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REVIEW ARTICLE

Polymeric micelle nanocarriers in cancer research

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Abstract Amphiphilic block copolymers (ABCs) assemble into a spherical nanoscopic supramolecular core/shell nanostructure termed a polymeric micelle that has been widely researched as an injectable nanocarrier for poorly water-soluble anticancer agents. The aim of this review article is to update progress in the field of drug delivery towards clinical trials, highlighting advances in polymeric micelles used for drug solubilization, reduced off-target toxicity and tumor targeting by the enhanced permeability and retention (EPR) effect. Polymeric micelles vary in stability in blood and drug release rate, and accordingly play different but key roles in drug delivery. For intravenous (IV) infusion, polymeric micelles that disassemble in blood and rapidly release poorly water-soluble anticancer agent such as paclitaxel have been used for drug solubilization, safety and the distinct possibility of toxicity reduction relative to existing solubilizing agents, e.g., Cremophor EL. Stable polymeric micelles are long-circulating in blood and reduce distribution to non-target tissue, lowering off-target toxicity. Further, they participate in the EPR effect in murine tumor models. In summary, polymeric micelles act as injectable nanocarriers for poorly water-soluble anticancer agents, achieving reduced toxicity and targeting tumors by the EPR effect.

Keywords nanomedicine, parenteral, poly(ethylene glycol), poly(lactic acid), reformulation

1 Introduction

Cancer is a major worldwide public health problem, and it became the leading cause of death in China in 2010. In 2015, it was estimated that there were about 4.2 million new cases of cancer and 2.8 million deaths in China. Lung cancer is the most common cause of cancer and cancer death [1], followed by stomach, esophageal and liver cancers. While incidence rates for cancer in China have stabilized for males, they have increased significantly for females, ca. 2.2% per year. Thus, while progress against cancer has been made over the years, incidence of cancer and mortality is high and will likely grow with population growth, aging and changing demographics. Cancer treatment usually involves combination of surgery, radiation therapy and/or drug therapy. Surgery and radiation are usually confined to local disease, whereas drug therapy is used for metastatic disease, spread to distant sites in the body. Given that 90% of cancer death has been attributed to metastases and systemic chemotherapy is very toxic, there have been major efforts in cancer research that seek to improve systemic cancer treatment, and they include drug combination strategies, targeting aberrations in cancer cell signaling, adjuvant therapies for surgery or radiation and anticancer nanomedicine, seeking reduced toxicity and synergistic anticancer efficacy [2–4].

Biomedical research in nanomedicine has attracted worldwide attention in the fight against cancer, drawing scientists, engineers and clinicians together in highly interdisciplinary research that has quickly moved from the bench to bedside in the last decade or two. While critics have questioned the slow pace in drug development and low clinical impact of nanomedicine in cancer research, it must be noted that similar criticisms were raised against monoclonal antibodies and immunotherapy, and they are now blockbuster drugs and major part of the armamentarium against cancer, including antibody-drug conjugates [5].

The aim of this review article is to update progress on anticancer nanomedicine, highlighting advances in polymeric micelles for drug solubilization, controlled release and drug targeting. There are many excellent review articles on anticancer nanomedicines that describe various nanocarriers including polymeric micelles, biological barriers and clinical progress [6–10]. In this review article, we cover recent progress on polymeric micelles as an injectable nanocarrier for poorly water-soluble anticancer agents.

Amphiphilic block copolymers (ABCs) assemble in water into various supramolecular nanostructures, e.g.,

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cylindrical micelles, spherical micelles and vesicles for drug delivery [11], and at a hydrophilic fraction > 50% and above the critical micelle concentration (CMC), ABCs assemble into a spherical polymeric micelle with nanoscopic dimensions (Fig. 1). Poly(ethylene glycol) (PEG) is often the shell-forming block, being used in drug delivery because of its proven safety profile and stealth-like nature due to its hydration, high mobility and thus protein repulsion [12]. The hydrophobic block varies considerably in drug delivery, e.g., poly(D,L-lactic acid) (PLA), poly(α-amino acid). Anticancer agents are loaded chemically or physically in the core of region of polymeric micelles (Fig. 1). In this way, drug solubilization can be accomplished for intravenous (IV) injection or infusion. Polymeric micelles have a capacity for single or multiple anticancer agents, acting as an injectable multi-drug nanocarrier by physical loading (i.e., multiple drug solubilization) and chemical loading, forming mixed polymeric prodrug micelles. In either case, chemical crosslinking may be an important tactic that helps to stabilize polymeric micelles against disassembly for prolonged circulation in blood.

