, neuropathy, thrombocytopenia) indicate that, despite the strong ADC pipeline, the technology still needs further investigation and improvement [251]. Lessons are to be learnt concerning linker design as several toxicities have been attributed to specific linkers [252–255]. Next, the ability of mAbs to effectively localize at the target site can be considered a point of discussion. Even though mAbs exhibit blood half-lives > 3 days, mAbs might extravasate too slow and not efficiently penetrate in tumor tissue, leading to heterogeneous distribution, with the highest concentration at the periphery [256–258]. Additionally, the number of drug molecules that can be conjugated to mAbs is limited. The drug-antibody-ratio (DAR) is usually 4 mol/mol [259,260]. As the average molecular weight of the used cytotoxins is 1000 Da and the molecular weight of an antibody is approximately 150,000 Da, loading capacities are typically even below 5%. This explains why initially developed ADCs based on regular chemotherapeutics, including taxanes, lack therapeutic efficacy and hence novel cytotoxic agents (e.g. MMAE and DM1) with substantially higher potency (i.e. up to factor 1000) are required for obtaining sufficient therapeutic effect in spite of low drug loading

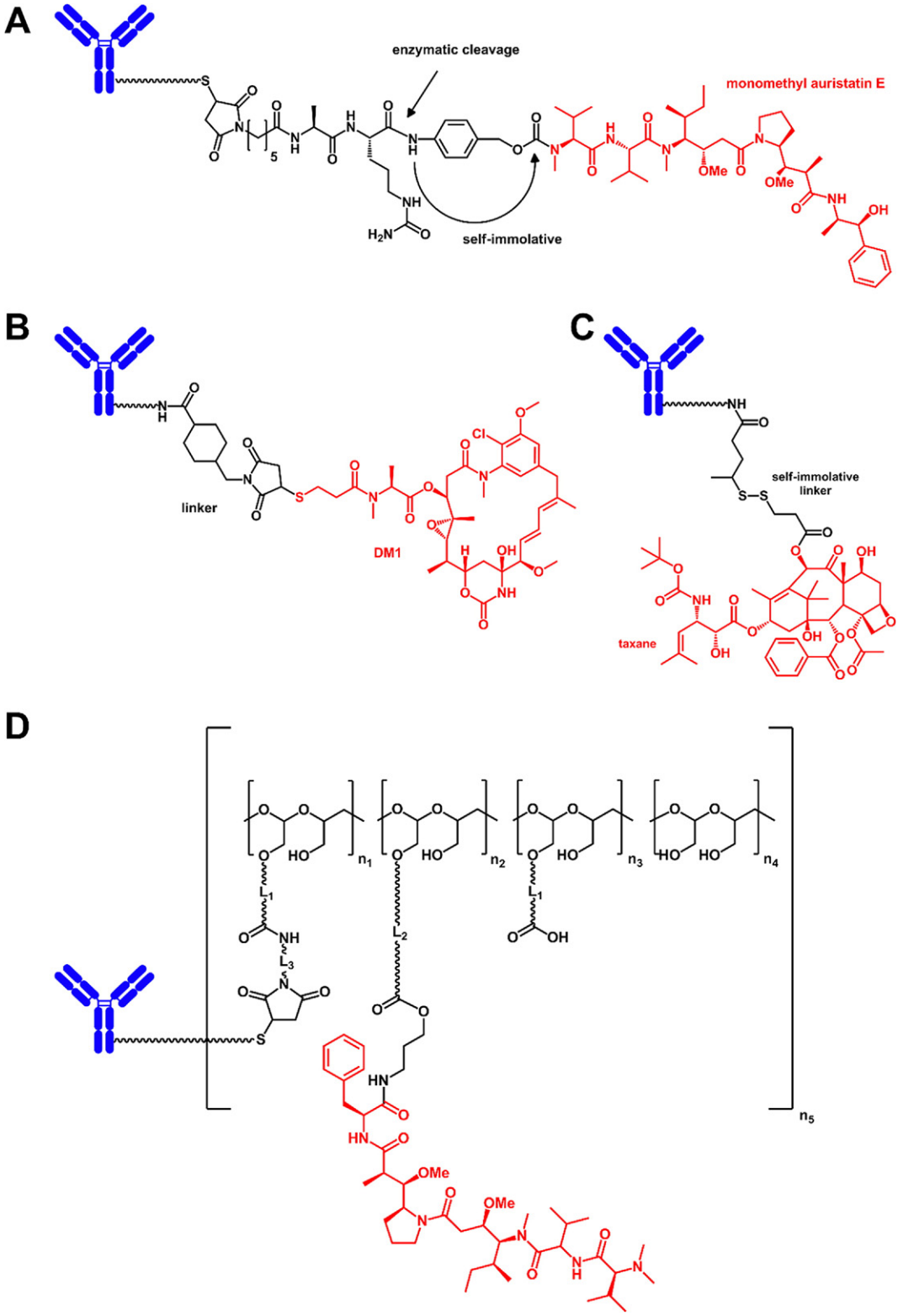

Fig. 8. Molecular structures of ADCs: brentuximab vedotin (A), trastuzumab emtansine (B), a novel taxane ADC (C), XMT-1522 (D).

[261,262]. However, this also implies that only a small fraction of premature drug release could result in severe systemic toxicity. The final and probably biggest issue from a healthcare point of view is that the power of the technology comes at a price. ADCs rank amongst the most expensive medicines on the market (e.g. the treatment cost of trastuzumab emtansine is approximately $ 1400 per dose) [245]. This is not only due to the high cost of mAb manufacturing, in particular the drug conjugation and the subsequent purification are a major challenges [263]. In order to allow widespread use in clinic, these costs would have to be lowered in the future.

A lot of promising efforts are being conducted to address these issues. New linkers are being developed based on rational design. The field is currently investigating the use of smaller antibody fragments such as diabodies, miniantibodies and small immune proteins (SIPs),