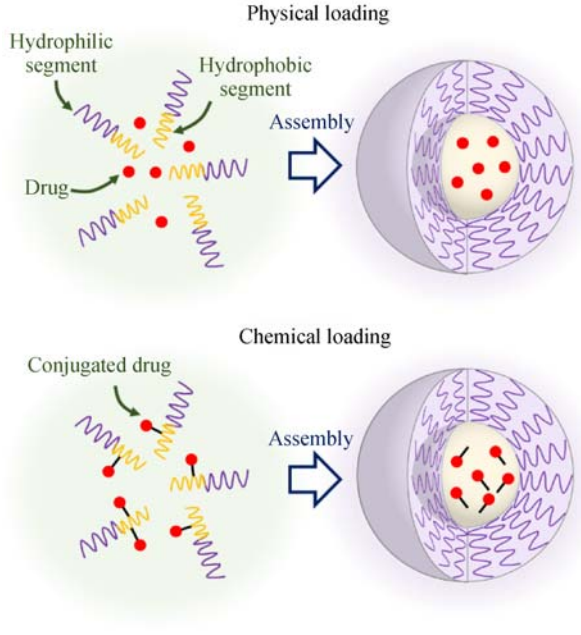

Fig. 1 Physical and chemical drug loading of polymeric micelles

Figure 2 outlines the scope of drug delivery via injectable nanocarriers, such as polymeric micelles. In step one, nanocarriers such as polymeric micelles are administered by the IV route, bypassing a drug absorption step. IV injection or infusion requires drug solubilization at > ca. 1.0 mg/mL, sterility and stability against drug precipitation to avoid embolism. In this situation, polymeric micelles replace toxic IV vehicles such as Cremophor EL and ethanol that are toxic in their own right, causing life-threatening hypersensitivity reactions despite pre-medication [13]. Unstable polymeric micelles that rapidly release anticancer agent in blood following administration do not play a major role in determining pharmacokinetics (PK) of anticancer agent. After release, poorly water-soluble anticancer agents tend to be protein bound, maintain solubility in blood and distribute widely (Fig. 2(A)). In a few cases, anticancer agents may accumulate preferentially at solid tumors on their own due to an overexpression of tumor-associated drug target, e.g., heat shock protein 90 (Hsp90) [14]. After polymeric micelle disassembly, ABCs may be removed from the body by renal clearance. Rapid disassembly of polymeric micelles results in a high plasma level of anticancer agent that is appropriate for log-kill, maximum tolerated dose (MTD) strategy [15], but may be associated with dose-limiting toxicity.

Stable polymeric micelles, given stability against disassembly (e.g., chemical crosslinking) and controlled release, evade innate immune defense, i.e., mononuclear phagocyte system (MPS) in the liver, spleen and bone marrow and circulate in blood for prolonged periods (Fig. 2(B)). As a result, drug distribution is minimized to non-target tissue, reducing off-target effects. To date, reduction in toxicity by injectable nanocarriers, best exemplified by liposomal doxorubicin (Doxil®) and reduction of its cardiotoxicity, has been the most important clinical contribution of anticancer nanomedicine [16]. Thus, replacement of toxic IV vehicles and reduction in off-target effects are certainly within the realm of possibility for polymeric micelles for old (i.e., reformulation and/or repurposing) and emerging anticancer agents in drug development that include novel signal transduction inhibitors.

Stable polymeric micelles accumulate preferentially at solid tumor through leaky vasculature by the enhanced permeability and retention (EPR) effect (Fig. 2(B)). In this case, the small size of polymeric micelles (down to ca. 30 nm) favors targeting of solid tumors by the EPR effect [6,17]. Ideally, drug release occurs after tumor accumulation and intracellular uptake, controlled by pH or lysosomal enzymes that cleave oligo(peptide) spacer group between drug and polymer. While there are numerous examples of tumor targeting of nanomedicines by the EPR effect in pre-clinical studies, clinical results on polymeric micelles are now just emerging.

2 Drug solubilization

Several different kinds of ABCs have been widely studied for drug solubilization that enables safe IV administration of anticancer agents, including PEG-*b*-PLA, PEG-*b*-poly(propylene glycol)-*b*-PEG (i.e., Pluronics®), PEG-*b*-poly(ε-caprolactone) (PEG-*b*-PCL) and PEG-*b*-poly(α-amino acid) (Fig. 3). Proven safety of ABCs is paramount for IV

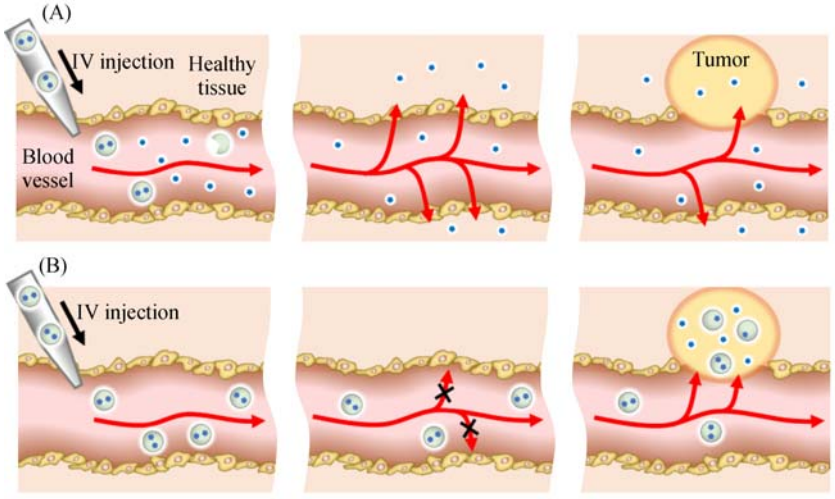

Fig. 2 Key steps in drug delivery via unstable and stable polymeric micelles. (A) Unstable polymeric micelle; (B) Stable polymeric micelle

administration, along with other major requirements that include sterility, stability, solubility and scale-up. ABCs tend to disrupt cellular membranes less than low molecular weight surfactants, such as Cremophor EL, and undergo renal clearance that limits accumulation in the body, increasing safety. While the primary driving force for drug solubilization is hydrophobic interaction, polarity and hydrogen bonding play parts in governing interaction between drug and core region of polymeric micelles. Thus, structural diversity of core-forming blocks of ABCs permits drug solubilization of a variety of poorly water-soluble anticancer agents; for example, camptothecin, paclitaxel, resveratrol and valspodar (Fig. 3), increasing water solubility by 10^3-fold. After IV administration, many polymeric micelles disassemble and rapidly release anticancer agents into blood (Fig. 2(A)), and their chief advantage as a nanocarrier is low toxicity relative to existing drug vehicles, such as Cremophor EL and ethanol found in first formulation of paclitaxel called Taxol® and many other anticancer agents in pre-clinical and clinical development.

2.1 Genexol-PM®

The low toxicity of polymeric micelles in drug delivery is best exemplified by paclitaxel-loaded PEG-*b*-PLA

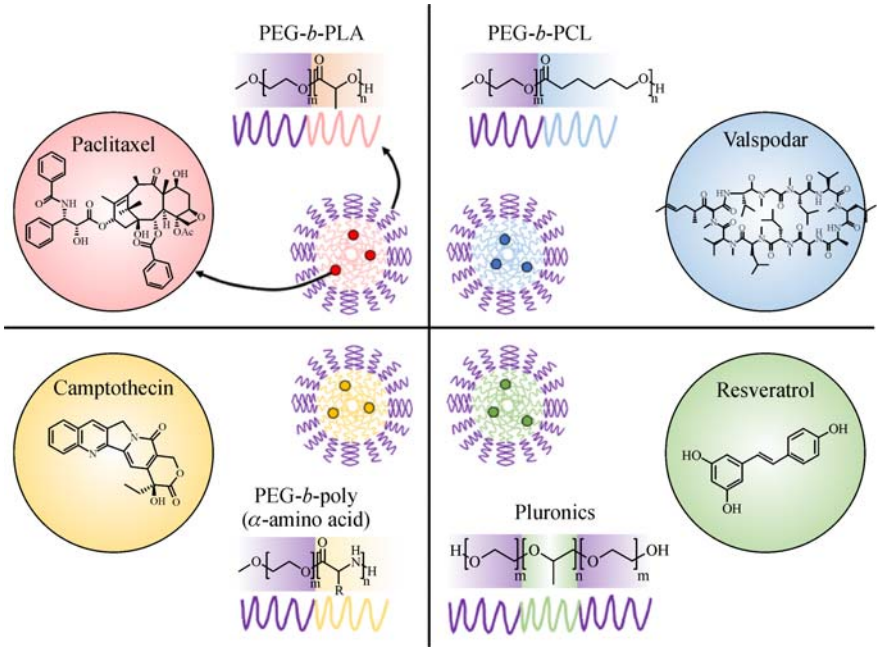

Fig. 3 Examples of polymeric micelles for drug solubilization

micelles, termed Genexol-PM® [18,19]. Paclitaxel stabilizes microtubules, induces G2/M arrest and causes cancer cell apoptosis. Taxol® has been used widely to treat lung, breast and ovarian cancers, noting sales of Taxol® in 2000 of ca. $1.6 billion. However, paclitaxel has low water solubility, ca. 0.3 mg/L, being relatively large (853 g/mol), hydrophobic and nonionizable (Fig. 3). At PEG and PLA blocks at 2000 and 1750 g/mol, respectively, PEG-*b*-PLA assembles into polymeric micelles containing paclitaxel at 20% wgt drug/wgt polymer. Paclitaxel gains water solubility > 1.0 mg/mL as small polymeric micelles, ca. 30 nm in diameter. Genexol-PM® is an approved product in Asia, developed by Samyang Biopharm in South Korea.

The MTD of Genexol-PM® and Taxol® in nude mice was 60 and 20 mg/kg, respectively, leading to higher antitumor efficacy in murine tumor models [18]. After IV injection of Genexol-PM®, paclitaxel appeared to be rapidly released from PEG-*b*-PLA micelles (Fig. 4), have a high peak plasma level (C_{max}), distribute widely and undergo rapid clearance by hepatic metabolism and biliary excretion. In a phase 1 clinical trial of Genexol-PM®, the MTD of paclitaxel was 300 mg/m^2 infused every 3 weeks, and dose-limiting toxicities were neuropathy and myalgia [20]. The MTD of paclitaxel as Taxol® is 175 mg/m^2 infused every 3 weeks. Out of 21 patients with advanced malignancies, there were 3 partial responses for Genexol-PM®. Consistent with rodent studies, paclitaxel as Genexol-PM® was cleared more rapidly than Taxol®, possibly contributing to a low incidence of myelosuppression.

Genexol-PM® was recently licensed by a US company, Sorrento Therapeutics Inc., and became Cynviloq™. Cynviloq™ has entered a phase 3 clinical trial versus nanoparticle albumin (*nab*)-paclitaxel (Abraxane®) [19]. Termed TRIBECA™ (TRIal establishing bioequivalence (BE) between Cynviloq™ and Abraxane® a comparative bioequivalence study has been designed to be a randomized, multi-center study of Cynviloq™ and Abraxane® at 260 mg/m^2 for female patients with metastatic or locally recurrent breast cancer. Abraxane® is approved for breast, non-small cell lung, and pancreatic cancers and it is in clinical trials for melanoma, ovarian and bladder cancers [21]. *Nab*-paclitaxel is an amorphous nanoparticle of paclitaxel, ca. 120 nm, coated and stabilized by serum albumin (Fig. 4). After IV infusion, amorphous paclitaxel dissolves rapidly due to the presence of serum albumin in blood, resulting in rapid clearance and short plasma half-life, $t_{1/2}$. After IV infusion of Abraxane® into pigs at 300 or 900 mg/m^2 over 30 min, no nanoparticles were detected in plasma by dynamic light scattering measurement, indicating rapid dissolution of amorphous paclitaxel [22]. Cynviloq™ and Abraxane® are less toxic than Taxol®, permitting dose escalation of paclitaxel and higher tumor levels. They do not require pre-medication that is needed for Taxol® to prevent hypersensitivity reactions. They are also similar in having a higher clearance, CL, and higher volume of distribution, V_d, than Taxol®, consistent with rapid release of paclitaxel (Fig. 4). In summary, Cynviloq™ represents a major milestone for polymeric micelles as an alternative paclitaxel nanomedicine without serum albumin, a blood product.