which could allow for more efficient distribution into tumor tissue [264]. Novel ADCs are developed based on highly potent second-generation taxanes (Fig. 8C) [265]. The latter show promising in vivo activity leading to complete tumor regression in a subcutaneous A431 xenograft mouse model [266]. Additionally, mAb decoration with drug-functionalized polymers is being investigated to increase cytotoxic payload [267]. For example, Mersana Therapeutics has developed an anti-HER2 ADC (XMT-1522) to which 3 to 5 hydrophilic, biodegradable polymer chains were conjugated, each bearing up to 5 auristatin molecules and an average payload of 15 molecules per mAb (Fig. 8D) [268]. XMT-1522 showed very potent activity in vitro against several breast, NSCLC, gastric and ovarian cancer cell lines. Complete regression was observed in vivo for both high and low HER2-expressing mouse xenograft models [269]. This could also be a viable approach for obtaining sufficiently potent taxane-based ADCs. Novel techniques including the use of algae as vector for protein expression are being explored to cut production costs [270]. Apart from these efforts, as for most biotechnological products, it is not likely that the price will dramatically drop in the very near future. This leaves opportunities open for other alternative active targeted therapies based on non-biotechnological, synthetic endeavors.

4.4. Small molecule-drug conjugates

Besides nanomedicines and ADCs, small molecule-drug conjugates (SMDCs) have shown great promise in targeted chemotherapy and the latter could provide a complementary platform for the treatment of tumors that are less susceptible to EPR-mediated drug delivery. Similar building blocks are exploited for SMDCs as for ADCs (i.e. drug, spacer molecule and targeting moiety). The latter however is not a mAb, but a synthetic, small molecule targeting ligand. Spacer design is crucial for the construction of SMDCs. The same chemical rationale is used as for ADCs, i.e. to achieve specific drug release in the tumor environment based on (self-immolative) acid-, redox- or enzyme-sensitive properties. SMDCs preferably encompass a rigid, hydrophilic spacer molecule to avoid intramolecular interference between drug and ligand [271,272]. Increasing hydrophilic properties also limits aspecific cellular uptake by passive diffusion, which is the predominant uptake mechanism of free hydrophobic drugs [273,274]. The molecular weight of the spacer molecule can influence the efficacy of SMDCs. For example, it was shown that folate-rhodamine conjugates with HMW PEG spacers (i.e. >5 kDa) do not exhibit fast and adequate tumor penetration [275]. On the other hand, SMDCs with LMW spacers (i.e. <2 kDa) are capable of passively diffusing into the tumor tissue more thoroughly and more rapidly than macromolecular carrier molecules [59,273,275,276].