3 Poorly water-soluble drug combinations in cancer research

Drug combinations are common in cancer treatment, and they are evolving from chemotherapy cocktails to drug combinations that involve standard-of-care chemotherapy and signal transduction inhibitors, aiming to shut down aberrant signal pathways shown to be involved in drug resistance [23]. Drug combinations also involve just signal transduction inhibitors that target aberrant signal pathways based on our increasing knowledge of cancer biology,

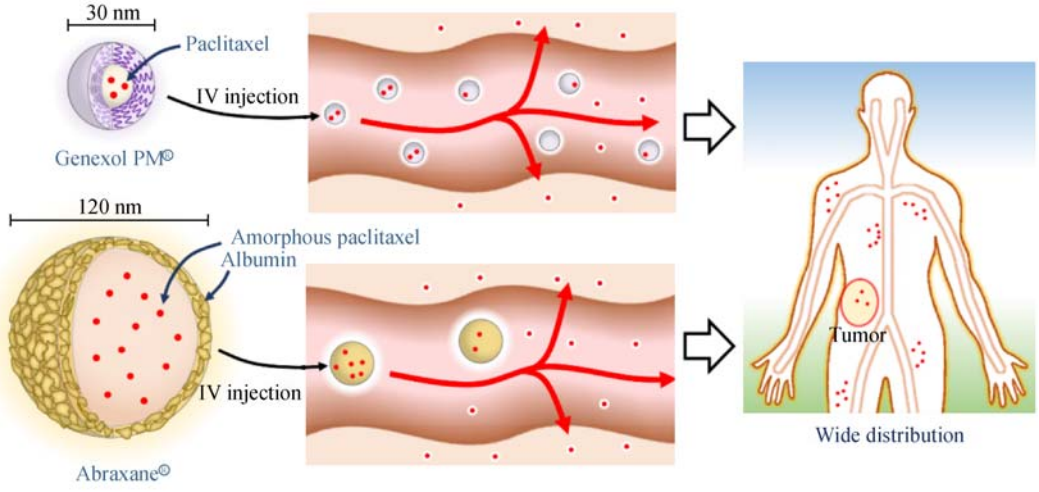

Fig. 4 Rapid release of paclitaxel after IV injection of Genexol-PM® and Abraxane®

aiming for greater selectivity and low toxicity relative to chemotherapy [24]. Chemotherapy and signal transduction inhibitors often lack sufficient water solubility for *in vivo* testing and require drug solubilization, and Cremophor EL is commonly used in pre-clinical and clinical development of anticancer agents. Unfortunately, it contributes to toxicity on top of toxicities of anticancer drug combinations that while often non-overlapping, still hinder dose escalation and clinical progress.

Fortunately, *nab*-technology and PEG-*b*-PLA micelles have emerged as alternatives to Cremophor EL in drug development. Besides *nab*-paclitaxel, there are *nab*-docetaxel (microtubules), *nab*-rapamycin (mTOR), *nab*-17-AAG (Hsp90) and *nab*-thiocolchicine (vascular targeting agent). Thus, *nab*-technology can be used in drug combinations involving these anticancer agents and may be aplied to anticancer agents in pre-clinical drug development. The major caveat is that anticancer agent has to be stabilized in an amorphous state by serum albumin. Besides paclitaxel, 17-AAG and rapamycin, PEG-*b*-PLA micelles have solubilized bicalutamide (androgen receptor), *β*-lapachone (NQO1), etoposide (topoisomerase II), sagopilone (microtubules), suberoylanilide hydroxamic acid (HDAC) and thiocoraline (DNA polymerase) [19].

Drug combination (co)-delivery with PEG-*b*-PLA micelles and/or *nab*-technology may involve sequential IV infusion or perhaps concurrent IV infusion of an admixture, assuming compatibility. For example, Genexol-PM® and a 17-AAG-loaded PEG-*b*-PLA micelle, defined as 2-in-2 micelles, may be safely mixed and infused together as an IV admixture. In contrast, Taxol® and tanespimycin, 17-AAG solubilized by a DMSO/lipid vehicle, were dosed by sequential IV infusion, and DMSO was toxic in a phase 1 clinical trial [25]. Besides single agent drug solubilization for drug combination, PEG-*b*-PLA micelles have a capacity as a nanocarrier for multiple poorly water soluble anticancer agents. Co-delivery can be accomplished as a single manufacturing process, followed by a single IV infusion, aiming for concurrent tumor exposure. In this scenario, toxicity of concurrent drug exposure is offset by PEG-*b*-PLA micelles replacing toxic solubilizing agents, such as Cremophor EL. In summary, PEG-*b*-PLA micelles and *nab*-technology are attractive options for poorly water-soluble drug combinations, owing to low toxicity relative to Cremophor EL, and it is expected that they will have a clinical impact in cancer as part of emerging drug combination strategies in drug development.

A PEG-*b*-PLA micelle has a capacity for 3 poorly water-soluble anticancer agents: Paclitaxel, 17-AAG and rapamycin (termed Triolimus), enabling concurrent IV infusion and co-delivery (Fig. 5). While, 17-AAG and rapamycin are largely cytostatic towards cancer cells, they help to increase apoptosis of cancer cells caused by paclitaxel [26,27]. Rapamycin inhibits mTOR that is aberrantly activated in many cancers including lung and breast, and rapamycin analogs have been approved and combined with paclitaxel along with trastuzumab in a phase 3 clinical trial [28], aiming for synergistic anticancer activity. However, several mechanism of resistance toward rapamycin have been identified, such as activation of upstream Akt and MAPK (Ras/Raf/Mek/Erk) signaling pathway [29]. Hsp90 inhibition by 17-AAG simultaneously degrades client proteins that rely on Hsp90 for correct folding and activity, and these client proteins include a major list on oncogenic proteins, such as Akt, Raf-1, ErbB-2, mutant p53 and HIF-1. Thus, co-delivery of paclitaxel, rapamycin and 17-AAG by PEG-*b*-PLA micelles represents a promising and rationale drug combination strategy for cancer treatment.