In certain respects, SMDCs have advantages over ADCs. First, the smaller size of SMDCs could allow for more effective penetration throughout the tumor mass [260,273]. The latter can be an important asset for treating less EPR-sensitive tumors and for passing dense membrane structures such as the blood brain barrier (BBB). Second, the stepwise synthesis of SMDCs allows for a more controlled and site-specific drug conjugation [260]. Third, the low molecular weight of SMDCs inherently results in a high drug loading capacity. Hence, beyond the conjugation of extremely potent drugs (e.g. MMAE and DM1), this technology is also suitable for conjugation of well-established chemotherapeutic drugs including taxanes. Fourth, small hydrophilic molecules exhibit fast renal clearance [277]. Though considered a drawback in EPR-mediated drug delivery, this property could pose advantages with regard to toxicity. Unless a technology possesses 100% binding specificity, which currently cannot even be achieved by ADCs, the longer the blood half-life, generally the higher the risk of side-effects [260]. As SMDCs generally saturate the target receptor within 5–20 min after intravenous injection [275], fast excretion of the dose fraction that is not bound to the target tissue would minimalize systemic side-effects [278,279]. To compensate for the fast renal clearance, a higher dosing might be required. Hence, a prodrug approach with tumor-specific, stimuli-responsive drug release would be preferred, as this can substantially increase MTD and hence allow administration of higher doses without risking high off-site toxicity.

9 SMDCs are currently in clinical trials, of which more than half in phase II or III [273], including GRN1005. The latter is a conjugate, composed of 3 PTX molecules linked to angiopep 2 by ester bonds, which are cleaved in lysosomal vesicles (Fig. 9A) [280]. Angiopep 2 is a peptide-based ligand, targeting low-density lipoprotein receptor-related protein 1 (LRP1), a cell-surface receptor involved in cancer metastasis. GRN1005 is currently undergoing a phase II trial for treatment of NSCLC with brain metastases and for breast cancer in combination with trastuzumab (Table 2)

As for nanomedicines, the hydrophobicity of anti-cancer drugs challenges the design and formulation of SMDCs. For example, a surfactant (i.e. Solutol HS15) is still required to obtain a clinically applicable formulation of GRN1005 [281]. These solubility issues could be resolved by designing spacers with sufficient hydrophilicity. Most of the spacer molecules currently used for SMDC-technology involve peptide sequences. The latter are typically synthesized by multistep solid phase peptide synthesis (SPPS). A more straightforward, alternative approach could be the one-step synthesis of a hydrophilic, LMW polymer spacer, directly onto the drug molecule. Polymerization strategies have been developed to achieve the latter. For example, degradable LMW PTX-PLA conjugates have been synthesized by direct ring-opening polymerization (ROP) of DL-lactide from PTX (Fig. 9B) [139]. Within our own research group, we have recently developed a hybrid system in which a customized, hydrophilic LMW polymer spacer (i.e. poly(*N*,*N*-dimethylacrylamide) (pDMA)) can be engineered onto PTX using reversible addition-fragmentation chain transfer (RAFT) polymerization (Fig. 9C) [282]. Additionally, the obtained highly water-soluble PTX-polymer conjugate can be post-modified by established thiol-maleimide coupling at the opposite polymer chain end. As the resulting compound meets the structural requirements of SMDCs, we are currently exploring the potential of this approach for engineering effective, water-soluble taxane-based SMDCs. Another aspect of SMDC-technology that might hamper clinical translation is the current limited availability of high-affinity small-molecule targeting ligands. This is not an issue in the field of ADCs, as high-affinity mAbs can be raised against virtually any target antigen [283]. The search for novel, high-affinity small-molecule ligands towards tumor-specific epitopes has indeed proven to be challenging [284,285]. However, this can be facilitated in the future by using predictive computational modeling methods [286] and DNA-encoded chemical library technologies, which in contrast to conventional high-throughput screening (HTS), allows for extensive parallel evaluation of chemical libraries in one reaction tube [287–289].

5. Discussion

A broad range of taxane nanomedicines have been developed and various preclinical in vivo studies have demonstrated that passive and active targeting is a great tool for significantly improving drug delivery. Surprisingly, only a few of these systems survived clinical trials [290]. This somewhat low number, along with the recent disappointing clinical results of NK105 and BIND-014, obliges the field to question the overall validity of targeted nanomedicines in clinical chemotherapy. A recently published meta-analysis on the preclinical performance of anti-cancer nanomedicines suggests that the field might have a delivery problem, as only 0.7% (median) of the intravenously injected nanoparticle dose was claimed to accumulate in tumors [291]. This publication triggered a major discussion amongst experts [292,293].

The majority of (taxane) nanomedicines currently residing in clinical trials rely on passive targeting. Their limited clinical translation thus far suggests that EPR-mediated delivery cannot always be taken for granted [294]. Several possible limitations in the regard of EPR effect have indeed been brought forward amongst experts. Two well-reported mechanisms that can counteract the EPR effect include dense stroma

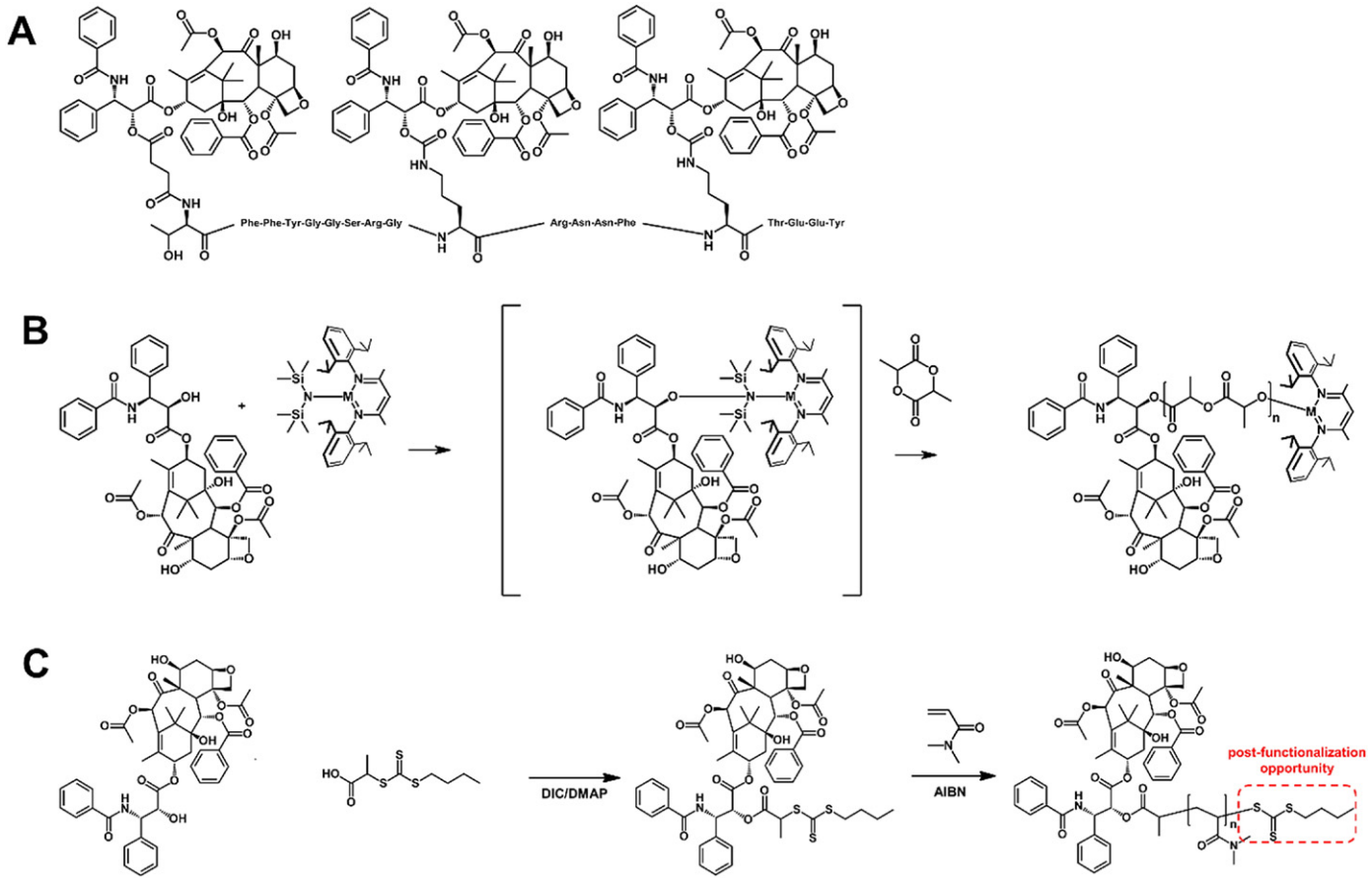

Fig. 9. Molecular structures of SMDCs: GRN1005 (A), PTX-PLA (B) and PTX-pDMA (C).

and high tumor interstitial fluid pressure (IFP) [295,296]. These features can impede the tumor-directed flow of nanomedicines. However, strategies have been explored to overcome these hurdles, for example by pretreatment or co-formulation with anti-stromal drugs and/or vascular endothelial growth factor (receptor) (VEGF(R)) inhibitors respectively [297–299].