At PEG and PLA blocks at 4000 and 2000 g/mol, respectively, PEG-*b*-PLA micelles loaded with paclitaxel, 17-AAG and rapamycin were about 40 nm in diameter, stable at room temperature against precipitation, and could easily achieve mg/mL of all 3 anticancer agents [30]. Remarkably, drug levels achieved as a 3-in-1 micelle were similar to the values achieved as 1-in-1 micelles, presumably owing to drug-drug interaction in cores of PEG-*b*-PLA micelles. Triolimus was synergistic against several human cancer cell lines based upon combination index analysis and was far superior to paclitaxel-loaded PEG-*b*-PLA micelles in A549 non-small cell lung cancer

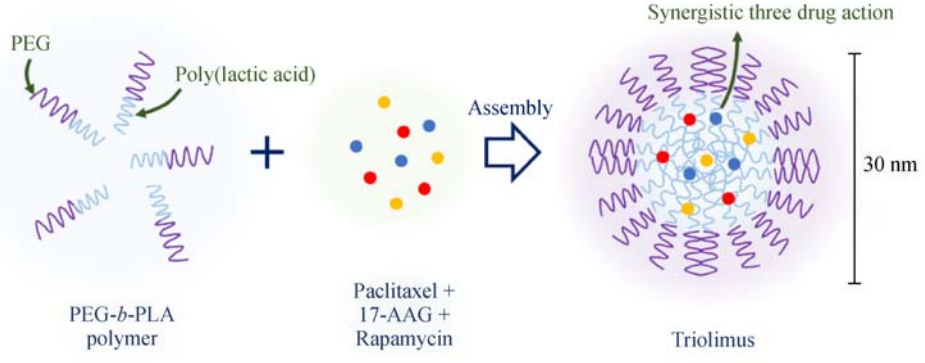

Fig. 5 Schematic illustration of Triolimus

(NSCLC) and MDA-MB-231 breast tumor models [31]. Notably, the MTD of Triolimus was 60, 60 and 30 mg/kg for paclitaxel, 17-AAG and rapamycin, respectively, versus 60 m/kg for paclitaxel-loaded PEG-*b*-PLA micelles, indicating non-overlapping toxicity profiles. More recently, Triolimus has been shown to be more effective than paclitaxel in murine tumor models of angiosarcoma, a rare cancer of blood vessels [19]. As a result, Triolimus has gained orphan drug status by the Food and Drug Administration (FDA) in the USA, providing financial incentives for the pre-clinical and clinical development for angiosarcoma and subsequently broader evaluation in other cancers.

Triolimus builds on progress of Genexol-PM® by offering a novel IV admixture of paclitaxel, 17-AAG and rapamycin, safely enabling a rationale 3-drug nanomedicine for clinical development. One limitation of Triolimus is rapid multi-drug release from PEG-*b*-PLA micelles after IV injection, consistent with earlier *in vivo* studies on Genexol-PM® [32]. In this case, drug interaction via metabolic enzymes, e.g., cytochrome P450, may increase drug levels and has to be characterized in the context of drug development. In summary, Triolimus represents the first example of a 3-in-1 nanomedicine for cancer treatment and moves us closer to the opportunity of concurrent tumor targeting of drug combinations.

4 Reduced off-target toxicity

While many injected or infused polymeric micelles containing anticancer agent are diluted in blood and rapidly disassemble, such as PEG-*b*-PLA and Pluronics® (Fig. 2(A)), there are several studies that show that disassembly of polymeric micelles may be kinetically-controlled versus thermo-dynamically controlled, leading to prolonged circulation in blood, reduction in off-target distribution and the EPR effect (Fig. 2(B)) [33–36]. Polymeric micelles may have highly hydrophobic cores that may be partially crystalline or glassy, and while dilution in blood favors disassembly, it may occur gradually over several hours. Coupled with slow drug release, long-circulating polymeric micelles have a major impact on the PK of anticancer agents in pre-clinical experiments, and there are good examples of polymeric micelles that have reduced tissue distribution and reduced off-target toxicity (Fig. 2(B)).

4.1 NK911

NK911 is an ABC that has PEG (5000 g/mol) and poly (aspartic acid) (30 units) with chemically bound doxorubicin (topoisomerase II), and it forms polymeric micelles that physically encapsulate doxorubicin (Fig. 6). While doxorubicin is not highly hydrophobic (log$P$ = 0.52), stability with respect to disassembly and drug release was imparted by π-stacking of doxorubicin besides hydrophobic interaction. As a result, NK911 was long-circulating in blood, reduced distribution of doxorubicin to non-target tissue and effectively delivered physically encapsulated ^{14}C-doxorubicin to solid tumors in a C26 colon adenocarcinoma xenograft model [33]. NK911 showed selective accumulation over doxorubicin at solid tumors versus in normal organs, such as the heart (site of cardiotoxicity): Tumor-to-heart ratio 24 h post-injection was 4.9 and 1.3, respectively. After 24 h, tumor level of ^{14}C-doxorubicin as NK911 and free drug was ca. 10% and ca. 1% dose per gram tumor, respectively.

In a phase 1 clinical trial, the area under the plasma curve (AUC) of NK911 was 2-fold higher than free doxorubicin at 50 mg/m^2 [34]. However, the plasma AUC was > 100-fold lower than Doxil®, and CL was 400-fold higher. Thus, exposure of doxorubicin as NK911 runs in between Doxil® and free doxorubicin in humans. It is noted that the NK911 evaluated in the phase 1 clinical trial was not identical to earlier pre-clinical versions of NK911 that contained dimeric doxorubicin that appeared to stabilize micelles against disassembly, but had to be removed due to precipitation upon prolonged storage. NK911 was well tolerated, producing dose-limiting neutropenia, moderate nausea and vomiting, but provided minor improvement in PK for doxorubicin, owing to the removal of dimeric species. NK911 entered a phase 2 clinical trial for pancreatic cancer, but it was terminated with no further

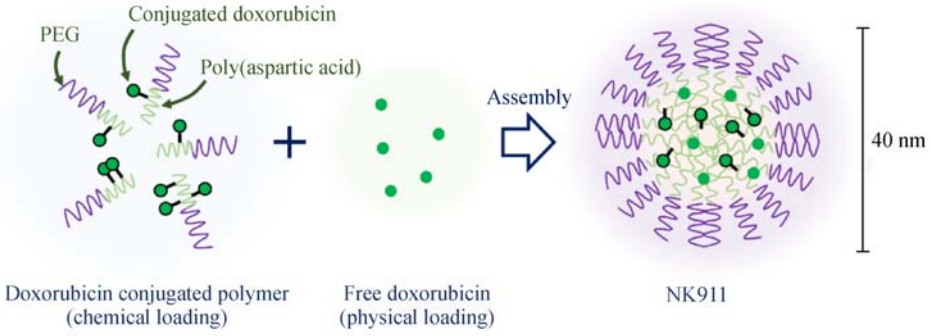

Fig. 6 Schematic illustration of NK911

update. In summary, NK911 is a good early example of a long-circulating polymeric micelle that reduced doxorubicin toxicity relative to free drug, but its termination points to production challenges in manufacturing for clinical trials.

4.2 PEG-*b*-PCL micelles

PEG-*b*-PCL micelles have been widely studied for drug delivery *in vitro* and *in vivo*, particularly for drug solubilization (Fig. 3), and prolonged circulation in blood has been evidenced in rodents [35,36]. PEG-*b*-PCL with PEG and PCL at 5000 and 5000 g/mol, respectively, formed 40 nm polymeric micelles that were dosed at 250, 2 and 0.2 mg/kg in mice. At 0.2 mg/kg, below CMC, PEG-*b*-PCL unimers were rapidly eliminated, probably by renal CL, and at > CMC, i.e., 250 mg/kg, PEG-*b*-PCL micelles were long-circulating in blood and showed low tissue distribution (Fig. 7). At 2 mg/kg, while below CMC, PEG-*b*-PCL micelles showed kinetic stability attributed to partial core crystallinity and displayed long blood circulation, although not as prolonged as at 250 mg/kg. In summary, PEG-*b*-PCL micelles are long-circulating in blood, but stability is dependent on dose relative to CMC.

PCL like PLA is a biodegradable poly(α-hydroxy acid), but is much more lipophilic, solubilizing highly lipophilic drugs, such as cyclosporine A. Besides being a potent immunosuppressive agent, cyclosporine A is also a potent inhibitor of P-glycoprotein, which is at least partially responsible for multi-drug resistance in cancer. However, cyclosporine A distributes widely into tissues such as the kidneys, causing dose-limiting renal toxicity. Further, cyclosporine A is poorly water-soluble and requires Cremophor EL for IV injection. In a PK study in Sprague-Dawley rats, PEG-*b*-PCL with PEG and PCL at 5000 and 13000 g/mol, respectively, formed 100 nm polymeric micelles containing 20% wgt drug/wgt polymer, resulting in ca 1.0 mg/mL water solubility [36]. PEG-*b*-PCL micelles increased the AUC of cyclosporine A by 6.1-fold over Cremophor EL and reduced V_d and CL by 10- and 7.6-fold, respectively (Table 1). Further, kidney distribution of cyclosporine A was reduced by 1.4-fold. Accordingly, PEG-*b*-PCL micelles reduced the renal toxicity of cyclosporine A in a repeat dose study in rats, whereas Cremophor EL (Sandimmune®) decreased creatinine CL as a marker of kidney toxicity [37]. More recently, a non-immuno-suppressive analog of cyclosporine A, valspodar, has been loaded into PEG-*b*-PCL micelles (Fig. 3) and shown to increase AUC by 1.8-fold and decrease V_d and CL by 1.5- and 1.3-fold, respectively [38]. Given that valspodar and cyclosporine A share structural similarity, it was surprising to find differences in their PK profiles in rats, pointing to difference in intermolecular interaction between the drugs and the PCL core and a difference in drug release rate. In summary, PEG-*b*-PCL micelles are long-circulating in blood and may cause dramatic changes in PK of lipophilic drugs such as cyclosporine A, increasing AUC and lowering V_d, CL and renal toxicity. However, changes in PK due to PEG-*b*-PCL micelles must be proven in rodents and cannot be based solely on *in vitro* experiments or based on structural similarity of drugs.

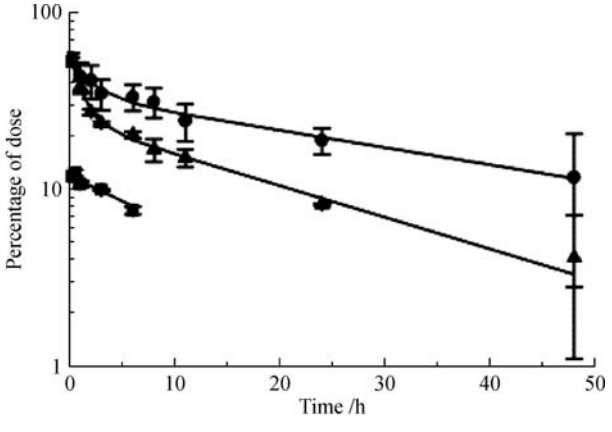

Fig. 7 The plasma clearance of PEG_{5000}-*b*-PCL_{5000} micelles in Balb/C mice (n = 3, SD shown as error bars) following intravenous injection at a dose of 250 mg/kg (●, concentration of copolymer above CMC upon dilution following administration) 2 mg/kg (▲, concentration of copolymer above CMC prior to administration but falls below CMC upon dilution) or 0.2 mg/kg (■, copolymer unimers). The plasma concentration data for all groups were fit using compartmental open models by Scientist software and are shown as solid lines [35]

5 Tumor targeting by the EPR effect

As proposed by Matsumura and Maeda [39], long-circulating macromolecules preferentially extravasate and accumulate at solid tumors through leaky vasculature, and an absence of functional lymphatic drainage impedes CL of macromolecules from solid tumors (Fig. 2(B)). The EPR effect has served as the primary justification for many nanomedicines studied in cancer research, and many of the physicochemical and biological factors governing tumor targeting by the EPR effect have been characterized [6–10]. While there is certainly solid evidence for the EPR effect by nanomedicines such as polymeric micelles in murine tumor models, these results have not translated into higher antitumor efficacy in clinical trials in humans [16]. Instead, a reduction in toxicity of anticancer agents by nanocarriers (vide supra) seems to be the primary outcome to date. While reasons for the limited clinical impact of long-circulating nanomedicines is being debated, factors such as tumor heterogeneity and high intratumoral fluid pressure are formidable barriers that certainly come into play and strategies beyond the EPR effect are warranted. Nonetheless, long-circulating polymeric micelles have entered clinical trials in humans, and they are a logical