The most likely reason for the limited clinical translation of nanomedicines is the overall intrinsic heterogeneity of human cancer disease, which is often not accurately reflected by the current, most commonly used preclinical models [300–304]. Due to their easy access and fast proliferation rate, in vitro cultured human cancer cell lines are frequently used for subcutaneous xenograft mouse models. However, extensively cultured cell lines are known to genetically adapt to their in vitro environment [305]. Hence, tumors generated from these cell lines in immunodeficient mice often present pathologies in vivo with little resemblance to the corresponding tumors occurring in humans. For example, due to the fast proliferation rate, the tumor vasculature is often underdeveloped and fenestrated and thus very suitable for EPR-mediated drug delivery. In patients however, the immune system can drastically slow down the proliferation rate, thereby generating tumors with normal physiological vasculature. This partly explains why nanomedicines perform only moderately in clinical trials, in spite of superior preclinical efficacy. Preluding imaging techniques could determine which population would be suitable for EPR-mediated drug delivery. Indeed, besides nanomedicines, pharmaceutical companies are equally investing in nanodiagnostics. For example, Merrimack Pharmaceuticals has recently used 30 nm iron oxide nanoparticles (Feraheme, AMAG Pharmaceuticals) in a clinical trial to screen patients on EPR effect before administrating their liposomal irinotecan formulation Onivyde (MM-398) [217,225,306,307].

Furthermore, preclinical evaluation of anti-cancer nanomedicines typically occurs in primary tumor models. In clinical setting, however, cancer is generally treated differently. Primary tumors are preferentially removed by surgical resection and/or radiotherapy [303]. Though occasionally conducted at presurgical stage as neoadjuvant therapy, chemotherapy is commonly applied when conditions are too delicate for the latter two techniques and for treatment of metastases [308]. Metastatic tissues are less featured by fenestrated endothelia as compared to primary solid tumors and very small metastases are often poorly vascularized [309,310]. It is clear that these characteristics do not allow for efficient passive targeting. Additional active targeting strategies should also be considered for more effective treatment of metastatic cancer disease and preclinical evaluation should be performed using more appropriate animal models [311].

Research efforts over the past 10 years resulted in development of clinically more relevant models, including the use of genetically engineered mouse (GEM) models. GEM mice can be designed to spontaneously grow orthotopic and hence anatomically relevant tumors. The latter could allow for better insights in the behavior of nanomedicines in a physiologically similar tumor environment as in patients. Furthermore, metastatic GEM models provide an added value in identifying what features of nanomedicines are required for effective treatment of metastatic cancer disease. Another important model is the patient-derived xenograft (PDX) model [312,313]. By extracting cancer cells directly from patients and live passaging in mice, greater concordance has been observed in terms of tumor architecture, histomorphology and global gene expression [314]. Expanding the patient population for PDX modeling also allows discovery of predictive biomarkers based on genomic and proteomic profiling of responders and non-responders [315]. This could allow for a better patient

screening, improve the success rate of nanomedicines in clinical trials and hence deliver a valuable contribution towards personalized medicine [316]. It is likely that inter-patient variabilities in EPR susceptibility could be detected in PDX models [317].

6. Concluding remarks

The progress towards more efficient and safer taxane anti-cancer nanomedicines has so far not been a very swift and efficient process in terms of clinical translation. Many ups and downs can be acknowledged. Does this imply that the assets of nanomedicines have been overestimated? Most probably not, but it would be unrealistic to expect a universal effective response in all cancer patients. Different types of nanomedicines all have their benefits and drawbacks. It is therefore useless to discuss which one is better than the other, but crucial to figure out which one or which combination is best for each individual patient. Human cancer disease is highly heterogenic and should therefore be taken more into account for future design and evaluation of novel targeted anti-cancer nanomedicines. As now more and more relevant animal models (i.e. PDX, GEM) are becoming available, it will be highly recommended to expand preclinical testing beyond the well-established cell line-derived xenograft models. This will probably deliver superior predictive values and could help in deciding whether or not to proceed to evaluation in patients. These approaches will also provide biomarkers in regard of drug response which can be used in patient screening for clinical trials. Additionally, companion nanodiagnostics (e.g. imaging nanoparticles) will be complementary tools to further screen tumors in patients and evaluate their susceptibility to passive and active targeting. By careful selection of suitable patients through personalized nanomedicine, a better assessment of the full potential of targeted nanomedicine will be possible [318]. Furthermore, the clinical response and hence approval rate of future anti-cancer (nano)medicines can be significantly improved.

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