Table 1 Non-compartmental pharmacokinetic parameters (± SD) of cyclosporin A after intravenous administration of cyclosporin A in polymeric micellar formulation in comparison to cyclosporin A in Cremophor EL (Sandimmune®) formulation[a)] [3]

	Cyclosporin A in Cremophor EL	Cyclosporin A in polymeric micelles
AUC_{0-24} /(μg·h·mL^{-1})	25.3 ± 7.64	167 ± 18.8[b)]
$AUC_{0-\infty}$ /(μg·h·mL^{-1})	32.7 ± 13.8	199 ± 20.9[b)]
$t_{1/2}$ /h	11.5 ± 4.58	9.40 ± 1.20
MRT /h	14.4 ± 6.62	9.24 ± 2.06
CL /(L·kg^{-1}·h^{-1})	0.195 ± 0.131	0.0255 ± 0.00319[b)]
V_{dss} /(L·kg^{-1})	2.33 ± 0.785	0.232 ± 0.0425[b)]

a) Micellar data is normalized to injected dose of Cremophor EL and presented as mean ± SD (n = 4); b) Denotes significant difference between groups

step in the progression to effective cancer treatment beyond the current scope of chemotherapy.

5.1 NK105

NK105 is based on an ABC that has PEG (12000 g/mol) and poly(aspartic acid) (8000 g/mol) modified with 4-phenyl-1-butanol, and its polymeric micelles physically encapsulate paclitaxel (Fig. 8) [40]. Various hydrophobic side chains were screened for paclitaxel, aiming for high drug loading and slow drug release. In this way, this ABC was tailor-made for paclitaxel. About 50% of the aspartic acids were modified with 4-phenyl-1-butanol, resulting in 23% wgt drug/wgt polymer and 85 nm paclitaxel-loaded polymeric micelles. In contrast with Genexol-PM®, dramatic changes in PK were revealed in pre-clinical experiments: 90-fold increase in plasma AUC and a 35-fold increase in tumor AUC versus free paclitaxel (Fig. 9). As a result, NK105 showed potent antitumor efficacy over free paclitaxel in a HT-29 colorectal xenograft model. It is noted that peripheral neurotoxicity is a major dose-limiting toxicity of paclitaxel, and this study showed reduced neurotoxicity of paclitaxel as NK105 versus free paclitaxel based on histochemical and physiological analysis. This result might be due to reduced distribution of paclitaxel as NK105 into non-targeting tissue, noting a V_d that was 100-fold lower than free paclitaxel.

In a phase 2 clinical trial in patients with previously treated, advanced or recurrent gastric cancer, NK105 was dosed at 150 mg/m^2 every 3 weeks without pre-medication [41]. Notably, the MTD of NK105 was lower than Genexol-PM® and Abraxane®, caused by myelosuppression. Major adverse effects from NK105 were grade 1 or 2 in severity. The AUC of paclitaxel as NK105 was 9-fold higher than Taxol®, dosed at 210 mg/m^2 every 3 weeks. Consistent with an earlier pre-clinical study, only 1 patient out of 57 experienced grade 3 neuropathy (1.8%). By contrast, 10% and 11% of patients on Taxol® and Abraxane®, respectively, experienced grade 3 or 4 neuropathy in a phase 2 setting. These results indicate a slow release nanocarrier of paclitaxel such as NK105 reduces peripheral neuropathy at the expense of myelosuppression. The overall response rate of patients with gastric cancer from NK105 was 25%, and median progression-free survival was 3.0 months. Compared to earlier phase 2 clinical trials on other anticancer agents that showed response rates at 12% to 32%, antitumor efficacy of NK105 was modest, considering earlier pre-clinical studies. A phase 3 clinical trial on NK105 is ongoing in patients with metastatic or recurrent breast cancer and will assess survival benefit.

5.2 PEG-*b*-poly(aspartate-hydrazone-doxorubicin)

To minimize drug loss during transit in blood, a PEG-*b*-poly(aspartate-hydrazone-doxorubicin) (p(Asp-Hyd-DOX)) with PEG at 12000 g/mol and 40 units of aspartic acid was coupled with doxurubicin by a pH-sensitive

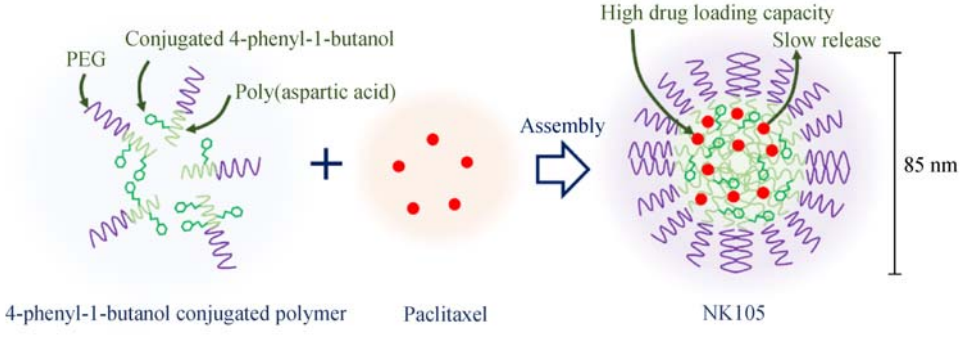

Fig. 8 Schematic illustration of NK105

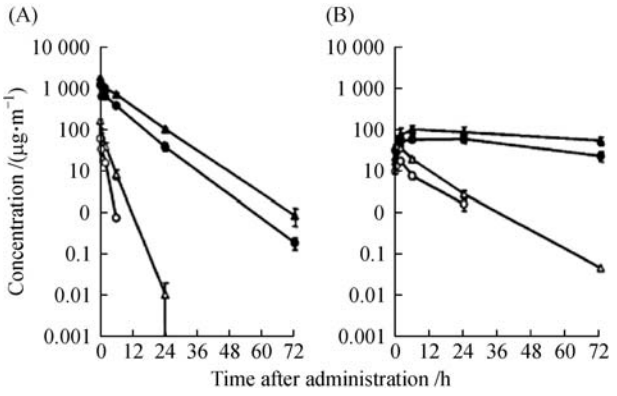

Fig. 9 Plasma and tumor concentrations of paclitaxel after single i.v. administration of NK105 or paclitaxel to Colon 26-bearing CDF1 mice. Plasma (A) and tumor (B) concentrations of paclitaxel after NK105 administration at a paclitaxel-equivalent dose of 50 mg/kg (●), NK105 at a paclitaxel-equivalent dose of 100 mg/kg (▲), paclitaxel 50 mg/kg (○) and paclitaxel 100 mg/kg (△) [40]

hydrazone bond at a 70% degree of substitution, and the ABC assembled into a polymeric micelle at ca. 65 nm in diameter (Fig. 10) [42]. PEG-*b*-p(Asp-Hyd-DOX) micelles were stable at physiological pH, but released doxorubicin at an acidic pH associated with late endosomes and/or lysosomes in cells (pH = 5). At 10 mg/kg, blood and tumor AUC of doxorubicin from PEG-*b*-p(Asp-Hyd-DOX) micelles increased 15- and 4-fold, respectively, relative to free doxorubicin, and distribution of doxorubicin to the heart was reduced [43]. Mice were safely treated with PEG-*b*-p(Asp-Hyd-DOX) micelles at 40 mg/kg, compared to 10 mg/kg for free doxorubicin (17% body weight change on day 30). Accordingly, doxorubicin at 10 mg/kg delayed tumor growth for 15 days, whereas PEG-*b*-p(Asp-Hyd-DOX) micelles at 40 mg/kg caused a 4% body weight change on day 30, delayed tumor growth for 28 days and caused complete cures in 3/6 mice. In summary, PEG-*b*-p(Asp-Hyd-DOX) micelles are long-circulating in mice, target tumors by the EPR effect and reduce off-target distribution by controlled, pH-sensitive release of anthracyclines.

More recently, several interesting tactics have emerged for this class of pH-sensitive polymeric micelles [44–46]. For a phase 1 clinical trial, epirubicin replaced doxorubicin as a less toxic anthracycline, and the core-forming p(Asp) block was partially substituted with hydrophobic benzyl groups to stabilize the PEG-*b*-p(Asp-Hyd-epirubicin) micelle, termed NC-6300 [44]. For stability in blood, hydrazone or disulfide crosslinked polymeric micelles have been synthesized, aiming for higher tumor accumulation [45,46]. For tumor targeting of a fixed ratio drug combination, mixed prodrug polymeric micelles based on PEG-*b*-p(Asp-Hyd-DOX) have been prepared [47].

6 Conclusions

Polymeric micelles are a major class of anticancer nanocarriers, owing to proven safety in humans and drug solubilization, and there are several examples that have shown reduced off target effects and tumor targeting by the EPR effect. PEG-*b*-PLA micelles clearly show advantages for drug solubilization over Cremophor EL, permitting dose escalation of paclitaxel, a key anticancer agent that is standard-of-care for many cancers. The diversity of ABCs permits drug solubilization for a range of anticancer agents and permits testing of emerging drug combinations without or with minor toxicity, contributed by ABCs. Kinetically-stable polymeric micelles such as PEG-*b*-PCL micelles are long-circulating in blood and may provide slow or controlled drug release, minimizing drug distribution to normal organs and lowering off-target toxicity. Thus, polymeric micelles may reduce toxicity in two major ways, being biocompatible and lowering off-target biodistribution. For anticancer drug combinations, these are important contributions as we seek synergistic anticancer efficacy while minimizing host toxicity.

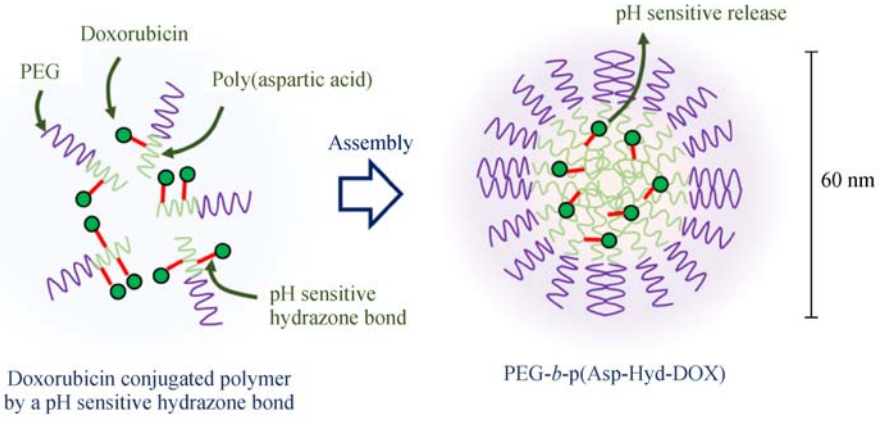

Fig. 10 Schematic illustration of PEG-*b*-p(Asp-Hyd-DOX)

The clinical impact of tumor targeting of polymeric micelles by the EPR effect, e.g., NK105, remains to be established. As shown for NK105, while peripheral neurotoxicity was reduced by long-circulating polymeric micelles that gradually release paclitaxel in blood, dose-limiting myelosuppression emerged, and the dose of paclitaxel could not be escalated beyond that of Taxol®. Thus, NK105 relies primarily on the EPR effect and not dose-escalation for anticancer efficacy, contrasting with Genexol-PM®. The best polymeric micelle for paclitaxel in humans is uncertain. Given the structural diversity of ABCs, controlled release and crosslinking, polymeric micelles can be tailor-made for poorly water-soluble anticancer agents. While clinical impact has been modest, long-circulating polymeric micelles will continue to be researched as versatile nanocarriers for drug targeting by the EPR effect. In future directions, targeting strategies will likely involve monoclonal antibodies as targeting ligands for polymeric micelles [48].

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